SELECTIVE DOPAMINE AGONISTS IN MAN AND THE MPTP-TREATED PRIMATE MODEL

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A thesis submitted to the Faculty of Medicine,
University of the Witwatersrand, Johannesburg,
for the degree of Doctor of Philosophy (Medicine)

Johannesburg: December 1991

To my wife Louise, thanking you for your encouragement,
wisdom and patience

To my parents, William Arnot and Rosemary Joy Temlett,
for their guidance and support over the years
and

To my children Richard, Julia, Jacqui-Louise
and especially the memory of Michelle,
(16-12-1990 to 15-3-1991)
who make it all worthwhile

ABSTRACT

Idiopathic Parkinson's disease remains one of the commonest neurodegenerative diseases known today. It causes incapacitating symptoms untreated but when given replacement neurotransmitters, principally levodopa or dopamine, corrects the major features of the illness. The fundamental cause of nigral dopaminergic cell decline remains unknown, is not principally genetic, but may be due to abnormal hepatic handling of neurotoxins. One such putative neurotoxin is MPTP which causes parkinsonism in man and primates. The MPTP-treated primate model is thus a useful model within which new drugs, including dopamine receptor agonists may be tested.

Levodopa remains the mainstay of successful pharmacotherapy in the treatment of Parkinson's disease. However the last decades have taught us that levodopa treatment with time produces problems of dyskinesias and unpredictable motor fluctuations. Hence alternate pharmacotherapy is sought to supplement levodopa or possibly to circumvent its necessity. The dopamine selective agonists are a major player in this strategy and this background encouraged

analysis of novel dopamine D-1 receptor compounds (SKF 38393 and CY 208-243) and dopamine D-2 receptor compounds ((+)-PHNO, CQA 206-241, LY 171555 and bromocriptine), in the MPTP treated primates and in man with idiopathic Parkinson's disease.

Clearly these agents all possess individual antiparkinsonian activity but suitable combinations were also sought. The reason this was done was to further explore the role of D-1 and D-2 dopamine receptor interplay at various stages in the natural progression of nigrostriatal decline.

One D-2 dopamine agonist, namely bromocriptine has been evaluated for more than 10 years. Patients were tested with this dopamine agonist in combination with levodopa in moderately advanced Parkinson's disease and found useful benefit as an anti-akinetic agent but few patients were able in addition, to stop their levodopa. A long acting levodopa preparation (Sinemet CR4) was also given to patients but both of these initial trials completed by 1987 suggested severe limitations and neither strategy reduced levodopa demand. Indeed the Sinemet CR4 trial patients required more total daily levodopa.

Selective dopamine receptor agonists and antagonists were sought to better evaluate the role of these compounds individually as well as assess concurrent dopamine receptor

subpopulations (in this thesis the D-1 and D-2 receptors). The MPTP-treated primate is a useful animal model in which to address some of these issues. MPTP-treated marmosets were thus used to test CQA 206-291 (D-2 selective) and CY 208-243 (D-1 selective) antiparkinsonian drug efficacy.

These novel compounds were tested with success in the MPTP-treated primate model hence (+)-PHNO, CQA 206-291, and CY 208-243 thereafter were applied to man with idiopathic Parkinson's disease, complicated by on-off fluctuations. These patients were taken off their levodopa while under supervision in hospital and once the drug was applied any intrinsic antiparkinsonian activity easily observed and recorded.

The D-2 agonists were similar in action to both bromocriptine and levodopa. They demonstrated safe antiparkinsonian motor activity and were associated with dyskinesias. The D-1 agonist CY 208-243, but not SKF 38393, was also a good anti-akinetic compound, may also produce dyskinesias in advanced Parkinson's disease, and synergises D-2 dopamine agonist drug action.

Since the discovery of the D-3 limbic dopamine receptor, and possibly other dopamine receptors, the field of drug development remains wide open to creation and testing of new selective compounds. What still remains unknown, is

the best combination of D-1, D-2 and now the D-3 subtype dopamine receptor stimulation. Many agents stimulate dopamine receptors in various percentages and one objective in this thesis was to seek a desirable strategy to best explore antiparkinsonian benefit without side effects. The MPTP-treated primate model showed that many of these novel dopamine agonists were potent. Since animal toxicological safety standards have prevented further clinical trials using many of these compounds, chemicals with similar structures, should be further developed to improve the therapeutic options best available in the therapy of Parkinson's disease.

ACKNOWLEDGEMENTS

I thank Professor C DAVID MARSDEN, (The National Hospital for Neurology and Neurosurgery and Institute of Neurology - London, UK), who supervised this thesis from its inception, for his enthusiasm and guidance over the years.

Professor VIVIAN FRITZ, (University of the Witwatersrand and Johannesburg Hospital - Johannesburg), who supported and encouraged my interest in research and who locally supervised me.

Professor HARRY REEF, (University of the Witwatersrand and Johannesburg Hospital), who teaches clinical neurology so very well and who initially stimulated my interest in movement disorders

Professor PETER JENNER, (King's College Campus and University of London - London, UK), for supervision and advice with the primate work.

My colleagues NIALL QUINN, RICHARD COLEMAN, PIANG-CHONG, WOLFGANG OERTEL, PHILLIP THOMPSON (LONDON)
and ALEC MING, SEAN LILIENFELD, JOHN KENT, ANDY BLUMENFELD, TANIA BILCHIK (JOHANNESBURG), who co-worked with me on various projects.

My wife LOUISE TEMLETT, who typed the manuscript; MRS ISABEL COOK, who typed references. MRS CAROL CARDOSO and MISS JILL VAN VEEN, proof read the chapters.

The photo illustration Unit of the University of the Witwatersrand, particularly ALISTAIR WEBB, CAROL CARDOSO, TERRY BORAIN, CHARLES MAY and ROY HOLLIDAY.

The South African Medical Research Council, for funding a post doctoral Scholarship in 1987-8, where time was spent under the auspices of PROF C DAVID MARSDEN, at London University and Kings College Hospital Medical School, The Maudsley Hospital and The National Hospital for Nervous Diseases, Queen Square, London;

and particularly the patients suffering from Parkinson's disease, who taught me so much and who allowed themselves to be studied and upon whom new unknown drugs were evaluated.

DECLARATION

Permission was granted in 1987 by the Home Office, United Kingdom (No: PIL 70/01273, 10th March 1987); and both the Human Ethics Committee (No: 14/3/87, 27th March 1987) plus Animal Ethics Committee (No: 88/145-5, 29th October 1988) of the University of the Witwatersrand, to complete these experiments.

This thesis is my own, unaided work.
It is being submitted for the degree of Doctor of Philosophy (Medicine) in the University of the Witwatersrand, Johannesburg, and has not been submitted before, for any degree or at any other University.

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16th December 1991.

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ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase
A	adrenaline
Aq	aqueduct of Silvius
C	caudate nucleus
CCK	cholecystokinin
CG	central gray
ChAT	Choline acetyl transferase
Cm	centrum medianum thalamus
CNS	central nervous system
COMT	catechol-O-methyl transferase
CPH	carboxypeptidase H
cp	cerebral peduncle
CPu	caudate-putamen
CQA 206-291	[5R(5ß, 8α)]-N,N-Diethyl -N-(1-ethyl-6-methyl-ergoline-8-yl); Sandoz, Basle
CR4	Sinemet CR4, Merck, Sharpe and Dohme, Montreal; Canada
CSF	cerebrospinal fluid
CY 208-243	(-)-(6aR)(12bR)-1,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3-ab] phenanthridine; Sandoz, Basle

Cx	cerebral cortex
DA	dopamine
DAB	di-aminobenzidine tetrahydrochlorine
DAT	disease of Alzheimers type
DOPAC	dihydroxyphenylacetic acid
D-1	dopamine type 1 receptor
D-2	dopamine type 2 receptor
D-3	dopamine type 3 receptor
DP	dorsal pallidum
d str	dorsal striatum
DYN	dynorphin
EP	entopeduncular nucleus
EMG	electromyography
F	frontal cortex
FD (or [^{18}F]FD)	radiolabelled flurodopa
g	grammes
GABA	gaba-amino-n-butyric acid
GAD	gaba-amino decarboxylase
GLU	glutamate
GP	globus pallidus
GPe	globus pallidus externa
GPi	globus pallidus interna

H	habenula
HPLC	high pressure liquid chromotography
HRP	horseradish peroxidase
^{3}H dopamine	tritiated dopamine
^{3}H haloperidol	tritiated haloperidol
^{3}H SCH 23390	tritiated SCH23390
^{3}H QNB	tritiated quinuclidinyl benzilate
^{3}H sulpiride	tritiated sulpiride
^{3}H cF	tritiated cis-flupenthixol
5-HT	5-hydroxy tryptamine
5-HIAA	5,hydroxy indoleacetic acid
HLA	human leucocyte antigen
HVA	homovanillic acid
I	intralaminar nucleus
ip	intraperitoneal
IPD	idiopathic Parkinson's disease
kg	kilogrammes
l	litre
LANT-6	lys-asn-neurotensin
L-DOPA	levodopa
Leu-ENK	Leucine Enkephalin
LY 171555	quinpirole
mg	milligrammes
ml	millilitre

M	molar
MAO A	monoamine oxidase type A
MAO B	monoamine oxidase type B
Met-ENK	Methionine Enkephalin
MPTP	N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
nm	nanogrammes
nM	nanomolar
NT	neurotensin
NPY	neuropeptide Y
NA	noradrenaline
PET	positron emission tomography
PNS	peripheral nervous system
PD	Parkinson's disease
P	putamen
pM	picomolar
6OHDA	6-hydroxydopamine
po	orally
pg	picogrammes
pH	acid-base content
(+)-PHNO	(+)-4-Propyl-9-hydroxynapthoxazine; Merck, Sharpe and Dohme, Essex, England.

R	raphe nucleus
RN	red nucleus
RF	reticular formation
S	subthalamic nucleus
sc	subcutaneous
SC	superior colliculis
s.coll	superior colliculis
SCH23390	(R(+)-8chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol)
SKF38393	2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine
SNc	substantia nigra pars compacta
SNl	substantia nigra pars lateralis
SNr	substantia nigra pars reticulata
SOM	somatostatin
STh	subthalamic nucleus
SUB P	substance P
TBS	tris-buffered saline
Th	thalamus
TH	tyrosine hydroxylase
t.p.c.	pedunculopontine nucleus
ug	microgrammes
uM	micromolar
VA	ventral anterior thalamic nucleus

VL	ventral lateral thalamic nucleus
VIP	vasointestinal polypeptide
VP	ventral pallidum
v str	ventral striatum
VTA	ventral tegmental area

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CHAPTER 1

HUMAN IDIOPATHIC PARKINSON'S DISEASE

1.1 History and clinical features

In 1817, James Parkinson described the clinical features of Parkinson's disease, or idiopathic Parkinson's disease (IPD) as it is more specifically known today. We recognise the tetrad of bradykinesia, (also akinesia and hypokinesia), rigidity, resting tremor and postural impairment as comprising its major features.

While the clinical features at presentation have not changed over the last two centuries, the impact of therapy, especially levodopa, has changed not only expected longevity but also individual quality of life. Drug related side effects have furthermore complicated the anticipated disease progression, limiting the use of some drugs and creating demands to find the combination of pharmacological agents that best treat the illness. Ideally one strives to improve mobility with least risk of adverse effects.

Charçot (1877) gave an accurate account of the clinical features expected in Parkinson's disease. He pointed out that the tremor was best seen while "resting" or when the patient was seated - which contrasts with cerebellar ataxia or intention tremors. While Parkinson himself did not pay much attention to rigidity, Charcot emphasised that truncal and limb rigidity was commonly present in Parkinson's disease. Furthermore normal muscular power was noted to accompany bradykinesia of speech, handwriting, walking and all associated movements. While bradykinesia is thought today to be the major feature of idiopathic Parkinson's disease, all patients eventually develop tremor and rigidity. Loss of equilibrium with propulsion, retropulsion and start hesitation may be present. Charcot's books illustrate the typical hand posture, flexed trunk, flexed upper limbs and knees and he in addition alluded to the progressive nature of the disease.

Gowers (1888) felt Parkinson's disease was more common in males. He recorded the onset of Parkinson's disease in younger patients and periodically familial cases. The characteristic resting tremor always became bilateral. Gowers mentioned an immobile and expressionless facies, a monotonous speech, finger and hand deformities and a typical gait with a stooped posture.

Kinnear Wilson (1940), in his textbook considered Parkinson's disease a rarity with the "classical triad" of tremor rigidity and flexed attitude. He drew attention to the insidious onset of symptoms, infrequent blinking and the inevitable progression of the illness. While Wilson separated akinesia (specifically the initiation of movement) from bradykinesia (rather the slowness of an executed action) he felt they originated from a similar pathogenetic mechanism. Wilson, as did Charcot, remained unconvinced that dementia was part of idiopathic Parkinson's disease - rather bradyphrenia represented "retarded execution" of the motor thought process.

Hoehn and Yahr (1967) in their survey of the illness, suggest that age of onset assumes a gasserian distribution with a median of 55 years (range 17-80). An incidence of 20 per 100 000 and prevalence of 187 per 100 000 has been suggested (Kurkland, 1958; Martila, 1987). A sex incidence ratio of 3:2 is found with males slightly predominating. While it is thought to be much rarer in Negroid (Reef, 1977) compared with Caucasian populations, this observation is not found in North American blacks (Schoenberg et al., 1982).

Typically the illness has an insidious onset, with slowness of movement and tremor the first noted symptoms. Commonly Parkinson's disease may begin unilaterally, deteriorate and

eventually become bilateral. Initially, while patients may not show a resting tremor, they all will demonstrate bradykinesia. Classically tremor is of 4Hz frequency (range 4-7Hz), which may disappear with active movement, but always has a resting component. Additionally some patients however may demonstrate a postural tremor. Bradykinesia may be referred to as lethargy, diminished drive, stiffness, dragging of limbs or difficulty getting out of chairs or turning in bed. These symptoms may all be accentuated by depression. Often the last symptom to develop in idiopathic Parkinson's disease is postural imbalance which is aggravated by loss of confidence and may be associated with falling.

Cognitive function in idiopathic Parkinson's disease is difficult to interpret. While as many as 30% of advanced Parkinsonian patients show slowing of cognitive function, many may have concomitant Altzheimers dementia or drug induced blunting of intellect (Mayeau, 1983). The dementia is described as "sub-cortical" in nature, implying that the thought and memory recall process is simply slowed down but not factually inaccurate. While Parkinsonian patients have no memory problem, rather slowness in recall (bradyphrenia) there may be detectable difficulties in spatial perceptions (Lees and Smith, 1983; Stern 1983). It is by no means inevitable that advanced parkinsonian patients will finally dement. Hence while between 10-20% patients,

by strict criteria will appear demented, this needs more careful clarification before assuming this is an integral part of Parkinson's disease.

1.2 Diagnosis of Idiopathic Parkinson's Disease

Idiopathic Parkinson's disease differs from symptomatic parkinsonism and the "parkinson's plus" syndromes (Table 1.1). The most common cause of the rigid akinetic state is idiopathic Lewy body positive Parkinson's disease, which in addition to the typical symptom triad, demonstrates levodopa responsiveness in the early stages. Idiopathic Parkinson's disease does not show early or striking abnormalities in cognitive function, significant oculomotor, pyramidal, sensory or cerebellar abnormalities. At necropsy idiopathic Parkinson's disease shows degeneration of the pigmented neuromelanin containing nuclei, due to the death of dopamine cells in the substantia nigra. Prior to the advent of the neurotoxin N-methyl 4-phenyl 1,2,3,6 tetrahydropyridine (MPTP), the illness, predominated in the elderly population, however young onset and even juvenile cases have been rarely documented (Wilson, 1940). Drug induced parkinsonism is a problem today with widespread use of dopamine antagonists (Table 1.2). Clearly today in the younger population other syndromes such as Wilson's disease, Hallervorden-Spatz

Table 1.1

The causes of symptomatic "parkinsonism" and the "parkinson's plus" syndromes

- Parkinson's disease (Lewy-body disease)
- Postencephalitic parkinsonism
  - Encephalitis lethargica (von Economo's disease)
  - Other viral encephalitidies
  - Syphilis
- Drug-induced parkinsonism
  - Dopamine receptor blockers
  - Inhibitors of dopamine synthesis
  - Dopamine storage inhibitors (Reserpine, Tetrabenazine)
  - Alphamethyldopa
  - Lithium
- Neuronal System Degenerations with variable occurrence of parkinsonian features
  - Multiple-System Atrophy
    - Olivo-ponto-cerebellar atrophy
    - Striatonigral degeneration
    - Shy-Drager syndrome
    - Familial parkinsonism with depression
  - Dominant olivopontocerebellar atrophy (multiple types)
  - Glutamate dehydrogenase deficiency
  - Progressive supranuclear palsy
  - Corticobasal degeneration
  - Hallervorden-Spatz disease
  - Primary pallidal atrophy (varied types)
  - Idiopathic dystonia-parkinsonism
  - Corticodentatonigral degeneration
  - Hemiatrophy with hemiparkinsonism
  - Guam disease (Parkinsonism-ALA-Dementia complex)
  - Alzheimer's or Pick's disease

Table 1.1 (continued)

Metabolic disorders

- Wilson's disease
- Acquired hepatocerebral degeneration

Toxic Encephalopathies

- MPTP-induced parkinsonism
- Carbon monoxide, Carbon disulphide, Cyanide, Methanol.

Pseudoparkinsonism; i.e., various disorders which on occasion may present parkinson-like features

- Hypo- and hyper-parathyroidism
- Normal pressure hydrocephalus
- Creuzfeldt-Jacob disease
- Gait apraxias (various types and causes)
- Chronic post-traumatic encephalopathy
- Cerebral arteriosclerosis
- Lacunar infarction
  - multi-infarct brain
- Brain tumour
- Subdural haematoma
- Syringomesencephalia
- Spinocerebellarnigral degeneration
- Parkinsonism with ataxia and neuropathy

Miscellaneous

- Juvenile dystonia-parkinsonism syndrome
- Idiopathic basal ganglia calcification
- Congenital lesions

Table 1.2

List of dopamine receptor antagonists or "neuroleptic" agents

GROUP	GENERIC NAME	TRADE NAME
PHENOTHIAZINES	chlorpromazine	largactil
	trifluopromazine	parstelin
	promazine	sparine
	thioridazine	melleril
	mesoridazine	serentil
	piperacetazine	quide
	fluphenazine	anatensol
		modecate
	trifluoperazine	stelazine
	perphenazine	trilafon
		etraphon
	butaperazine	repoise
	prochlorpreazine	clopazine
	metotrimeperazine	levoprome
	promethazine	phenergan
	acetophenazine	tindal
	triethylperazine	torecan
BUTRYOPHENONES	haloperidol	serenace
	benperidol	anquil
	droperidol	innovar
	oxiperomide	
	trifluperidol	psicoperidol
DIPHENYLBUTRYL-PIPERIDINE DERIVATIVES	pimozide	orap
	penfluridol	
	fluspirilene	
	chlopimozide	
THIOXANTHENES	flupenthixol	fluanxol
	chlorprothixene	taractan
	clopenthixol	
	thiothixene	navane
	zuclopenthixol	clopixol

Table 1.2 (continued)

"DEPOT" PREPARATIONS	zuclopenthixol	clopixol depot
	fluphenazine deconate	moducate depot
	haloperidol deconate	haldol depot
	flupenthixol deconate	fluanoxal depot
	pipothiazine deconate	piportil depot
DIBENZODIAZEPINES	clothiapine	etomine
	clozapine	leponex
SUBSTITUTED BENZAMIDE	sulpiride	eglonyl
	metoclopramide	maxalon
PHENOTHIAZINE DERIVATIVES USED AS ANTI-EMETICS	promethazine	phenergan
	pericyazine	neulactil
	prochlorperazine	stugeron
	dixyrazine	esucos

disease, juvenile Huntington's and other illnesses, that may be associated with the rigid-akinetic state, need exclusion. Young onset of disease, lack of initial levodopa responsiveness, "non-Parkinsonian" neurologic signs, early dementia or postural hypotension with severe autonomic dysfunction requires further investigation to exclude alternate conditions.

1.3 Aetiology of idiopathic Parkinson's disease

Genetic or environmental exposure is thought to cause dopaminergic cell death in the substantia nigra. Early in the 1980's genetic factors were considered less likely since it was shown that the concordance rate between an affected twin suffering from idiopathic Parkinson's disease and the matching twin, even though they often grew up in a similar environment, was not increased. Furthermore when comparing monozygotic to dizygotic twins there was no greater risk regarding their likelihood of developing idiopathic Parkinson's disease at a later date (Ward et al., 1983). Hence while estimates of 10-15% of idiopathic Parkinson's disease sufferers may have a particular family history, this data indicates that there is no important Mendelian inherited predisposition to Parkinson's disease (Ward et al., 1983; Duvoison, 1981; Eldridge 1984).

Also in the 1980's the neurotoxin MPTP was identified as causing neuropathological, neurochemical and clinical features of parkinsonism in humans and primates. If MPTP, or MPP^+ (Chiba et al., 1984) can kill dopamine cells selectively, the search to find an "MPTP-like" toxic environmental insult becomes very important. However after many years no such toxin, in spite of the many compounds tested, has emerged (Tanner, 1989).

While it appears idiopathic Parkinson's disease is not inherited following Mendelian genetic patterns the possibility remains that individuals may metabolise toxins at differing rates. This hypothesis suggests that the liver detoxifying enzyme systems, which are genetically dictated, may handle a toxic insult by differing cellular mechanisms and rates thus allowing a potential toxin to be more harmful in a selected group of slow detoxifiers. Evidence has recently emerged that among idiopathic Parkinson's disease sufferers there is slower and differing hepatic conjugation systems (Williams et al., 1989), regarding the handling of debrisoquine and cystene. This data however requires independent confirmation.

The method MPP^+ toxicity occurs is known to invoke the monoamine "B" (MAO "B") enzyme system. This enzyme not only converts MPTP to MPP^+ via MPDP, but is also responsible for levodopa breakdo. . Normal MAO "B" levels differ

in various racial groups, increase with age advance and may in some way be responsible for a susceptibility to develop parkinsonism. Two indirect pieces of evidence lend support to different MAO "B" enzyme activity and individual susceptibility. Firstly, smoking which depletes MAO "B" centrally is thought to be less common among idiopathic Parkinson's disease sufferers. Secondly, selegiline (Eldepryl or Deprenyl) an antiparkinsonian drug and MAO inhibitor may have a neuroprotective effect in idiopathic Parkinson's disease (Tetrud and Langston, 1989; Shoulson et al., The Parkinson's Study Group 1989). Furthermore in the MPTP-primate model of parkinsonism it has been shown that selegiline, a MAO "B" inhibitor, prevents MPP+'s toxic mitochondrial insult (Heikkala, 1984; Langston 1984). A combination of both individual genetic predisposition and a subsequent secondary environmental insult to dopamine cells is emerging as a possible aetiologic cause of idiopathic Parkinson's disease (Langston and Calne, 1985).

The rationale of using a monoamine oxidase "B" blocking drug was originally proposed to reduce exogenous levodopa breakdown by inhibiting oxidative deamination of dopamine hence amplifying the therapeutic result and also theoretically inhibiting disease progression by interference with dopamine cell mitochondrial insult (Birkmayer et al., 1975).

Since selegiline selectively inhibits the MAO "B" enzyme it does not produce adverse effects found with other non-selective MAO-inhibitors. Selegiline is metabolised to methamphetamine and amphetamine (Reynolds et al., 1978), these catecholamines are able to stimulate the dopamine receptors (Knoll, 1978). Clinical trials show potentiation of levodopa (Birkmeyer et al., 1975; Yahr et al., 1978) especially in patients who have lost levodopa responsiveness. Others reporting an antidepressant effect of selegiline (Eisler et al., 1981; Mendlewicz and Youdim, 1983) were less impressed with the antiparkinsonian potential of selegiline versus placebo. Some patients received selegiline with success in improving motor fluctuations (Yahr, 1978; Rinne et al., 1978; Schacter et al., 1980), but not regarding freezing (Lees et al., 1977). There is no evidence that monoamine oxidase "B" inhibitors reduce nigral dopamine cell death in human idiopathic Parkinson's disease (Yahr, 1988).

Another theory is that a toxic metabolite such as Fe^{2+} may progressively accumulate within the substantia nigra and that subsequent free radical formation may be damaging. Evidence for this latter possibility is that excess free iron is found and the iron binding protein ferritin is, contrary to expectation, reduced in idiopathic Parkinson's disease compared with age matched controls.

Free radical suppression with Vitamins E and C and other antioxidants may slow disease progression (Fahn and Shoulson et al., 1989).

A further aetiologic possibility has been proposed. An *in utero* MPTP-like toxic influence may damage the developing central nervous system thereby producing subclinical nigral cell damage that will only become apparent with expected age attrition in the future (Marsden, 1990).

Evidence against the environmental hypothesis is the lack of obvious clusters of Parkinson's disease outbreaks, the observation that disease prevalence is not changing and except for the influenza induced encephalitis-lethargica association with parkinsonism no viruses have been consistently associated with Parkinson's disease. Furthermore conjugal Parkinson's is rare in spite of marriages lasting many decades, suggesting that the spouse of the affected individual, while concomitantly subjected to environmental toxic insults, is at no greater risk.

In summary while the aetiological mechanism in idiopathic Parkinson's disease remains unknown it is likely to be multifactorial. The susceptible individual, exposed to a subsequent environmental insult which cannot be suitably detoxified, remains speculative.

1.4 Biochemistry of idiopathic Parkinson's disease

While the neuropathology in idiopathic Parkinson's disease is well defined (Jellinger, 1986) the biochemical mechanisms of human idiopathic Parkinson's disease remain incompletely understood. The earliest established fact to emerge and stimulate biochemical research was striatal dopamine depletion (Ehringer and Hornykiewicz, 1960). This section will briefly review the established important biochemical markers thought to be important in idiopathic Parkinson's disease (McGear et al., 1984; Agid et al., 1987).

1.4.1 The nigrostriatal dopamine system

Topographical dopamine loss, with rostro-medial striatal greater than ventro-lateral striatal cell decline (Fahn et al,. 1971; Kish et al.,1986, Ehringer and Hornykiewicz, 1960;) concomitantly parallels nigral dopamine cell loss (Bernheimer et al.,1973; Javoy-Agid et al., 1981). This is especially so in the putamen but must be treated with caution since it arises from homogenised human tissue and direct HPLC measurements of dopamine in sampled areas. However these results have been reproduced in many centres. Indirect confirmation comes from decreased stria-

tal tyrosine hydroxylase and DOPA decarboxylase enzyme activity (Lloyd et al., 1975; Rinne et al., 1979); decreased specific aminergic nerve terminal radiolabelled markers (Schoemaker et al., 1985); decreased dopamine metabolites (specifically DOPAC and HVA) in SN and striatum (Bernheimer et al., 1973; Lloyd et al., 1975) and finally reduced levels of HVA in the urine and CSF of patients (Barbeau et al., 1961; Weil-Malherbe and van Buren, 1969; Olson and Roos, 1968).

1.4.2 The mesolimbocortical system

Man suffering from Parkinson's disease, shows normal dopaminergic cells and higher dopamine concentrations in the mesencephalic ventral tegmental areas compared with the lateral substantia nigra Javoy-Agid et al., 1981). Cortical and limbic target sites show normal amounts of dopamine in early idiopathic Parkinson's disease (Price et al., 1978; Scatton et al., 1983) suggesting that the "limbic" dopaminergic pathway is less severely involved in Parkinson's disease. It should be noted that the mesolimbocortical pathway is sparse (±500 times less dense) when compared with the nigrostriatal pathway and anatomically supplies different target sites (Scatton et al., 1983). There is also evidence that in advanced Parkinson's disease, the mesolimbocortical pathway degenerates, since

40-60% decreases are found in these dopaminergic target sites (Price et al., 1978; Scatton et al., 1983). Morphological evidence suggests some VTA dopamine cell loss, reduced TH staining and reduced dopamine concentrations (Javoy-Agid et al., 1982 and 1984; Uhl et al., 1985). It remains difficult to estimate how much noradrenergic input from the VTA accounts for the target zone limbic dopaminergic findings. The mesolimbocortical system is not entirely normal in human idiopathic Parkinson's disease (Agid et al., 1987).

The hypothalamic, neuroendocrine dopamine systems and brainstem is 60% depleted in parkinsonians compared with controls. Spinal cord concentrations of dopamine are unaffected (Scatton et al., 1986).

In summary it appears, with the possible exception of the spinal cord, that all dopaminergic systems may be depleted (<60%) in Parkinson's disease. However the nigrostriatal system, especially the putamenal radiations, are the worst affected (<20%).

1.4.3 Compensatory biochemical mechanisms

Adjustments both pre and postsynaptically occur following decreased dopaminergic input into the neostriatum. The activity of surviving dopaminergic neurones is commonly

estimated by calculating the HVA/dopamine ratio. The dopamine metabolite HVA reflects the amount of dopamine degradation thus indirectly allowing an indication of overall dopamine turnover. Dopamine receptor density, both D-1 and D-2, and their biochemical affinity reflect postsynaptic dopamine pathway status. Evidence that surviving dopamine neurones work harder arises from the observation that DOPAC and HVA content in postmortem tissue is consistently less than the reduction of dopamine (Hornykiewicz, 1979; Scatton et al., 1984). This functional hyperactivity occurs when 50-60% cells of the SNc are destroyed, markedly increases to 90% cell loss and falls off once less than 10% cells remain (Agid et al., 1973; Hefti et al., 1980, Zigmond et al., 1984). Small lesions, destroying less than 50% of nigral dopamine cells, thus remain asymptomatic with physiological compensation. Once greater than 90% cell loss, the functional physiological hyperactivity will fail. Thereafter insufficient nigral cell dopamine output will need to rely upon dopamine postsynaptic receptor hypersensitivity (Creece and Snyder, 1979). At this point motor signs and behavioural changes occur (Melamed et al., 1982). This increase in dopamine output occurs principally in the nigrostriatal tract, less in the mesolimbocortical tract and not in the corticostriatal tracts. The implication of this is that either:

a) The mesolimbocortical tract is not damaged or these neurones cannot physiologically increase their metabolic rates (Javoy-Agid et al., 1984).

b) Dopamine turnover is also increased in the SNr (Bokobza et al., 1984) but remains decreased compared with normal controls. This results in functional modification of striatal dopamine metabolism since dopamine itself influences the release of dopamine ipsilaterally (Cherany et al., 1981) and probably contralaterally (Björklund and Lindvall, 1984).

In addition other systems such as the thalamus and brainstem tectum, may be influenced by decreased dendritic dopamine in the SNr (Rinvik et al., 1976).

Evidence that the dopamine target receptors functionally adjust arises from a combination of biochemical (Creece and Synder, 1979; Guerin et al., 1985), pharmacological (Ungerstedt, 1971) and electrophysiological (Ohe et al., 1970, Carlsson et al., 1957) studies. Contrasting reports of the D-2 receptor status have appeared, shown to be increased (Lee et al., 1978) decreased (Reisine et al., 1977) or dependant upon the stage of illness, to be increased or normal (Lee et al., 1978; Rinne et al , 1981). Since this depends on the sites sampled, Agid pools the available data, the consensus of which indicates increased D-2 receptor density in putamen (dorsal striatum), but not

caudate or nucleus accumbens (ventral striatum) (Agid et al., 1987). Density of D-2 receptors apparently does not change with advance of the disease (Guttman et al., 1986). D-1 receptor density is also controversial, being increased (Nagatsu et al., 1978), or decreased (Reiderer et al., 1978; Shibuya, 1979) when judged by dopamine sensitive adenylate cyclase. Radioligand binding studies show either unchanged D-1 receptors in putamen (Pimoule et al., 1985), or increased D-1 receptors (Raisman et al., 1985; Rinne et al., 1985). Interpretation of data also depends upon the influences of levodopa treatment (Raisnam et al., 1985; Rinne et al., 1985). Receptors should be evaluated with caution in the substantia nigra, but D-2 receptors are thought to remain normal while D-1 receptor density decreases (Rinne et al., 1985).

In conclusion, the remaining nigral dopamine cells become functionally hyperactive to maintain nigrostriatal output. Once these forwarding dopamine cells fail, both postsynaptic D-1 and D-2 striatal receptor types multiply becoming more active. This temporarily restores basal ganglia function and this physiological adaptation may be influenced or even reversed by levodopa (Lee et al., 1978; Rinne et al., 1981) or dopamine agonist therapy (Riederer et al., 1983). This theory is not accepted by all (Bokobza et al., 1984). Agid proposes a staged biochemical degeneration process. Initially the nigral dopamine cells com-

pensate with increased dopamine output, then partial decompensation occurs when the postsynaptic dopamine receptors become hypersensitive but do not increase in number. Finally decompensation (with >90% nigral cell body loss) where dopamine output falls and a consequential increase in D-2 (and D-1) receptor numbers and activity occurs in parallel with advancing symptoms. While drugs may reverse all stages of the disease, physiological adaptation will ultimately fail once sufficient SNc dopamine cells die (Hornykiewicz, 1966 and 1979; Agid et al., 1973 and 1987).

1.4.4 Degeneration of other ascending systems

Pathologically, the ventral mesencephalon (substantia nigra, compacta, reticulata more than the VTA) and other brainstem nuclei are abnormal. The locus ceruleus, raphe nuclei and the nucleus basalis of Meynert all degenerate (Jellinger, 1986). The ascending noradrenergic, serotoninergic and cholinergic systems thus are also likely to be involved in Parkinson's disease (Agid et al., 1987).

Noradrenergic system

Two ascending NA pathways are identified, one dorsal from

locus ceruleus to neocortex and limbic forebrain, one ventral from lower brainstem to hypothalamus and nucleus interstitialis stria terminals. The locus ceruleus is the fulcrum, providing input into both the ventral and dorsal tracts of the human brain. This is similar to the rodent regarding noradrenergic distribution (Farley et al., 1976). Noradrenalin is 40-70% below normal in the neocortex and limbic areas of the nucleus accumbens, amygdala and hippocampus (Agid et al., 1987). Cell loss in the locus ceruleus, decreased target site noradrenaline concentrations and their metabolites suggest degeneration of this system (Riederer et al., 1977). Adrenergic system receptors change as a consequence, the beta-1 receptors which increase, while alpha-2 receptors decrease. The beta-2 and alpha-1 receptors remain unchanged (Cash et al., 1984). Increases in beta-1 receptors may be due to post-synaptic hypersensitivity and decreased alpha-2 receptors may reflect presynaptic localisation of adrenergic terminals (Agid et al., 1987). The central noradrenergic pathways are variably damaged in Parkinson's disease, which may explain the intellectual deterioration of some patients. This is mainly found to affect the dorsal tract (relatively sparing the ventral NA tract) thereby markedly reducing noradrenalin in the frontal cortex.

Serotoninergic system

Two serotoninergic systems are identified in animals, the ascending mesencephalic raphe nuclei to the forebrain and the descending pontine raphe nuclei to the spinal cord (Steinbusch, 1984). Serotonin and its major metabolites 5-hydroxyindole-acetic acid (5-HIAA) are found in greatest concentrations in the substantia nigra, striatum, amygdala and spinal cord (Reiderer and Winketich, 1976; Scatton et al., 1984). Serotonin is reduced (50-60%) in the forebrain (basal ganglia, hippocampus and cerebral cortex), raphe nuclei, spinal cord and hypothalamus (Berkheimer et al., 1961; Fahn et al., 1971; Reiderer and Winketich, 1976; Scatton et al., 1983 and 1986; Conte-Devolx et al., 1985). Scatton et al (1984) found normal values in the amygdala, accumbens, VTA, SNr, cingulate and entorhinal cortices in parkinsonians compared with controls. Serotonin receptors (5-HT1 and 5-HT2 types) do not change in Parkinson's disease in the cortex, striatum (Perry et al.,1984) but increased 5HIAA/serotonin ratios in the surviving serotonin neurones are found (Agid et al., 1987). The impact of serotoninergic systems in man with Parkinson's disease is uncertain, but these mechanisms do not affect motor activity (Chase, 1972) or tremor (Goldstein et al., 1969). They may however influence learning behaviour (Green and Heal, 1985; Hardy et al., 1985), depression and cognitive function (Van Praag, 1982; Cash et al., 1985).

Cholinergic systems

The cholinergic systems in the brain may be subdivided into the short neurones intrinsic to brain nuclei and longer fibres which connect the basal forebrain and brainstem to the subcortical structures and cortex. Striatal cholinergic neurones comprise less than 1% of the striatal micro-architecture. Further details of the intricate cholinergic pathways and their interconnections are unknown. The cholinergic cells in the basal forebrain have major projection sites to the olfactory bulb, cerebral cortex, amygdala, hippocampus and thalamus. Neocortical fibres originate in the nucleus basalis of Meynert and from the pedunculopontine nucleus. Hippocampal fibres originate from the septum and diagonal band of Broca.

The cholinergic marker catecolaminotransferase (CAT) appears normal in the striatum in Parkinson's disease (McGeer, 1976; Javoy-Agid et al., 1980) but has been reported to be low in some studies (Lloyd et al., 1975; Resine et al., 1977). Pathological agreement that the striatum appears microscopically normal in idiopathic Parkinson's disease and normal CAT activity suggest cholinergic pathways to be less consistently damaged in idiopathic Parkinson's disease, a finding confirmed by cholinergic striatal muscarinic receptor studies (Ruberg et al., 1982). Integrity of the striatal cholinergic neurones is remarkable since they are

intimately linked with dopaminerigic tracts. Striatal cholinergic influence which is stimulatory, in contrast with dopamines inhibitory tone, would allow tonic functional basal ganglia hyperactivity (Agid et al., 1987). Levodopa or anticholinergic receptor drugs may thus restore the balance. Within the realm of the intellectual functioning in Parkinson's disease, the cholinergic system must claim neurochemical priority and has resulted in many contrasting views (Brown and Marsden, 1984; Quinn et al., 1986; Mayeau et al., 1983; Rossar et al., 1982; Dubois et al., 1985). Neurochemically CAT activity is decreased in specific cortical areas (frontal, entorhinal, cingulate and occipital) (Ruberg et al., 1982). The cortex shows fewer cholinergic cells and combined with significant cell body loss in the substantia innominata, support the concept that the innominatocortical cholinergic system is severely damaged in idiopathic Parkinson's disease. The greatest cholinergic chemical defects are found in demented parkinsonians (Ruberg et al., 1982; Dubois et al., 1983; Perry et al., 1983) and even early in the course of idiopathic Parkinson's disease some cholinergic cell loss may be present (Dubois et al., 1983 and 1985; Perry et al., 1985; Nakano and Hirano, 1984). If this degeneration is a primary degenerative component of idiopathic Parkinson's disease and not a drug effect, it would support the notion that "dementia" is a feature of the parkinsonian syndrome.

Acetylcholinesterase (AChE), the enzyme that breaks down ACh, is decreased in the cortex of demented idiopathic Parkinson's disease, compared with control patients (Perry et al., 1985). The significance of this remains unknown. Muscarinic cholinergic receptors density, demonstrated with ^{3}H-QNB, is increased in the cortex in patients with idiopathic Parkinson's disease, but not in the striatum (Rüberg et al., 1982; Dubois et al., 1985). Anticholinergic drugs may influence this finding but no significant difference was observed in idiopathic Parkinson's disease patients who had not received these compounds (Agid et al., 1987). Anticholinergic drugs are known to interfere with memory in both normal and parkinsonian patients (Drachman, 1977; Sadeh et al., 1982; Dubois et al., 1982). Demented subgroups are especially sensitive to anticholinergic drugs (De Smet et al., 1982). In senile dementia of the Altzheimers type (DAT), intellectual impairment correlates well with decreased cortical CAT activity which reflects innominatocortical and septohippocampal cholinergic tract degeneration (Perry et al., 1978). The major difference in DAT is the associated cholinergic fibre degeneration (Hardy et al., 1985) and for the same degree of cholinergic deficiency, the intellectual fall off is much greater in DAT, compared with idiopathic Parkinson's disease (Perry et al., 1985). Cognitive impairment and memory disorders (aphasia, ataxia, apraxia or agnosia) do not occur in Parkinson's disease (Mayeau and Stern, 1983). It could be the slowing of the thought process that occurs

(bradyphrenia) with frontal clinical and neuropsychiatric abnormalities rather than the dementia that emerges as the cause of cognitive abnormalities in Parkinson's disease (Brun and Englund, 1981; Terry et al., 1981).

In conclusion the striatal cholinergic interneurone network is well preserved in contrast to degeneration of the substantia innominate and subcortical cholinergic tract systems, which creates important differences between idiopathic Parkinson's disease and other demented patients.

Gamma aminobutyric acid (GABA) pathways

This neurochemical is widespread in the brain (McGeer at al., 1984) and striatopallidal, striatonigral, pallidothalamic pathways, striatal and even cortical interneurones use GABA as their neurotransmitter (Fonnum et al., 1978; McGeer and Mc Geer, 1975). Dopaminergic and GABAergic systems interact in the striatum and substantia nigra where dopamine is thought to regulate GABA release (Lehmann and Langer, 1983). The marker enzyme for GABA, GAD is 20-40% reduced in the striatum, substantia nigra and cerebral cortex in Parkinson's disease (Bernheimer and Hornykiewicz, 1962; McGeer et al., 1971; Rinne et al., 1974; Javoy-Agid, 1982). Caution in interpretation of postmortem tissue is recommended due to enzyme instability (Agid et

al., 1987), but when very closely matched idiopathic Parkinson's disease may not differ from controls (Manfort et al., 1985). Hence cholinergic and GABAergic cortical neurones may well be normal in idiopathic Parkinson's disease (Manfort et al., 1985; Laaksonen et al., 1975; Kish et al., 1986). Tissue GABA levels are consistently above normal in the putamen and below normal in the cortex (Hornykiewicz et al., 1976; Rinne et al., 1979; Perry et al., 1983; Kish et al., 1986) and normal or subnormal (Bonnet et al., 1986; Manyam 1982, Teychenne et al., 1982) in cerebrospinal fluid. GABA receptors, as identified by the radioligand [^{3}H]GABA distribution are normal in the striatum, but reduced in the SN in Parkinson's disease (Lloyd et al., 1977; Rinne et al., 1978).

1.4.5 Neuropeptides in idiopathic Parkinson's disease

Neuropeptides in the brain in idiopathic Parkinson's disease remains a relatively unexplored field (Agid, 1987; Javoy-Agid et al., 1983 and 1986). CCK8 is 30% decreased in the SN (Studler et al., 1982); substance P is decreased in the SN and pallidum (Mauborgne et al., 1983); Met-enkephalin and Leu-enkephalin are decreased in the pallidum and SNc (Taquet et al., 1983 and 1985). Neurotensin, TRH, VIP, dynorphin are normal (Agid, 1987).

A summary of the current state of neurotransmitters in idiopathic Parkinson's disease (Fig 1.1)., suggests the core deficit is the depleted nigrostriatal dopamine tract, then consequently many other pathways become disturbed. All dopaminergic pathways are affected to different degrees and in addition the noradrenergic, serotoninergic, GABAergic and cholinergic pathways are variably disturbed. The place and significance among the classical neurotransmitters of the neuropeptides remains undefined.

1.5 Natural history and therapy of Parkinson's disease

Ever since Horniekiewicz and colleagues established that the primary chemical defect in idiopathic Parkinson's disease is striatal dopamine deficiency (Horniekiewicz, 1966) it is logical to attempt to replace dopamine or its precursor levodopa centrally to treat the disease. While a reciprocal relationship exists with acetylcholine, central nervous system dopamine is also influenced by other catecholamines, GABA and serotonin. Theoretically all drugs used to treat Parkinsons's disease either stimulate dopamine release, inhibit its breakdown, facilitate the stimulation of the dopamine receptor or adjust cerebral

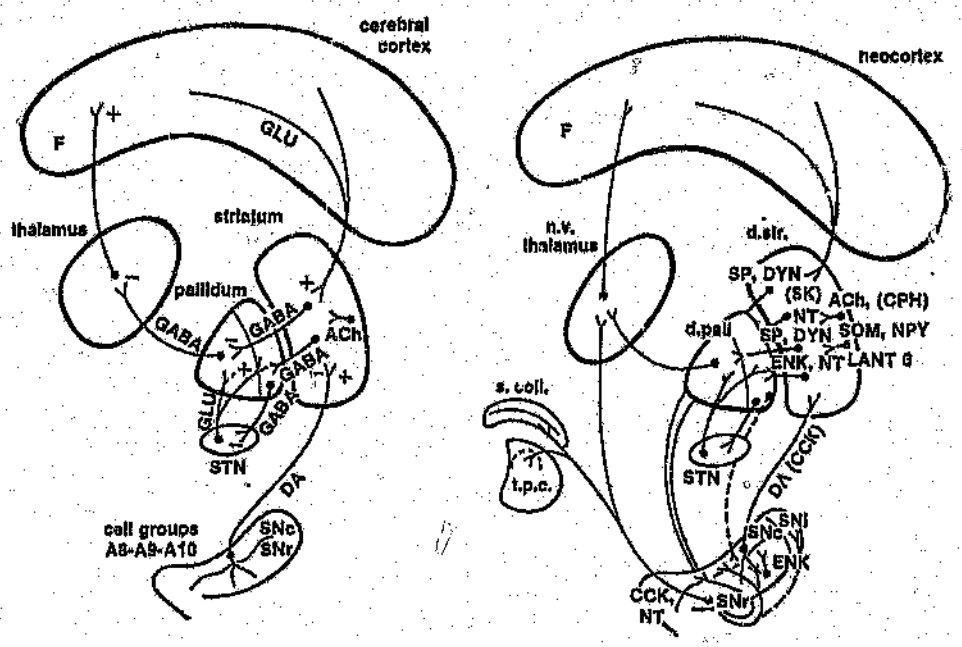


Fig 1.1

The classical neurotransmitters and neuropeptides of the basal ganglia

Key: Neuro-transmitters

ACh:	acetylcholine
CCK:	cholecystokinin
CPH:	carboxypeptidase H
DYN:	dynorphin
ENK:	enkephalin
GLU:	glutamate
GABA:	gamma-aminobutyric
LANT 6:	Lys^8-Asn^9-neurotensin
NPY:	neuropeptide Y
SOM:	somatostatin

Anatomical site

d.str.	dorsal striatum
d.pall:	dorsal pallidum
F:	frontal lobe
n.v. thal:	ventral tier nuclei of thalamus
s.coll:	superior colliculus
SNc:	substantia nigra pars compacta
SNl:	substantia nigra
SNr:	substantia nigra pars reticulata
SOM:	somatostatin
STN:	subthalamic nucleus
SNr:	substantia nigra pars reticulata
tpc:	pedunculopontine nucleus

neurochemistry in such a way as to facilitate dopaminergic activity. No alternate drug enjoys the impact that the dopamine precursor levodopa has had on the therapy of idiopathic Parkinson's disease.

Astute clinicians in the nineteenth century documented the course of the illness, but limited epidemiologic techniques at that time, make direct comparison with today's patient impossible. Since one ethically could not compare randomised groups with and without levodopa today the best method we have is comparison with well documented progression studies before the widespread use of levodopa (prior to 1967) and prospective studies thereafter. Crucial to this issue is the question of when in the disease course levodopa has been introduced, to what cumulative dose and finally what the influence of adjuvant dopamine agonists or monoamine "B" oxidase inhibitors might be.

The most important therapeutic strides in the treatment of idiopathic Parkinson's disease have been levodopa, dopamine agonists and potential neural transplantation. One could thus examine the natural history of the illness pre-levodopa, post-levodopa and at some future date, subanalyse the post-levodopa period reviewing the impact that additional dopamine agonists and transplantation will have made.

1.5.1 Pre-levodopa era

In the early nineteenth century Parkinson began to divide the disease up into various stages and Charcot, Buzzard and Gowers all stressed the inevitable progression and deterioration of the disease (Gowers, 1888). "Paralysis agitans does not directly cause death, the advanced age of the subjects renders its duration and its influence in shortening life difficult to determine ..." the longest case observed was "10 years, but known for 20 years before that". Stressful and anxious events were noted. Death from exhaustion, bed sores or intercurrent respiratory infections was noted (Gowers, 1888).

Brain emphasised that the Parkinsonian syndrome (both idiopathic and post-encephalitic) is always progressive, having different rates of progression, but an average life-span of 10 years from diagnosis may be expected (Brain 1955).

Schwab (1960) and Hoehn and Yahr (1967) recently put the natural history of Parkinson's disease pre-levodopa into best perspective. The gloomy prospect of inevitable progression, a bedridden state by 7-8 years, complete dependency upon others and finally death from respiratory infections and malnutrition only affected some patients (Schwab, 1960). Schwab showed a subset of patients able to

work 25 years later and suggested differing rates of patient progression. Prognostically akinesia, memory loss, individual personality, diminished drive, depression and bilateral signs within the first few years suggested more severe disease progression. Neurodegenerative illnesses have an insidious onset with slow progression, hence it is difficult to date the onset of idiopathic Parkinson's disease. Tremor remains the most common presenting complaint (70%), pre and post-levodopa simply because it is noticed by patients and their families (Mjones, 1949). What is often overlooked, but emerges with close questioning, is bradykinesia, cramping, malaise and lethargy, loss of dexterity, deterioration of handwriting, reduced facial expression, impaired balance and posture, depression, speech dysarthrias and sleep disturbances. These symptoms are more difficult to measure objectively. Recognition of these associated findings leads to increased awareness and earlier diagnosis and treatment today.

The natural progression of untreated idiopathic Parkinson's disease was reported by Hoehn and Yahr in 1967. (Appendix II)

STAGE 1: Unilateral findings with mild disease

Symptoms begin in one extremity, usually the dominant upper limb followed by the ipsilateral leg, proceeding to the

opposite side of the body. While the illness may initially be noticed for 1-3 years unilaterally patients eventually develop bilateral symptoms. Electrophysiological studies show slowing on the clinically unnoticed side years before signs and symptoms emerge.

STAGE 2: Bilateral disease, with axial involvement

Both sides of the body show bradykinesia or tremor but postural reflexes remain preserved. While asymmetrical signs remain, axis, face, flexed trunk, slowing of gait and diminished "associated" movements relentlessly progress over the next 3-6 years.

STAGE 3: Disequilibrium, bilateral parkinsonism signs and moderate disease

Progression occurs despite therapy pre-levodopa this stage was more easily identifiable by the feeling of unsteadiness, specifically upon turning. Retropulsion, lateropulsion and anteropulsion with festination, freezing and start hesitation all appear. This stage takes 7-10 years from the onset of visible clinical signs to develop. Most patients, while found to have uncertain equilibrium, remain functionally independent.

STAGE 4: Disability in daily activities, and severe illness

Once dressing, bathing or feeding become difficult the generalised and now advanced disease is considered stage 4. Without levodopa this stage takes approximately 10 years.

STAGE 5: Bed or chair bound patient, with advanced Parkinson's disease

Within 14 years most patients denied levodopa will be totally incapacitated. While many survive a further 10-20 years in this state the danger of pressure necrosis, infection, nutritional imbalance and dehydration will threaten their lives (Ford 1960).

The mean rate that an expected individual would progress from stage 1 through 5 would be 3,6,7,9 and 14 years respectively (Hoehn and Yahr, 1967). This results in 28% patients being incapacitated without levodopa between 0-5 years, 61% in 5-10 years and 83% with disease duration 10-15 years. While levodopa cannot prevent disease progression it clearly has affected the number of incapacitated people. Today only 9% are incapacitated within 5 years,

22% 5-10 years and 51% 10-15 years follow up (Hoehn and Yahr, 1967).

Only two drugs, anticholinergics and amantadine, have had any impact upon symptom modification, especially tremor, before levodopa. These drugs while not prolonging life, changing morbidity or mortality (Hoehn and Yahr, 1967) did provide a degree of symptomatic relief.

1.5.2 Anticholinergics

Belladonna alkaloids were initially employed by Ordenstein (1867), a student of Charcot, and until the introduction of levodopa was the principle drug employed to treat parkinsonian symptoms. After atropine and hyoscine (natural alkaloids) synthetic anticholinergic drugs were introduced in the 1940's. The pharmacokinetics of trihexyphenidal (Artane), show a half life of 1.7 ±0.2 hours (normal controls) but more recently 3.7 ±0.3 hours in patients with dystonia (Burke and Fahn, 1982). It would hence appear that anticholinergics should be given four to six hourly. The mechanism of action invoked is the blockade of central muscarinic acetylcholine receptors (Duvoison, 1967). Unfortunately no double blind studies

are reported evaluating different anticholinergics but overall they have declined in popularity as antiparkinsonian agents. Anticholinergics are widely used to alleviate tremor (Lang, 1984), but the most effective response has been in the alleviation of rigidity (Strange, 1965) and postural deformity (Parkes et al., 1974). Akinesia, in contrast, does not respond well to anticholinergics. Once initiated anticholinergic drug withdrawal is difficult (Hughes et al., 1971; Horrocks et al., 1973; Goetz et al., 1981). Long term anticholinergic administration may induce acetylcholine receptor hypersensitivity (Ruberg et al., 1982) in the cortex and striatum which may be aggravated by levodopa administration (Weintraub et al., 1971; Duvoison, 1967).

Neuropsychiatric side-effects make this group of drugs less attractive. Since cholinergic systems mediate memory (Davies and Maloney, 1976) anticholinergics may aggravate any cortical cholinergic pathway or receptor deficit already present in parkinsonian patients (Ruberg et al., 1982; Whitehouse et al., 1983). Acute confusional states (De Smet et al., 1982) added to memory dysfunction and delayed recall (Syndulke et al., 1981) are alarming and contraindicate these drugs in patients with any pre-existing intellectual decline. The place these drugs have in non-demented and younger patients is uncertain but current thought is to use anticholinergics only when other

drugs cannot be used (Yahr, 1986).

Cholinergic agonist drugs have likewise proved disappointing but were theoretically tried to diminish levodopa complications (Miller, 1974; Klawans et al., 1975; Barbeau et al., 1(9).

1.5.3 Amantadine

The benefit of amantadine hydrochloride in idiopathic Parkinson's disease was demonstrated but its mechanisms of action still remains to be determined (Schwab et al., 1969). Evidence that amantadine augments striatal dopamine release increases cell release of dopamine and possibly increases dopamine synthesis is incomplete (Parkes, 1974). Anticholinergic side effects such as dry mouth, constipation, urinary retention and visual focusing difficulties all suggest cholinergic mechanisms (Schwab et al., 1972). Acute overdose may be reversed by physostigmine (Casey, 1978). Amantadine, in contrast to anticholinergics, improves all features of Parkinson's disease and furthermore potentiates anticholinergic improvement. It probably has a mixture of dopaminergic as well as cholinergic effects (Lang, 1984).

1.5.4 Post-levodopa era

Cotzias (1967 and 1969), was so impressed with the results of levodopa that he suggested this therapy might halt disease progression. The impact this compound made upon clinicians in the 1960's made levodopa the most important milestone of the century regarding therapy of idiopathic Parkinson's disease.

Levodopa treatment regimens by Birkmeyer and Hornykiewicz (1962), were confirmed by other centres (Barbeau, 1962; Calne et al., 1973; Yahr 1975) but reality set in once levodopa complications appeared (Barbeau, 1976; Marsden and Parkes, 1977). Loss of levodopa responsiveness, involuntary movements, dystonia and on-off motor fluctuations became major problems. The consensus was, however, that levodopa response was dramatic, improved quality of life and prolonged life span to >14 years from the time of diagnosis. In spite of this improved quality of life the disease continued to progress relentlessly. Controversy regarding observed versus expected mortality rates occurred ranging from 1.9 (Sweat and McDowell, 1975) to 6.79 (Markham et al., 1974; Muenter et al., 1984) in different studies. Barbeau subanalysed akinetic and severely affected patients with idiopathic Parkinson's disease on 6 years of levodopa and reported an observed to expected

mortality ratio of 2 to 4. Recently Hoehn, using similar methods to their earlier study, compared the progression of patients with idiopathic Parkinson's disease, but now on levodopa from 1968-1982. Using similar epidemiological tools she showed a delay in Hoehn and Yahr stage deterioration by 3-5 years in most treated patients with a reduced mortality factor of 1.5-3, thereby approaching normal mortality. However 29% of patients on levodopa had involuntary movements after 3 years (Hoehn, 1985). Stern and Hardie serially observed 178 patients (1969-1972) on levodopa therapy found that at six year review, an overall mortality ratio of 1.5:1 (Hardie, 1984).

Subanalysis of those not able to tolerate levodopa for more than 2 years was 2.38 to 1, but those tolerating levodopa all 6 years was 1 to 1. Hence the most recent data suggests that those patients well controlled on drug therapy should have a normal life expectancy (Shaw et al., 1980). Continued follow up after 12 years found an increased mortality of 2.59. (Curtis et al., 1984). Hence levodopa, if tolerated, protects for 1-6 years but benefit lessens thereafter.

1.5.5 Levodopa

Levodopa (L-3,4-dihydroxyphenylalanine) is a naturally occurring neutral amino-acid produced along the synthetic pathway that converts L-tyrosine to noradrenalin. The enzyme tyrosine hydroxylase synthesises endogenous dopamine in the adrenal medulla, mesencephalon and other dopaminergic sites. Endogenous and exogenous levodopa are converted to dopamine by the enzyme L-aromatic amino-acid decarboxylase (L-AAAD) (Fig 1.2), usually shortened to dopadecarboxylase. Levodopa may alternately be metabolised by COMT which is found in liver cells and erythrocytes. COMT and monoamine oxidase are two central nervous tissue enzymes responsible for dopamine breakdown. Two forms of MAO, namely MAO "A" and "B", exist. Utilisation of monoclonal antibody techniques in man have shown cytosolic MAO "A" in the nigrostriatal dopamine containing neurones, serotoninergic and glial cells. The same region contains MAO-type "B", but rather in the mitochondria (Denny et al., 1982; Levitt et al., 1982). Monoamine oxidase type "B" is specifically inhibited by deprenyl while the MAO "A" is inhibited by clorgiline Heikkila et al., 1979; Pierce and Roth, 1984). Brain COMT is exclusively found in glial cells (Kaplan et al., 1979). The breakdown products (Fig 1.2) of dopamine are DOPAC, 3-methoxytyramine and HVA. Central dopamine is stored in dopamine vesicles, released

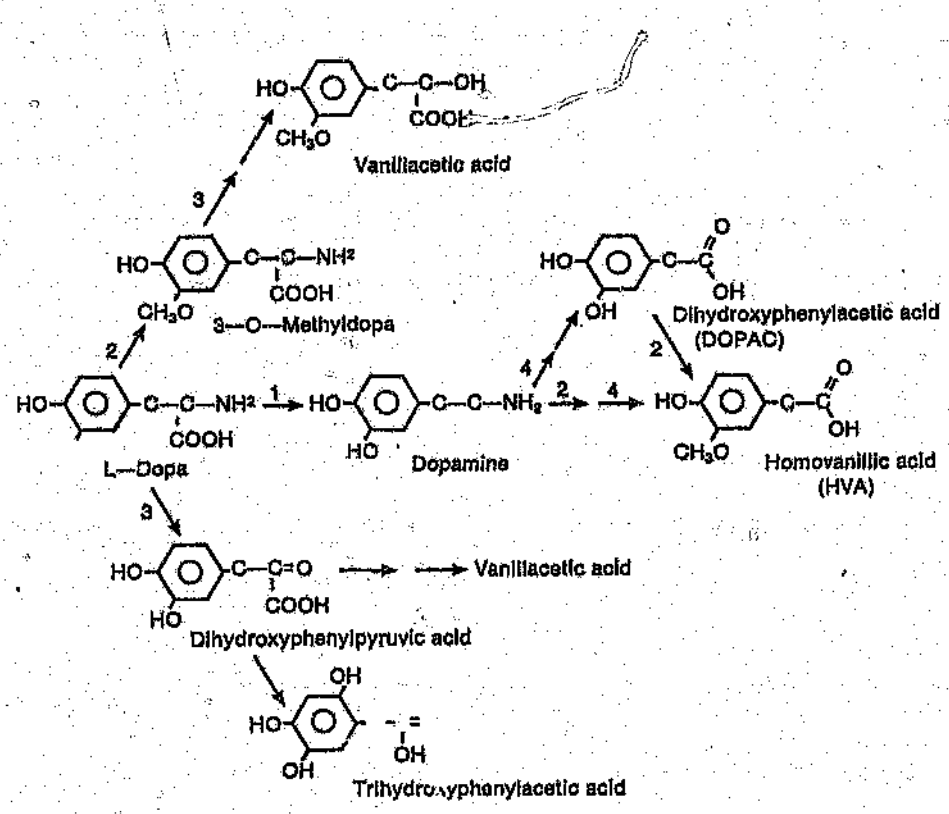


Fig 1.2

Dopamine metabolism and breakdown products within the body

from the dopaminergic presynaptic terminals activating the D-1 and D-2 receptors.

Following oral levodopa administration, parkinsonian symptoms are improved within 30 minutes. Improved tolerance of levodopa occurs once oral preparations are combined with a decarboxylase inhibitor.

The pharmacokinetic problems of levodopa could be subdivided into extracerebral and intracerebral events.

ORALLY, levodopa is absorbed from the proximal small intestine (Sasahara et al., 1981) using a saturable stereospecific amino-acid intestinal cell system (Wade et al., 1973). Meals that delay gastric emptying reduce and retard plasma levels of levodopa (Nutt et al., 1984). Anticholinergic drugs potentiate this delay (Fermaglich, 1972). The distribution of levodopa is biphasic, as with most drugs (Nutt et al., 1985), it is essentially all free with little protein binding (Hinterberger and Andrews, 1972). Levodopa then crosses the blood-brain barrier via a stereospecific, saturable, bidirectional, neutral aminoacid transport system (Wade and Katzman, 1975). The amount of levodopa that crosses into the brain is a function of serum levodopa and other amino acid concentrations (Pardridge, 1977). Levodopa competes with other neutral amino-acids and simultaneous ingestion may cause transient

clinical deterioration, in spite of adequate blood levodopa levels (Nutt et al., 1984).

Biotransformation of levodopa occurs at multiple sites, including the intestinal mucosa. As much as 50% of the oral dose is decarboxylated while being digested in the liver, kidney and other tissues (Rivera-Calimlin et al., 1970; Tyce, 1971; Cotler et al., 1976; Saskhara et al., 1981). N-methylation (using COMT) occurs mainly in the liver resulting in 3-O-methyldopa which has a long half life of >15 hours (Sharpless et al., 1972). Accumulation of 3-O-methyl-dopa occurs with constant dosing which has a much greater capacity to cross the blood-brain barrier (Wade and Katzman, 1975). 3-O-methyl-dopa has minimal antiparkinsonian activity but may produce side effects. Eighty percent of levodopa will eventually appear in the urine, the majority (75%) within the first 24 hours (Morgan et al., 1971). The remainder is excreted in the faeces (Tyce and Owen, 1979), oxidised to melanin or is transaminated in mitochondria (Fellman and Roth, 1971). L-AAAD inhibitors called "decarboxylase inhibitors", do not penetrate the brain and peripherally inhibit conversions of levodopa to dopamine. This allows 80% reduction of the oral dose giving an equivalent central benefit and simultaneous reduced peripheral side effects (Reid et al., 1972; Mars 1973). Decarboxylase inhibitors reduce gut biotransformation and thus improve bioavailability (Nutt et al.,

1985). One problem is that higher peak levodopa doses occur which may increase the incidence of dyskinesias and psychiatric disturbances.

INTRACEREBRALLY, levodopa is converted to dopamine in the striatum, 45-90 minutes from ingestion. Dopamine then acts upon postsynaptic striatal dopamine receptors to produce motor effects (Shoulson et al., 1975,. The rate and extent of conversion of levodopa to dopamine and the rate of inactivation of dopamine dictate the efficacy of any given levodopa dose. Striatal L-AAAD is located in the nigrostriatal terminals and with the advance of Parkinson's disease the concentration of this enzyme declines (Hornykiewicz 1975; Melamed et al., 1980). In advanced Parkinson's disease the decline of nigrostriatal input not only affects endogenous dopamine promotion but patients also experience less effective responsiveness to exogenous levodopa.

Since levodopa is considered the best drug to reverse the symptoms of idiopathic Parkinson's disease and restore expected life span to normal (Yahr 1976; Diamond et al., 1976) the question arises as to when to introduce it? (Lees 1986; Fahn 1984). It has been suggested that due to anticipated levodopa related side effects that levodopa be delayed until the functional needs of the patient warrant the risk and then only using the lowest possible dose (Calne 1986).

1.6 Complications and limitations encountered with longterm levodopa therapy

If all remained well regarding efficacy of levodopa in the long term we would require neither new medication nor different therapeutic strategies. Recognised complications of long term use have emerged (Marsden 1976, 1977) with the most troublesome being motor fluctuations and unpredictable failure to respond to regular levodopa doses. Motor fluctuations take many forms.

WEARING-OFF (END-OF-DOSE) DETERIORATION

As many as 50% of patients on levodopa for 2-5 years develop end-of-dose deterioration (Marsden and Schacter, 1981; Fahn, 1982; Marsden et al., 1982). Mobility follows changes in plasma levodopa levels which improve by 15-60 minutes following ingestion of levodopa and within 180 minutes gradually wear off. Fluctuations emerge as the duration of action of levodopa decreases. The likely explanation for the wearing off phenomenon is the progressive alteration of cerebral pharmacokinetics of levodopa (Spencer and Wooten, 1987). The prerequisites of this phenomenon are advanced idiopathic Parkinson's disease or

rapidly progressive parkinsonian syndromes such as young onset parkinsonism or MPTP-induced parkinsonism (Langston and Ballard, 1984). Wearing off correlates with temporary falling blood levodopa levels (Shoulson et al., 1975) and may be rapidly reversed by D-2 agonist apomorphine injection (Duby et al., 1972; Stiebe, 1987 and 1988) or continuous intravenous levodopa infusion (Shoulson et al., 1975; Nutt et al., 1984).

Idiopathic Parkinson's disease progresses in spite of effective therapy. What therefore is difficult to separate is natural disease progression from levodopa-related adverse effects. It is helpful to examine suspected levodopa side-effects to see whether they occurred or were reported before levodopa was utilised. An absence of these phenomena in the pre-levodopa era would suggest their occurrence is related to treatment rather than illness progression. Motor fluctuations such as dyskinesias, on-off fluctuations and some neuropsychiatric psychoses were not reported before the introduction of levodopa and hence they may well be unique to the deteriorating levodopa treated state or directly attributed to the drug. Freezing, dystonias and even wearing off phenomena, while they may be aggravated by levodopa, may equally be part of expected disease progression. Increasing nigrostriatal decompensation morphologically, structurally and biochemically would suggest a given dose of levodopa will

be progressively less effective. The rate of deterioration varies amongst patients. It is observed that whenever and at whatever stage levodopa is commenced, most patients will eventually experience loss of efficacy. If this was due only to available nigrostriatal dopamine levels then those more severely affected patients would develop wearing off sooner than the patient with mild idiopathic Parkinson's disease, relative to the initiation of levodopa therapy. This is not so and all patients regardless of severity at the onset of levodopa administration appear to run into wearing off problems at between 3-5 years following levodopa initiation. Could levodopa itself then be influencing the deterioration? Postmortem studies of receptor sensitivity in the levodopa treated state suggest down regulation of striatal dopamine receptors compared to similar untreated individuals. Levodopa may blunt the physiological compensation to the ultimate detriment of the sufferer. Furthermore exogenous levodopa when broken down, may stimulate free radical damage of the nigral dopamine cells which may further accelerate striatal dopamine decline (Agid et al., 1973).

Clear cut levodopa related side-effects such as oscillations in motor performance and dyskinesias are more of a therapeutic problem. Involuntary movements may become so severe as to force levodopa dose reduction with commensur-

ate worsening of bradykinesia. Patients eventually remain rigid throughout the day with a few scattered periods of mobility associated with excessive dyskinesias. Initially and often the first to appear are DYSKINESIAS, thereafter OSCILLATIONS IN MOTOR PERFORMANCE.

At least three different types of dyskinesias are recognised:-

a) peak dose

b) biphasic

c) "off" period dystonias

PEAK DOSE DYSKINESIAS affect up to 80% of Parkinsonian patients on levodopa for > 6 years (Lees, 1988). The dyskinesias are choreic or choreo-athetoid, correlate with maximal plasma and presumably central levodopa levels and begin 20-60 minutes after the previous dose. These incapacitating movements affect the head and neck, upper limbs, less commonly the legs, trunk and respiratory musculature. They become more generalised and often are worse on the side that developed symptoms initially. They typically get progressively more severe with time and of longer duration. Patients tolerate these dyskinetic periods well because the less acceptable alternative is a rigid-akinetic state in which the patient cannot move. Eventually, however, a reduction in levodopa becomes mandatory.

BIPHASIC DYSKINESIAS are less common (15% of patients after 6 years on levodopa) but more resistant to therapy. These occur at two (or more) phases in each interdose period with an intermission of mobility between. These movements are usually choreic but may be dystonic, ballistic or even myoclonic. They bear no correlation to levodopa serum levels, are not predictable, may occur throughout the body and occur more frequently in the young onset idiopathic Parkinson's disease sufferer. Occasionally another dose of levodopa may help and the mechanism, while unknown, may be due to a central dopamine receptor state fluctuation in advanced illness.

MOTOR FLUCTUATIONS may take the form of end-of-dose or wearing off deterioration already discussed, which will respond to an increased dose of levodopa, combinations with dopamine agonists or the addition of mono-amine "B" enzyme inhibitors. More difficult to manage are rapid on-off fluctuations. Another observation, possibly a variant of on-off, are the "yo-yo" oscillations which rapidly occur within a few minutes, resembling a series of false starts.

SEGMENTAL RESPONSIVENESS has also been rarely reported where a part of the body remains immobile and another part mobile with or without accompanying dyskinesias. Only the on-off motor fluctuations are partially treatable by regular intravenous levodopa preparations, subcutaneous or

slow release levodopa administration.

Commonly between six and ten years on levodopa the following scenarios may be noted:

a) A steadily increasing dose is required to maintain mobility.

b) The duration of action of a single dose decreases, sometimes accompanied by a prolonged latency to onset time.

c) Eventually dyskinesias accompanying the mobile periods, followed by on-off motor fluctuations and a less predictable response to levodopa.

d) Levodopa (albeit less effective with time) remains useful therapy. Even in advanced idiopathic Parkinson's disease some benefit will be observed.

e) Eventually in spite of successful therapy patients become chair and bed bound. Indistinct speech, inability to write and eventually total inability to dress, feed and bathe independently will occur.

The longer the duration and the higher the overall dose of levodopa therapy the more frequent and severe are the dyskinesias. Once these motor fluctuations present, a much lower dose of levodopa with or without dopamine agonists may perpetuate them.

While not fully explained the answer to dyskinesias and on-off, probably lies in the altered striatal dopamine receptor status. Years of exogenous levodopa not only regulate dopamine receptors but make them unpredictable in their response to dopamine. While peak dose dyskinesias may relate to serum and CSF dopamine levels, clearly biphasic dyskinesias do not. Commonly patient who develop wearing off eventually develop unpredictable on-off motor oscillations.

Failure to respond to levodopa may present with regular failure of afternoon doses, random dose failure or progressive global failure. Erratic dose responses must be approached from exploration of defective ingestion, absorption, metabolism and finally excretion mechanics. High protein diet, malabsorption and other drugs concomitantly administered often turn out to be the culprits of erratic levodopa failure (Wooten, 1987).

1.7 Dopamine receptor agonists

1.7.1 Dopamine D-2 receptor agonists

Levodopa efficacy declines with time. Since this effect is due to a combination of disease progression and to levodopa effects, alternate definitive therapy is required. Three options are to incorporate a monoamine "B" blocking drug to boost levodopa response; to modify the levodopa dose; or finally to stimulate the striatal dopamine receptor. Dopamine agonists, especially the D-2 dopamine receptor type, provide the best alternative to date (Lieberman et al., 1987 and 1990).

The main pathology in idiopathic Parkinson's disease is SNc dopamine cell depletion with decreasing nigrostriatal afferent input (Bernheimer et al., 1973) into the neostriatum. This encourages dopamine postsynaptic receptor multiplication and increases striatal D-2 (and D-1) receptor affinity (Ungerstedt et al., 1971). Direct stimulation of these receptors provides a sensible opportunity to correct striatal and basal ganglia function thereby correcting parkinsonian symptomatology.

Theoretically the best opportunity to utilise dopamine receptor agonist drugs is when the receptors are still sensitive to endogenous dopamine but not so sensitive that dopamine, levodopa or dopamine agonists produce unpleasant side effects thought to be mainly D-2 mediated (Rinne et al., 1985).

Studies using oral apomorphine (Cotzias et al., 1976; Corsini et al., 1979), piribedil (Dourish et al., 1983, Chase 1974; Sweet 1974), n-propylnorapomorphine (Cotzias and Papavasilious et al., 1976) were all limited by unacceptable side effects. Other ergoline derivatives showed minimal efficacy namely CF 25397, CQ 32084, CM 29712 (Teychenne 1977; Birket-Smith 1978 and Rinne, 1983).

The introduction of bromocriptine (Corrodi et al., 1973; Calne et al., 1978 and Teychenne et al., 1986) proved most useful as adjunctive therapy to levodopa (Lees and Stern, 1978) but may also be employed de-novo in mild disease (Lees and Stern, 1983; Rinne et al., 1986 and 1990) or to help smooth out motor fluctuations (Lees and Stern, 1981). Difficult, sometimes painful, dystonias may respond well to bromocriptine (Le Witt, 1986; Lander et al.,1979;).

In the early stages it may be sufficient to use dopamine agonists alone but eventually all patients require additional levodopa therapy (Rinne et al., 1990). Aside from

the advantage of delaying the introduction of levodopa and hence drug related adverse effects, bromocriptine is attractive in that while in high enough dosage it produces dyskinesias, the longer half life of the drug (180 minutes) allows smoother drug peaks which are thought to be more physiologically beneficial to the dopamine receptors (Chase, 1986). Bromocriptine monotherapy allows control of symptoms at approximately 10-25 mg/day orally (Lees and Stern, 1981; Lieberman, 1980; Teychenne et al., 1986). When to introduce dopamine agonists remains controversial. Two schools of thought exist. If the notion that levodopa induced fluctuations and end-of-dose unresponsiveness is related to overall levodopa dosage and duration of treatment (Fahn and Bressman, 1984; Meunter et al., 1984; Rajput et al., 1984; Melamed, 1986;) then alternate drugs such as early dopamine agonists, anticholinergics or amantadine should be given for as long as possible to delay these complications. The possibility that levodopa could aggravate the disease progression would strengthen this argument. In contrast it is argued that one should not deny the most effective therapy to patients in their productive years. If it could be shown that dopamine agonists, especially when employed with levodopa, delays the emergence of levodopa complications then a case for early use of dopamine agonists could be made. Dopamine agonists are not without risk. Emesis, nausea, malaise, orthostatic hypotension, neuropsychiatric side-effects commonly occur

with bromocriptine (Le Witt, 1987) but rarer complications of digital vasospasm (Wass, 1976) with limb gangrene, peptic ulceration (Delposa, 1976) angina, infarction, skin reactions (erythromelalgia, pedal oedema, vasculitic rashes), retroperitoneal and pulmonary fibrosis (Demonet et al., 1986) have all been reported (Le Witt, 1987).

The synergistic mechanism of action of bromocriptine and levodopa originates from animal observations (Goldstein et al., 1985). Bromocriptine's effects are enhanced by the presence of dopamine in the nigrostriatal synapses. If dopamine is not present in nerve terminals, for example in the rodent treated with reserpine or alphamethyl-para tyrosine, bromocriptine will not produce characteristic postsynaptic dopamine agonist behaviours. Dopamine with dopamine agonists may change the receptors conformation in such a way that the receptor becomes more receptive to stimulation by converting the D-2 dopamine receptor from the low to the high affinity state. Bromocriptine cannot differentiate between low versus high affinity receptor states and will only in combination with dopamine, stimulate the D-2 receptors (Goldstein et al., 1985). The resultant benefit, especially upon tremor, of bromocriptine plus levodopa in man with idiopathic Parkinson's disease was better than levodopa at higher doses alone (Rinne et al., 1980).

Dopamine D-2 receptor agonist evaluation is difficult as some dopamine agonists are partial agonists; some antagonists to the D-1 dopamine receptor; some are active agonists on normoreceptive dopamine receptors and some act only upon postsynaptic receptors, while others act both pre and postsynaptically. Furthermore the dopamine receptor population changes with disease progression, dependent to a degree upon the residual SNc dopamine cell population. Only well controlled double-blind, random crossover and placebo matched studies can answer the questions that remain regarding the stage and dose of dopamine agonist to employ. What is emerging however, is that low dose dopamine agonists with low doses levodopa produce the best combination (Marsden et al., 1990). Generally levodopa should be introduced once decreased functional mobility interferes with quality of life and the dose requirement increased to 400-600 mg/d. Thereafter bromocriptine should be incorporated into the treatment regimen, increasing the dose to 10-20 mg/day/po. Combination trials, including a group who receive deprenyl (European and North American study groups) are in progress. Following its introduction in 1972 bromocriptine has been the most widely studied dopamine agonist drug.

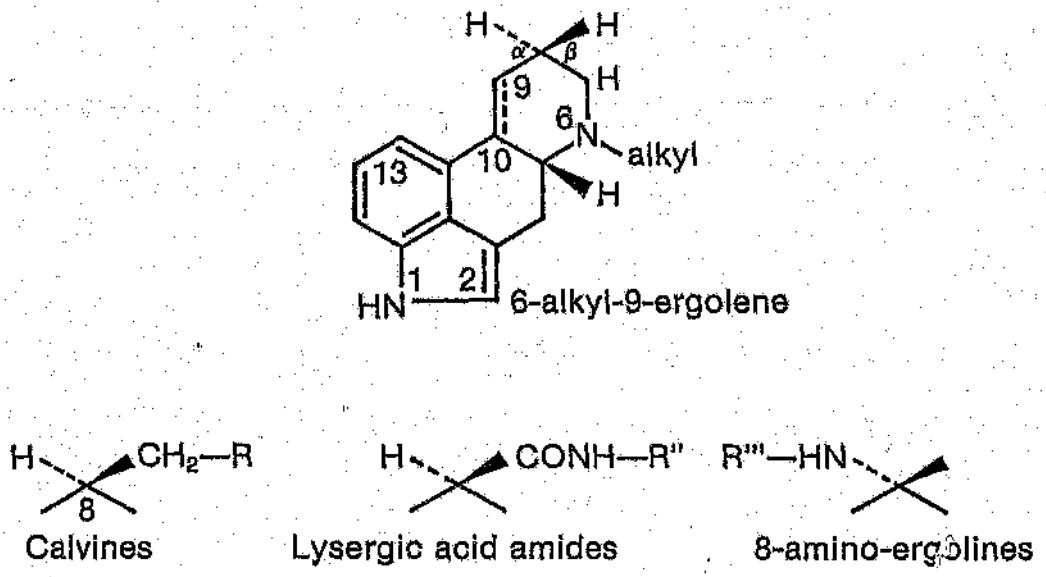


Fig 1.3

Structure of dopamine agonist drugs

Clavines	Lysergic acid amides		8α-amino-ergolines
	R'' = aliphatic	R'' = substituted cyclol (ergopeptines)	
Methergoline	Methysergide	Bromocriptine	Lisuride
Lergotrile	Methyl-ergometrine	Dihydro-α-ergokryptine	CH 29-717
Pergolide	Ergometrine	Dihydro-ergotoxine (1)	CQ 32-084
CF 25-397	(Ergonovine)		

(1) New non-propriety names: co-dergocrine (BAN), ergoloid mesylate (USAN)

Table 1.3

List of the dopamine agonist drugs currently available

1.7.1.1 Bromocriptine

Bromocriptine pharmacokinetics will be used as the prototype of drugs in this group, other dopamine agonists (Table 1.3) are considered in contrast to it.

Ingested orally, the ergot derivatives, including bromocriptine, are absorbed in the proximal small intestine, thus are best taken and tolerated with meals. While gastric contents and delayed gastric emptying will retard bromocriptine absorption, adverse intestinal effects such as nausea and vomiting are best avoided on a full stomach. Once absorbed the drug has a half-life of 4 hours. Bromocriptine is partially protein bound and is thus mainly in its free state, bioavailable in serum and able to cross the blood brain barrier in proportion to the serum concentration. Binding of agonists takes place directly at the central D-2 dopamine receptor sites (and central D-1 dopamine receptors) to render its anti-parkinsonian effects. The combined effect of peripheral DA-2 blood vessel receptors which facilitate vasodilatation and central vasomotor centre effects will drop the blood pressure. Metabolism occurs in the liver where the drug is detoxified, rendering 90% of the metabolites present in the urine within 24 hours.

Optimal dose is dependant upon the stage of Parkinson's disease and patient tolerance. Today the trend is 10-20 mg/day overall dose, in 4-6 divided doses commencing with small incremental doses of 1,25-2,5 mg/12 hourly. Higher doses, >30 mg/day have increased dose related side effects and marginal additional antiparkinsonian benefit. Levodopa may be introduced when required (Teychenne et al., 1986). Disadvantages of bromocriptine include its expense, lower maximal efficacy than levodopa as monotherapy, idiosyncratic side effects and dose-related adverse effects.

The majority of side effects related to bromocriptine may be overcome by either slow build up of therapeutic dose or by a dose reduction (Le Witt, 1987). The commonest side-effects encountered are gastro-intestinal (nausea, vomiting, malaise and anorexia) but neuropsychiatric (hallucinations, delusions, obsessions, paranoid psychosis, mania, confusion, vivid dreaming, anxiety or restlessness) or orthostatic hypotension frequently limit use of this drug. Other side effects described include drowsiness, constipation, fatigue, dry mouth, light-headedness, diplopia and failure to accommodate, insomnia, nasal stuffiness, palpitations, abdominal cramping, hair loss, diarrhoea, angina and myocardial infarction, intestinal bleeding, Raynaud's and digital gangrene, face puffiness, oedema, and a variety of movement disorders (sleep myo-

clonov , dykinesias, painful dystonia, blepharospasm or hiccups) (Le Witt, 1987). Pretreatment with domperidone often improves intestinal complaints (De Bono et al., 1976; Quinn et al., 1981). Orthostatic hypotension may respond to dose reduction and gradual drug build up with time. While theoretically possible, cardiac arrhythmias, sudden death and myocardial infarction are rare and often occur on an idiosyncratic basis (Tanner et al., 1985; Kurlan et al., 1986). Skin rashes may occur (erythromelalgia, violaceous capillary networks, ankle oedema and vasculitis) with high doses and are often reversible upon drug withdrawal (Eisler et al., 1981; Olanow et al., 1986). Retroperitoneal (Demonet et al., 1986) mediastinal and pulmonary fibrosis, while described with other ergot compounds, such as methysergide, have rarely been reported in chronic bromocriptine use. Over the last decade many studies have been completed with bromocriptine alone and in combination with levodopa.

While controversial a few indications for bromocriptine appear widely accepted:

a) To reduce established levodopa complications (such as painful dystonias, dyskinesias, end-of-dose deterioration or "on-off" motor fluctuations. (Lieberman et al., 1987; Kebabian et al., 1979; Le Witt et al., 1983).

b) To allow lower dose levodopa administration but

obtain sufficient anti-parkinsonian benefit (Lieberman et al., 1986; Lees 1978).

c) Initially in early therapy of idiopathic Parkinson's disease as monotherapy to delay the limitation of le odopa (Stern 1989; Le Witt and Calne, 1982; Rinne, 1987 and 1990; Staal-Schreinemachers et al., 1986; Rascal, 1984).

Theoretical indications that require confirmation are:

d) Bromocriptine in combination with levodopa to delay the onset of levodopa-related side effects (Lieberman 1976; Rinne, 1987; Stern, 1986).

e) High dose bromocriptine as monotherapy instead of levodopa with more severe disease, (Lieberman et al., 1979; Teychenne et al., 1978).

1.7.1.2 Legotrile

This semisynthetic alkaloid of the ergot group, is D-2 dopamine receptor selective (Lemberger, 1974) and when employed up to 20 mg/day demonstrated encouraging antiparkinsonian activity (Lieberman et al., 1975). Minimal dyskinesias were seen and further study using a mean of 49 mg/day orally, showed improvement in half the patients treated and was able to reduce levodopa require-

ments by ±10% (Lieberman et al., 1979). Mental changes similar to bromocriptine were noted (Klawans et al., 1978; Teychenne et al., 1978). Unfortunately hepatotoxicity halted further human studies.

1.7.1.3 Lisuride

Lisuride, a D-2 dopamine receptor agonist with selective D-2 receptor postsynaptic activity and some D-1 receptor antagonism, is a semi-synthetic ergot alkaloid (Cote et al., 1979). It is more potent than bromocriptine also acting as a 5-hydroxy-tryptophan receptor antagonist. Lisuride has been tested alone (Le Witt et al., 1982; Agnoli et al., 1983; Lieberman et al., 1983) compared with levodopa (Le Witt et al., 1983; Parkes et al, 1981) used in combination with levodopa to reduce levodopa dose and side-effects (Lieberman et al., 1983; Lang et al., 1983; Obeso et al., 1986; Ulm, 1983) and compared with bromocriptine (Lieberman et al., 1983). Lisuride, being water soluble, may be administered intravenously to alleviate motor fluctuations or dyskinesias as well as dystonia (Parkes et al., 1981; Quinn et al., 1985;) and myoclonus (Obeso et al., 1986). Side effects are similar to other dopamine agonists but the neuropsychiatric complications are alarming (Le Witt et al., 1983). Lisuride,

administered 0.05 to 0.15 mg/kg intravenously shows effects within 15 minutes and benefits that last 2-3 hours (Parkes et al., 1981). Oral administration induces prompt clinical response which parallels blood lisuride levels (Le Witt et al., 1982 and 1983). Lisuride is completely absorbed but has a significant degradation due to the hepatic first pass effect, which reduces the absorption by up to 80% (Burns et al, 1981). The half-life of lisuride is 1.5 to 3.5 hours and 90% of its metabolites appear in the urine within 24 hours.

Terguride trans-dihydrolisuride, a modification of lisuride, with similar potency but longer half-life, has also been tested as an antiparkinsonian drug (Wachel, 1983).

1.7.1.4 Pergolide

Pergolide mesylate is a potent semisynthetic ergot alkaloid, with mixed D-1 and D-2 receptor agonist activity and has a half life of 6 hours (Goldstein et al., 1980; Lemberger et al., 1980). The dose range is 0.1-7.2 mg/day orally in divided doses, and may be used as monotherapy or as an adjunct to levodopa (Ilson et al., 1983; Lang et al., 1982; Lieberman et al., 1990). Compared with

bromocriptine, pergolide is more potent, of equal or better efficacy, has similar side-effects (Le Witt et al., 1983; Lieberman et al., 1983) and like bromocriptine the effects, while additive to levodopa, are not sustained beyond a couple of years (Lieberman et al., 1987).

1.7.1.5 Mesulergine

The 8-alpha amino ergoline and selective D-2 receptor agonist CQ 32-084 was given to animals (Markstein, 1983) and humans (Burton et al., 1984; Pfeiffer, 1985; Jellinger, 1982). Doses ranged from 2-60 mg/day orally. Mesulergine has been withdrawn due to rodent testicular carcinogenic effects.

1.7.1.6 CQA 206-291

The ergot derivative CQA 206-291 (5R [5-alpha 8-beta 10-alpha]-N-N-diethyl-6-ergoline-8-yl) sulfamide hydrochloride is chemically similar to its predecessor mesulgerine, the active metabolite of which is CQ 32-084.

CQA 206-291 is active upon D-2 dopaminergic and 5-HT2 serotinergic receptors, using rat striated slices and displacement of [^{3}H]spiroperidol and [^{3}H]clonidine respectively (Jaton and Eng, 1987). Further evidence of D-2 activity comes from:

a) Electrically mediated rat striatal acetylcholine release following CQA 206-291 and inhibition of dopamine effect upon acetylcholine following CQA 206-291.

b) Increased striatal level of HVA after CQA 206-291 administration, a similar observation having been reported with mesulgerine.

c) Unilateral 6OHDA rodents which showed contralateral turning following CQA 206-291 similar to apomorphine. This was completely inhibited by the D-2 antagonist sulpiride (86% reduction) and less so by the D-1 antagonist SCH 23390 (31% reduction). No apomorphine like stereotipy was induced in normal rats.

d) CQA 206-291 reversed the MPTP-induced parkinsonian features of squirrel monkeys.

e) Finally prolactin secretion and lactation was inhibited less potently than with mesulgerine but more potent than with bromocriptine (Jaton et al., 1987).

Evidence of D-1 dopamine receptor activity is controversial. Biochemically dopamine-stimulated adenyl cyclase was not influenced by CQA 206-291 or CQ 32-084 but SCH 23390 unexpectedly affected CQA 206-291 induced rodent rotation, a feature suggesting some D-1 receptor effect. Cardiovascular effects in volunteer studies show decreased orthostatic hypotension and nausea compared with bromocriptine.

CQA 206-291 is well absorbed, with greater than 50% of the oral dose entering the bloodstream. A significant first pass hepatic effect is observed. The half-life is approximately 8.6 hours, terminal half-life of biphasic peak and excretion occurs in the urine (50% in 24 hours) and up to 30% in the faeces.

The potential of CQA 206-291 in animal models has led to preliminary studies in healthy human volunteers. The drug was well tolerated to 0.5 mg orally (single dose), without nausea or postural hypotension (Spector, 1986). Therapeutic indications are still to be defined in idiopathic Parkinson's disease, but following bromocriptine experience "de novo" initiation of therapy in early Parkinson's (Hirt and Gugerli, 1988; Roscol et al., 1988) and additional to levodopa as adjuvant therapy (Hirt and Notter, 1987; Luef et al., 1988) have been recorded.

CQA 206-291 has antiparkinsonian activity in advanced idiopathic Parkinson's disease (Temlett et al., 1988 and 1989; Fabre et al., 1988).

1.7.1.7 (+)-PHNO

(+)-4-propyl-9-hydroxynaphthoxazine ((+)-PHNO) is structurally different from other dopamine agonists (Fig 1.3) and in vivo dose for dose is the most potent D-2 agonist available today (Martin et al., 1984). (+)-PHNO is exquisitely postsynaptic D-2 dopamine receptor selective in that:

a) It inhibits in vitro [^{3}H]apomorphine and [^{3}H]spiperone binding to rat striatal homogentes. It also has some 5-HT1 receptor and alpha-2 adrenoreceptor activity (Martin et al., 1986).

b) Hypothermia and postural changes in unilaterally lesioned mice have been reported.

c) Rodent stereotypies, gnawing, licking and sniffing, rotation in unilaterally lesioned animals similar to apomorphine, is also blocked by priming with haloperidol, but not reserpine or alpha-methyl-paratyrosine. This suggests PHNO is a direct acting postsynaptic dopamine agonist drug.

d) Enuresis occurs in dogs.

e) Failure to stimulate retinal cAMP, suggests little D-1 activity.

f) Rapid reversal of parkinsonism in the MPTP-treated common marmoset was observed (Jenner at al., 1986; Nomoto et al., 1987) when given orally, intravenously, interperitoneally, subcutaneously or transcutaneously.

Skin or parenteral potency is 7 times greater than oral potency suggesting a significant hepatic metabolism with the "first pass" effect following intestinal absorption (Coleman et al., 1990). Animals vomited and became hyperactive confirming the potent D-2 receptor effects also found in human idiopathic Parkinson's disease sufferers (Coleman et al., 1987).

1.7.2 Dopamine D-1 receptor agonists

1.7.2.1 SKF 38393

2,3,4,5, tetrahydro-7,8-dihydroxy-1-phenyl-H-3-benzazapine (SKF 38393) identified by Pendleton et al., 1978 is a selective and unique D-1 dopamine agonist (Setler et al., 1978). In vitro studies suggest D-1 dopamine receptor agonism in that cAMP in homogenised caudate was stimulated by SKF 38393. Less cAMP was liberated following dopamine, compared to SKF 38393 . The effects of dopamine and SKF 38393 were not additive and fully inhibited by dopamine antagonists such as chlorpromazine and bulbocarpine (Setler et al., 1978). Pharmacologically SKF 38393 is a partial agonist. Dopamine turnover studies in striatal and limbic structures suggest that apomorphine (D-2 action) and not SKF 38393 (D-1 action) alters the rate of depletion of dopamine once tyrosine hydroxylase is inhibited. Basal dopamine levels are unaffected. In vivo studies show no emesis in dogs, dose related contralateral rotation in unilateral 6OHDA rats similar to apomorphine, the absence of stereotypies, increased rodent prolactin release and finally that SKF 38393 has potent effects on the renal artery (Pendleton, 1978; Settler et al., 1978). Furthermore pimozide (a potent D-2 antagonist but weak D-1

antagonist) (Pendleton et al., 1978; Clement-Carrier et al., 1974) had no effect in blocking rotation behaviour or upon the renal vasculature of unilaterally 6OHDA lesioned animals. Limited clinical studies exist utilising SKF 38393. Completed studies show disappointing results in human idiopathic Parkinson's disease. The MPTP-treated primate did not respond predictably to SKF 38393 (Nomoto et al., 1985; Braun et al., 1987). Metabolites of SKF 38393 may inhibit D-2 receptor expression (Nomoto et al., 1985; Close et al.,1985; Temlett et al., 1988;).

1.7.2.2 CY 208-243

A recently manufactured D-1 receptor agonist CY 208-243 (Markstein et al., 1987) has all the expected biochemical characteristics of a D-1 selective agonist. CY 208-243 displaces [^{3}H] dopamine binding (a D-1 and D-2 marker) in rodent striatal slices in micromolar concentration while displacing [^{3}H] spiperone (D-2 selective marker) in nanomolar amounts. CY 208-243 stimulates cyclic AMP formation. Selective D-1 activity is suggested by the observation that striatal dopamine turnover or electrically stimulated acetylcholine release remains unaltered. CY 208-243 does not produce stereotipy, emesis or reduce serum prolactin (Markstein et al., 1987; Temlett et al., 1988)

but does produce potent contralateral turning in unilaterally lesioned 6OHDA rodents. Limited studies in man (Temlett et al., 1989) suggest that D-1 agonists may have antiparkinsonian effects in advanced parkinsonian patients and even produce dyskinesias thought to be D-2 receptor mediated (Agid et al., 1988; Temlett et al., 1989).

CY 208-243 will not be studied further due to hepatotoxicity in man (Lataste, 1987; personal communication). The impetus of subhuman primate data and the early human data have initiated development of other drugs with similar chemical structures to facilitate exploration into the antiparkinsonian effects of other agents that mediate their activity by the D-1 dopamine receptor.

1.7.3 The place of dopamine agonists in idiopathic Parkinson's disease

All the dopamine D-2 receptor agonist drugs, have antiparkinsonian efficacy. Table 1.4 ranks the potency, arranged according to biochemical data, of some of the agents available today. The drugs are best employed in combination with lower dose levodopa to alleviate fluctuations in advanced disease and possibly "de novo" as primary therapy. Overall levodopa dose is reduced by up

Table 1.4

Spectrum of the dopamine agonists ranked according to their D-1 and D-2 potency

MOST D-2, LEAST D-1 ACTIVITY

(+)-PHNO
-LY 171555
CQA 206-291
LISURIDE
PERGOLIDE
MESULERGINE
CF 25-397
CH 29-717
AMINOTETRALIN 5,6-diol-(±)
AMINOTETRALIN 6,7-diol-(±)
APOMORPHINE-R-(-)
N-PROPYL-APOMORPHINE
DOPAMINE
3-PPP-(+)
SKF 38393
CY 208-243

MOST D-1, LEAST D-2 ACTIVITY

to a third when these dopamine D-2 receptor agonists are used in combination. Dopamine agonists may only sustain meaningful antiparkinsonian benefit for 18-24 months.

Reasons advanced for diminishing efficacy are:

a) Diurnal fluctuations complicate patient evaluation and unless in hospital with dose monitoring are evaluated by "on-off" diaries which lead to variable results (Lieberman et al., 1987).

b) Psychological factors and placebo effects are most notable early, with new therapies.

c) Ergot dopamine agonists are mainly hepatically metabolised and patients may change absorption, metabolism, clearance and overall pharmacokinetics with time.

d) "Down-regulation" of postsynaptic D-2 dopamine receptors which become less responsive with disease progression.

e) Endogenous dopamine decline may make dopamine agonist effect less effective with time.

f) As the overall potential to respond in advanced idiopathic Parkinson's disease is limited, comparisons in surveys should adjust results to the time period for which

the population has suffered the illness.

Finally the low incidence of dyskinesias, dystonias and possibly the reduced chance of levodopa enhancing disease progression have established the place of dopamine agonists in the current therapy of Parkinson's disease. Testing of novel dopamine agonists such as delayed release forms, long acting dopamine agonists, or transdermal applications will take place in the future.

1.8 New delivery systems

1.8.1 Slow release levodopa compounds

Early in the progression of idiopathic Parkinson's disease ordinary preparations of levodopa suffice and it is only after six to ten years therapy that levodopa related problems emerge. Motor fluctuations have been shown to respond to intravenous infusions of levodopa (Shoulson et al., 1975; Nutt et al., 1984; Hardie et al., 1984; Quinn et al., 1984) which has led to the suggestion that levodopa administration in an "unphysiological" manner may eventually damage dopamine receptors. To improve receptor sensitivity slow acting and slow release formulations are being devised.

At least two slow release methods have emerged, an enteric coated oral capsule and a skin patch. Recent evidence shows that with both Madopar HBS and Sinemet CR 3,4 and 5 (all enteric coated formulation) the ideal of once or twice daily medication is insufficient for most patients (Duvoison, 1989). A combination of normal Sinemet with the slow release formulation is usually required because the latency to onset is too long after ingestion of the slow release tablets (Bush et al., 1984; Quinn 1989). Many patients benefit, especially those who suffer nocturnal akinesia and difficulty turning when recumbent.

1.8.2 Transcutaneous drugs and skin-patches

Novel routes that avoid tablet taking have been used in the therapy of motion sickness, angina pectoris and hypertension by utilising the skin and subcutaneous fat "sump" (Price, 1981; Shaw, 1980; Armstron, 1980; Lyrenas, 1981). Common to all these drugs is their lipid-water solubility profile, a factor which has made the usual antiparkinsonian drugs unsuitable. The dopamine agonist (+)-PHNO has changed this since it is water and lipid soluble, not irritant to skin and a potent D-2 selective dopamine receptor agonist (Coleman et al. 1989). Application of (+)- PHNO in a plaster fixed to the skin of

rats (Grandes-Perez et al., 1986), primates (Coleman et al., 1989) and man (Coleman et al., 1989) all show absorption of the drug and improvements in parkinsonian status. Advantages of the transcutaneous route of administration are that the hepatic "first pass" effect upon intestinally absorbed drugs is avoided allowing more potent antiparkinsonian effects which, if slowly released, could potentially alleviate many levodopa related motor fluctuations. Hence patients already receiving benefit from levodopa may be supplemented with (+)-PHNO to smooth out inevitable motor fluctuations that sometimes parallel serum levodopa levels.

1.8.3 Subcutaneous apomorphine

The mixed D-1 and D-2 dopamine receptor agonist, apomorphine, has been successfully employed to treat levodopa dependant parkinsonian patients who develop motor fluctuations especially "on-off" phenomenon (Stibe 1987, 1988; Poewe et al., 1988).

Either a subcutaneous infusion, via a continuous pump or a portable injection, may be used periodically to prevent "off" periods (Frankel et al., 1990). Benefit in urinary dysfunction (Christmas et al., 1985), "off" period pain and dystonia (Frankel 1990) and biphasic dyskinesias have

also been reported (Duby et al., 1972). There is a short lag between apomorphine injections and rising blood and cerebrospinal fluid drug levels, indicating the drug crosses the blood-brain barrier very well. Clinically the apomorphine also appears to have a long half-life which allows mobility long after serum levels fall (Gaucher et al., 1990). How exactly this dopamine agonist works and whether the dopamine receptor actually needs to be stimulated continuously is unknown. Apomorphine is well tolerated and no significant drug tolerance occurs. Reversible neuropsychiatric side effects however are common.

1.8.4 Polymeric brain implants

Following the demands made upon researchers from the impetus of dopaminergic cell implantation techniques, researchers have begun looking for alternate sources of cell lines or artificial dopamine sources to be implanted within the brain. Dopamine copolymer matrix devices may be one method of delivering dopamine into the ventricular cerebrospinal fluid or parenchymally to the striatum itself (McCrae et al., 1988; Freese et al., 1989).

This technique has only been employed in rodents but does provide a useful theoretical basis for further development.

1.8.5 Nasogastric and intravenous infusions

Developed as a more physiological and regularly administered levodopa dose regimen the techniques of intravenous but also nasogastric levodopa administration are impractical to employ routinely. However patients with motor fluctuations will clearly be better controlled by infusion pumps. The attraction of dopamine agonists administered in this way ((+)-PHNO, lisuride and apomorphine) still need to be developed to their full potential. Fluctuating motor responses (especially "on-off" phenomenon) are thought to be due to altering striatal dopamine (both D-1 and D-2 receptors) sensitivity. Chronic intermittent levodopa administration commonly causes alteration in these receptors. In addition high protein meals, erratic bowel absorption, delayed gastric emptying and alteration of serum pharmacokinetics may all aggravate the central dopamine receptor fluctuations (Nutt, 1984). Long term continuous and slower release dopamine administration is thought to be more physiological than chronic intermittent oral therapy (Mouradian et al., 1989). Problems with long term continuous dopaminomimetics are still emerging, such as neuropsychiatric side-effects and drug tolerance with less effect serially of similar drug dosage. A further possible site of levodopa or dopamine agonist administration would be their administration into the CSF directly.

1.9 Transplantation in idiopathic Parkinson's disease

The concept of transplantation has been discussed over the last century (Dunn, 1917; Weiss, 1950). Dopamine producing cell implants began in 1979 with two research groups showing that dopamine cells survive and function in rat models (Björklund and Stenevi, 1979; Perlow et al., 1979). The notion that placement of catecholamine producing cells may functionally and behaviourally change parkinsonism was tested in rats with unilateral 6OHDA nigrostriatal lesions (Björklund et al., 1980; Freed et al., 1980; Dunnett et al., 1981 and 1983; Sladek et al., 1988), non-human primates (Redmond et al., 1986; Barkey and King, 1986) and finally in man with Parkinson's disease (Backlund et al., 1985; Madraso et al., 1987; Hitchcock et al., 1988). Two sources of dopaminergic tissue are used - the patient's adrenal medulla or fetal mesencephalon. Fetal tissue was shown to grow (Freed et al., 1980), send out axons across damaged neurologic tissue (Arbuthnott et al., 1985; Mahalik et al., 1985; Freund et al., 1985), excrete neurotransmitters and neuropeptides (Freed et al., 1983) which become metabolically active (Schmidt et al., 1982; Rose et al., 1985; Strecker et al., 1987). Due primarily to ethical difficulties in obtaining fetal material the emphasis was placed on adrenal medullary cell transplantations (Freed et al., 1981 and 1983; Madraso et al., 1987). Over 200

adrenal transplants (Lancet editorials, 1988-1990) have been performed in man with idiopathic Parkinson's disease. Review of the literature suggests that the technique bears a 10% overall mortality and does not demonstrate objective evidence of benefit (Goetz et al., 1989; Sladek et al., 1988; Allan et al., 1986; Landau, 1990). Hence adrenal transplants have been enthusiastically supported, over evaluated in man, but sadly show no evidence of success and thus should be abandoned.

Although less than 20 fetal mesencephalic transplants have been carried out in man, experimental fetal tissue transplantation appears more encouraging. The procedure has significant ethical implications (Mahowald, 1987). Animal data has utilised the 6OHDA nigrostriatal lesioned rat (Ungerstedt et al., 1970) and the MPTP-treated primate (Burns et al., 1983) to address the issues involved in cell growth, survival, interaction and function. With (Lund et al., 1987; Mason et al., 1986) or without immunosuppression human fetal cells are not rejected, up to 9 months (Widner et al., 1989) and grow in different species, such as human cells in rats or rat cells in mice (Brunden et al., 1983). Mesencephalic fetal cells, unlike adrenal cells, do not require nerve growth factor for long term survival, even attempting to send out axon terminals to contact other intrinsic cells (Krieger et al., 1982).

Stereotactic techniques (Backlund et al., 1985) compared with the open procedure, exposing the caudate nucleus (Madrasso et al., 1987) needs to be refined but is safe and feasible. Proof of functional, electrophysiological or *in vivo* improvement in hu an IPD is not currently evident. Studies report temporary improvement (Lindvall et al., 1989; Madrasso et al., 1987) but long term data beyond two year follow up remains disappointing.

Re-evaluation of animal data and longer follow up of those patients already successfully transplanted is required before further transplants are initiated (Lancet, 1988). The overall verdict in fetal transplantation is cautious optimism (Lancet editorial, 1987) but remains very controversial (Hitchcock et al., 1988; Landau, 1990).

Since we do not know the suspected environmental toxin that causes nigral cell toxicity and most neurologists today do not advise experimental striatal graft implant surgery, the best pharmacological management of idiopathic Parkinson's disease remains the only practical choice.

Levodopa problems have created a need to explore the role of the dopamine agonist drugs and to create well tolerated, potent compounds that are both D-1 and D-2 specific. Better definition of the role of monoamine inhibitors, slow release preparations, transcutaneous and subcutaneous drug delivery is concurrently being sought.

CHAPTER 2

THE BASAL GANGLIA AND DOPAMINERGIC PATHWAYS

2.1 Anatomy

Anatomically the basal ganglia comprise a group of subcortical grey matter masses. Their interconnecting pathways are derived from the embryonic diencephalon, mesencephalon as well as telencephalon and consist of the caudate nucleus, putamen (the combination referred to as the corpus striatum or neostriatum), globus pallidus, substantia nigra, subthalamic nucleus, parapontine reticular formation, various limbic components (cingulate gyrus, striae terminalis, nucleus accumbens) and the amygdaloid nuclear complex. Lentiform nucleus refers to the putamen externally and inner globus pallidus itself subdivided into an external segment (GPe) and internal segment (GPi). The central structure of the basal ganglia is the striatum. The striatum receives input from most areas of the cerebral cortex, the midline intralaminar thalamic nuclei and the substantia nigra zona compacta (SNc).

FUNCTIONALLY however, we need to expand the above nuclei to include parts of the embryonic diencephalon and mesencephalon, because the subthalamic nuclei, substantia nigra pars compacta (SNc) and pars reticulata (SNr), thalamus, pontine reticular formation, raphe nucleus and superior colliculus all interconnect intimately with the anatomic basal ganglia. The various pathways of the functional basal ganglia Fig.2.1 could be broken down into:

a) striatal input (afferent)

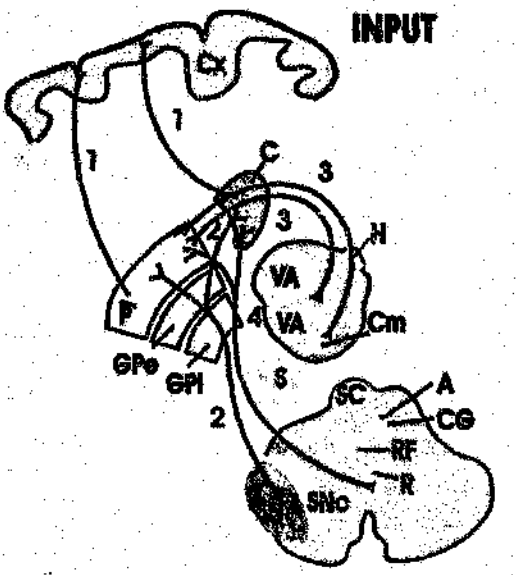
b) interconnections

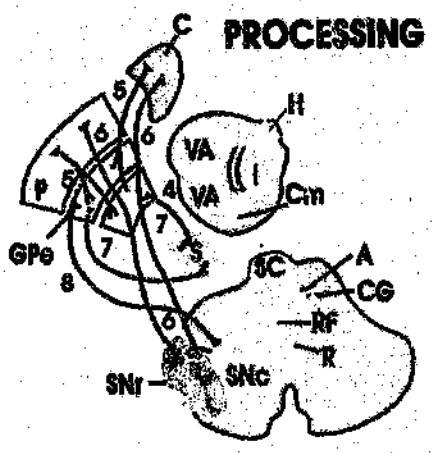
c) striatal output (efferent) systems

(McGeer et al., 1987)

Cortical structures, such as the supplementary motor cortex (SMA) or Brodman's area 6, of the frontal lobe are also integrated and crucial to normal functioning of this extrapyramidal network of nuclei. A motor act begins with idea formulation or praxis in the frontal cortex. Fibre groups within the corticostriatal tracts target the neostriatum or corpus striatum. The tract is massive, glutamate mediated neurochemically, excitatory to striatum and somatotypically arranged. The striatum receives less significant input from the midline thalamic nuclei, the centromedian and intralaminar nuclei and two brainstem nuclei namely the raphe nucleus and pars reticulata. These three afferent tracts to the striatum are respectively called thalamostriate and mesencephalostriate tracts being

MAJOR PATHWAYS OF THE BASAL GANGLIA





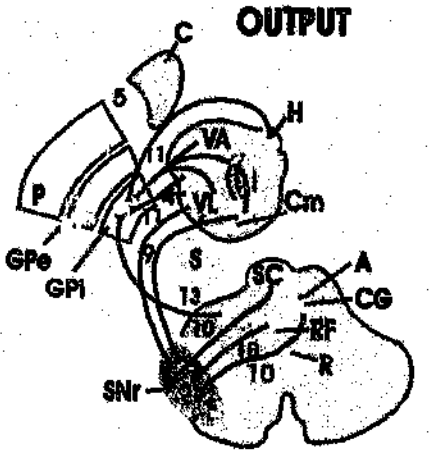


Fig 2.1

The major pathways of the basal ganglia:
(a) input circuits (striatal afferents)
(b) processing circuits
(c) output circuits (striatal efferents)

(See page 86 for detailed key)

Figure 2.1 Major pathways of the basal ganglia: key

A. Input circuits to the basal ganglia plus the nigro striatal feedback loop.

B. Processing circuits within the basal ganglia.

C. Output circuits of the basal ganglia.

1. Corticostriatal
2. Nigrostriatal
3. Thalamostriatal
4. Raphestriatal
5. Striatopallidal
6. Striatonigral
7. Pallidal-subthalamic-pallidal
8. Pallidonigral
9. Nigrothalamic
10. Nigrobrainstem
11. Pallidothalamic
12. Pallidohabenula
13. Pallidotegmental

CX: cerebral cortex
C: caudate nucleus
P: putamen
GPE: external globus pallidus
GPI: internal globus pallidus
S: subthalamic nucleus
SNC: substantia nigra, pars compacta
SNR: substantia nigra, pars reticulata
VL: ventral lateral thalamic nucleus
VA: ventral anterior thalamic nucleus
I: intralaminar thalamic nucleus
CM: centrum medianum thalamus
SC: superior colliculus
RF: reticular formation
CG: central gray
R: raphe system
A: aqueduct of Sylvius

(McGeer, 1987)

neurochemically also mediated by glutamate (Kim et al., 1976; McGeer et al., 1977). The thalamostriate are probably mediated by cholinergic input (Carpentier, 1981). The all important nigrostriatal tract is known to contain dopaminergic terminals (Beckstead et al., 1979). The striatal complex contains many different neurones. the commonest are the medium spiny neurones comprising 80-90% of the cells, which while utilising GABA (Fonnum et al., 1978; Nagy et al., 1983) also contain other neurotransmitters (Graybiel and Ragsdale, 1983). Axons and dendrites from these cells receive input from the dopamine containing nigrostriatal tract. Large cholinergic interneurones, called large spiny striatal neurones are also found in the striatum (Phelps et al., 1985). Smaller somatostatin and neuropeptide Y-containing interneurones are less commonly observed (Di Figlia and Aronin, 1982). Recently "striosomes" representing islands of dopamine cell populations are demonstrated in the striatum (Graybiel et al., 1983). Functionally these striosomes selectively receive reciprocal input from the SNc and frontal cortex (Gerfen et al., 1982). The remaining "matrix neurones" stain differently to striosomes and receive input from SNr and ventral tegmental area (VTA) (Jimeng and Graybiel, 1985). Collectively the information thus far transmitted is funnelled and converged through the interconnecting pathways from the caudate and putamen via striatal efferent tracts mainly from the median spiny neurone field through

the external, then the internal globus pallidus. The internal globus pallidus and pars reticulata of the substantia nigra are the two major output zones of the basal ganglia network. The subthalamic nuclei connect reciprocally (Kitai, 1981) with the internal globus pallidus but do not receive input from the external globus pallidus (Carpentier, 1981). The neurochemicals within these nuclei are numerous but include GABA (Fonnum et al., 1978), acetylcholine (Agid, 1987) and other neuropeptides (Graybiel, 1990; Young and Penny, 1983). Finally output from the internal globus pallidus and pars reticulata occurs, via GABAergic inhibitory pathways, called ansa and fascicularis lenticularis. The subthalamo-thalamic fasciculus connections to the ventral nuclear tier of the thalamus are situated principally in the lateral and anterior thalamus nucleus ventralis (VLo). In addition a "comb" system of fibres traverse the internal capsule to connect basal ganglia to the thalamus (Webster, 1990). Thereafter the thalamus radiates to the pre-frontal motor cortex and via other tracts to the brainstem (Yoshida et al., 1966). The nigrostriatal dopaminergic tract connects SNc to the caudate and putamen thereafter through GPe to the GPi. The SNr also projects to the thalamus (ventromedial thalamus) and brain stem (superior colliculi and reticular formation). The striato-pallidal complex influences the motor cortex, less directly the brainstem and the spinal cord motor apparatus.

Analysis of this intricate network suggests that information from the frontal cortex is rapidly transmitted via many modifying steps to the striatal "small spiny neurones" and then via interneurones back to the pyramidal system. The cumulative striatal signal which arrives at the thalamus is balanced by additional input to adjacent but different thalamic nuclei from the cerebellar hemispheres. A delicate balance is thus set up to govern overall thalamic influence upon the pyramidal system and thereby execution of movement. There is no known important direct output below the mesencephalon to the motor system, so the thalamus must influence the pyramidal system via ascending systems to the cortex. More complex than the above motor and extrapyramidal network is the influence of the limbic system, which governs mood, affect, emotion and stress. Intricate connections exist between the mesolimbic areas and the brainstem, nucleus accumbens, hypothalamus and other cortical and subcortical structures. Including the basal ganglia, these structures are collectively referred to today as the mesolimbocortical radiations. While it is clear that the control of movement is one of many functions of the basal ganglia, exactly which component of overall movement is still controversial. For example bilateral destruction of the caudate nucleus does not always affect movement. Several projec- tions from the basal ganglia to the cortex occur to areas which do not appear involved with movement control. Currently it is thought that the basal

ganglia integrate "higher centres" (such as sensory, cognitive, limbic and motivation) to the motor system (Everts, 1981). Whatever output the cortex and thalamus induces upon the striatal spiny neurones (which make up 90% of striatal microarchitecture), concurrent dopaminergic input from the nigrostriatal tract, arising from the pigmented neuromelanin containing dopaminergic cells of the SNc is crucial to striatal function. Dependant upon exactly where this dopaminergic influence is focused, dominant effects may influence the signal ushered by the collective pool of medium spiny neurones (Smith and Bolam, 1990). Idiopathic Parkinson's disease is the primary example of a deficient nigrostriatal dopaminergic tract, consequent upon cell death in the substantia nigra pars compacta. The result of excessive inhibitory input into the thalamus is rigidity and akinesia. Replacement with the neurotransmitter L-dopa corrects this state and explains why the afflicted patient notices tremendous benefit as judged by improved execution of movement. Additional dopaminergic pathways originating from the ventral tegmental nucleus situated medial to the midbrain's substantia nigra, influence the various limbic nuclei, including nucleus accumbens, lateral hypothalamus and selected areas of cerebral cortex. While not directly responsible for movement these systems influence motivation to move and may be responsible for excessive side-effects due to levodopa and other compounds when treating the deficient nigrostriatal output state. Additional input

from raphe nuclei (serotoninergic) and locus ceruleus (noradrenergic) is also present.

2.2 Physiology

Any hypothesis concerning the true function of the basal ganglia must incorporate what is clinically established in Parkinson's disease, Huntington's disease and clinico-pathologically correlated case studies (Marsden, 1982). We know from lesioning experiments in animals and pathology case studies in man, that defects in the basal ganglia are associated with disorders of muscle tone and diminished movement. Exactly which part of the basal ganglia machinery and network is responsible for specific abnormalities, remains elusive. For example, large destructive lesions, usually bilateral, are required to produce bradykinesia and rigidity in primates. Small, accurately placed lesions leave no motor deficit (Lee, 1984). Overall weakness is not a characteristic of disturbed basal ganglia function but speed, planning, sequencing, fluidity and execution of movement are all affected.

Alexander et al, described at least five parallel functioning loops passing through the basal ganglia. They subdivided these specific functioning loops as:

a) A motor circuit, projecting to the suplementary motor cc :ex (SMA, Area 6)

b) An oculomotor circuit, projecting to frontal eye fields (Area 8)

c) An anterior cingulate circuit receiving input from the hippocampus, amygdaloid and limbic systems.

d) A dorsolateral prefrontal projection.

e) A lateral orbitofrontal prefrontal projection.

Knowledge is limited in the latter three categories as researchers tended to concentrate upon the motor pathways (Alexander et al., 1986). Clearly many basal ganglia functions exist outside the domain of motor physiology (Alexander et al, 1986; Marsden, 1982). Many models of the basal ganglia have been proposed.

It is possible that the idea to move begins in the frontal cortex, along with integrated input from the sensory motor cortex, premotor cortex and posterior parietal lobe, all of which are mediated through the striatum. Widespread cortical impulses are directed subcortically, focused through the thalamus and directed back to the supplementary motor cortex. It is known that patients with frontal cortical atrophy, whatever the aetiology, exhibit symptoms of slowness, slow thought process and initiation, apraxias

and may show "paratonic rigidity" (Gegenhalten) which is remarkably similar to parkinsonian symptomatology (Everts and Wise, 1984). Specific degeneration of the frontal cortex is not a significant pathological finding in parkinsonian patients and hence the defect that causes bradykinesias must reside subcortically, probably in the basal ganglia. When comparing the subcortical basal ganglia motor control mechanisms with those of the cerebellum, two striking differences emerge. Firstly, there is little direct sensory input to the basal ganglia in contrast to the two ascending spinocerebellar tracts that ascend the spinal cord and influence the cerebellar hemispheres. Furthermore, extensive projections conveying joint sensation, from tendon, muscle and skin receptors ascend both the posterior columns and spinocerebellar tracts to influence the cerebellum. Secondly, the output from the internal segment of the globus pallidus, while targeting the thalamus in the same ventro-lateral and ventro-anterior area, remains separate from the cerebellar input which is destined for the primary motor cortex. There are specific cerebellospinal pathways, such as the reticulospinal and vestibulospinal pathways, which in addition to thalamic influence may modify motor output. The cerebellar connections have more direct access and influence upon not only the primary motor cortex, but also the brainstem and spinal cord compared with the basal ganglia. Why this relationship exists is unknown, but the dual cerebellar and

basal ganglia input allow specific control of the pyramidal output and specific execution of motor actions are carefully governed and checked continually.

2.2.1 Clinical evidence of normal basal ganglia function

Early this century Kinnier Wilson began asking questions regarding basal ganglia such as: What is the origin of motor control and what components of this complex system break down in Parkinson's disease? Marsden suggested in 1981 that evidence still pointed to the functioning basal ganglia (as opposed to anatomic) as the origin of motor control and hypothesised that the basal ganglia are responsible for the automatic execution of learned motor plans (Marsden, 1982). To understand the implications of this, one is invited to do battle with the physiology of just how we move! In idiopathic Parkinson's disease, however, motor slowness overshadows any defect in sensory, cognitive, intellectual or perceptual findings. While patients may complain of burning, uncomfortable paraesthesia, and sometimes even pain, no objective sensory deficit is demonstrated. Some patients show evidence of dementia. The majority of idiopathic Parkinson's disease patients remain intellectually intact, as James Parkinson himself suggested "...the senses and intellect being uninjured".

Based upon the observation that akinesia (bradykinesia) is fundamental to the diagnosis of Parkinson's disease which is a good example of abnormal functioning of the basal ganglia, comparison of parkinsonian patients with normal controls provides a useful platform to analyse the various components of movement.

Concentration upon normal basal ganglia function in contrast to the parkinsonian patient, will simply draw comparisons, suggesting where movement breaks down. Humans move in many different ways and each movement may be broken down into automatic, repetitive or voluntary components which may be slow (ramp) or rapid (ballistic).

Electrophysiology has demonstrated that each movement requires:

a) the idea (inherent thought or automatic stimulus response)
b) the perceptual judgement
c) memory of the sequence
d) plan of action
e) execution of the motor plan
f) checking of the movement with modification
g) stopping the movement or repetition (Marsden, 1982).

Concepts governing initiation of movement will not be dis-

cussed further but may be relevant when considering "paradoxical kinesia" so well known in parkinsonian patients. Movement depends upon the degree of practice or degree of memory of the motor plan, which eventually becomes automatic. What comprises the "automatic" part of voluntary movement is still controversial (Phillips and Porter, 1977).

Ballistic (rapidly executed) movements are short lasting, energetic and the stimulus will halt long before the limb achieves its objective. Marsden refers to this as "open-looped" type programme in that it is preprogrammed and occurs so fast that not enough time transpires for it to be checked or regulated by peripheral stimuli. EMG's show agonist contraction, then silence while the antagonist muscle fires and finally the agonist incrementally fires again (Hallet and Marsden, 1979).

Ramp (slowly executed) movements are slow, continually corrected by feedback control and in contrast are called "closed-loop" movements. Most movements will have the characteristics of both ramp and ballistic movement characteristics, but the former are typically mediated via the basal ganglia and the latter via the cerebellum (Kornhuber, 1971). Memory and learning enter into the equation since people will correct movements faster than their initial attempts when following a target or visual

trajectory upon an oscilloscope screen. Synergist muscles, prepare a platform from which a movement is executed. Synergists are crucial to the execution of balance and actions. These muscles are also thought to be controlled by the basal ganglia (Purdon-Martin, 1967). Purdon-Martin called these synergist muscles the "proximal postural fixators". EMG recordings show these muscles to anticipate a given movement and if defective destabilise the foundation from which a given movement is executed.

Structure of movement may be broadly subdivided into planning the motor program and execution of the plan (Allen and Tsukahara, 1974; Marsden, 1981). Once the action is conceived, "where", "when", and "how" to act to achieve the task must be internally programmed. "When" implies timing, "where" involves planning and "how" is crucial to basal ganglia functioning. Learned automatic pathways are thus utilised in a fashion similar to a computer which "RUNS THE PROGRAMME". Running the programme also involves fine control, modification if needed and finally termination once achieved. Brooks modified Allen and Tsukahara's concepts whereby the programme plan executes an idea through the combined influences of the basal ganglia, lateral cerebellum and frontal association cortex which is conveyed by a signal to the motor cortex whereby movement may be finally executed. The cerebellum (specifically the intermediate cerebellum or pars intermedia) then modifies

execution of the movement by integration of peripheral proprioceptive information. It has been proposed that motor programmes exist as a "set of structured commands" established and memorised before a movement begins and may allow the entire sequence to be performed without peripheral feedback" (Keele, 1968; Brooks 1979; Traub et al., 1982.)

2.2.2 Parkinson's disease and abnormal basal ganglia function

Having put forward various levels, that if defective would slow down movement, what can parkinsonian patient studies tell us regarding the initial decision to move, during slow ramp movements or fast ballistic movements?

Marsden argues that the preparation for movement is normal and minimally slowed. Evidence for this comes from a study of the readiness potential recorded over the frontal cortex (Beritschafts-potential) by surface EEG recordings. An EEG spike, which is found by computer-linked back averaging, is maximal over the precentral frontal area and contralateral to the limbs that actively move. This EEG spike is not recorded following passive limb movement. While some have found delay in the timing of this potential (Shibaski et al., 1978) most researchers have not (Marsden, 1982). All reports agree that the amplitude of the readiness potential is reduced in idiopathic Parkinson's disease (Traub et al.,

1980). Initiation of movement is apparently slowed in parkinsonian patients, yet this is discrepant with observations by Wilson who suggested simple movements were not significantly slowed (Wilson, 1925). Further breakdown of initiation of movement into "reaction" time and "execution" time becomes necessary and the suggestion is that both aspects of timing are prolonged, often independently (Everts et al., 1981). Movement execution time is more consistently prolonged and correlates with the akinesia observed clinically (Everts et al., 1981).

Studies utilising EMG's of various synergistic muscles examining the correlation of agonist and antagonist muscle movement reveal normal patterns in idiopathic Parkinson's disease. Studies of this aspect utilising reciprocal inhibition and H reflex studies in the forearm show no difference between normal control and idiopathic Parkinson's disease patients. Synergist muscles stabilise patients automatically since synergist muscle activity is normal in parkinsonian patients, they are unlikely to be of major importance to the striatum (Wilson, 1925). Perhaps the globus pallidus is the centre for postural control integrating kinaesthetic, labyrinthine and visual information but this hypothesis remains to be confirmed (Martin, 1967).

2.2.2.1 Closed-loop (Slow, ramp) reflexes

Slow movements are reasonably accurately executed in

parkinsonian patients since kinaesthetic input, especially visual perception, is not disturbed. Stretch reflexes (both H reflexes and tendon jerks) are normal but tonic vibration reflexes are increased in parkinsonian patients (McLellan, 1973). Exteroceptive spinal polysynaptic reflexes elicited by percutaneous electrical stimuli, involving excessive IA afferent input, are exaggerated (Rondot and Metral, 1973). Since parkinsonian patients have difficulty relaxing, this finding is thought to be equivalent to excess alpha-gamma linkage (Burke, 1977). Long latency stretch reflexes (Phillips, 1969; Marsden et al., 1973 and 1982) have been suggested to represent a transcortical stretch reflex. Tatton and Lee (1975) first showed abnormally long latency stretch reflexes in parkinsonian patients compared with normal controls, especially when studying ramp compared to relatively normal ballistic movements (Mortimer and Webster, 1979; Chan et al., 1979).

2.2.2.2 Open-loop (fast, ballistic) reflexes

This is where the crux of parkinsonian motor deficit and disability resides. While parkinsonian patients may compensate with slow ramp movements they cannot recruit with ballistic mechanisms accurately. Many authors have demonstrated this (Wilson, 1925; Flowers 1976; Hallet et al., 1977 and 1980) with parkinsonian patients "slowly creeping"

toward the required position at a slow, steady, accurate rate in contrast to that observed with rapid movement speed. EMG's show failure to generate the required initial agonist burst, inducing further agonist bursts, which increase with time. Since the initial agonist burst is thought to be the one that develops with practise into an accurate movement, it is not surprising that parkinsonian patients are slow to start ballistic movements. In addition, clinical observations show they are delayed learners and cannot perform multiple tasks, (Schwab 1954 and 1964). Study of patients who suffer from "on-off" motor fluctuations very clearly demonstrate that when "off", akinetic and rigid, they recruit agonist muscles slowly and inaccurately. The same patient on therapy, mobile and "on" performed the same task quickly, accurately and employing the correct initial EMG recorded burst of activity (Marsden et al., 1977). Marsden summarises this concept by hypothesising that the defect could be one of MOTOR PLANNING with perceptual judgement and motor execution relatively intact. Motor plan, as distinct from motor programmes which are concurrently linked by a hierarchic motor control system, is abnormal in Parkinson's disease in that the parkinsonian patient cannot execute simultaneous or sequential programmes. Marsden concludes "the automatic execution of learned motor plans" planning is "automatic" (because the basal ganglia normally functions subconsciously), "execution" (indicating the basal ganglia

sequence of motor programmes to achieve the end motor plan) and "learned" (because of the effects observed with careful practise) (Marsden, 1982).

2.3 Microphysiologic evidence of normal basal ganglia function

When one takes the physiological concepts one step further and examines the microphysiology at a cellular level, matters become more complex since we rely upon electrical stimulation techniques and single cell recordings employing micropipette technique. However it is still relevant to study cellular physiology of the target zones from the striatum to learn how the striatum itself functions. Limitations in technique aside from accurate anatomical placement, are that one cannot know exactly within which cell the pipette tip is placed. Since at least 9 distinct striatal neurones are present on golgi impregnation studies and more than a dozen neurotransmitters identified (Bolam, 1990), it would be important to be sure from which cell one was recording. Cells are classified according to their site, size, dendritic spines and immunochemical characteristics (Table 2.1). The most common striatal cell, is the medium sized, densely spined neurone that contains GABA, substance P and encephalin neurotransmitters. Larger, colinergic, spiny cells are rare on Golgi preparations but only found in the ventral striatum and send projections to the substantia nigra. Interneurones, uti-

Table 2.1

Different types of identified neurones in the neostriatum

	Projection sites	Neurotransmitters
OUTPUT NEURONES		
Medium size densely spiny neurons	Globus pallidus Substantia nigra Other striatal neurons	GABA Substance P Enkephalin
Large aspiny neurons	Substantia nigra	ACh
INTERNEURONS		
Cholinergic neurons		
GABAergic neurons		
Somatostatin neurons		

(Bee, 1987)

lising GABA, acetylcholine and somatostatin neurotransmitters form several morphologic types on Golgi preparations. These cells are thought to connect medium spiny neurones to one another and thereby amplify in a forward and co-ordinated way an impulse travelling from striatum inwards to globus pallidus. Pipette probes are statistically likely to reach the medium, spiny, GABAergic neurone visually but obviously difficult to confirm. Inputs from the cerebral cortex terminate upon many cells including the medium, spiny cells. Dopaminergic input from the SNc synapses upon the same neurone pool. Evidence suggests the inhibitory influence of dopamine occurs more critically upon dendritic shafts and the cell soma itself on striatal neurones while corticostriatal input is distally received upon dendritic boutons which set overall striatal tone (Nieoullar et al., 1983; Smith, 1991).

Data that has emerged should be interpreted with three cautionary points prior to acceptance of results that follow (De Long et al., 1979).

Firstly, one supposes that the recording micro-electrode is in the medium, spiny cell. Only if correctly placed will it then reflect the main traffic through the striatum and globus pallidus. Secondly, neurones in areas of the basal ganglia show a less than precise relationship to specific body movements. Hence one could cannulate the correct cell

and perform an action but get no impulse because that cell only responds to some other action. Thirdly, specific dopaminergic islands within the majority of cholinergic and GABAergic striatal neurones have been identified (Graybiel et al., 1988). Should a micro-electrode enter one of these "striosomes" in the striatum it would predominantly reflect nigrostriatal dopaminergic contributions and hence invalidate data acquired by this method.

Microphysiological studies suggest a striking difference between the input centre (striatum) and the output centres (GPi or SNr). The output zones intrinsically have a high level of spontaneous activity with sustained high frequency discharges (De Lan, 1979), in contrast to the input zones such as the putamen which is electrically silent at rest but shows intermittent electrical bursts which correlate with contralateral limb movement (Critcher, 1984). Only 50% of neurones in the putamen or globus pallidus fire and different cell populations are thought to exist for extension versus flexion across a joint. Direction of movement is preserved within the striatum while a large reserve of cells are not routinely employed for simple movements. Timing of these impulses is not tightly controlled but a marked increase in cell firing occurs at the onset of the movement. More cells are recruited when an opposing force is applied. In summary, some cells monitor initiation of movement, some direction and yet

others amplitude of a given voluntary movement. Once electrodes read "down-stream" in the output zones (GPi and SNr) there is continual electrical transmission towards the thalamus. While the impulse is not started in the striatum, there is a behavioural preparation that suggests electrical evidence within the putamen that allows adequate preparation for movement. This has been termed a "motor set" which seems to prime the system in anticipation of a given movement (Everts, 1984). This was neatly shown on a primate model, the micro-electrode placed within the putamen. Following an auditory signal the animal would need to move a lever to obtain a juice reward. Putamenal activity, which correlates with observed limb movements, indicates the "motor set" neuronal activity. This priming activity diminished once the signal was repeatedly not followed by the usual juice reward. This suggested the anima. learned not to expect a reward and the putamen cells were set back to baseline resting state. Somatotopic orientation of neurones has been suggested with the SNr carrying head and neck fibres and the GPi serving the arm medially and the leg laterally (De Long, 1979). Putamen cells while thought also to be somatotypically represented are more difficult to separate by these techniques.

Microstimulation of these areas has produced conflicting results, but while stimulating the caudate produces no contralateral movement, stimulating the putamen sometimes

produces electromyographically detected contralateral movements (Alexander and De Long, 1985).

2.4 Normal dopaminergic pathways

Dopamine was first demonstrated microscopically in storage vesicles in peripheral tissue, not initially neuronal cells but rather within peripheral mast cells (Falck et al., 1959). Later dopamine was demonstrated in nerve cells of snails (Dahl et al., 1962), mammalian retina (Haggendal, 1963) and in striatal projections from the mesencephalon (Andén et al., 1964; Bertler et al., 1964; Dahlström and Fuxe, 1964) and limbic forebrain (Andén et al., 1965; 1966).

Neurotransmitters and their receptors may be divided into three broad subgroups (McGeer et al., 1977) depending upon their receptor mechanisms and biochemical characteristics (Table 2.2). The amino acid neurotransmitters have inophoretic receptors that instantaneously act via ionic membrane channels. Examples of these in the basal ganglia are glutamate (excitatory) or GABA (inhibitory). The second class of neurotransmitter exemplified by acetylcholine (excitatory) or dopamine (inhibitory) take longer to exert their influence because they act via a second messenger system, such as cAMP or cGMP. These second

Table 2.2

Neurotransmitters and receptor types in the brain

Transmitter Type	Concentration Range (nanomoles/gn tissue)	Major Receptor Type
Amino acids	2-14 X 10^3	ionotropic
Amines	1-25	metabotropic
Peptides	10^{-3} to 10^{-6}	unknown

Receptor Type	Locus of action	Typical Response Time (sec)
ionotropic	membrane ion channels	10^{-3}
metabotropic	second messenger	10^{-1}
genotropic	transcription of specific mRNA	10^3 - 10^6

(McGeer 1987)

messengers are within cell cytoplasm, hence postsynaptic and more generalised in functional specificity. Examples of this are the dopamine D-1 receptors which are linked to dopamine specific cyclic AMP. Least is known of the peptide and genotrophic cell receptors. Signals that occur in the region of cell nuclei produce messenger RNA (mRNA) which transcribes specific cell to cell signals. However the impulse takes a long time, because protein manufacture is required and thereafter cytoplasmic mRNA production. Examples of these genotrophic class receptors are the steroid receptors, and in the basal ganglia the endorphins, enkephalins, cholecystokin, somastatin, oxytocin or vasopressin which continue to intrigue workers in the field (Agid, 1987; McGeer 1987).

Table 2.3 presents the current concepts of these neurotransmitters divided into the three subtypes described, including overall input and output of basal ganglia nuclei themselves. Many of these pathways influence each other directly or indirectly and indeed more than one chemical may be present in similar cells. Hence cell death in one area will not only reduce local neurochemicals produced at that site but may influence, via their synaptic terminals, other areas even indirectly affecting different neurotransmitter subgroup systems.

Crucial to our understanding of dopaminergic pathways has

Table 2.3 Neurotransmitters of the basal ganglia

Transmitter	Interneurons [ ] or Paths between nuclei
Type I	
Gutamate/Aspartate	Cortex to CP, SN Subthalamus to GP, SN Tegmentum to SN
GABA	[CP], CP to GP, SN, EP GP to EP, CP, SN, (subthalamus) EP to thalamus, tegmentum, habenula, Raphe to CP NAc to GP, (SN) SN to CP, thalamus, SuC, tegmentum, midbrain (Cortex to SN)
Type II	
Acetylcholine	[CP], VnGP to CP GP to CP Tegmentum to SN
Noradrenaline	LC to CP, SN
Adrenaline	LC to CP, (SN)
Dopamine	SN to CP, GP, spinal cord, amygdala, olfactory system, subthalamus, Raphe to CP
Serotonin	Raphe to CP, SN
Histamine	Hypothalamus to CP
Type III	
Dynorphin	CP to GP, SN, EP
Enkephalin	CP to GP, EP, SN
Tachykinins (SP and SK)	CP to GP, SN, EP, VnGP
Cholecystokinin	Cortex to CP Amygdala to CP Contra. SN to CP (NST to SN) ([CP] or CP to ?)
Somatostatin	[CP], (Amygdala to CP) EP to habenula
Oxytocin	Hypothalamus to SN
Vasopressin	(Hypothalamus to SN)
Neuropeptide Y	[CP]
IB-236	(CP or CP to ?)
Neurotensin	[CP] and CP to SN

been the development of suitably sensitive histologic and tracer techniques. Immunohistochemical stains can stain with great sensitivity catecolaminergic (including dopaminergic) colinergic, serotoninergic, GABAergic and glutamate containing cells, axon pathways and terminals. These comprise not only the classical cell body neurotransmitters but in addition many neuropeptides (CCK 8, substance P, VIP, somatostatin, enkephalin, neurotensin) all of which may be individually stained. Retrograde tracer studies with immunofluorescence can trace out pathways, from these cell body groupings.

Dahlström and Fuxe (1964) described the A1 to A12 dopamine and catecholaminergic cell groups to which others, A14-16, have been subsequently added from rodent model data. The largest assembly, namely the A8, A9 and A10 cell groups, are the mesencephalic dopamine cell system, equivalent to the nigrostriatal tract origin in man, which links the SNc (dopamine cell bodies) to the striatum (axon cell terminals). The diencephalic cell system (Group A1 to A14), links the spinal cord, midbrain, hypothalamus and pituitary gland but is much smaller containing only 10% of the number of dopamine cells compared with the nigrostriatal tract (Björklund and Lindvall, 1984). Dopamine cells and their projections comprise 80%, the remaining 20% are mainly noradrenergic. Three quarters of these dopamine cells are located in the mesencephalon. Transplantation advances

involving foetal studies have produced data that is compatible with the proposed existence of similar pathways in primates. One important difference, namely the mesocortical dopaminergic pathway which links the midbrain dopamine cells to the mesocortex, is however found (Parent et al., 1984). The much larger cerebral cortex (telencephalon) in man compared to other primates and especially to lower animals, identifies an evolutionary trait of mammalian development and adaptation. This stresses a crucial difference that should be remembered to avoid erroneous conclusions when extrapolating rodent data to primates, including human primates. Central serotoninergic systems remain comparable despite phylogenetic advances (Olson and Sieger, 1972), the cell bodies of which reside in the mesencephalic and pontine raphe nuclei which in turn send ascending axons to influence the ascending forebrain or descending axons to influence the spinal cord (Steinbusch, 1984).

Mammalian central monoaminergic systems are highly complex and widely collateralised. Single cell bodies may thus influence widespread areas, exerting diffuse influence over many different neurones (Parent et al., 1984). The relatively inexpensive rodent model has been widely researched to study dopaminergic pathways and mechanisms. One may thus individually examine the following:

2.4.1. topographical anatomy of DA cell bodies

2.4.2. fibre tracts containing dopamine

2.4.3. dopaminergic terminals from fibre tract projections

2.4.4. dendritic fields of nigral dopamine cell bodies

2.4.5. functional integration of dopamine neurones and tracts.

2.4.1 Topographical anatomy of dopamine cell bodies

Reviews and rodent atlases (Dahlström and Fuxe, 1964; Fallon and Moore, 1978; Swanson, 1982; Björklund et al.,1975 and Halasz et al., 1977) specifically staining dopamine cells with tyrosine hydroxylase immunocytochemistry indicate that the majority of dopamine cells reside in the lateral SNc (A9 region) with little overlap to A8 (pars lateralis) and A10 (ventral tegmental) areas. This collectively is called the MESENCEPHALIC DOPAMINE CELL SYSTEM.

Separate from the mesencephalic cell system there are four cell body origins of the DIENCEPHALIC DOPAMINE CELL SYSTEM (Fig 2.2).

a) The <u>tuberal cell group</u> (A12) which projects to the pituitary and hypophyseal complex.

b) The <u>caudal diencephalic cell group</u> (A11) in caudal

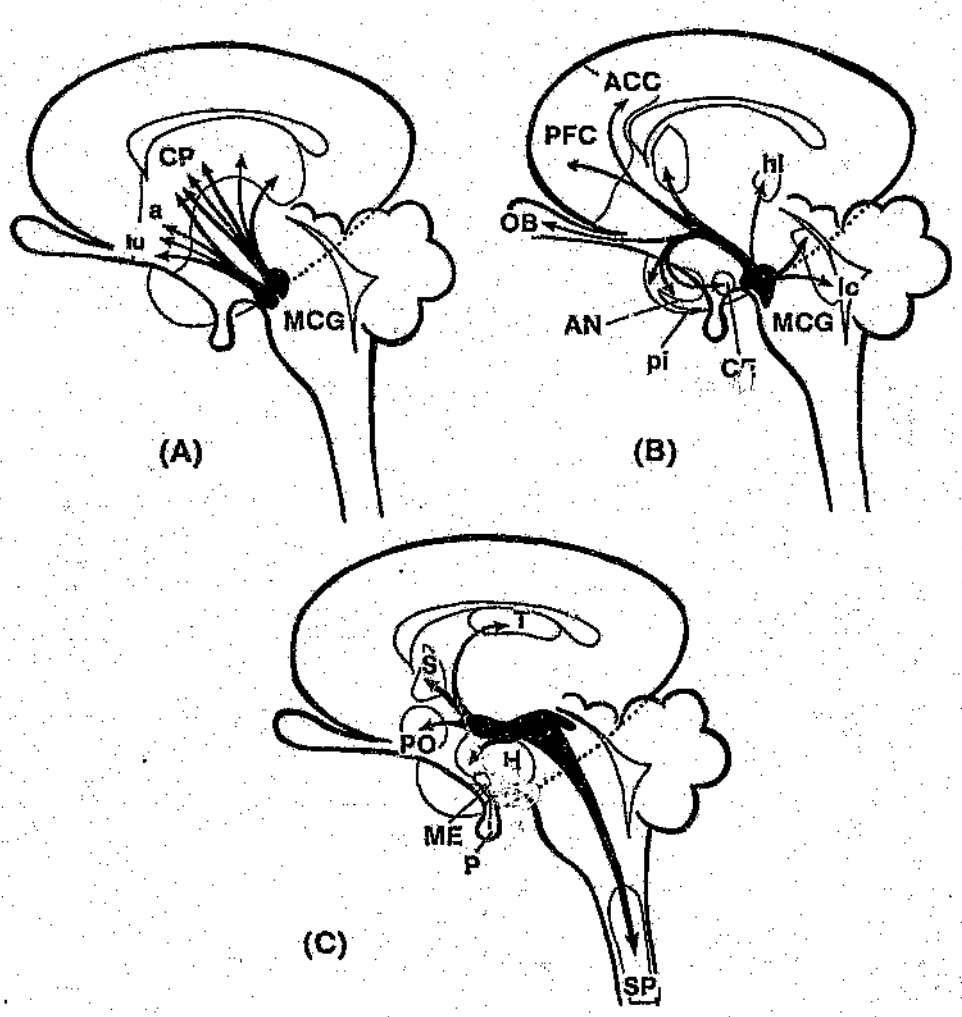


Fig 2.2

Schematic representation of the dopaminergic systems of the brain
(a) mesostriatal
(b) mesolimbocortical
(c) diencephalic

Key:	a:	nucleus accumbens	OB:	olfactory bulb
	ACC:	anterior cingulate cortex	PFC:	prefrontal cortex
	AN:	amygdaloid nuclei	P:	pituitary gland
	CE:	entorhinal cortex	pi:	piriform cortex
	CP:	nucleus caudatus-putamen	PO:	preoptic area
	H:	hypothalamus	S:	septal area
	hl:	lateral habenular nucleus	SP:	spinal cord
	lc:	nuclues locus ceruleus	sl:	lateral septal nucleus
	MCG:	mesencephalic DA cell groups	T:	thalamus
	ME:	median eminence	tu:	tuberculum olfactorium

Table 2.4

Major dopaminergic systems of the brain, their cells of origin and their projections

System	Cells of origin	
1) Mesotriatal system	Substantia nigra	(A9)
	tegmental area	(A10)
	retrorubral nucleus	(A8)
Projections		
Striatal areas, particularly		
globus pallidus		(A9)
nc.accumbens	(A10, [A8] medial A9)	
nc.caudatus-putamen		(A9)
ventral part		(A8)
anteromedial part	(A10 [lateral part])	
olfactory tubercle	(A10 [lateral part])	
interstitial nucleus of the stria terminalis		(A10)
islands of Calleja		(A9, [A10])
subthalamic nucleus		(A9)

System Projections	Cells of origin	
2) Mesolimbo-cortical system	Ventral tegmental area, limbic and substantia nigra, retrocortical areas and rubral nucleus	
Projections		
Limb and cortical areas particularly		
olfactory bulb		(A10, medial A9)
anterior olfactory nuclei		(A10, medial A9)
lateral septal nucleus	(A10 [medial part])	
piriform cortex		(A10, medial A9)
amygdala		(A10, medial A9)
ventral entorhinal cortex	(A10 [lateral part], [A8])	
suprarhinal cortex		(dorsolateral A10)
pregenual anteromedial cortex	(A10 [medial part])	
supragenual anteromedial cortex		(A9)
peripheral cortex and temporal association cortex		(lateral A10, A9)
lateral habenular nucleus		(medial A10)
locus ceruleus		(A10, A9)

Table 2.4 (continued)

System	Cells of origin	Projections
3) Diencephalospinal	Dorsal and posterior hypothalamus, zona incerta, caudal thalamus (A11)	Spinal cord
4) Periventricular system	Mesencephalic periaqueductal gray, periventricular gray of caudal thalamus (A11)	Periaqueductal gray, medial thalamus and hypothalamus
5) Incertohypothalamic system	Zona incerta, periventricular hypothalamus (A11, A13, A14)	Zona incerta, anterior medial preoptic and periventricular hypothalamus, septum
6) Tuberohypophyseal	Arcuate and periventricular hypothalamic nuclei A12 and A14)	Median eminence, par nervosa and pars intermedia of the pituitary
7) Periglomerular dopamine neurons	Olfactory bulb (A16)	Dendritic processes into olfactory glomeruli
8) Retinal dopamine system	Mainly in the inner nuclear layer of the retina	Local dendritic projections

Table 2.5

Subdivisions of dopaminergic projection systems from the mesencephalic cells

System	Origin	Terminal area
Nigrostriatal system (o)	Substantia nigra	Nc.caudatus-putamen (dorsal striatum)
Mesostriatal system (n)		
dorsal part	Substantia nigra	Nc.caudatus-putamen (dorsal striatum)
ventral part	Ventral tegmental area	Nc.accumbens, tuberculum olfactorium, bed nucleus of the stria terminalis (ventral striatum)
Mesolimbic system (o)	Ventral tegmental area	Nc.accumbens, tuberculum olfactorium, bed nucleus of the stria terminalis, septum, amygdala
Mesolimbocortical system (n)	Ventral tegmental area Medial substantia nigra	Septum, amygdala, piriform, entorhinal, prefrontal and anterior cingulate cortex, habenula and limbic brain stem regions

periventricular caudal thalamus and posterior-dorsal hypothalamus.

c) The dorsal hypothalamic cell group (A13) found in the medial part of zona inserta, ventromedial to the mamillothalamic tract and

d) The rostral periventricular cell group (A14) scattered in the hypothalamus from anteri r commisure to median eminence.

The OLFACTORY BULB and RETINA comprise the final and recently described subdivision (A16) of DA cells, containing comparatively little endogenous dopamine.

2.4.2 Fibre tracts containing dopamine

Two main catecholaminergic (CA), tracts the noradrenergic central tegmental tract (including the noradrenergic dorsal tegemental bundle) and the dopaminergic dorsal periventricular system are found in the brainstem. Medulla, pons, mesencephalon and diencephalon fibre bundles ascend and descend, finally assembling to form three output projections.

a) nigrostriatal pathway
b) ventral periventricular system
c) medial forebrain bundle (Ungerstedt, 1971; Lindvall and Björklund, 1974).

a) Nigrostriatal dopaminergic tract (NST)

The DA fibre tract, so crucial in the pathogeneses of all parkinsonian models, connects SNc to the striatum (Anden et al., 1966; Ungerstedt, 1971; Lindvall and Björklund, 1974; Hokfelt, 1974 and Nuta et al., 1974). Fibres run from the lateral pars compacta (A8), ascend medially, collecting a few VTA (A10) axons along the way, to sharply turn rostrally and form the H-2 fields of Forel, incorporate the substantia innominata, dorsolateral to the medial forebrain bundle. They skirt the subthalamic nucleus, bend sharply above the crus cerebri then rostrally into the internal capsule passing the globus pallidus and eventually fan out into the thalamus (VLo). The most ventral fibres of the nigrostriatal tract continue rostrally in front of the anterior commisure along with the medial forebrain bundle to the limbic system entering the ventromedial caudate-putamen complex. This allows topographical distribution of limbs within the striatum.

b) Ventral periventricular system

This system ascends and probably descends dorsally along the periaqueductal grey matter linking medulla oblongata to diencephalon. In addition ventral projections to the hypothalamus exist. The function of this tract is unknown.

c) Medial forebrain bundle (MFB)

This mixed noradrenergic and dopaminergic limbic pathway connects the reticular formation of the brainstem to the diencephalon and telencephalon. Dopamine fibres from MFB run close to the NST then divide into lateral and medial fibres. Lateral fibres go to the amygdala, piriform, perirhinal cortex and entorhinal cortex. Medial fibres which are the bulk of the MFB continue rostrally, terminating in nucleus accumbens, olfactory tubercle, diagonal band. This follows the septum over the external capsule to the prefrontal cortex. Possibly a portion of this tract also projects into the head of the caudate-putamen complex.

2.4.3 Dopaminergic terminals from fibre tract projections

Considering the cell body as the origin of a pathway the brain's principal dopaminergic tracts could be subdivided into:

a) A mesencephalic system (to striatal, limbic and cortical areas)

b) A periventricular system (influencing the short diencephalon connections, but also long descending projections)

c) The short diencephalon neurones (to hypothalamus and hypophesis)

We will only pursue in this discussion those dopaminergic projections affecting the striatum, limbic system and cortex - the mesotelencepahlic system (Table 2.4 and Fig 2.1). Rodent data (Björklund and Lindvall, 1984) primate data (Battista et al., 1972; Garver and Sladek, 1975) and human data (Nobin and Björklund, 1973; Pickel et al., 1980; Pearson et al., 1983) collectively suggest the hypothesised mesotelencephalic diagrammatic systems.

2.4.3.1 The mesolimbocephalic system

Previously the nigrostriatal tract (A9 origin) was separated from the mesolimbic tract (A10 origin) but since their target areas overlap a new terminology is demanded.

Björklund and his colleagues suggest the:

Mesostriatal system

DORSAL part SNc to caudate-putamen (ie: dorsal striatum)

VENTRAL part VTA to accumbens nuclei, olfactory tubercle, amygdala and the bed nucleus of stria terminalis (i.e. ventral striatum)

Mesolimbocortical system

VTA to septum, amygdala, piriform and entorhinal cortex

Medial SN to prefrontal, anterior cingulate cortex, habenula and limbic brainstem (Björklund and Lindvall, 1984)

Hence conceptually the nucleus accumbens and olfactory tubercle become part of the neostriatum (Heimer and Wilson, 1975) more specifically part of the ventral striatum.

2.4.3.2 Mesostriatal system

Retrograde radiolabelled tracers placed in the caudate-putamen appear not only in SNc (A9) but also to a lesser degree A8 and A10 areas. Topography is maintained along an oblique longitudinal axis throughout the striatum from rostromedial (SN and VTA) to dorsal (striatum) and caudo-lateral to ventral (Veening et al., 1980). Hence the A10 (VTA) cells and adjacent (SNc) A8 also radiate to the main to the dorsal striatum (Nuta et al., 1978; Swanson, 1982).

2.4.3.3 Mesolimbocortical system

The olfactory bulb receives medial A9 and A10 input, (Fallon and Moore, 1978); the septum originates from A10 cells (Lindvall et al., 1978), the piriform cortex and finally the amygdala from medial A9 and A10 areas (Björklund and Lindvall, 1984).

The DA innervation of the entorhinal cortex comes from cell bodies in the VTA (A10) (Lindvall et al., 1974; Fuxe et al., 1974). Hippocampal innervation is as yet unidentified. The neocortical projections are complex but biochemical studies (Thierry, 1973) and histochemical methods have shown the frontal neocortex DA innervation comes from three origins:

a) anteromedial
b) suprarhinal and
c) supragenual systems (Lindvall et al., 1978).

All three begin in the A10 ventral tegmental nucleus. The projection field to the frontal cortex coincides exactly with afferents to the frontal cortex (prefrontal cortex) from the mediodorsal thalamus (Divac et al., 1975; Björklund et al., 1978). Horse radish peroxidase (HRP) retrograde tracer studies from the prefrontal cortex terminals in primates, suggest the VTA and SNc as the cell body origin (Porino and Goldman-Rakic, 1982).

Topographical arrangement from the ventral to dorsal frontal lobe correlates with medial (VTA) to lateral (SNc median part) midbrain cells.

2.4.4 Dendritic projections of the nigral dopamine cells

Specific staining of dopamine cells shows DA in the cell body, axon, axon terminal but also in the dendrites of the dopaminergic pathways (Björklund et al.,1978; Geffen et al., 1976). Dendrites of SNc cells have long, slender dendrites that ramify horizontally within the SNc and ventrally to the SNr (Ramon y Cajal, 1955). Approximately

30% of total dopamine is found in the SNr originating from a few scattered cell bodies but mainly from dendritic processes (Fahn, 1976; Cheramy et al., 1981). Dopaminergic nigrostriatal transmission may be influenced by effects upon these SN dendrites in a number of different ways. This is reviewed (Cheramy et al., 1981), but in summary:

a) Dopamine released from dendrites may tonically inhibit dopamine cells themselves. This negative feedback could reduce cell firing of the specific cell and also surrounding nigral cells (Korf, 1976).

b) Dendritic dopamine may modulate GABA release on striatonigral afferent fibres (Dray et al., 1976; Reubi et al., 1977) or GABAergic interneurones (Van Der Heyden et al., 1980).

c) Dendritic dopamine may activate SNr efferent neurones.

This phenomenon may either be direct or indirect via attenuation of GABAergic fibres (Waszczak and Walters, 1983).

These mechanisms would facilitate "cross-talk" between nigrostriatal neurones on the two sides of the brain suggesting increased dopamine on one side would reduce the contralateral dopamine release. Dopamine is stored in the dendritic network in rough endoplasmic reticular (in

contrast to vesicles elsewhere). Release of dopamine, involving ion channels and synaptic junctions, may not apply to dendrites themselves (Wassaf et al., 1981; Cheramy et al., 1981). Non-junctional release of dendritic dopamine hence could result in a prolonged influence modulating the SN (20-40 minutes).

2.4.5 Functional integration of dopamine neurones and tracts

It is easily appreciated that the core dopaminergic pathway connecting SNc to the neostriatum (dorsal striatal complex) may influence and indeed is crucial to widespread global control of brain function. The total number of those dopaminergic cells has been estimated at 800 000 (McGeer et al., 1977) in man. Lesions of those specific dopamine cells, their striatal afferent or efferent connections or depletion of their neurotransmitters produce a model showing an akinetic, rigidity, flexed posture and sometimes resting tremor. Rodents in addition show catalepsy, sensory inattention and in extreme bilateral states, adipsia with aphagia (Ungerstedt, 1974; Stricker and Zigmond, 1976; Robbins and Everett, 1982).

Pharmacological intervention and responsiveness promotes the view that the mesencephalic dopamine system acts as a "level-setting" or "permissive" influence upon the pyramidal system and ultimately behaviour response.

Striatal control remains pre-eminent in this system and removal of dopaminergic input produced inhibition of normal striatal influence (Sticker and Zigmond, 1976).

Medication to restore this input in lesioned animals restores most symptoms and a hope of the future could lie in a successful grafting of dopaminergic tissue into the brain to restore most the balance which has been upset in idiopathic Parkinson's disease (Björklund et al., 1981; Dunnet et al., 1982).

2.5 Basal ganglia research objectives of the future

Understanding basal ganglia function has been furthered by integration with known clinical observations following degenerative illnesses such as Parkinson's or Huntington's diseases. While no pathology affects all the basal ganglia equally, Marsden has argued that the parkinsonian patient is the best human example and the MPTP-primate parkinsonian model is the best animal model we have today to learn the physiology of the basal ganglia (Marsden, 1984). Further consideration of the human with idiopathic Parkinson's disease and the MPTP-treated primate model of parkinsonism will be dealt with in separate chapters.

Assuming these models are the best available today, what specific symptomatology should be examined? Parkinsonian

tremor (typically 4Hz, resting type) may be influenced by other types of tremor and furthermore is not a consistent sign in the MPTP-treated primate. Defects in the ventral tegmental area and dentato rubral thalamic tracts produce similar tremors. Doubt about the peripheral, spinal, supraspinal or combined mechanisms of tremor make it an unlikely candidate to further explore physiology of the basal ganglia.

Rigidity while invariably present in all patients and animals with parkinsonism, reflects release of hyperactive spinal pathways undoubtedly influenced by both the cerebellum and basal ganglia. Hughlings Jackson referred to tremor and rigidity as positive symptoms due to the release of distant mechanisms once disinhibited by the damaged basal ganglia. The two major negative symptoms released by abnormal basal ganglia are:

a) Disturbed postural control mechanisms.

b) Bradykinesia and the inability to execute normal voluntary movements.

Hence future study to learn how the normal basal ganglia operates should concentrate upon these two aspects, especially akinesia, which are likely to yield the most informative clues when studying the parkinsonian patient. Bradykinesia correlates well with biochemical parameters

such as striatal dopamine depletion (Hornykiewicz, 1966; Rinne and Sonnien, 1972; Bernheimer et al., 1973). Hence bradykinesia may well link clinical observations with electrophysiology and ultimately cell biology in the parkinsonian state. Unfortunately bradykinesia remains difficult to quantitate accurately both clinically and in behavioural models.

2.6 Conclusions

Having considered the anatomy, biochemistry, physiology of the normal and abnormal basal ganglia, then their interconnections, it would appear that bradykinesia should be the main feature upon which to judge the functioning of the basal ganglia. Breakdown in normal basal ganglia function occurs in IPD mainly in the ability to run accurately and automatically well recognised and memorised motor plans.

The basal ganglia are anatomically principally the location of most of the dopamine receptors in the brain.

Consideration of these dopamine receptors, their place, function, number, type and influence upon the basal ganglia and the dopaminergic pathways that influence them will now be considered.

CHAPTER 3

DOPAMINE RECEPTORS

3.1 History and current classification of the dopamine receptors

In 1960 loss of striatal dopamine in parkinsonian patients was reported (Hornykiewicz, 1961; Barbeau 1960). The next step was to determine where dopamine originated, upon which receptor this classical neurotransmitter was working and what influence dopamine had on striatal functioning.

A decade after the successful use of levodopa (Cotzias et al., 1967), the differentiation of two dopamine receptors, namely D-1 and D-2 occurred (Kebabian and Calne 1972; Seeman 1974 and 1975). The D-1 dopamine receptor, linked to adenyl cyclase may today be measured by its ability to bind a D-1 selective ligand such as [^{3}H]SCH 23390 (Billard et al., 1984; Hess et al., 1986). The D-2 receptor, unlinked or possibly negatively coupled to adenyl cyclase (De Camilli et al., 1979) is measured by displacement of [^{3}H]dopamine by [^{3}H]haloperidol (Seeman et al.,

1975) and more recently by specific binding of [^{3}H] spiperone, a D-2 selective marker.

Receptors are macromolecules, usually proteins, which are either located upon cell membranes, or within the cytoplasm or cell nucleus. The dopaminergic receptor is a membrane situated protein, which when stimulated, transmits a chemical signal that results in an electrical or a chemical impulse which propagates messages to other cells. Minute quantities of the neurochemical substance will cause a cell to respond in a precise way. Dopamine agonist drugs, in suitable pharmacological dosage, simulate the ligand molecule, endpgenous dopamine, at these receptor sites to elicit the same postsynaptic receptor cell response.

Dopamine receptors are one of the most widely studied receptor populations in the human brain. Huntington's disease, schizophrenia, tardive dyskinesia, Gilles de la Tourette syndrome and even Altzheimers dementia have been studied as they all share an abnormal functional dopaminergic component (Seeman, 1987).

Although dopamine receptors respond to other chemicals, they remain most sensitive to dopamine and least sensitive to other neurotransmitters and catecholamines. Drug effects thought to be receptor mediated imply the drug should express its efficacy in the dose range that correlates closely with concentrations used to elicit a

biochemical or electrical in vitro brain response, otherwise other chemicals which are only effective at very high dosage, would inappropriately trigger receptor response thereby transmitting inappropriate messages. In Parkinson's disease degenerating nigrostriatal dopaminergic systems physiologically compensate until more than 80% of dopamine cells in the SNc die, thereafter parkinsonian signs occur, in parallel with the decline in striatal dopamine. Striatal dopamine decline suggests the striatal postsynaptic dopamine receptors must in some way be responsible for parkinsonian symptomatology. The adaptive mechanism that results is mediated by receptor changes both pre and postsynaptically, especially in the putamen (Hornykiewicz, 1979). Firstly presynaptic dopamine turnover rate in the parkinsonian nigrostriatal pathway and striatum becomes accelerated, as suggested by increased HVA:dopamine ratios (Bernheimer et al., 1973). However this compensatory response is limited and still requires endogenous dopamine production from a steadily degenerating cell population within the SNc. Hence in advanced idiopathic Parkinson's disease increased dopamine output cannot be a major compensatory influence.

Secondly and more important, is the upregulation of postsynaptic dopamine receptors (Lee at al., 1978; Guttman and Seeman, 1986). Postsynaptic dopamine receptors may adapt in one of two ways. They may either increase their sen-

sitivity (qualitative) or increase their number (quantitative).

Dopamine receptor research was complicated by the proposed existence of both D-3 and D-4 dopamine receptors (Seeman, 1980 and 1982). Consensus was reached when it became appreciated that the D-1 and D-2 receptors were able to operate at different affinity states (Leef and Creece, 1984; Seeman et al., 1984 and 1985; Grigoriades and Seeman, 1984). Laboratory subclassification of central dopamine receptors is principally pharmacological and based mainly upon the concept of differing sensitivity to concentrations of dopamine agonists and antagonists. Biochemically drugs such as dopamine, spiperone, SCH 23390, sulpiride and many others, are easily radiolabelled to provide good radioligand differentiation of the various types and location of dopamine receptors.

Evidence suggesting two receptors was crystallised by Kebabian into two main subdivisions (Spano et al., 1978; Garau et al., 1980). The D-1 receptor responded to dopamine by increasing synthesis of cAMP by stimulation of dopamine sensitive adenyl cyclase (Kebabian et al., 1972; Brown and Markman, 1973; Iversen, 1975). The enzyme dopamine-sensitive-adenyl-cyclase is found in insects, gastropodes, primates and the human brain (Clement-Cormier et al., 1974). Human caudate nucleus slices contain this

enzyme (Kebabian et al., 1972) and therefore striatal post-synaptic receptors can regulate the enzyme adenyl cyclase.

While dopamine was the most potent catecholamine active at this receptor site, other analogues were examined. It became clear that there was more than one dopamine receptor. Firstly apomorphine, known to be a potent D-2 and D-1 dopamine receptor agonist, was thought to mimic the effects of dopamine and was tested on the striatal system, producing only partial agonist effects. Apomorphine fully antagonises dopamine receptors in cell free homogenates (Kebabian et al., 1972) and intact cell preparations (Brown and Makman, 1977).

Secondly, known central dopamine competitive antagonists which are a characteristic of many antipsychotic drugs, had variable effects depending upon their chemical configurations. Clinically active compounds were potent dopamine antagonists, while structural isomers of the same compounds were inactive both clinically and biochemically (Miller at al., 1974). Finally, the impression of <u>in vivo</u> dopaminergic function analysed in behaviourally 6OHDA rodent models precluded dopamine sensitive adenyl cyclase in the brain as the only enzyme which mediated striatal dopaminergic effect. At least two dopamine receptors, one of which was linked to cAMP, were proposed. Thereafter various experiments suggested striatal dopaminergic effects

that were not linked to adenyl cyclase. These reactions became known as D-2 mediated dopaminergic effects. Prolactin, for example, was released from pituitary preparations without changes in cAMP (Mar, 1976; Thorner, 1977), apomorphine was shown to mimic the inhibition of dopamine on the pituitary (MacLeod et al., 1974; Smalstig et al., 1974) and these effects were antagonised by selective dopaminergic antagonists (MacLeod and Lehmeyer, 1974; Caron et al., 1978). Further evidence of a "non-cAMP dependant dopamine receptor" came from radiolabelling of agonists and antagonists of the dopamine receptor in the pituitary mammotroph (Calabro and MacLeod, 1978; Creese et al., 1977).

Cholera toxin was utilised in experiments to confirm that cyclic AMP had no role in dopaminergic receptor release of prolactin. Cholera toxin activates adenyl cyclase in pituitary cell cultures <u>in vitro</u> either stimulating or maintaining prolactin release. Decreased prolactin would occur if the mechanism was linked to D-1 dopamine receptors, thus cAMP is unlikely to be responsible (Rappaport and Grant, 1974). A known dopaminergic mediated pathway utilising prolactin inhibitory factor is released from the hypothalamus to inhibit prolactin release from pituitary mammotrophs. Thereafter D-2 receptors were looked for in the striatum. The dopaminergic nigrostriatal pathway is destroyed in the rodent by injecting 6-hydroxydopamine (6OHDA) into the vicinity of the SNc.

Presynaptic nigral receptors are destroyed and postsynaptic striatal receptors become supersensitive, but the level of adenyl cyclase was unaltered in the SN (Kebabain and Stoof, 1976) or striatum (Krueger et al , 1976). D-1 receptors are thought to remain relatively unaltered following 6OHDA rodent nigrostriatal lesioning. Conversely kianic acid lesions of the striatum, known to destroy striatal neuronal cell bodies while sparing the dopaminergic cell terminals cause significant loss of striatal dopamine sensitive adenyl cyclase with normal striatal dopamine concentration (Hattori and McGeer, 1977; Di Chiara 1978). Neither the autoreceptors of the nigral cell bodies nor the striatal terminals are dependent upon adenyl cyclase (Kebabian and Calne, 1979), which together form a subpopulation of dopamine receptors now known as the D-2 type. The above ideas, derived from lesioning experiments, were consolidated by additional pharmacological evidence to suggest several types of dopamine receptors.

Pharmacological observations that several antagonists were not equally effective upon the different dopamine receptor tissue populations were reported (Meltmeyer et al., 1978; Calaf 1973; Yamuchi et al., 1977; Bunney et al., 1975), for example metoclopramide and sulpiride do not effectively block striatal adenyl cyclase (Spono et al., 1978; Roufogalis et al., 1976; Peringer et al.,1979). Dopamine has variable displacement potencies in different tissues,

nanomolar dopamine concentrations are required in the anterior pituitary and higher concentrations are effective in striatal neurones (Iversen et al., 1976). In contrast dopamine receptors linked to adenyl cyclase are stimulated by micromolar quantities of dopamine in the striatum (Kebabian and Greengard, 1971) and similarly micromolar concentrations of dopamine are required to stimulate bovine parathyroid hormone release (Brown et al., 1976). The parathyroid gland has become the standardised D-1 dopamine receptor pharmacological model and source. Apomorphine, like dopamine, is a potent D-2 agonist (Cannon, 1978). When sympathetic presynaptic terminals (Steinsland et al., 1978) and autoreceptors of the nigrostriatal tract are examined, they are mostly D-2 receptor type (Iversen et al., 1976). Apomorphine, as it is not a pure D-2 dopamine receptor agonist, may even demonstrate antagonist pharmacological effects upon D-1 receptors such as dopamine stimulated accumulation of cAMP in bovine parathyroid preparations. Striatal dopamine receptors, a mixed D-1 and D-2 population may show apomorphine agonist responsiveness at low concentrations but overall antagonistic activity at higher concentrations (Kebabian et al., 1972).

Pharmacological studies utilising many drugs thus confirm the existence of more than one dopamine receptor subtype (Table 3.1; modified from Kebabian and Calne, 1979).

Table 3.1

Elements of the "two-dopamine" receptor hypothesis of Kebabian and Calne

Name	D-1	D-2
Cyclase linkage	Yes	No
Location of prototype receptor	Bovine parathyroid	Mammotroph of anterior pituitary
Dopamine	Agonist (umolar potency)	Agonist (nmolar potency)
Apomorphine	Partial agonist or antagonist	Agonist (nmolar potency)
Dopaminergic ergots	Potent antagonist (nmolar potency) Weak agonist (umolar potency)	Agonist (nmolar potency)
Selective antagonist	SCH 23390	Metoclopramide sulpiride
Radiolabelled ligand	*cis*-flupenthixol	Dihydroergocryptine

Dopamine agonists such as bromocriptine, lisuride and legotrile are potent D-2 agents and have antiparkinsonian properties. High concentrations are needed to mimic dopaminomimetic D-1 action by accumulating cAMP. Bromocriptine inhibits striatal D-1 receptors (Markstein et al., 1978). Haloperidol, a butyrophenone, is a potent antipsychotic agent and dopamine receptor antagonist. It is a potent D-2 antagonist yet also has some D-1 activity shown by its ability to weakly antagonise adenyl cyclase. Other antipsychotic agents, for example cis-fluphenthixol (a thioxanthene) and (+)butaclamol do not display such differential dopamine receptor affinity and hence are more D-2 selective (Clement-Cornier et al., 1974; Karobath 1974; Cannon 1978).

A third method of investigating dopamine receptors came from radiolabelling of dopamine and dopamine receptor ligands. These methods have also characterised D-1 and D-2 receptors. Tritiated spiperone is the choice label of the D-2 striatal receptor. The D-1 receptor labels [^{3}H]-2-fluphenthixol and [^{3}H]piflutixol (Hyttel, 1978 and 1981) previously used have recently been superseded by [^{3}H]SCH 23390 which is highly selective to the D-1 receptor (Hyttel, 1983; Iorio et al., 1983). Seeman emphasised the point that receptors are better defined by their rank order of antagonist potencies. Receptors are thus not defined by the type of [^{3}H]-ligand which attaches but rather by the rank order of antagonist which

displace the agonist attached to the specific receptor (Fig 3.1) (Seeman and Grigoriades, 1987). Radioligand displacement studies suggest D-1 receptor sites are characterised by picomolar concentrations of [^{3}H]SCH 23390 and also by micromolar [^{3}H]dopamine and [^{3}H]spiperone concentrations. Very low sensitivity to [^{3}H]sulpiride or other substituted benzamides such as [^{3}H]metoclopramide is noted.

In contrast to the D-2 receptor, the D-1 receptor has a much higher affinity for drugs associated with tardive syndromes. They have picomolar sensitivity for spiperone and either nanomolar affinity for dopamine (low affinity state), or micromolar affinity for dopamine (high affinity state). Spiperone, a dopamine D-2 receptor antagonist, but not SCH 23390, a D-1 selective antagonist, equally recognises the low and high affinity state of the D-2 receptor.

Dopamine, being unselective, cannot differentiate the four types of receptor status. It has only been with the development of selective dopamine receptor antagonists, the selective D-1 marker being SCH 23390 and D-2 marker being sulpiride, that biochemical differentiation of central dopamine receptors is better understood (Seeman, 1987). It remains unclear where the dopamine receptors are anatomically and microanatomically located or what functional inter-relationships exist between the different receptor populations.

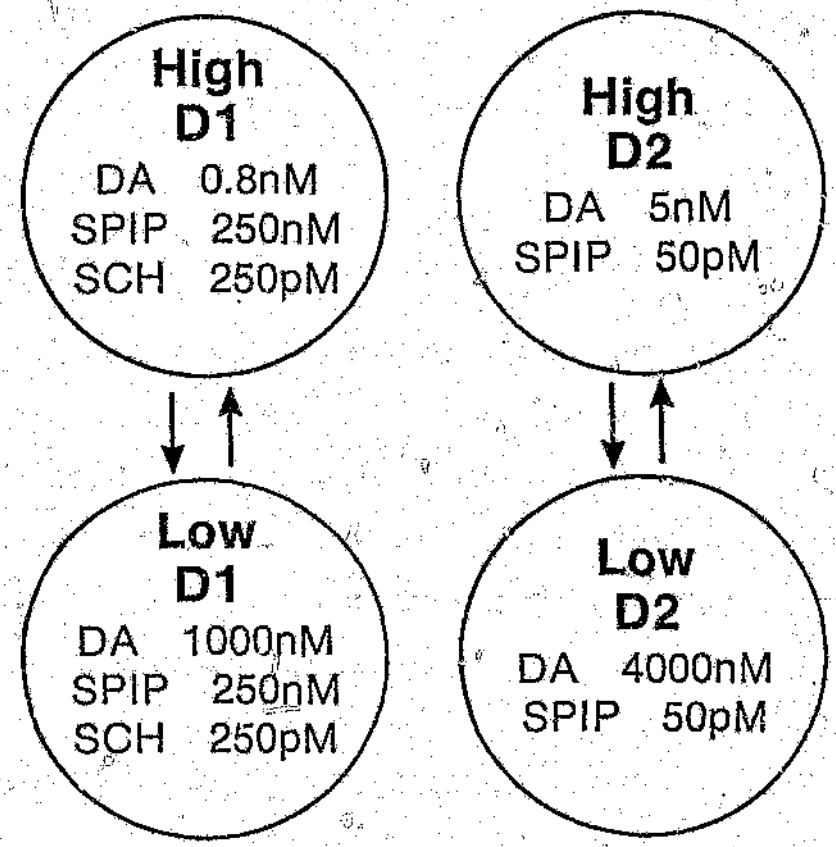


Fig 3.1

The two major dopamine receptors, their characteristics and their high versus low affinity states (from Seeman, 1987)

Specific probes that label the D-1 and D-2 dopamine receptor at a cellular level do not exist but radioligand receptor assays utilising the various selective agonist and antagonist radiolabelling drugs may indicate the anatomical areas where receptor populations are found. Combining this technique with specific anatomical neurotoxic or mechanical lesioning methods has the potential to confirm ideas concerning receptor sites in the nervous system. For example, dopamine is found in the striatum, as suggested by radioligand [^{3}H]dopamine radioligand uptake. Lesions that reduce nigrostriatal transmission, such as MPTP or intranigral 6-OHDA, produce both D-1 and D-2 dopamine receptor hypersensitivity which increases striatal receptor responsiveness to endogenous dopamine.

Increased [^{3}H]dopamine uptake or increased antagonist displacement, when incubated with dopamine, is seen in striatal slices. Conversely lesions, such as kianic acid, which destroy the striatal cell bodies and some dopamine receptors, create substantial D-1 linked adenyl cyclase loss but do not diminish striatal dopamine (Crease et al.,1977).

The possibility that there is more than one type of dopamine receptor, operational at different affinities, complicates the issue. The exact situation of striatal

dopamine receptors remains unknown. Cloning of the D-2 and other receptors, utilising molecular hybridisation technology, may lead to the design of probes which accurately determines specific receptor sites (Wooten et al., 1990).

3.2 Evaluation of dopaminereceptors

3.2.1 Biochemical receptor binding studies

D-1 and D-2 brain receptors are identical to dopamine receptors found on peripheral blood vessels, DA1 and DA2. This thesis however will be confined to discussion of the central nervous systems dopamine receptors. Dopamine receptors are best defined by the rank order with which agonists inhibit the attachment of a radiolabelled ligand which competes with the agonist at the receptor binding site (ie: competitive antagonist agent) (Seeman and Grigoriadies, 1987). Dopamine is more potent than noradrenaline, which in turn is more potent than serotonin, at this specific receptor site. While noradrenaline and serotonin may influence D-1 and D-2 receptors they only do so at very much higher concentrations. Binding of [^{3}H]spiperone to anterior pituitary mammotrophs is inhibited more readily by dopamine than any other catecholamines or serotonin (George

et al., 1985). To emphasise this point then it is not so much that the [^{3}H]ligands binding ability itself (in this example [^{3}H]spiperone) but more accurately the degree to which the agonist itself (in this example dopamine) displaces the receptor specific radiolabelled antagonist. Brain striatal slices, containing both dopamine and serotonin receptors will bind [^{3}H]spiperone at both receptor sites. Frontal cortex preparations bind [^{3}H]spiperone but this is much more displaceable by serotonin than dopamine agonists (Leysen et al., 1978). It would be an apparently simple matter with preknowledge of the specificity of the ligand, along with the receptor agonists and antagonists, to accurately quantitate the combined receptor numbers and affinity in any given tissue sample. It was noted, however, that the individual receptor may function in different affinity states. The dopamine agonist ADTN ((+-)-6,7-dihydroxy-2-amino-tetralin) displaces the D-2 maker [^{3}H]spiperone in three distinct phases (Seeman, 1987) and hence the D-2 (high) and D-2 (low) affinity state was proposed instead of D-2 and D-4 receptors respectively (Seeman and Grigoriades, 1987; Sokoloff et al., 1985). The same principle applies to analysis of the D-1 receptor (List and Seeman, 1982; Leff and Creese, 1984) and even the beta-adrenoreceptor (De Lean et al., 1982; Sibley and Creese, 1983). The proposed D-3 receptor could be correlated with the D-1 (low) affinity state (Fig 3.1). Since the dissociation constant (K value)

differs by three orders of magnitude with dopamine displacement between the high and low affinity D-2 receptor states, one should examine the radioligand displacement results for spiperone (D-2 specific and K value of 250nm in both receptor affinity states) and SCH 23390 (D-1 specific, K value of 250pm in both states) (George at al., 1985; Hamblin et al., 1984). Since dopamine is not specific for D-1 and D-2 differentiation a rank order profile could be estimated including all the widely employed dopamine agonists. Ratios of D-1 over D-2 Kd selectivity, only considering the high affinity receptor states are shown in Fig 3.2. New agents could be plotted similarly with (+)-PHNO being most highly D-2 selective and CY 208-243 most D-1 selective recently available (Horn et al.,1975; Horn at al., 1985; Setler et al., 1978; Markstein et al., 1986). Methodologically the absolute density of dopamine receptors may be estimated by high speed ce fugation techniques generating 11 000g forces, employing teflon-glass rather then polytron homogenisation beads, with or without filters (List and Seeman, 1986; Seeman et al., 1984). Non-specific binding to other receptors needs consideration (accounting for 10-20% error) but high speed centrifugation and fat soluble [^{3}H]ligands reduce these errors.

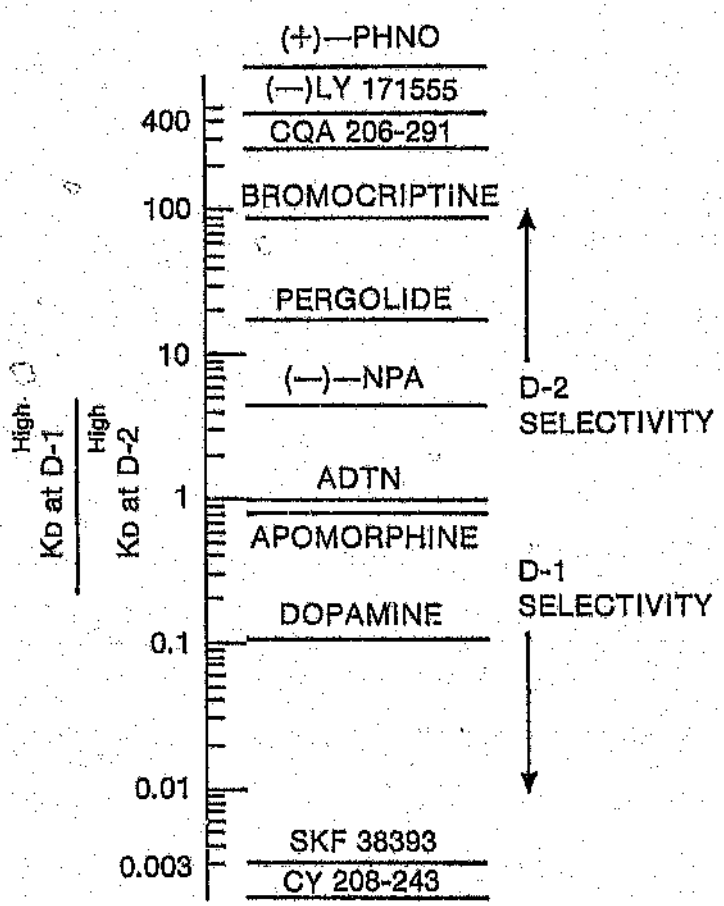


Fig 3.2

Rank order of D-1 and D-2 dopamine agonists

3.2.2 Electrical methods

Electrical observations in the early seventies suggested two different dopaminergic effects each of which could be selectively blocked (Strugler et al., 1974; Heiss and Hoyer, 1974). Cools and Van Rossum suggested two dopamine receptors based on electrical evidence, namely DAe (excitation) and DAi (inhibition) responses (Cools and Van Rossum, 1976).

Inconsistent results and a paucity of different tissue cultures and anatomical sites tested, did not facilitate widespread acceptance of D-1 and D-2 dopamine receptors until pharmacological data strengthened these observations. Single neurone extracellular cell recordings from the substantia nigra (Bunney et al., 1973; Aghajanian and Bunney, 1977) and more recently from the output zones of the SNr and external globus pallidus (Carlson et al., 1984; Walters et al., 1984) provide useful data on collective basal ganglia output and function.

Substantia nigra (SNc) dopamine cell studies

Nonselective dopamine agonists such as apomorphine inhibit SNc dopamine neurones (Walters et al., 1979; Skilbol et al., 1979) an effect unaltered by lesions of the nigrostriatal pathway (Baring et al., 1980). Direct iono-

phoretically applied dopamine or apomorphine also reduces SNc cell firing rates (Aghajanian and Bunney, 1977). This inhibition of SNc dopaminergic cells reflects direct blockade of the dopamine autoreceptor in the SNc. The D-1 agonist SKF 38393 and antagonist SCH 23390 did not affect SNc cell firing rate while LY 171555, a potent D-2 selective agonist and other D-2 agonists such as pergolide or lisuride, inhibited SNc electrical cell activity. The effects of apomorphine and LY 171555 were completely reversed by subsequent use of haloperidol (Carlson and Walters, 1987). This model suggests a SNc autoreceptor that is D-2 mediated and provides an accurate model to evaluate known pharmacological agents.

Globus pallidus (and SNr) cell studies

Selecting cells to study "downstream" from the dopamine receptors and the striatum, gives collective basal ganglia output information. Modification of this collective output may be influenced by a direct dopaminergic stimulus to the external globus pallidus (Lindwall and Björklund, 1979). Direct application of dopamine to this area has little effect on more then half the cells cannulated (Walters and Bergstrom, 1984). The remaining pallidal cells are either stimulated or inhibited, by about 20%, from baseline recordings, hence the direct dopaminergic path is probably not important. D-amphetamine and especially apomorphine

attenuated GABA's effects in the pallidal cells (Walters et al., 1984). Rodent pallidal cell firing rates to D-2 agonists (apomorphine, lisuride and pergolide, LY 171555 and LY 141865) are increased, blocked or reversed by haloperidol (Walters et al., 1982). In contrast the D-1 agonist SKF 38390 had no effect on pallidal cell firing rates (Carlsen et al., 1984).

It was observed that while apomorphine and LY 171555 had similar SNc dopamine autoreceptor effects they differed in their effects upon pallidal cell firing rates (Carlson et al., 1984). This concept may invoke a functional interaction of D-1 and D-2 receptors in the striatum to achieve maximal pallidal output (Mailman et al., 1984; Malloy and Waddington, 1984; Carlson et al., 1984). The D-1 antagonist SCH 23390 had minimal effect upon pallidal cell output but did attenuate apomorphine induced stimulant effects. Pretreatment with SCH 23390, before apomorphine, significantly reduced the expected apomorphine induced increase in pallidal cell firing.

In summary there is fairly convincing evidence that a functional interaction may well exist between the dopamine D-1 and D-2 receptors as judged by electrical pallidal output studies utilising the rodent model.

3.2.3 Behavioural methods

Knowledge of normal and lesioned behavioural patterns in rodents, mice, primates and other animals often serves as a useful test battery to safely test drugs thought to be active antiparkinsonian agents, prior to their introduction to human studies. While it is not possible to directly relate behavioural drug effects on dopaminergic systems and their receptors *in vivo*, multiple *in vitro* methods support the observed behavioural patterns. The advent of PET scans promises to integrate the *in vitro* hypothesis forwarded with what is observed in animal models including ultimately man. Reviews examining the methodology and various animal models are well described (Fog, 1972; Kelly, 1977; Niemegeers and Jansen, 1979; Costall and Naylor 1979; Pycock, 1980 and Arnt, 1987). This section will summarise behavioural influences upon dopaminergic systems and scrutinise the results of administration of dopamine agonist and antagonist drugs carefully. Accepting two types of dopamine receptor (Kebabian and Calne, 1979) along with the concept of pre and postsynaptic dopamine receptors (Usdin and Bunney, 1975; Langer et al., 1979) examination of the motor effects of these models could be approached under the following subsections:

a) Test models utilised to study dopamine mediated behaviour.

b) Regional differentiation of behavioural effects.

c) Interactions of multiple dopamine receptors, utilising normal versus abnormal behavioural dopaminergic systems.

Dopamine mediated behaviour

Striatal, limbic and cortical dopaminergic systems all contribute towards motor control. The rodent model, utilising specific neurotoxins and lesioning techniques, provides a useful model to assess increased or decreased dopaminergic system pathways.

Increased dopamine functional pathways

Dopamine receptor agonists which are centrally active produce diverse motor manifestations. Biphasic locomotor activity (decreased at low doses, increased with high doses) which is not selective to prasynaptic or post-synaptic receptor function occurs (Costal et al., 1981; Ungerstedt et al., 1983; 1985; Bradbury et al., 1984). Stereotypic behaviour (sniffing, rearing, head movements,

licking and biting) ensues with increased agonist dosage (Costall and Naylor 1975, 1980). Mice develop less marked stereotypies (Costall et al., 1982), but readily climb the cage (Protias et al., 1976), exhibit yawning behaviour (Mogilmeka and Klimek, 1977; Yamada and Farukawa, 1980) often with penile erection and genital grooming. Emesis however may be produced by dopamine D-2 receptor stimulation outside the blood-brain barrier, specifically the area postrema (Harding et al., 1985; Martin et al., 1982). Sexual behaviour, food intake, reward related tasks, self stimulation of specific brain areas, self administered drugs (Fibiger and Phillips, 1979) and drug discrimination (Colpaert et al., 1976; Nielsen, 1985) represent other behavioural dopaminergic tracts less well defined or specific in rodent models.

The most useful rodent model is the combined unilateral brain lesion and the circling behaviour that results (Ungerstedt, 1967; Pycock, 1980). While turning may be produced in naive rats (Glick, 1985) the unilateral brain lesion exaggerates asymmetry and rotation (Ungerstedt, 1971). Over the last 15 years two rodent models have been tested widely. Lesions of the substantia nigra or median forebrain bundle with 6-hydroxydopamine (6-OHDA) produce damage to striatal afferents specifically the ascending nigrostriatal tract but also the mesolimbic tract, culminating in contralateral rotation. This phenomenon is

accentuated by dopamine agonist drugs operating upon supersensitive dopamine receptors in the denervated striatal side (Ungerstedt, 1971, Costall et al., 1976). Dopamine releasing agents such as amphetamines, stimulate the normal striatum more then the lesioned side resulting in ipsilateral circling (Ungerstedt 1971, Oberlander et al., 1979).

Utilisation of a different lesioning technique, that of striatal kianic acid, by electrolytic lesions or destruction of the SNr, causes spontaneous rotation towards the ipsilateral side mediated by preferential dopamine stimulation of the non-lesioned striatum (Costall and Naylor, 1975; Marshall and Ungerstedt 1977; Schwarcz et al., 1979). Hence the circling rodent model is very useful to study dopaminergic nigrostriatal pathways which may be damaged in selected areas and responds in time to known drugs in a predictable fashion.

Decreased dopaminergic functional pathways

Spontaneous behaviour is depressed following dopamine antagonist drugs, but behavioural responses are similarly seen with inhibition of nigrostriatal input following 6-OHDA, alpha-methyl-p-tyrosine or MPTP. Akinesia and catalepsy occur in rodents, which are reversed by antimuscarinic agents which possess extrapyramidal effects

(Arnt et al., 1981). Conditioned behaviours including selected avoidance, reward levers, self stimulation reactions, aphasia, adipsia and sensory neglect may all occur with depleted dopamine pathways (Ungerstedt, 1971).

Regional differentiation of behavioural effects

Specific focal lesions in the brain following electrocoagulation, physical transection or chemical ablation, is one method of studying the effects of damage to cell groups including the fibres that pass through the area. Another method is to inject drugs intracerebrally and if reproducible and accurate, these drugs may stimulate or inhibit cells locally. Both techniques are limited by problems such as lesion site confirmation, accuracy of injection site and the dose needed intracerebrally. This requires comparison with what physiologically crosses the blood brain barrier and is thus active (Arnt, 1985).

Locomotor and stereotypic activity

Of the mesolimbostriatal and nigrostriatal dopaminergic pathways only the nigrostriatal dopamine path is involved in stereotypic behavioural patterns. Evidence for this occurs from observations following the administration of

intracerebral drugs at different stereotactic sites (Fog, 1972; Jackson et al., 1975; Costall and Naylor, 1976) but also following 6-OHDA lesion studies combined with systemic apomorphine or amphetamine (Asher and Aghajanian 1974; Kelly et al., 1975; Costall et al., 1977, Iversen 1977). Mesolimbocortical pathway analysis is better assessed by studying rodent locomotor activity. Lesions of the nucleus accumbens alone are insufficient to reduce mobility in contrast to combined mesolimbic and striatal lesions (Fink and Smith, 1980; Winn and Robbins, 1985). Hyperactivity and oral stereotypies may follow high drug doses of dopamine or dopamine agonists (Jackson and Kelly, 1984). As seen in the section on dopamine pathways, the mesolimbic and nigrostriatal systems are linked functionally which confounds behavioural expression.

Circling behaviour

Striatal influence regulates postural symmetry and rotational behaviour (Pycock, 1980). Unilateral dopamine pathway manipulation will affect rotation while the nucleus accumbens amplifies the postural bias (Kelly and Moore, 1977; Pycock and Marsden, 1978). Intrastriatal injection of dopamine (Setler et al., 1978) or peripheral administration of dopamine or dopamine agonists in a unilateral 6-OHDA nigrostriatal lesioned rodent produces stronger drug induced contralateral circling compared with spontaneous

behaviour. No obvious difference is detected when injecting the striatum dorsally to ventrally (Arnt, 1987). Circling behaviour is not restricted to striatal dopaminergic pathways since high doses of dopamine agonists produce contralateral rotation after injection not only into the striatum but also into the SNr in normal animals (Andrews and Woodruff, 1982; Kelly et al., 1984). In the unilateral 6-OHDA model dopamine agonists administered into the striatum, accumbens, amygdala, ventromedial thalamus, superior colliculus, periaqueductal grey matter, lateral habenula, all produce circling behaviours (Starr and Summerhayes, 1982). No firm evidence to date exists that the striatum alone topographically mediates dopamine mediated circling. Whatever the limitation, the overall model of dopamine mediated circling remains useful to assess peripherally administered drug behaviours.

Catalepsy

The striatum is thought to be important for cataleptic properties of dopamine antagonist drugs (Fog, 1972; Dunstan et al., 1981) even though employing relatively high doses of drugs. It was therefore surprising that -(-)sulpiride, given into the striatum or nucleus accumbens in sufficient dosages to prevent apomorphine-induced stereotypies, did not produce catalepsy (Schul-Kruger and Arnt, 1987). This suggests dopamine striatal receptors are blocked, since

separate striatal or nucleus accumbens lesions do not produce catalepsy while combined lesions do (Koob et al., 1984).

Conditioned Behaviour

Jackson showed that avoidance reactions induced by alpha methyl-p-tyrosine were reversed by dopamine applied either to striatum or nucleus accumbens (Jackson et al., 1977). Dopamine antagonists administered intracerebrally to the striatum, septum or amygdala produced avoidance reactions but administration in other areas were ineffective (Sherman et al., 1982). While the nucleus accumbens is a major nucleus, how specific the stimulus must be and where exactly is not yet known.

Adipsia, aphagia and animal death in the bilateral 6-OHDA rodent with bilateral severe sensory neglect is observed following widespread bilateral nigrostriatal dopaminergic lesions (Ungerstedt, 1976; Iversen, 1984; Schultz, 1982). Hence practically, the bilateral 6-OHDA rodent model is not a useful one, since animal maintenance becomes impossible.

Behavioural evidence of multiple dopamine receptors

While the dopamine receptor behavioural theory preceded biochemical and electrical data (Cools and Van Rossum, 1976; Costall and Naylor, 1975), pharmacological and biochemical data facilitated more logical interpretation. Dyskinesias induced by intrastriatal dopamine or dopamine agonist application were antagonised by most dopamine antagonists including pirozide, oxiperomide and toapride (Costall and Naylor, 1975 and 1979). All D-2 dopamine agonists (D-2) produced dopamine induced hyperactivity. To allow meaningful interpretation of this data further consideration of D-1 and D-2 behaviour should subdivide agonist or antagonist effects into normal, normosensitive dopamine receptors versus abnormal, hypersensitive dopamine receptors. Abnormal receptors occur in idiopathic Parkinson's disease, lesioned animals and MPTP models.

Dopamine agonists, normosensitive receptors

Apomorphine influences both D-1 and D-2 dopamine receptors (Seeman, 1980; Hyttel, 1980) and cannot evaluate discriminatory functions of these receptors. However it is very useful to study overall dopaminergic function especially in unilateral circling models.

Selective D-1 and D-2 agonists are more informative but while many D-2 agonists exist (Arnt and Hyttel, 1985) mini-

mal data is available regarding selective D-1 agonists. Data from SKF 38393 (Setler et al., 1978) and more recently CY 208-243 is limited (Markstein et al., 1987). The relative potency difference between different drugs make comparisons difficult, but Table 1.4 and Fig 3.2, indicate estimates of various D-1 and D-2 agent potencies.

The D-1 agonist SKF 38393, while able to induce contralateral circling in 6OHDA rats, does not, even at very high doses, produce stereotyped behaviour (Setler et al., 1979). This, plus the observation, that it does not cause emesis led researchers to define its profile as D-1 selective (Drew et al., 1982). SKF 38393 is weak behaviourally since its administration produces no desire to explore (Costall et al., 1981; Martin and Bendesky, 1984), circling is absent in hemisectional rats (Arnt, 1981) and SKF 38393 fails to inhibit the discriminative stimulus properties induced by amphetamines in rats (Nielsen, 1986). In primates, self administration of cerebral stimuli was not maintained following SKF 38393 administration, an observation which occurs with apomorphine administration (Woolverton et al., 1984). In contrast we know D-2 agonist actions, inhibitory and stimulatory, elicit predictable behavioural patterns. Apomorphine is most widely studied along with levodopa which similarly has both D-1 and D-2 agonist properties. Bromocriptine, lisuride, pergolide, (+)3PPP, LY 141867 and LY 171555 are all predominantly D-2

active dopaminomimetic agents.

Central to this discussion is the functional and behavioural role of the D-1 receptor alone or in combination with the D-2 receptors. SCH 23390, a specific D-1 antagonist, attenuated the central effects induced by SKF 38390 in rats (such as sniffing, rearing, grooming and increased locomotion) suggesting that the D-1 receptor must play some role in this behaviour, until recently thought to be all D-2 mediated. Metoclopramide blocked only rodent rearing and locomotion (Molloy and Waddington, 1987). A specific behavioural role is possible for the D-1 receptor but further data is required (Waddington, 1988). The D-1 receptor is more likely to exert its influence indirectly upon the D-2 receptor. Perioral rodent dyskinesias were also induced by D-1 (SKF 38393) and D-2 (LY 141865) agonists and blocked by a D-2 receptor antagonist, such as spiperone or sulpiride (Rosengarta et al., 1983). Vacuous chewing behaviour was reported in aged rats, who presumably have less D-2 receptors, compared with young animals after SKF 38393 (Malloy et al., 1985).

When examining the D-1 receptor inhibition of D-2 behaviour, SKF 38393 did not inhibit maximal stereotyped amphetamine or apomorphine-induced behaviour (Arnt, 1987). SCH 23390 does not influence D-2 mediated behaviour at low

doses but in MPTP-treated primates may well do so when greater doses are used (Temlett et al., 1987).

Intracerebral administration of SKF 38393 produces typical dopamine agonist responses such as contralateral circling in unilateral 6OHDA rats (Setler et al., 1978; Gower and Mariott, 1982). Administration of SKF 38393 into the nucleus accumbens, stimulates locomotion (Friedman et al., 1979), increases climbing behaviour (Costall et al., 1984) and produces strong contralateral circling in naive mice (Biziere et al., 1985). In summary SKF 38393 has a strong agonist profile when centrally administered.

Dopamine antagonists, normosensitive dopamine receptors

D-2 selective antagonists (butyrophenones, dipheny-butyrophenones and benzamides) (Hytell, 1980) and mixed D-1 and D-2 antagonists (thioxanthenes, thiepins and phenothiazines) (Hytell, 1978 and 1981) have been known to produce antipsychotic and behavioural effects. Most of these correlate with D-2 receptors especially the anti-stereotypic, cataleptic and avoidance-inhibitory reactions (Creese et al., 1976; Niemegeers and Jannssen 1979; Seeman 1980; Arnt 1982; Hayashi and Tadoro, 1984). D-1 selective antagonists, SCH 23390 and SKF 83566, (Hyttel 1983; Iorio et al., 1983; O'Boyle and Waddington 1984; Arnt 1985) recently introduced, also had behavioural effects similar

to those previously thought to be D-2 specific. Especially interesting was the observation that SCH 23390 blockade (comparable to spiperone) of D-2 agonists, using pergolide, LY 171555 or RM 24213 induced circling or stereotypic behaviour (O'Boyle et al., 1984; Arnt, 1985 and 1986). This was confirmed with SKF 83566C (O'Boyle and Waddington, 1984; Arnt, 1985). Motility experiments in rats separate SCH 23390 from spiperone, where spiperone but not SCH 23390 reversed the sedative effect of apomorphine and 3-PPP (Maj et al., 1977; Gessa et al., 1985) suggesting a lack of D-1 receptors controlling dopamine release (Hyttel, 1984). While the D-1 and D-2 receptors are found together in the brain, where and how they influence one another and their ultimate output is reflected by a functional D-1 and D-2 interaction. Intracerebral administration of SKF 38393, SCH 23390 and -(-)sulpiride blocked spontaneous climbing behaviour of mice following SKF 38390 (Arnt, 1985), antagonised rodent stereotypic following apomorphine (Costall et al., 1984) and finally circling mice behaviour in response to SKF 38393. Sulpiride blocks locomotor stimulation induced by SKF 38393 applied to the nucleus accumbens (Freedman et al., 1979). Evidence suggests that D-1 and D-2 receptors function similarly but independently while manipulation of either subpopulation of receptor will influence the other. Each receptor may be individually influenced, the best behavioural expression must represent some unknown functional synergism that exerts ultimate

influence on basal ganglia output.

Abnormal dopamine receptors

Early work on SKF 38393 induced contralateral circling behaviour, with equal efficacy to apomorphine, was reproduced by SKF 38393 in 6OHDA rats with unilateral lesions administered either parenterally or intrastriatally (Setler et al., 1976). While it is known that various striatal receptor populations occur in striatal differentiation, this phenomenon was originally thought to be predominantly D-2 mediated. SKF 38393, a selective D-1 agonist, was therefore felt to be acting upon receptors that had "lost" their D-1 specificity (Costall et al., 1976). However in time SKF 38393 was shown to mediate its effects upon the supersensitive D-1 receptor (Setler et al., 1976; Gower and Marriott, 1982). This hypothesis was strengthened when SCH 23390 selectively blocked all the SKF 38390 induced rodent circling but did not alter those changes produced by LY 171555 or pergolide (Arnt and Hyttel, 1984 and 1985). Haloperidol and pimozide were ineffective in blocking SKF 38393's circling effects (Gower and Marriot, 1982). Partial antagonistic effects against apomorphine following SKF 38393 was observed, (Herrera-Marchitz et al., 1984; Arnt and Hyttel, 1985) a biphasic peak rotation pattern was noted following apomorphine, of which only the second component of the biphasic response

was abolished by SCH 23390 (Coward 1983; Herrera-Marchitz and Ungerstedt, 1984). SKF 38393 also affects the second phasic peak suggesting it is the latter peak that is D-1 mediated with the former peak being primarily a D-2 phenomenon (or D-1 plus D-2). When selective D-2 antagonists were applied to the observed behavioural effects of 6OHDA rodents, following D-1 and D-2 agonists, there was a 500 fold difference bet n observed inhibition of pergolide or LY 171555 in contrast to SKF 38393 (Arnt and Hyttel, 1984). Apomorphine was partially inhibited by D-2 antagonists, especially the first peak response (Coward, 1983; Arnt and Hyttel, 1985). Haloperidol inhibits apomorphine-induced circling but this depended upon whether they were "high spontaneous rotators" (who did not respond) or "low rotators" who responded (Fenton et al., 1985).

These results may be explained by two separate D-1 and D-2 receptor site systems both producing similar circling results (Arnt, 1987). Multiple studies have confirmed behavioural specificity of SKF 38393 upon D-1 receptors compared with pergolide or LY 171555 (Arnt, 1987). It is unknown whether D-1 and D-2 receptors are present in parallel within the same system or whether different anatomical receptor sites ultimately mediate the same final result. The possibility that the D-1 and D-2 systems function in parallel arises from evidence to suggest:

a) The maximal effect of mixed D-1 and D-2 receptor agonist apomorphine is not greater than the D-1 receptor agonist SKF 38393 alone (Arnt and Hyttel, 1985).

b) Similar neuronal substrates which mediate rodent circling in both normal and supersensitive striatal receptors suggest a single output network is selectively influenced by both D-1 and D-2 receptor input either separately or in combination.

Differential time courses may exist regarding D-1 and D-2 receptor hypersensitivity. Dopamine effects are maximal 2 days following rodent 6OHDA lesions, apomorphine 2-4 days, but SKF 38393 or D-1 effects are maximal on days 9-11 (Arnt, 1987). The time course of SKF 38393's maximal effects post-lesion correlate with increases in D-2 receptor densities (Stannton et al., 1981) but not with D-1 receptor density (Hyttel et al., 1983). Early D-1 adaptive effects, before D-1 receptor hypersensitivity occurs, may be D-2 receptor mediated. The long term effects of D-1 and D-2 antagonists have not been shown in rodents but D-1 receptor density with normal D-2 density may begin to increase 3 weeks after treatment with SCH 23390 (Creese and Chen, 1985).

Dopamine presynaptic (autoreceptor) and postsynaptic receptor differentiation.

When it was noted that at low doses dopamine agonists depress locomotor activity and electrical cell firing (Carlsson, 1975; Skirboll et al., 1979; White and Wang, 1984), the existence of dopamine receptors in different affinity states or sites was proposed. The possibility of dopaminergic sedative side-effects and the coincident discovery that these same receptors mediate control of dopamine synthesis led to the concept of "presynaptic" dopamine receptors (Shalaby and Spear, 1980; Arnt, 1983). These receptors are better labelled "autoreceptors", since they exert postsynaptic effects as well (Arnt, 1987). 3-13-hydroxy-phenyl-n-n-propylpiperidene (3-PPP) was the initial autoreceptor agonist with minimal postsynaptic effect (Hjorth et al., 1981, Martin et al., 1981). Drugs of this group are easily differentiated from postsynaptic D-1 or D-2 agonists in rats since they do not produce stereotypic or circling behaviour in normal rats and lack reversal of reserpine and methyl paratyrosine-induced akinesia. Biochemical clues suggest they are D-2 receptors with a higher sensitivity to agonists and little response to SKF 38393. One should be careful to avoid stimulation of alpha-2 adrenoreceptors which also produce motility inhibition (Delini-Stula et al., 1979). Concomitant use of a selective alpha-2 adrenoreceptor antagonist such as

idazoxan in experiments avoids this problem (Dettmar et al., 1983). Few dopamine antagonists have been shown to reverse apomorphine-induced drowsiness, but sulpiride and spiperone may do so (Maj et al., 1977; Sumners et al., 1981; Costall et al., 1980; Arnt et al., 1983). While sulpiride preferentially blocks postsynaptic dopamine receptors; metoclopramide and molindone block the auto-receptors (Alander et al., 1980). Interpretation of these results is made difficuilt, by the necessity of a critical dose range that needs to be employed, so that only autoreceptors but not postsynaptic receptors are affected (Costall et al., 1982 and 1983). Yawning behaviour in naive rats may be the best model to test autoreceptors, but then only with alpha-2 adrenoreceptor blockade (Gower et al., 1984) following dose response studies (Protias et al., 1983; Schultz, 1982). Behavioural effects of autoreceptors partially mimic those of neuroleptic drugs, potentiate neuroleptic drugs to some extent when concomitantly employed at high doses and should simulate full dopamine agonist drugs (Arnt, 1987).

The supersensitive striatal receptor models show marked contralateral turning (Martin et al., 1981; Arnt, 1982; Oberlander and Boissier, 1983) following 3-PPP. Since contralateral turning behaviour is thought to be a post-synaptic phenomena, this was surprising as 3-PPP in high doses antagonises the normal postsynaptic D-2 receptor

(Arnt et al., 1982; Hjorth et al., 1983). Other auto-receptor agonist drugs only stimulate normal rats to turn contralaterally (Arnt, 1984). Since the dose required for the above is similar to that producing hypomotility, emesis and yawning, the suggestion has been proposed that post-synaptic receptors in the 6OHDA lesioned animals change and become similar to autoreceptors. Antagonistic studies of 3-PPP induced circling, using D-2 selective antagonists indicate typical D-2 responsiveness (Arnt, 1982; Christensen et al., 1984). Thus instead of distinct pre and postsynaptic D-2 receptors, a single receptor able to change its affinity has been proposed (Carlsson, 1983; Arnt and Hyttel, 1984; Clarke, 1985). Endogenous dopamine could then dictate the functional state, or tone, of the D-2 receptors (Eriksson et al., 1983; Arnt and Hyttel, 1984). The state of the autoreceptor in long term neurolept treated animals has been called into question as with long term dopamine depletion they may become functionally inactive (Arnt, 1987). Behavioural models do provide a test bed against which many drugs and hypotheses relating to dopaminergic systems may be tested. Progress in the understanding of dopamine receptors, their subtypes, distribution and their functional interaction is dependent upon intelligent application of these available models. Conclusions derived from rodent data are increasingly being tested in the MPTP-treated primate model (Glodstein et al., 1984). Recent definition of selective D-1 and D-2

compounds has validated and tested the response of these behavioural models, in particular the 6OHDA circling rodent which continues to provide useful pointers to the functioning dopaminergic components of the basal ganglia. The intricate functional linkage between the D-1 and D-2 receptors in the normosensitive and hypersensitive state remains undefined. The different functional states of the D-2 receptors continue to intrigue scientists and the autoreceptor versus postsynaptic D-2 state needs further work to understand how the D-2 receptor functions. Finally the relative interaction between different dopaminergic pathways such as cortical versus limbic, is also integrated and the feedback systems (which may involve autoreceptors) form a cell messenger network, starting from a dopamine cell in the SNc, exerting its influence upon the whole basal ganglia, including receiving feedback upon itself.

3.2.4 Histological and anatomical distribution of dopamine receptors

Kebabian suggested at least five possible receptor sites (Fig 3.3) (Kebabian and Calne, 1979).

D-2 types: (independent of cAMP)

a) Cortical neurones projecting to the striatum

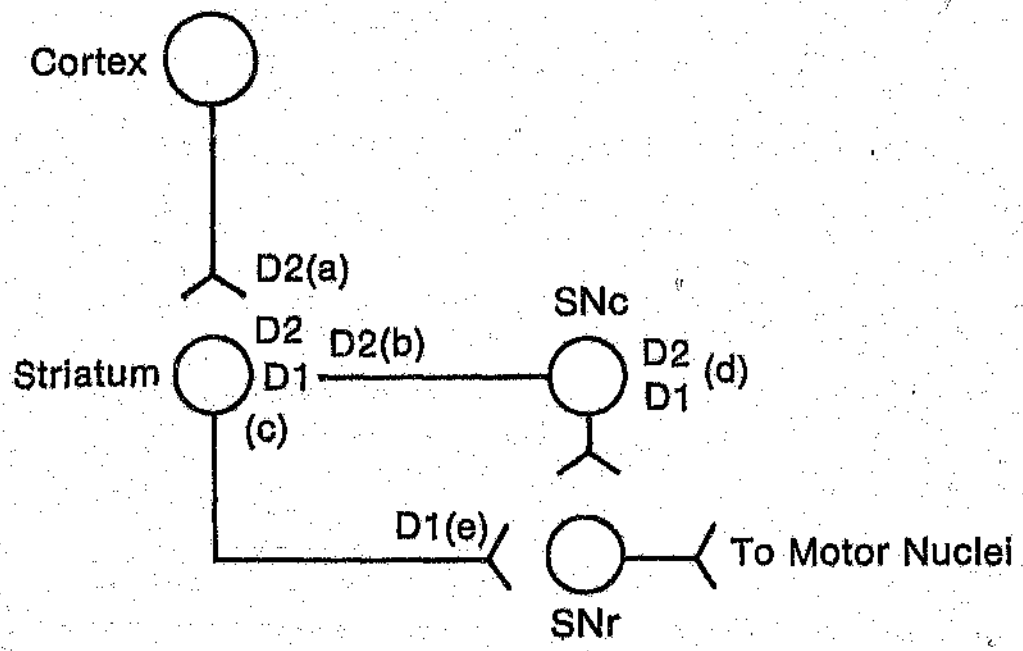


Fig 3.3

Schematic representation of D-1 and D-2 receptor localisation on the rat nigrostriatial system. (a) Although controversial, D-2 receptors appear to be located presynaptically on the glutamatergic fibres of the corticostriatial pathway. (b) D-2 receptors are located presynaptically on the dopaminergic fibres of the nigrostriatial pathway. (c) Both D-1 and D-2 receptors are found on the intrinsic neurons of the striatium. (d) Both D-1 and D-2 receptors are located on the cell bodies of the nigrostriatial pathway (SNc). (e) The vast majority of the D-1 receptors of the SNr are located presynaptically on the GABAergic fibres of the striatonigral pathway.

b) Autoreceptors in the striatum, regulating neurones functionally and identified by tyrosine hydroxylase

c) Intrinsic caudate-putamen interneurones, also ? D-1 type

D-1 types: (dependant upon dopamine sensitive cAMP)

d) Autoreceptors in the substantia nigra (SNc), also D-2 type

e) Striatal neurones that project back to the substantia nigra (SNr), (striatonigral pathway)

In several animal species, man included, the striatal D-1 receptor appears to outnumber the D-2 receptor by a factor of 6 to 1 (Hyttel and Arnt, 1986). Dopamine receptors are not found in a homogenous distribution in the striatum but rather a complex compartmentalisation has been proposed. Histochemical techniques suggest striatal striosomes which contain high densities of dopamine islands within a diffuse acetylcholinesterase rich staining background (Graybiel and Ragsgate, 1978). Further evidence of discreet zones by autoradiographic demonstration of [^{3}H]spiroperidol labelling of striatal D-2 receptors, showing different striatal zone densities has been reported (Joyce et al., 1986). The hypothesis that the dopaminergic and cholinergic striatal interconnections influence one another support what is known and observed in the treatment of

parkinsonian patients, when employing both levodopa and anticholinergic medications, both of which have additive and independent antiparkinsonian effects. The micro-anatomical site of dopamine receptors is unknown, but possibilities include the striatal interneurone, medium spiny neurones, axon terminals of the corticostriatal projecting neurones, the dopamine terminal of the cell body or finally, the presynaptic autoreceptors.

While D-1 and D-2 dopamine receptors are characterised by radioligand studies the combination of binding markers to receptors and histologic analysis may be the best method to elucidate where on the cell, which cell subpopulation and in which basal ganglia nuclei the dopamine receptors reside. Autoradiography may quantify receptor density further and also be helpful in identifying anatomical location. While the D-1 and D-2 receptor anatomic location and even density has been characterised using many of these techniques, the exact cytoplasmic site and which striatal cell eludes detection (Palacios et al., 1988).

In the late 1960's sensitive histochemical techniques for visualisation of dopaminergic systems (reviewed by Moore and Blom, 1978) and recently receptor localisation utilising the light microscope became a reality (Kerhar et al., 1986). Labelling of dopamine receptors <u>in vivo</u> (Kerher et al., 1986) and <u>in vitro</u> (Palacios and Warnsley, 1984)

and rodent identification of the D-2 receptor using the [^{3}H] spiperone ligand *in vivo* proved difficult (Kehar et al., 1978; Klemm et al., 1979) and the *in vitro* technique with autoradiography was thought more practical (Young and Kehar, 1979).

D-2 receptor distribution

Quantitative autoradiography suggests that the D-2 dopamine receptors are predominantly located on the intrinsic striatal neurones (Trugman 1986). Dopamine receptors may be visualised via the light microscope and autoradiographic techniques (Young, 1979). Corticostriatal pathway lesions produce striatal neurone degeneration with no effect upon overall D-2 receptor density. In contrast, striatal lesions which destroy intrinsic cell bodies while sparing afferent nerve terminals, result in a large decrease in striatal D-2 receptor density. Circumstantial evidence exists that the D-2 receptor is at least found on the intrinsic striatal neurones. Other states may still exist and application of a specific antibody or protein directed against the receptor site is eagerly awaited.

Autoradiographic techniques, labelling the D-2 receptor with either an agonist or antagonist, show densities greatest in the caudate-putamen, olfactory tubercle and

nucleus accumbens in roderts (Palacios et al., 1981; Gehlert and Warmsley et al., 1985). The substantia nigra (SNc and SNr), ventral tegmental area, olfactory bulb (Nock et al, 1986; Charuchinda et al., 1987; Palacios and Payos, 1987), intermediate plus lateral pituitary (Lightman et al., 1982; Payos et al., 1985) also contain D-2 receptors. A rostrocaudal and ventrodorsal receptor gradient has been observed (Dubois and Scatton, 1985). The globus pallidus and thalamus contain very few dopamine receptors but the cerebral cortex, hippocampal formation and even the cerebellum contain significant D-2 receptors (Gehlert and Warmsley, 1985). Radiolabels may only mark high affinity D-2 receptors and due to variable densities utilising different agonists one needs to interpret radioligand results with caution. In postmortem human brain tissue, high D-2 receptor densities are found in the caudate-putamen, nucleus accumbens, olfactory tubercle, anterior pituitary and lateral globus pallidus and with less density in the substantia nigra (SNc) and hippocampus (Camps et al., 1989).

D-1 receptor distribution

D-1 dopamine receptor radiolabelled markers show high D-1 densities in the caudate-putamen, nucleus accumbens, olfactory tubercle, entopeduncular nucleus, SNc, SNr and globus

pallidus. Overall there is a much higher density, indicating numerically more D-1 compared with D-2 receptors in the rat brain. The cerebellum, ventral tegmental area and amygdala show no D-1 receptors (Dubois et al., 1986; Dawson et al., 1986; Kebabian et al., 1986; Savasta et al., 1986). Human D-1 receptors are found in high concentrations in the caudate, putamen, olfactory tubercle, nucleus accumbens, medial globus pallidus, substantia nigra (SNc and SNr) and, as expected, in the hippocampus and several cortical areas. The densities in these areas is much lower and nonexistent in the human medial globus pallidus, pituitary and cerebellum (Cortes et al., 1989). Human postmortem brain slices have confirmed observations that a life long diminution in D-1 and possibly D-2 receptor density occurs, indicating age related dopaminergic system degeneration (McGeer et al., 1977; Palacinos et al., 1988).

In conclusion, the autoradiographic techniques described are helpful but do not allow sufficient cellular resolution to further delineate the specific cell upon which the dopamine receptor is situated. Inferential studies using known lesioning techniques are helpful. Kianic acid intrastriatally will kill striatal neurones and deplete D-1 and D-2 receptor densities suggesting these receptors could be sited upon the cell body or dendrite of intrinsic striatal interneurones.

Trugman found with striatal kianic acid that all D-2 receptors disappeared, in contrast to only 5% reduction reported using membrane-binding assays (Schwartz et al., 1978; Trugman et al, 1986).

Secondly, specific lesions of the medial forebrain bundle and interruptions of the striatonigral tract (electrolytic lesions) cause decreased striatal and nigral D-1 receptors, however no decrease in striatal dopamine receptor density occurs following rodent decortication (Trugman et al., 1986). This correlates with biochemical studies (Spano et al., 1977) which show depleted SNr adenyl cyclase in experiments lesioning the descending striatonigral pathway. D-2 receptors in the SNc are less affected by striatal or medial forebrain bundle lesions but diminish following 6-OHDA in the SN known to destroy dopamine SNc cells and ascending nigrostriatal tracts. Hence D-2 receptors exist in the SNc and are probably located upon the dopaminergic cell bodies. Limited striatal D-2 receptor effects seen with medial forebrain lesioning and striatal lesions could be explained if the D-2 receptors are located in the nigrostriatal nerve terminals and not the striatal neurones.

Ultimately, better microscopic labelling with new probes such as a labelled monoclonal receptor antibodies or genetic probes for mRNA and greater membrane magnification may possibly define the exact cell housing the dopamine

receptors. Furthermore the potential of combining probes, marking receptors, utilising immunocytochemical markers of neurotransmitters, may localise the area of common staining and thus suggest possible functional neurotransmitter linkage within single cells (Kuhar, 1981).

3.2.5 Confirmation of receptor location by positron emission tomography

In vivo positron emission tomography (PET) studies have demonstrated radioactive tracer uptake concentration regionally within the brain in a number of neurologic illnesses (Calne et al., 1985). The most promising radio-isotope studies utilised have been [^{18}F]flurodopa (Furian et al.,1975) to subanalyse the presynaptic dopamine systems and postsynaptic dopaminergic function using the neurolept ligand 3-N-methylspiperone (NMSP) which binds to D-2 receptors (and serotonin receptors) (Wagner et al., 1983). Nonspecific binders such as [^{18}F]haloperidol, (Welch et al., 1983), [^{11}C]chlorpromazine (Comar et al., 1979) or [^{11}C]raclopide (Farde et al., 1986) may also provide useful data.

Presynaptic receptors

Flurodopa (FD), normally accumulates in the striatum following intravenous administration. Animal model studies show reduced striatal 5FD accumulation following reserpine and high affinity of D-2 markers for the striatal dopamine receptor systems. In man, semiquantitative interpretation comparing ratios of striatal versus cerebellum or cortical activity, both of which contain low D-2 dopamine receptors, show a normal age related decrease in striatal FD uptake (Martin et al., 1986). This correlates with the substantia nigra cell decline with age (McGeer et al., 1977). Hemi-parkinsonian models are useful as one can compare the striatum on either side in the same animals that provide necropsy specimens. Six patients with unilateral parkinsonism showed increased [^{18}F]FD in the contra-lateral striatum (Garnett et al., 1983 and 1984; Martin et al., 1984 and 1986). Diminished striatal FD percentage uptake on the ipsilateral striatum, suggesting reduced endogenous dopamine release was noted (Lenders et al., 1986), indicating physiological compensatory mechanisms that subclinically reduce dopaminergic input in an attempt to mask the signs of dopaminergic insufficiency on the opposite side. The loss of FD uptake is especially decreased in the posterior striatum, which primarily represents the putamen. Patients suffering from motor fluctuations, particularly the "on-off" type, showed

rapidly reduced striatal FD radioactivity following their usual levodopa medications (Lenders et al., 1986). Possible interpretation of this may be that "on-off" patients have lost their dopamine storage capacity. While both non-fluctuating advanced parkinsonian patients and those suffering "on-off" motor fluctuations showed reduced overall striatal FD uptake, it was the rapidity of FD displacement by levodopa (Sinemet) that identified the "on-off" subgroup of patients (Lenders et al., 1986). Dietary amino acids also reduce FD uptake in healthy subjects suggesting amino acid interference with levodopa crossing the blood-brain barrier (Lenders et al., 1986). MPTP and Guam dementia patients also show diminished striatal FD uptake even though they may still be subclinical in their parkinsonian symptomatology (Calne et al., 1985; Martin et al., 1986).

Postsynaptic dopaminergic receptors

The postsynaptic dopamine receptors and pathways may be studied by specific D-1 and D-2 selective ligands, the specificity of these markers is a major limitation at present. While biochemical markers such as SCH 23390 and sulpiride exist, they have not yet been successfully radio-labelled (Sedwill et al., 1986). Ideally the ligand must cross the blood-brain barrier successfully, be selective to the receptor but not influence it, be distinguishable by

PET spatial analysis and yield few metabolites (which may retain the radiolabel and give false information) and finally should be cleared rapidly from the blood (Young et al.,1986). Hence the ligand should only occupy 1-5% of the receptors, enough to identify them by PET techniques but not more than will disrupt or influence normal receptor functioning. No firm or repeated data has emerged regarding postsynaptic dopaminergic function as determined by PET but in future this technique will provide a useful method of examining receptor dynamics in vivo (Mailman et al., 1984; Malloy and Waddington 1984; Carlson et al., 1984).

3.3 The place and relevance of dopamine receptors in human idiopathic Parkinson's disease

Since the clinical features and natural progression of IPD in both the untreated and levodopa treated state is now well described what, if anything, may be done to alleviate or prevent the disease? Aetiological aspects of nigral dopamine cell death continue to be researched and until better understood the disease will continue to appear. Hence effective long term therapy is demanded and since pharmacological problems themselves dictate different treatment strategies many varying opinions exist.

One such option is the dopamine receptor agonists. Since at least two different dopamine receptors exist in the basal ganglia, selective drugs would have to be evaluated as to what specific antiparkinsonian properties they possess. Indeed should any dopamine agonist not be purely D-1 or D-2 receptor specific then the degree to which it affects the other receptors would be crucial to best judging its individual efficacy. Various methods mentioned in this chapter allow *in vitro* interpretation of dopamine receptor stimulation. However it is only behavioural data from rodents, but more reliably, data from primates and human IPD that allows confirmation of drug efficacy and tolerability. Only once this is shown can a dopamine receptor agonist be tested in subjects with incapacitating parkinsonism.

There are at least three reasons why the dopamine agonists stand a good chance of success. Firstly, it is physiologically reasonable that stimulation of these striatal dopamine receptors with levodopa or any agonist, will initiate the intercellular second messenger system to successfully initiate basal ganglia influence. This principle chemically normalises nigrostriatal input into the system.

Secondly, some dopamine agonists do not require endogenous or exogenous levodopa to be effective anti-akinetic agents. With steadily falling endogenous levodopa in the brain due

to the inevitable parkinsonian progression, drugs that require levodopa to be effective will decline in potency with time. Any drug may potentiate intrinsic levodopa chemical dynamics but those that can act independently of levodopa are theoretically more attractive. Furthermore, since levodopa metabolism may provide toxic products that may perpetuate further nigral cell decline, these agents that function away from the nigra may be a better choice. Any drug or metabolic action at the nigra should, if anything, prevent further damage by sequestering or detoxifying harmful free radicals.

Thirdly, the dopamine receptor is only one of many classical neurotransmitter systems in IPD that is affected. Becoming more apparent are the interconnections between these central chemical cascades within the human and animal brain. Affect one system, (in IPD mainly the dopaminergic system) and with time other non-dopaminergic systems will also become abnormal. Hence attempted correction of the dopamine nigrostriatal system will influence not only other dopaminergic but also other non-dopaminergic pathways.

Manipulation of the dopamine receptor populations with dopamine agonists is therefore physiologically appropriate, stands a good chance of working in human IPD and has already been successfully applied in MPTP primates and man.

New drugs facilitate development of novel agents that may with experimentation be found to revolutionise the management of the illness.

In this thesis, one of the aims is to review the benefit of a novel D-1 selective agent CY 208-243, by examining it alone, in combination with other known receptor agonists and antagonists and finally with levodopa itself.

CHAPTER 4

N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine — THE MPTP-TREATED PRIMATE MODEL

The neurotoxin N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) has produced the closest animal model currently available to human idiopathic Parkinson's disease (Jenner and Marsden, 1986). While the MPTP non-human primate model is imperfect, it has allowed researchers to pursue environmental toxic insults that may, in a susceptible person, produce parkinsonism.

Between 1977 and 1982 young drug addicts were accidentally exposed to MPTP, contained in new "synthetic heroin". A short cut in the production of this designer drug by synthesis at too high a temperature, contaminated the drug with MPTP resulting in a parkinsonian syndrome affecting young adults (Davis et al., 1979; Langston et al., 1983; Lewin 1984). As many as 300 young Californian adults may have been exposed to MPTP by inadvertent intravenous drug abuse (Snyder and D'Amato, 1986).

This chapter examines the current belief addressing the mechanism of action, biochemical consequences, anatomical selective damage and finally the behavioural changes of the MPTP-treated animal model. Special attention is given to the marmoset primate model since this model was primarily used to study the dopamine agonists, in this thesis.

4.1 Mechanism of action of MPTP

After MPTP administration, a portion of which crosses the blood brain barrier, a highly toxic water soluble pyridinium ion MPP^+ (1-methyl-4 phenylpyridinium) may be detected both in peripheral and brain tissue. MPP^+ has a long half-life (of 48 - 100 hours) and it continues to increase in the substantia nigra for up to 72 hours, thereafter diminishing in concentration (Irwin, 1985). Tissue concentrations of MPP^+ fall more rapidly outside the blood brain barrier. Other cyclic tertiary amides are biodegradable by the P450 cytochrome system in hepatocytes, but it would appear that once MPTP is concentrated in mitochondria (Singer, 1987) it is converted to MPP^+ by way of 2,3-$MPDP^+$ (Fig 4.1) (Chiba, 1984). The enzyme MAO "B", converts MPTP to 2,3-$MPDP^+$. MAO "B" is found in abundance in hepatocytes, brain astrocytes and glial cells. Minimal MAO "B" is found in dopamine selective nigrostriatal cells.

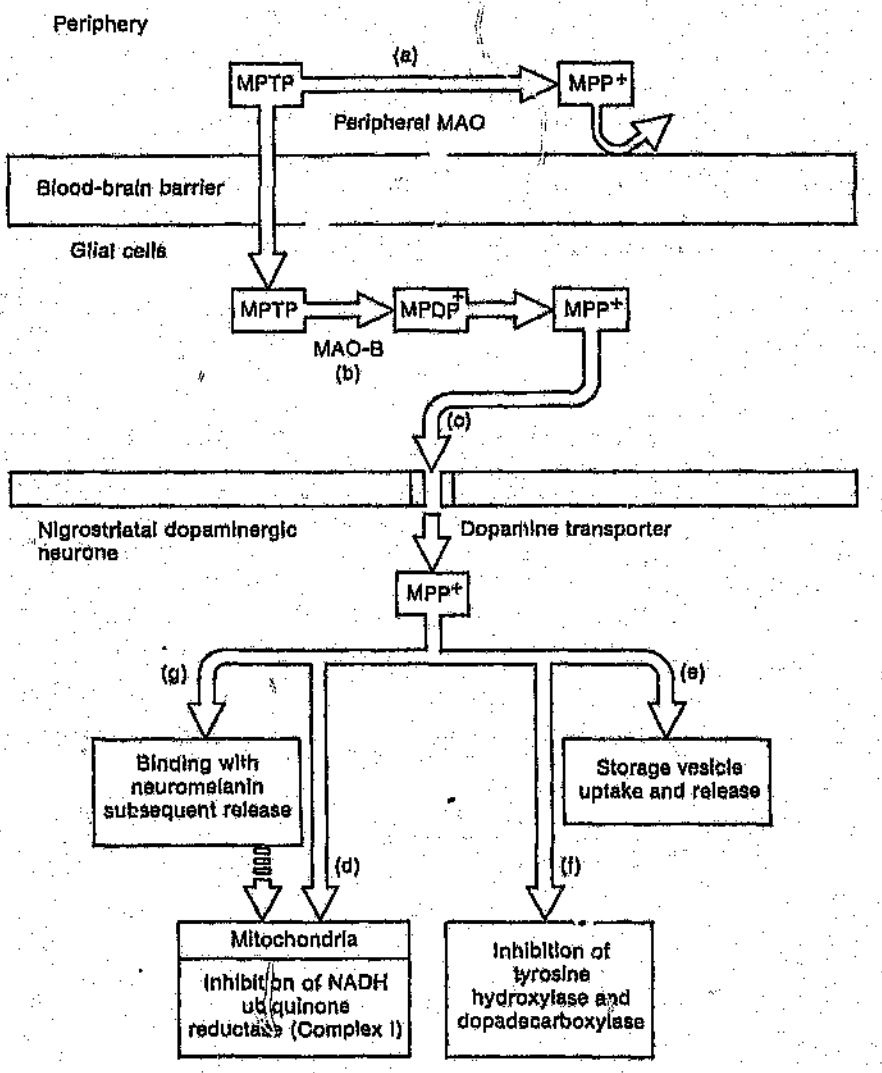


Fig 4.1 Metabolism of MPTP within the brain

Possible scheme of MPTP toxicity:

(a) MPTP is metabolised by gut, liver and blood-brain barrier (BBB) MAO. MPP^+ does not cross the BBB. First-pass metabolism renders oral MPTP much less neurotoxic than parenteral administration.

(b) Glial cells containing MAO "B" convert MPTP to MPP^+. (MAO "A" oxidises MPTP much more slowly and nigral MAO "A" concentrations are very low compared with MAO "B".

(c) MPP^+ is taken up by a specific dopmaine transporter protein.

(d) MPP^+ inhibits Complex I *in vivo*. New evidence suggests that this is also critical *in vivo*. Impaired cellular respiration produces cell death.

(e) MPP^+ is taken up into dopamine storage vesicles and stimulates vesicle release.

(f) MPP^+ inhibits dopamine synthesising enzymes; together with (e) this causes acute dopamine depletion.

(g) MPP^+ binds with neuromelanin, which may act as a storage pool. MPDP, 1-methyl-4-phenyl-2,3-dihydropyridinium; NADH, reduced nicotinamide adenine dinucleotide.

The selective MAO "B" blockers, paragaline or selegiline, are able to block the MPTP insult (Heikkila et al., 1984 and 1985). MPP^+ has been shown to enter dopaminergic neurones, via the dopamine uptake system (Javich, 1985), with similar but competitive affinity to dopamine, resulting in selective dopamine cell death. In rodent striatal synaptosomal preparations, MPP^+ is taken up by dopamine cells with the same affinity as dopamine. Uptake of MPP^+ is similarly reversible and competitively blocked by MAO "B" uptake inhibitors. Pharmacological rank order uptake of blockers is similar for both MPP^+ and dopamine. This data suggests they are taken up in a similar site and by a similar mechanism. Evidence suggests that while MPTP itself (and 2,3-$MPDP^+$) is not toxic, MPP^+ is highly toxic to dopaminergic nigrostriatal cells (Fig 4.2). Recent studies suggest MAO "B" enzyme activity is present in cells in the substantia nigra, but not in sufficient quantity to explain the selective MPP^+ accumulation. At least part of the MPP^+ accumulation is thought to be produced by glial cells. MPP^+ is then released to damage nigrostriatal dopaminergic cells. MPP^+ may firstly be taken up by dopaminergic striatal terminals and retrogradely transported to the cell body situated in the substantia nigra.

Alternatively the toxin may directly destroy the cell body in the substantia nigra. On reaching the dopaminergic

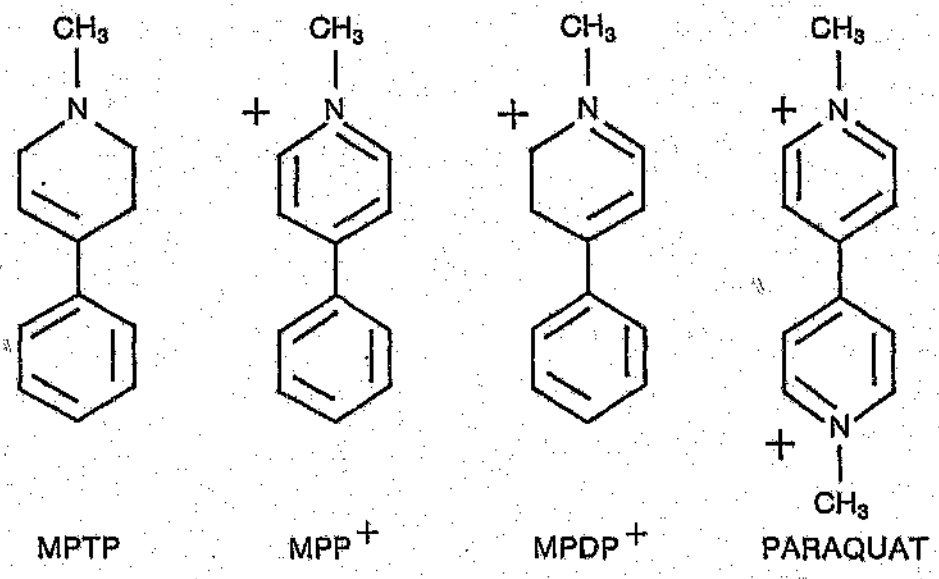


Fig 4.2

Structure of MPTP, its metabolites and the common herbicide paraquat

cell, MPP^+ inhibits ADP-stimulated oxidation of NAD-linked mitochondrial substrates (Nicklaus et al, 1985). An energy dependant carrier of positive ions concentrates MPP^+ within the mitochondria, well above the concentration required to totally inhibit NADH dehydrogenase (Ramsay, 1986). While MPTP, $MPDP^+$ and other 4-phenyl-pyridines also inhibit NADH oxidation, it is the accumulation of MPP^+ that makes it the most likely toxin responsible for dopaminergic cell death.

The postulated mechanism of cell death is that MPP^+ is concentrated in mitochondria and subsequently inactivates NADH-linked complex I, the complex required for mitochondrial respiration. This leads to interference in mitochondrial cell energy metabolism, depletion of ATP and altered calcium homeostasis within cells (Vyas et al., 1986). NADH inhibition causes inhibition of oxidative phosphorylation and depletion of ATP supply, which is cytotoxic.

Simultaneously MPP^+ synthesis by glial cells creates excessive free radicals which alter oxidative stress in the environment finally interfering with membrane lipid peroxidation (Rossetti et al., 1988). Free radicals such as superoxide, hydrogen peroxide, heavy metal ions (iron and zinc) may all damage mitochondrial complex I or neuromelanin containing nigral cells (Dexter et al., 1989). Once nigrostriatal cells die they cannot regenerate.

Natural attrition of neurones, with age, may in time compound susceptibility to MPP^+. All primates are susceptible to MPP^+, while some non-primate laboratory animals (especially the rat, but also dogs and cats) show demonstrable resistance to MPTP. Moreover while striatal dopamine is reduced in dogs and cats, they remain resistant to MPP^+, and their parkinsonism may not be permanent. Rodents not only resist MPP^+'s toxic influence, but also do not deplete striatal dopamine and do not show chronic nigrostriatal cell loss or motor dysfunction.

As MPTP is peripherally metabolised and crosses the blood-brain barrier in similar rates in primates and rats, the species difference is thought to be due to longer retention, accumulation and toxic insult of MPP^+ itself in the primate brain. This hypothesis is confirmed by longer retention of radiolabelled MPP^+ in the primate compared with the rodent brain (Irwin, 1985). This finding may be related to primate neuromelanin which is absent in lower animal species.

In summary it appears that MPP^+ is lethal to specific dopaminergic substantia nigra cellular structures. MPP^+ needs to be accumulated by the presence of MAO "B", a process which may also require cellular neuromelanin.

4.2 Neurochemical consequences of MPTP

The biochemical consequences of MPTP's neurotoxic insult have been recorded in the rat (Chieuch et al., 1984; Boyce et al., 1984; Bradbury et al., 1984), mouse (Heikkila et al., 1985), dog (Parisis et al., 1985), cat (Schneider et al., 1986), frog (Barbeau et al., 1985) and limited information exists in the primate models.

The common marmoset showed diminished dopamine and its major metabolites HVA, DOPAC in the caudate, putamen and nucleus accumbens, 10 days following the acute insult (MPTP 1-4mg/kg/day for 4 days), but by 40 days the nucleus accumbens had recovered dopaminergic function. Diminished caudate-putamen dopamine, HVA and DOPAC in the rhesus and squirrel monkey, reflects diminished input from nigrostriatal dopaminergic pathways. Normal dopamine in the ventral tegmental area and nucleus accumbens, is thought to reflect normal mesolimbostriatal dopaminergic pathways (Burns, 1983; 1985 and Langston, 1984). The greater the dose of MPTP administered, the greater the caudate-putamen reduction in dopamine and dopamine metabolites (Jenner, 1984). Tritiated dopamine uptake in synaptosomal striatal preparations was reduced at higher MPTP doses. No specific [^{3}H]spiperone binding to the caudate-putamen was found acutely after 6 weeks when the animals were behaviourally

stable parkinsonian models. Norepinephrine, serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA) were minimally changed in the acute stages, but normal when analysed later. No major neuropeptide changes including Leu-enkephalin, Met-enkephalin, substance P and CCK 8 were observed.

4.3 Histological changes found following

The predominant histological finding following MPTP administration is cell body loss from the substantia nigra zona compacta. This predominantly affects dopamine (A9) cells, while some cell loss also occurs in the ventral tegmental area (A10). However the medial SN is not as severely affected as the lateral substantia nigra. Locus ceruleus, substantia innominata and raphe nucleus remain unaffected. Tyrosine hydroxylase (TH) immunohistochemistry remains a useful marker of dopaminergic cells and shows histologic cell loss. Other cells which do not stain for TH have also been shown to be ·aged in the substantia nigra. Immunohistochemical markers of substance P, met-encephalin, dynorphin and neurotensin levels remain normal following MPTP administration. Eosinophilic inclusions have been noted in cells of the SNc (Forno, 1986). The presence of Lewy bodies, so important in establishing the pathological diagnosis of human idiopathic Parkinson's disease, remains

elusive in the primate MPTP-treated model (Forno et al., 1986).

4.4 MPTP: Behavioural effects in the primate and especially the common marmoset model

Evidence that the MPTP model is valid must include behavioural improvement following use of drugs that are known to have antiparkinsonian activity. The rodent, cat, dog and amphibian species are poor models for MPTP-induced behavioural analysis, therefore attention has been focused upon non-human primates. Animals including the rhesus, squirrel, cyanmolgus, African Green monkey (vervet) and common marmoset monkeys have been studied behaviourally. Rhesus and squirrel monkeys showed an acute behavioural syndrome following the second and subsequent dose of MPTP, comprising a stuporose state lasting up to one hour (Langston, 1984; Burns, 1983). After repeated MPTP doses persistent motor deficits and behavioural signs emerged and animals remained akinetic, flexed, rigid with decreased appetite and vocalisation. Levodopa and bromocriptine reversed these behavioural effects. Common marmosets similarly developed parkinsonism but in individual animal susceptibility varied. A dose titration, according to the animal age and response from day to day, was thus employed to render all animals equally parkinsonian. Most animals

required a cumulative dose of 7-10 mg/kg ip., over 3-5 days (Nomoto et al, 1986). Acute reactions similar to those described in the rhesus species followed the second and subsequent doses of MPTP. This rendered the animal unstable, sleepy, prostrate, sometimes dystonic and usually unrousable for up to one hour. Subacute motor deficits subsequently occurred after 2-3 doses of MPTP, in the form of bradykinesia, rigidity of limbs and trunk, flexion, loss of vocalisation and sometimes a postural tremor. The animals required assisted feeding in this stage. Chronic motor deficits, which remained stable up to six months appeared after six weeks (Temlett et al, 1989: Appendix V). This was indicated by a return of appetite, with bradykinesia, flexion of the trunk and slow climbing behaviour or perch stability. Balance remained unstable, the animals lacked co-ordination but could jump from perch to perch when stimulated. Variable degrees of recovery have been observed and generally the younger the animal the quicker the motor recovery (Jenner et al., 1984). The MPTP-treated marmoset responds predictably to known drugs such as levodopa, D-2 dopamine receptor agonists and antagonists when administered by mouth, subcutaneously or parenterally (Nomoto et al, 1987) and hence they provide a good reference against which new drugs may be tested. Not only may the animals' posture and balance be assessed but their overall motility may also be objectively measured using photocells and computer assisted recordings.

The rodent model may employ a unilateral nigrostriatal lesion and consequent rotating behaviour, first spontaneously and then following drugs that influence dopamine release. Similar behaviour has been reported following unilateral intercarotid infusion of MPTP in primates (Calne et al, 1989). Primates developing rotating behaviour, are thought to represent striatal imbalance between the affected compared with the the normal side of the brain (Kopin, 1986).

How close is the MPTP-treated primate model to human idiopathic Parkinson's disease? This question is relevant if we are to rely upon MPTP primate data to reflect motor behaviour in parkinsonism. Neuropathological studies show MPTP to be very selective to the substantia nigra dopaminergic cells. In human Parkinson's disease, but not in the MPTP-treated primates, the locus ceruleus is abnormal. Aged animals especially treated with long term low dose MPTP may show MPTP damage of this nor-adrenergic pathway (Kopin, 1986; Mitchell, 1985). However selective damage that occurs acutely following MPTP-treatment is not pathologically similar to IPD. No firm evidence of Lewy bodies, so important to the pathological confirmation in human illness, has emerged. Histologically, eosinophilic "Lewoid bodies" were recently reported in human MPTP-induced parkinsonism, but they remain absent in the MPTP-treated primates (Forno, 1982). Squirrel monkeys,

especially aged ones, on long term MPTP treatment, show eosinophilic inclusions in areas where Lewy bodies may be expected to be found. Perhaps the chronic administration of MPTP over many years may produce a model that better approximates the human and yields the vital neuropathological markers of Parkinson's disease thus validating the MPTP animal model.

The MPTP-primate model including the MPTP-treated common marmoset used in this thesis, while the best model of human idiopathic Parkinson's disease available, remains imperfect. While biochemically and behaviourally very similar to human idiopathic Parkinson's disease, histologically human Parkinson's disease is in contrast more widespread in its cellular insult.

4.5 MPTP's relevance to human idiopathic Parkinson's disease

Accepting the limitations of the MPTP-treated model of parkinsonism, it is still the best model available and has already answered many pathogenetic mechanisms particularly regarding clues to nigral cell death.

Caution in extrapolating data derived from MPTP primates is underlined by the fact that no Lewy bodies have been seen in the acutely lesioned model. This pathological hallmark is mandatory in human "idiopathic" PD. Without the Lewy body one thinks of the patient as suffering from one or another form of "parkinsonism" and not IPD.

Secondly, the MPTP neurotoxin is very specific in the damage it causes to the nigrostriatal system. This analogy does not follow in human IPD, since patients are well known to reveal degeneration of other dopaminergic and non-dopaminergic pathways. Hence the MPTP-model is too selective, but this may be an advantage. Much of the neurochemical nightmare that encompasses IPD is because of variable involvement in other central areas.

So, provided one bears in mind that the MPTP-treated primate is an acute and selective model one may utilise to maximum advantage the principal deficit in IPD - namely degeneration in the nigrostriatal system. Other side-effects of new drugs may only emerge once finally employed in man with IPD. This policy is followed routinely in science in any event, researches employing data derived from parkinsonian animal experiments as a prerequisite to human testing. The MPTP primate has simply added an exciting new dimension. One such new possibility is the testing of novel drugs and compounds. Another is the

possible benefit of infusion of chemicals, mesencephalic or adrenal medullary cell implants.

The MPTP primate model was found a reliable predictor of potency of known compounds, hence was used to test unknown potential compounds that showed *in vitro* promise. Finally when statistically successful in the MPTP primate the experiments allowed sequential ethical testing in humans with IPD.

With time one suspects that the MPTP-treated primate will become more widely employed both because it is a useful test-bed for transplants and new drugs but also to find clues in the aetiology of Parkinson's disease.

CHAPTER 5

AIMS OF THESIS

While levodopa remains the most effective drug therapy available today in idiopathic Parkinson's disease, the major problem to emerge has been the adverse effects thought to be medication related. To overcome the motor fluctuations and dyskinesias associated with long-term levodopa is an important objective and if achieved would allow more effective levodopa therapy. At best pharmacotherapy remains palliative. Ultimately intracerebral transplantation of dopamine producing cells may attempt to cure the illness and beyond that ideally one would hope to identify the aetiological factors that produce the disease.

Until this is done we remain confronted with, and challenged to, determine the best pharmacological approach in the management idiopathic Parkinson's disease. The role of dopamine agonist drugs in addressing these issues continues to be investigated.

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In determining the role of dopamine agonists in the therapy

of idiopathic Parkinson's disease, both humans suffering from the illness and the MPTP-treated common marmoset model of parkinsonism were analysed. The thesis is divided into three subsections:

a) Initially the well tested drugs and their limitations encountered in trials will be reviewed. Bromo- criptine was employed as adjuvant therapy to levodopa to evaluate whether one could maintain clinical stability and reduce total levodopa requirements (Chapter 6, trial 1).

A long acting levodopa compound, Sinemet CR4, was then tested to assess potential benefit offering the striatal receptors a slower and more physiological continuous dopamine receptor stimulation (Chapter 6, trial 2).

b) Primates lesioned with MPTP, will then be employed to evaluate novel selective dopamine agonists. The need for these drugs is apparant because literature review shows that while many dopamine D-2 agonists exist, few are selective and little data exists utilising D-1 selective compounds (Chapter 7, experiments 1 to 5).

c) Finally, those compounds that showed promise in the MPTP-treated primate experiments, were then applied to

man suffering from idiopathic Parkinson's disease (Chapter 8; trials 3,4 and 5).

All the experiments performed utilising new dopamine agonists will thus be subdivided into those applicable to the non-human MPTP-treated primate model and those experimental compounds utilised in man.

5.1 Animal experiments

The role of the various dopamine receptors in parkinsonism were tested by employing various D-1 and D-2 selective dopamine receptor agonists either alone, in combination with levodopa or in combination with each other.

Drug naive marmosets were treated with the neurotoxin MPTP, stabilised and once considered sufficiently parkinsonian they were tested against known drugs to confirm levodopa responsiveness and validate the model.

Thereafter known drugs (levodopa with carbidopa, LY 171555, bromocriptine and apomorphine) were administered to demonstrate a dose-response curve to each individual drug.

Once these known drug effects were reliably reproduced to correct parkinsonism , unknown compounds were employed.

CY 208-243, a partial selective D-1 dopamine receptor agonist was employed to assess the functional contribution of D-1 dopamine striatal receptors in MPTP parkinsonism. While D-2 selective agonist drugs are known to correct parkinsonian features, little is known of the D-1 receptors role motor movement. A new agent, (+)-PHNO, a potent D-2 agent, was administered to assess efficacy and dose responsiveness in the MPTP-treated primate.

Thereafter (+)-PHNO was administered in combination with CY 208-243 to investigate the functional interaction between the D-1 and D-2 receptors. Finally levodopa was employed alone, in variable doses and combinations with CY 208-243 or (+)-PHNO to assess the effect of D-1 or D-2 selective agents upon levodopa induced motor response.

It was clear from the literature that levodopa alone, while beneficial, produced problems in the long term. Bromocriptine adjuvant therapy suggested that low dose levodopa could be maintained and theoretically reduce fluctuations and dyskinesias. Hence, these drugs alone, in variable dose and combinations were tested behaviourally, to establish clues to the best drug combination.

Supporting biochemistry and histopathology, where relevant, will be utilised to ensure the model was suitable and could be compared with existing data in the marmoset and other

primate MPTP-treated models.

5.2 Human experiments

Animal data suggested these new drugs should be further investigated in man with idiopathic Parkinson's disease. The known profile of natural progression and the influence of levodopa therapy, all necessitate that new drugs be developed to better treat the illness. The main theme addressed in this thesis is the role of selective dopamine agonists in human Parkinson's disease. Specifically what place do the D-1 agonists play in human illness and how might they reduce levodopa side-effects? Furthermore does the D-1 dopamine receptor agonist have a role in improving known benefit of D-2 agents? Hence the following experimental phases tested many new and differing experimental regimens dependent upon the stage of development of the dopamine agonist.

Bromocriptine was initially employed in patients with idiopathic Parkinson's disease, to assess the ability to reduce overall levodopa requirements and thus circumvent levodopa related or other potential problems. The optimal dose of bromocriptine was sought with concomitant levodopa dosage reduction, while keeping the patient stable with a constant or improved Parkinsonian motor status.

Sinemet CR4, a slow release levodopa compound, was thereafter tested in man. The aim was to release levodopa slowly utilising an enteric coated matrix to maintain the serum drug levels and mobility. Following a theoretical Sinemet CR4 benefit suggested in the literature, the ability to reduce drug fluctuations that occur with intermittent conventional levodopa will be tested. Patients with unacceptable motor fluctuations will therefore be analysed, initially on their usual levodopa dosage then following a change to the long acting compound alone to establish motor benefit, dose frequency necessary and side-effects.

A new D-2 agonist, CQA 206-291, was administered to a group of patients with severe illness and "on-off" fluctuations (Chapter 8, trial 3). The efficacy of CQA 206-291 was tested at serially incremental dosage. Once safety of the drug is established a further study will be conducted in two further groups of patients.

CQA was given in combination with existing levodopa (Chapter 8, trial 4). The benefit and optimal dosage of CQA 206-291 was sought to allow reduction in overall levodopa requirement. This separate, double blind placebo matched trial, utilising CQA 206-291 was initiated in patients with moderate idiopathic Parkinson's disease. Those patients with less than optimal levodopa therapeutic response

such as wearing off or dyskinesias, on maintenance levodopa, was selected to evaluate drug efficacy, tolerability and saftey.

The D-1 partial agonist, CY 208-243, was thereafter employed alone, in advanced idiopathic Parkinson's disease patients, with "on-off" fluctuations, (Chapter 8, trial 5), to assess the overall efficacy of D-1 agonist drugs. Since the other D-1 selective agonist SKF 38393 in man is unimpressive the benefit of this new compound will be tested upon patients following levodopa drug withdrawal.

Multiple drug trials utilising selective D-1, D-2 and mixed D-1 plus D-2 agonist drugs were tested in patients with idiopathic Parkinson's disease. While the animal model may well suggest which dopamine agonist drugs are useful and in which combinations, the final arbitrator should be human idiopathic Parkinson's disease patients.
Bromocriptine, as an adjunct to levodopa, in attempt to reduce overall levodopa dosage, was felt would limit adverse effects. However bromocriptine cannot be taken by all patients, since side-effects and a weak antiparkinsonian activity was noted and is well described.

These drugs, all shown to have therapeutic efficacy have been reported and published (Appendix III to VIII) and this thesis finally sets out to integrate these reports and discuss them collectively.

CHAPTER 6

INITIAL HUMAN STUDIES

6.1 Trial 1: Bromocriptine as adjuvant to levodopa therapy (Appendix III)

BROMOCRIPTINE

6.1.1 Methods

Forty two patients with idiopathic Parkinson's disease of either sex (22 male and 20 female) aged 30-75 years (median 64,8) were studied as outpatients after giving informed consent. All were moderately severe Hoehn and Yahr stage 2 - 4, off medication and demonstrated stable levodopa responsiveness for at least 2 months prior to recruitment. Their disease duration was 13.4 (SEM 2.1) years and at the start of the study levodopa dosage (plus peripheral decarboxylase inhibitor) ranged from 300-1750 (mean 640) mg per day, orally. Patients continued taking anticholinergics and amantadine throughout the study period. None had previously been on dopamine agonists (Table 6.1).

Table 6.1

Patients and their personal attributes at bromocriptine trial entry.

	LEVODOPA plus BROMOCRIPTINE	PLACEBO
Number	22	18
Age (years)	64.3	65.3
Sex (M:F)	13:9	8:10
Duration of illness Mean (yrs)	13.4	13.3
SEM	1.4	2.1
Levodopa requirement (mg/day) Mean	669.3	622.2
SEM	66.6	69.9
Smoking habit Smoker	3	1
Ex-smoker	10	8
Never	9	9
Hoehn and Yahr Stage II	9	6
III	11	10
IV	2	2

SEM = Standard Error of the Mean

Phase I of the study was conducted double-blind keeping the levodopa dosage constant with the addition of bromocriptine (Parlodel) gradually increasing the dose from 2,5 to 20 mg per day over 5 weeks (n=23) or placebo matched (n=18) in identical looking capsules over the same period.

Those patients who, upon unblinding, had been randomised to receive placebo, then underwent an additional six week build up of bromocriptine so that at the completion of phase I all patients were receiving 20 mg per day, in four equally divided doses. Two patients who could not tolerate the bromocriptine progressive build up could not enter the next phase of the study.

Phase II of the study was open parallel in all patients (n=40), the objective being to reduce levodopa requirements at weekly intervals until the bromocriptine an levodopa combination failed to control symptoms and reduce objective parkinsonian scores. Thereafter the dose of the dopamine agonist could be increased until the patients' symptoms and signs of parkinsonism were once again controlled. Once stabilised, further reduction of levodopa and when necessary increases in bromocriptine was initiated over the six week period. The overall objective of this phase was to reach the greatest tolerated bromocriptine dose (up to 50 mg/day) with the lowest matched levodopa dosage that kept the patient stable and mobile without unacceptable side

effects. All patients were seen at weekly intervals, in outpatients, having previously been instructed on how to take their medication at home throughout the week.

At the beginning and end of the study a complete history and physical examination was undertaken by a physician, chest X-rays, urine and blood tests were performed. Each week the same researcher assessed the patients and rated them according to the Hoehn and Yahr stage, Webster, Columbia and the North Western University disability scales in the mornings. In addition the patients were asked to keep diaries and encouraged to report how the week progressed regarding side-effects, involuntary movements and overall activity. Vital signs, adverse reactions, compliance checks and other concomitant medication was noted throughout the period.

Full psychometric evaluations took place initially and each fortnight in phase II of the study. Psychological tests used included the Beck Depression Inventory, vocabulary and similarity sub-tests of the Weschler Adult Intelligence Scale, Bentons Controlled Word Association Test, Rey's Auditory Verbal Learning Test, Rey's Complex Figure test and the Mini Mental State Examination. An expanded version of the Mini Mental State Examination (maximum score of 80) was used at the beginning and completion of each phase of the trial.

Statistical computations were performed on the Statistical Analyser System (SAS, Version 5) on an IBM mainframe computer and regarded significant at the 5% level. Associations upon trial entry between levodopa plus bromocriptine versus levodopa plus placebo groups were assessed by ordinary chi squared analysis. Intragroup comparisons of patient status at beginning versus end of the double-blind phase employed the McNemar chi squared test for non-independence samples with the Yates correction for continuity. The Fischer Exact Test was employed for sparse 2x2 tables and for larger contingency tables the Mantel-H test of significance was used. Means comparisons by the paired students t-test for parametric data and time trends for vital signs, dosage requirements and mean disability scores were analysed by covariance and multiple regression.

6.1.2. Results

Double-blind bromocriptine versus placebo match

The two randomised groups were comparable in personal attributes and parkinsonian disability status as judged by the four rating scales at the onset of the study. Similarly levodopa related complications and clinical status were analysed in the groups and were comparable (Table 6.1)

At the completion of Phase 1 of the study, one group (n=22) was on their usual levodopa plus bromocriptine 20mg per day and the other (n=18) their usual levodopa dosage plus placebo. Two patients in the levodopa plus bromocriptine group were withdrawn due to unacceptable postural hypotension and mental confusion which resolved once the dopamine agonist was withdrawn.

Analysis of the two groups, the levodopa plus 20mg bromocriptine versus these on levodopa placebo, clearly showed the benefit of the dopamine agonist. The Hoehn and Yahr scale did not change dramatically, as judged off medication, but the major features (bradykinesia, tremor, rigidity and postural stability) individually and also when the Columbia or Webster scores were accumulated, showed a statistically valid difference in the scores. Hence bromocriptine compared with placebo was effective as an anti-parkinsonian agent when added to stable levodopa in patients with moderately severe IPD.

More dyskinesias were also noted, as one would expect, but "on-off" motor fluctuations and freezing remained statistically constant. However what was surprising was the observation that while dyskinesias overall were more common in intensity, severity and in multiple body area these did NOT correlate with progressive dose increase. For example dyskinesias, while worse on bromocriptine at

week 2 (dose of 7.5 mg/day) got better overall to week 3 (dose of 10 mg/day) and were worst at weeks 4 and 5 (dose 15-20 mg/day).

Dystonias were stable in the placebo group throughout follow-up, but were reduced in intensity and frequency in the bromocriptine group (Table 6.2).

Open phase build-up bromocriptine with levodopa reduction

The objective of steadily building up the bromocriptine dose (20-50 mg/day) while reducing levodopa by 25% to 50% over six weeks, was accomplished in the majority of patients while maintaining a stable parkinsonian status (Fig 6.1, and Table 6.3).

While clinically stable regarding parkinsonian status the optimal levodopa plus bromocriptine combination occurred at approximately 30% levodopa reduction, to a mean of 640 mg per day levodopa and 26 mg per day bromocriptine (Table 6.3). Neuropsychiatric complications (n=3), symptomatic postural hypotension (n=7), and worsening dyskinesias (n=4) were the most commonly encountered side effects on the drug combination all of which disappeared as the overall dose of levodopa was reduced (Table 6.4)

Table 6.2

Complications of therapy in patients taking levodopa plus bromocriptine (A) at baseline (week 1) and at the end of the double-blind period.

Week		1		6	
Number		A n=22	B n=18	A n=20	B n=18
Dyskinesia	Absent	55	66	61	73
	Present	45	34	39	27
	Mild	68	73	83	86
	Severe	32	27	17	14
Dystonia	Absent	72	70	70	70
	Present	28	30	30	30
Freezing	Absent	55	63	60	74
	Present	45	37	40	26
On-off fluctuations	Absent	90	93	94	93
	Present	10	7	6	7

Table 6.3

Levodopa dosage (plus PDI) versus bromocriptine in the open phase of the trial.

Week	Mean Levodopa	(Range) (mg/day)	Mean Bromocriptine	(Range) (mg/day)
1	640	(250-1750)	5	
2	640	"	7.5	
3	640	"	10	
4	640	"	15	
5	640	"	20	
6	640	"	22	(20-25)
7	526	(0-1625)	26	(20-35)
8	528	(200-1500)	28	(20-40)
9	490	(0-12500)	30	(20-40)
10	466	(0-1250)	33	(15-45)
11	423	(0-1250)	35	(15-50)
12	383	(0-875)	37	(15-50)

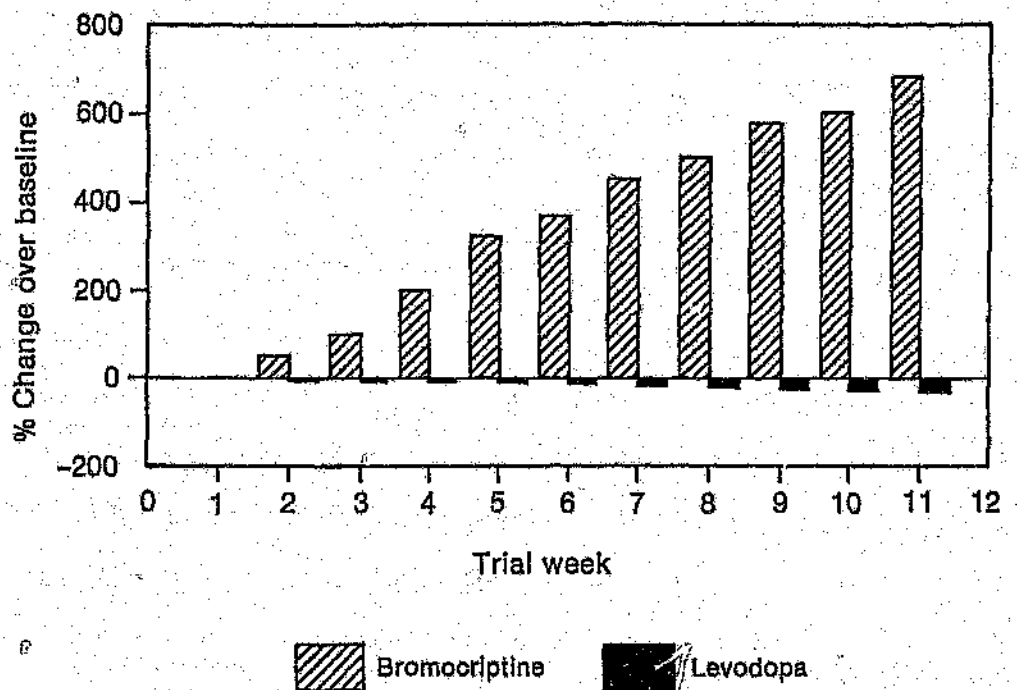

Fig 6.1

Mean percentage build up of bromocriptine from baseline with reduction in levodopa from week six.

No patient had to be withdrawn from the study during phase II due to persistent side effects. Side effects attributable to bromocriptine dosage increase (dizziness, postural hypotension and mental confusion) disappeared with time, but in some instances limited the maximal dosage achieved. Adverse effects were encountered in 25 patients (11 male, 14 female) at a mean of 802,3 mg per day (10 mg/kg/day) levodopa plus PDI in combination with 18,4 mg per day mean bromocriptine. Twenty two patients of the same group that ultimately developed side effects, when reanalysed at maximal levodopa reduction and bromocriptine increased to give stable parkinsonian rating scores, were free from side effects ($p<0,0001$). These patients who could tolerate dose limiting side effects (n=17) were increased to >40mg per day bromocriptine with reduction in levodopa plus PDI to 432 mg per day. This overall occurred with a 55% levodopa dose reduction. No significant additional benefit, however, above that obtained at a bromocriptine dose of 26mg per day was observed. Therefore for all patients in this study, whether they experienced side-effects or not, the optimal dose of bromocriptine was on average 26 mg po. per day.

Numerous psychological tests were employed on the same day as the rating scales were used to assess motor status. No significant mental status differences could be detected at

Table 6.4

Adverse effects in patients taking bromocriptine and levodopa in the open phase of the trial.

TYPE OF EVENT	SEVERITY	NUMBER OF PATIENTS	NUMBER OF EVENTS
HYPOTENSION	Mild	6	6
	Moderate	5	6
	Severe	2	3
DIZZINESS	Mild	2	2
	Moderate	5	5
	Severe	2	3
HALLUCINATIONS	Mild	2	4
	Severe	1	2
CONFUSION	Mild	1	4
	Severe	2	10
OTHER (Nausea, vomiting, syncope, concentration difficulty, dry mouth, impaired balance and increased libido.		4	9

the end of the trial compared to the baseline assessment. Mental status paralleled stable motor status throughout the study (Table 6.5). Good preservation of vocabulary, verbal fluency and memory was present but disturbances in the constructional function as measured by the Rey Complex Figure Test consistently occurred.

6.1.3. Discussion of bromocriptine

This study shows, as have others, that the combination of bromocriptine with levodopa allows maintenance of clinical rating scales reflecting parkinsonism. The optimal bromocriptine dose was 25 mg po. daily, allowing ±30% reduction in overall levodopa requirements. Although bromocriptine may be tolerated to as much as 150 mg po. daily, this study suggests little additional benefit beyond 25 mg daily, in this group of moderately severe IPD.

As expected adverse bromocriptine side-effects were dose related but were often transient and the patients developed tolerance to side-effects and usually they disappeared with reduction of dose. Bromocriptine, however, clearly reduces those side-effects thought to be levodopa related. While dyskinesias are more common compared to placebo, a dose dependant increase in severity, frequency and body area

Table 6.5

Mini-mental status scored at weeks 1 and then 12 in all patients to compare outcome with the end of the trial (n = 40).

	Week 1	Week 12
Mean score	64.4	66.1
(SEM)	(2.4)	(1.4)

Mean score out of maximum of 80

affected could not be demonstrated. Indeed patients while often developing dyskinesias initially with bromocriptine buildup developed tolerance to them and were better tolerated with time. Dystonias, including early morning dystonia, were improved on bromocriptine compared with placebo when added to stable maintenance levodopa. Even when administered in high doses, bromocriptine did not result in biochemical, haematologic, urinary or EEG abnormalities necessitating stopping the drug.

Finally neuropsychiatric stability throughout the trial on bromocriptine was noted. Those abnormalities detected in all phases of the study were primarily dissolution of construction with preserved memory and verbal function skills, which reflected limitations induced by Parkinson's disease. Since individual differences in mental status occurred, it appears advisable to include Mini-Mental State Score in the present rating scales.

A recommended dose of bromocriptine (25 mg po. per day) with a 30% levodopa reduction in moderately severe IPD patients appears to maintain mobility with reduced risk of levodopa side-effects.

This study was published in the South African Medical Journal (see Appendix III).

6.2 Trial 2: Sinemet CR4 (long acting levodopa) replacement of levodopa therapy (Appendix IV)

SINEMET CR4

6.2.1. Methods

Twenty patients with IPD were treated in an open label study to assess the benefit of Sinemet CR4 (Merck, Sharp and Dohme; Montreal, Canada) in moderately advanced disease (Table 6.6). Thirteen male and 9 female patients, aged 46-76 years (median 64), Hoehn and Yahr stage 2-4 off medication, with a duration of Parkinson's disease 1.5-40 years (median 14.3), all on levodopa plus carbidopa (Sinemet) 350-1375 (mean 706) mg/day total dose, were entered into the study once informed consent was obtained. None were on dopamine agonist drugs. While all were receiving optimal levodopa when entered, many had developed variable motor fluctuations (wearing-off in 20, dyskinesias in 13 and "on-off" fluctuations in 5); that required additional therapy. Each Sinemet CR4 tablet contains 200 mg levodopa and 50 mg carbidopa, suspended in a polymeric matrix, designed to release levodopa over 3-6 hours in an attempt to produce stable plasma levels following oral ingestion. Since the study was open labelled the dose of CR4 was adjusted throughout to obtain maximum benefit while stabilising the CR4 compound for the usual Sinemet.

Table 6.6

Characteristics of patients entered into the Sinemet CR4 trial and baseline.

Patient	Sex	Age (yrs)	Disease durat (yrs)	Levodopa dosage (mg/day)	Other drugs (mg/day)
IM	F	69	2	600	Benzhexol (6) Amantadine (200)
RS	F	70	15	1000	Amantadine (200)
AR	F	74	2	400	Dissipal (150)
AS	M	46	11	550	Amantadine (200) Bromocriptine (8)
CK	M	76	2	1000	Amantadine (200) Artane (3)
SK	M	54	20	350	Amantadine (200) Bromocriptine (22) Biperidin (6)
JP	M	68	40	350	Bromocriptine (30) Artane (10)
KJ	M	69	2.5	600	Amantadine (200) Dissipal (150)
LG	M	64	15	700	Amantadine (200) Bromocriptine (10)
OH	M	69	20	500	Artane (4)
CD	F	67	10	400	Bromocriptine (10) Amantadine (200) Artane (6)

Table 6.6 (continued)

Patient initials	Sex	Age (yrs)	Disease duration (yrs)	Levodopa dosage (mg/day)	Other drugs (mg/day)
AM	M	55	24	500	Amantadine (200) Bromocriptine (20) Artane (6)
DO	F	35	1.8	750	Bromocriptine (22) Amantadine (200)
NH	M	65	10	1375	Bromocriptine (10)
RH	M	72	18	750	Dissipal (150)
LvR	M	58	14	1200	Bromocriptine (20)
HM	M	72	10	1250	Amantadine (200) Artane (12)
BW	M	66	11	1125	Amantadine (200) Dissipal (50)
OA	F	54	25	400	Bromocriptine (10) Artane (6)
TC	F	57	10	600	Artane (6) Amantadine (200)
GG	M	72	20	550	Bromocriptine (15) Artane (6)
DN	F	57	12	600	Amantadine (200) Artane (6)

This study had two phases. A four week baseline period, wherein the patient's usual levodopa and other medications, amantadine and or anticholinergics were continued. During this period patients had routine general and neurological examinations, ECG's, urine analysis, biochemistry and full blood count and were finally rated utilising the Hoehn and Yahr and North Western University scoring scales. Diaries with a modified PDS score while "on" and mobile during the day were completed by the patients.

Thereafter the patients entered a 52 week treatment phase where they all had their conventional Sinemet changed for the equivalent dose of the slow release CR4 compound. The dose of CR4 was then altered at weeks 1,2,4,8,12,24,36 and 52 to allow maintenance of, or improvement, upon their baseline scores. All other medications were continued unaltered, the CR4 was administered three times daily with meals and patients were asked to complete diaries on at least one day of the week throughout the trial.

6.2.2 Results

Over 12 months only 11 patients of the original 20 were able to complete the study and remain exclusively upon the CR4 treatment. Hence 45% of the group required return to conventional levodopa therapy. Two patients died of unrelated causes, one from an acute myocardial infarction (at

week 22) and one from pneumonia and septicaemia (at week 48). Three had a poor therapeutic response and were converted to their usual Sinemet at 12 weeks. Four more, while experiencing some response, could not sustain benefit and required an additional quicker acting levodopa between 24 and 50 weeks.

Statistically while these 18 patients could be compared at 12 weeks to baseline parameters, only 16 could be examined to 24 weeks, 14 to 36 weeks and 11 to 52 weeks.

No significant changes in the Parkinson's rating scales or as judged by their diaries over the 52 weeks period was noted in spite of the fact that increased attention and trial placebo effect made most feel best at 12-24 weeks on CR4. In the 11 patients who completed the trial, while keeping patients stable by increasing overall CR4 dosage and decreasing interval of drug administration, significantly fewer doses were required (Table 6.7). This resulted in a mean reduction of 4.3 to 3 levodopa doses each day, at 3 months, but was unfortunately not sustained ($p<0.05$). Moreover overall levodopa dosage beginning at 495 mg/day increased to 705 mg/day CR4 after the year, indicating a 14% increase in levodopa requirements. One observation of concern is that from week 12 to 54 the duration of CR4 long-acting levodopa efficacy declined from 7.89 hours to 5.74 hours.

Table 6.7

Details of 11 patients who completed the full year conversion from Sinemet to Sinemet CR4.

	Base-line	Week			
		12	24	36	52
Hoehn and Yahr Stage 1	0	0	0	0	0
2	1	1	1	1	1
3	7	10	10	9	8
4	3	0	0	1	2
North Western University Score	10.27	7.63	7.72	8.54	9.18
Mean L-dopa dose (mg/day)	495	691	700	700	705
Mean number of doses (d^{-1})	4.28	3.04	3.91	4.09	4.18
Mean interval between doses (hrs)	5.61	7.89	6.14	5.86	5.74

Table 6.8

Dosage of Sinemet and/or Sinemet CR4 Baseline for 52 weeks on all patients.

Patient Initial	Baseline Sinemet	WEEK 12	24	36	52
		SINEMET CR4 (Levodopa only)			
IM	600	600	600	600	600
RS	1000	1300	1300	1300	1300
AR	400	300	300	600	600
AS	550	700	700	700	700
CK	1000	1200	1200	1200	1200
SK	350	600	600	500	650
JP	350	500	500	500	500
KJ	600	500	600	600	600
LG	700	1000	1000	900	900
OH	500	500	600	600	600
CD	400	500	500	500	500
Mean	586.4	727.3	736.4	736.4	740.9
AM	500	800	900	900	- -
DO	750	900	- -	- -	- -
NH	1375	- -	- -	- -	- -
RH	750	1000	- -	- -	- -
LvR	1200	1800	1800	1200	- -
HM	1250	- -	- -	- -	- -
BW	925	- -	- -	- -	- -
OA	450	600	600	- -	- -
TC	600	- -	- -	- -	- -
GG	550	800	800	1000	- -
DN	600	800	800	- -	- -
Means	813.6	957.1	980	1033.3	Nil

When questioned, on completion of the study, of the 11 patients who completed 52 weeks on CR4, only 4 preferred the slow release formulation to conventional Sinemet.

6.2.3. Discussion of Sinemet CR4

Advantages documented were the reduced number of dykinesias, wearing-off phenomena, "on-off" fluctuations and peak dose dystonias reported by some patients who experienced levodopa related problems. Unfortunately this was often at the expense of mobility. Most notable was the benefit recorded nocturnally - all patients reported increased and maintained nocturnal mobility in bed and relative ease when getting up at night.

Some adverse effects were reported by patients on CR4 alone and when taking their usual levodopa plus carbidopa regimens. Dose related side-effects attributable to CR4 were neuropsychiatric (hallucinations and confusion), peak dose dyskinesia and dystonia, mainly early morning in type, constipation and, in one case, hypersomnolence. The major problem noted by patients was the slower action of onset. Upon taking their early morning dose, patients took longer to become mobile, which was interpreted by the patient as the CR4 tablets being "less potent". Moreover single dose failures were not uncommon (60% patients), especially those patients with duration of illness longer than 8 years.

6.3 Limitations of current approaches in therapy

Levodopa, the most successful and long tested compound in man with IPD, has at least two marketed long acting preparations. Madopar HBS (levodopa plus benzerazide) and Sinemet CR4 (levodopa plus carbidopa) are available, have theoretical attractions in that they stimulate dopamine receptors in a slower more sustained fashion, but are insufficient therapy in many patients. In this thesis the long acting Sinemet CR4 showed that not all patients tolerate the switch from conventional release Sinemet, but more importantly outline that it has a slower (but expected) onset of action and requires more daily levodopa in total.

A selected group of patients, particularly those with nocturnal akinesia undoubtedly benefit. Since the sustained release preparations physiologically reduce serum levodopa peaks. Many clinicians today choose the long acting preparation from the start. Still to be evaluated is the possibility of reduced motor fluctuations and dyskinesias. A problem still present is the fact that more not less overall levodopa is required for mobility. Therefore it was clear that while levodopa remains the most useful antiparkinsonian agent it is inevitable to run into

levodopa related side-effects some of which may be delayed by adjuvant bromocriptine utilisation. The slow release formulation of levodopa such as Sinemet CR4 likewise is theoretically attractive but is not suitable in all patients.

With these problems of pharmacotherapy in the background a search for novel dopamine receptor agonists was initiated, either D-1, D-2 selective or mixed D-1 and D-2. The stimulation of the mesostriatal but not mesolimbic dopamine receptors, appeared to have the best chance of being a successful antiparkinsonian drug. Novel dopamine agonists were available, namely CY 208-243, (a D-1 selective partial agonist) and CQA 206-291 (D-2 selective). They were tested first in the MPTP-treated primate model, then in man with complicated advanced IPD. Research in this thesis then addressed two main issues in the dopamine receptor agonists that were found effective and safe.

Firstly, the drug efficacy compared with levodopa and bromocriptine was examined. Secondly, various combinations of D-1 selective and D-2 selective agents was examined to detect any possible functional interaction between the various dopamine receptor subpopulations. Always in mind was the potential benefit of being able to reduce overall levodopa maintenance requirements and where possible even to find an agent potent enough to be used as monotherapy to

replace levodopa. Bromocriptine is safe, is best used as adjuvant to levodopa rather than de novo, and at a mean dose of 25 mg/day, in divided doses. However many patients lose the initial synergistic benefit derived and require re-increasing the levodopa or changing to an alternate dopamine agonist.

While other D-2 agonists are available (lisuride, PHNO, apomorphine and legotrile) some are D-1 and D-2 selective (pergolide and mesulgerine). None has been as widely tested as bromocriptine and many are being increasingly employed (lisuride, pergolide and apomorphine) in clinical practice. Most of these choices of dopamine agonist stimulate multiple dopamine receptors, making analysis of their individual benefit difficult to interpret. Many new compounds developed in animals were never released for human trials due to toxicity and safety restraints (meselgurine, leagotrile and PHNO).

Hence these selective and novel agents were chosen to test initial safety, then drug efficacy and optimal dosage. At a later stage adjuvant use with levodopa of CQA 206-291 and in the long term the hopeful reduction in levodopa related motor fluctuations was sought. Chapter 7 describes the primate work and Chapter 8 the subsequent human trials.

CHAPTER 7

NON-HUMAN PRIMATE EXPERIMENTS

7.1 Preparation of the Model

Adult common marmosets (Callithrux Jacchus) of either sex, aged 3-8 years, weight range 260-365 grams, received MPTP (2 mg/kg sc., daily for 5-7 days) to a cumulative dose of 10+/-0.8 mg/kg sc., until they were persistently parkinsonian (Fig 7.1). MPTP hydrochloride (Research Biochemical Inc.) was dissolved in sterile 0.9% saline and filtered through a 0.22um bacterial filter the day of treatment. Following the second and subsequent injections, the animals became tremulous, rigid, jerky, sometimes developing dystonic postures and became unaware of the outside environment. This effect usually lasted 60-90 minutes, thereafter the animals became more alert and responsive to stimuli. Repeated injections produced persistent motor deficits characterised by marked bradykinesia, flexed trunk and limbs, rigidity, postural imbalance and perch instability, reduced spontaneous vocalisations and

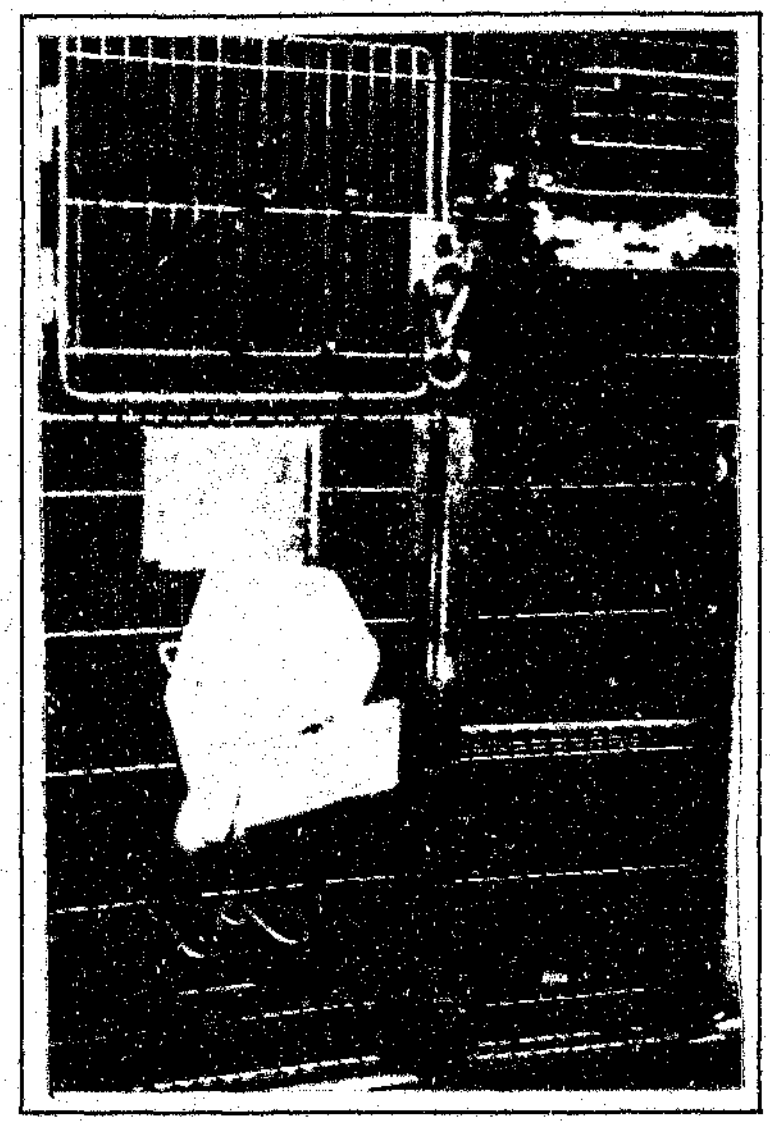

Fig 7.1

Photograph of the usual cage in which the primates were kept individually or in pairs. When housed together both animals received MPTP-treatment simultaneously.

sometimes a postural tremor. Resting tremor was rarely observed and then only in some of the animals. Hydration and nutrition was supported by syringed oral fluid feeds plus Mazure "marmoset jelly" food cubes and fruit were allowed ad libitum. After three weeks the primates were once again able to feed and groom themselves as they recovered from the acute MPTP effects. Between six weeks and six months post-treatment the animals all remained parkinsonian and suitable for the study of the effects of the administered drugs. Twenty four animals were treated with MPTP in 3 groups of 8 each. The best 16 animals were selected and used in the experiments as those consistently parkinsonian and following treatment, responded predictably to levodopa plus decarboxylase inhibitor.

7.2 Assessment of the Model

Individual animals were placed into a cage identical to that in which the paired animals were normally housed (Fig 7.2). The cage, in a separate noise and disturbance free environment, was fitted with 8 infrared photocells. Four were focused horizontally across the floor of the cage, 1 along each of the two perches and 1 from front to back of the cage above each perch (Fig 7.3). This photocell arrangement allows animal movement to be

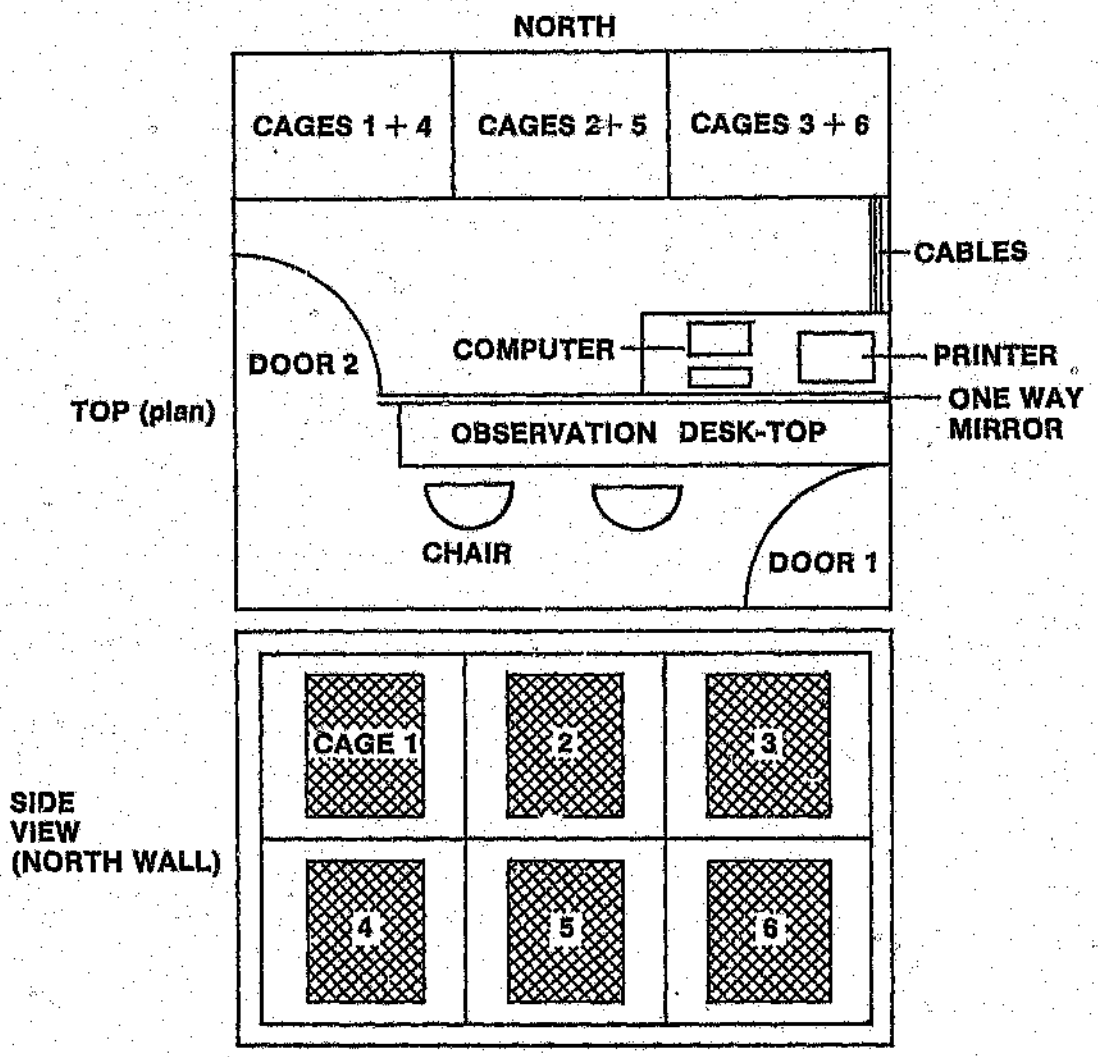

Fig 7.2

Plan of the room housing the monitoring cages in the specifically designed and isolated area for the experiments.

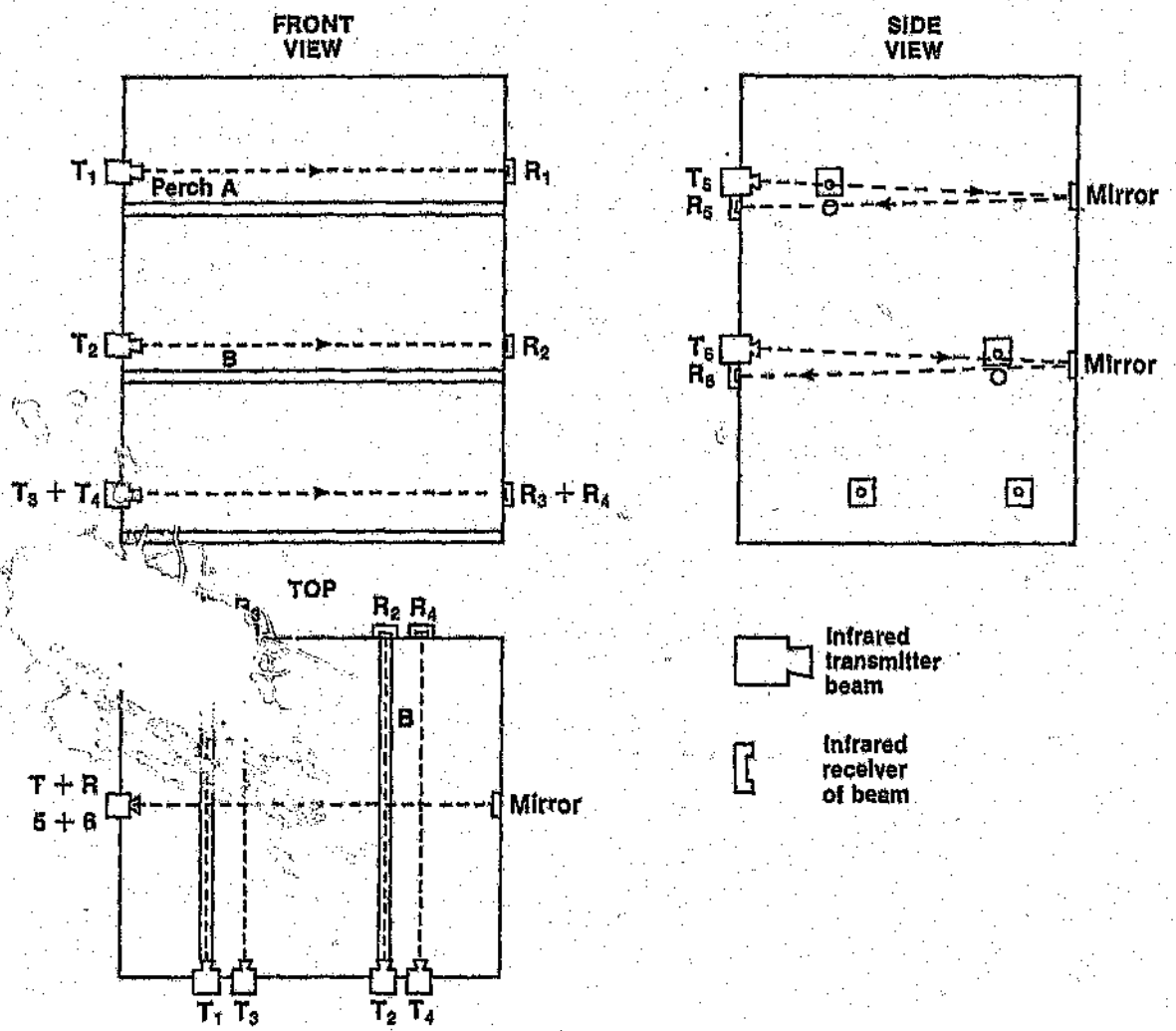

Fig 7.3

Drawing of a single cage to show the position of the perches within the cage and the infra-red photocell transmitters and receivers in each individual monitoring cage.

monitored in all directions. Motility counts were accumulated over 10 minute segments, after 30 minutes acclimatisation, for at least 180 minutes following drug administration.

A clinical appreciation and score of motor deficits was obtained by observer rating of videotapes and recorded throughout the experiment and subsequently analysed. This was done so as to leave the animal undisturbed and the scoring was done both from behind a one-way mirror and upon review of the video data. The following components of behaviour were scored:

1. EYES

Attention focused toward the camera, amount of blinking, oculomotor movements and alertness

Normal	0
Abnormal	1

2. POSTURE

Trunk, limbs and tail, undisturbed.

Normal	0
Abnormal trunk	+1
tail	+1
limbs	+1
flexed on the floor of the cage	4

3. BRADYKINESIA

Overall animal motility, undisturbed

Normal	0
Mild slowing	1
Moderate bradykinesia	2
Akinetic	3

4. VOCALISATION

Normal	0
Reduced	1

5. BALANCE

When placed on a perch

Normal	0
Unstable	1
Spontaneous falls	2

6. TREMOR

Absent	0
Present	1

7. FUR CONDITION

Indicative of the animals' ability to groom and feed themselves

Normal	0
Dirty	1

Assessment of these items would score a normal marmoset 0 and a maximally disabled one 13. When multiple scoring took place following drug administration the lowest score was taken.

7.3 Drugs and solutions

MPTP

N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (Research Biochemicals Inc.), was dissolved in sterile 0,9% normal saline and administered subcutaneously on consecutive days (2 mg/kg sc.) until the animal was parkinsonian.

L-Dopa

L-Dopa for injection (4.0 ml L-Dopa ampoules 5 mg/ml; pH 2,5; Hoffman La Roche, Basle) was mixed with 0,5M sodium phosphate buffer (0,864 ml; pH 7,4) and aqueous solution of 10% Tween 80 (0,364 ml; pH 6,5). The final pH of L-dopa was 6.0 and the final solution filtered through a 0,2 um bacterial filter to maintain sterility. Oral pretreatment with the decarboxylase inhibitor, carbidopa (12,5 mg/kg po. alphamethyldopa hydrazine; Merck, Sharp and Dohme) suspended in 1% methylcellulose, 30 minutes before the L-dopa (dosage range 3,125 to 25 mg/kg ip.) was administered.

(+)-PHNO

(+)-propyl-9-hydroxynapthoxaline; (Merck, Sharp and Dohme) was dissolved in sterile 0,9% saline and then adjusted to a pH 7,0 before injecting the animals with this D-2 selective dopamine agonist. Subcutaneous doses (2-4 ug/kg) were employed.

CQA 206-291

[5R 5-beta 8-alpha 10-alpha] N-N-Diethyl-N-(1-ethyl-6-methyl-ergoline-8-yl) sulphamide hydrochloride; (Sandoz Inc., Basle) was dissolved in 0,9% sterile saline and administered (0.1 - 1 mg/kg sc.).

SKF 38393

2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride; (Smith, Kline and French) was dissolved in sterile 0,9% saline in a darkened container that was warmed and shaken (pH 6,5). SKF 38393 (10-20 mg/kg) was administered intraperitoneally.

CY 208-243

(-)-(6aR) (12bR)-4,6,6a,7,8,12b) hexahydro-7-methlindolo (4,3-ab)-phenanthradine; (Sandoz Inc., Basle) was mixed with an equal weight of tartaric acid and dissolved in sterile 0,9% saline (pH 4.0) and subsequently adjusted with 0,1 M NaOH to pH 5,5.

SCH 23390

1-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3 benzazepine-7-ol hememaleate; (Schering) was dissolved in sterile 0,9% saline, shaken and warmed to allow dissolving of the powder base (final pH 6,5). Animals received SCH 23390 (2 mg/kg) sc., which was administered one hour prior to CY 208-243 (1 mg/kg sc.).

Sulpiride

Sulpiride (Delagrange, France) was dissolved in a minimum quantity of 2% sulphuric acid and diluted to volume with sterile 0,9% saline. The final solution was adjusted using 0,1M HCl (pH 7,0). Sulpiride (10-20 mg/kg ip.) was administered one hour prior to CY 208-243 (1 mg/kg sc.). Appendix IX, shows the chemical structures of the compounds employed in this thesis.

7.4 Experimental controls

Where possible the animals remained undisturbed throughout the period in the photocell motility cages. The day before all drug experiments the same animals received vehicle control injections. Drug vehicle was administered at the same pH, route, volume and time intervals when multiple injections were administered. The animals were not fed during the monitoring period, but were allowed free access to water bottles filled prior to their introduction into

the monitoring cages. An acclimatisation of 30 minutes was usually allowed to settle the animals. Following injections the monitoring of motility began within 10 minutes and video recordings were made continually.

7.5 Fate of the animals and their histopathology

The mortality within a month of MPTP-treatment was 10% of a group of 24 animals. Three animals were killed due to significant morbidity considered to prejudice the animal, between 3 and 6 months and all the brains subjected either to biochemical or perfused with cold 4% paraformaldehyde, for histological and immunocytochemical analysis. The remaining animals, when they recovered, were given a second MPTP-treatment with a view to ultimately transplanting the animals with fetal mesencephalic dopaminergic tissue in a separate experiment. Upon the second treatment the animals became parkinsonian very much more quickly requiring only 6 mg/kg MPTP. After the experiment 2 animals which developed a "wasting syndrome" were anaesthetised, perfused, and their brains fixed in 4% paraformaldehyde, for histologic verification of the MPTP primate model.

Upon rapid removal of the marmoset brain, the brain was fixed in 4% paraformaldehyde at room temperature for 7 to

10 days, 1 to 2 days in sucrose solutions to make firm, then cut into coronal blocks for cryostat sectioning of 40um thick sections.

Fig 7.4 shows the photomicroscopic pictures of the SNc of a normal control animal(No: 129) (Fig 7.4 a), an MPTP-treated marmoset (No:107) with severe dopamine cell depletion (Fig 7.4 b) of the SNc, and finally (Fig 7.4 c) following re-treatment with MPTP on partial recovery (animal No:157). All these samples were stained following the dopamine agonist experiments, utilising anti-tyrosine hydroxylase polyclonal antibodies (Eugene Tech, USA) employing the Sternberger indirect imunocytochemical technique (Sternberger, 1979).

7.6 General methods

All animals given MPTP were allowed 3-4 months to recover the acute syndrome and once stable were monitored regarding spontaneous movement. This was compared to the pre-MPTP treatment status. All animals became profoundly bradykinetic but with assistance, maintained body weight and hydration. Regular animal weights and veterinary checks were demanded by the UK code of animal ethics and when, in con

Fig 7.4 (a)

Photomicrograph of the substantia nigra of a control marmoset (No. 129) stained with the polyclonal antibody tyrosine hydroxylase (Eugene Tech International Inc, Allendale, New Jersey) and the indirect peroxidase-anti-peroxidase immunocytochemical technique.

Magnification x 30

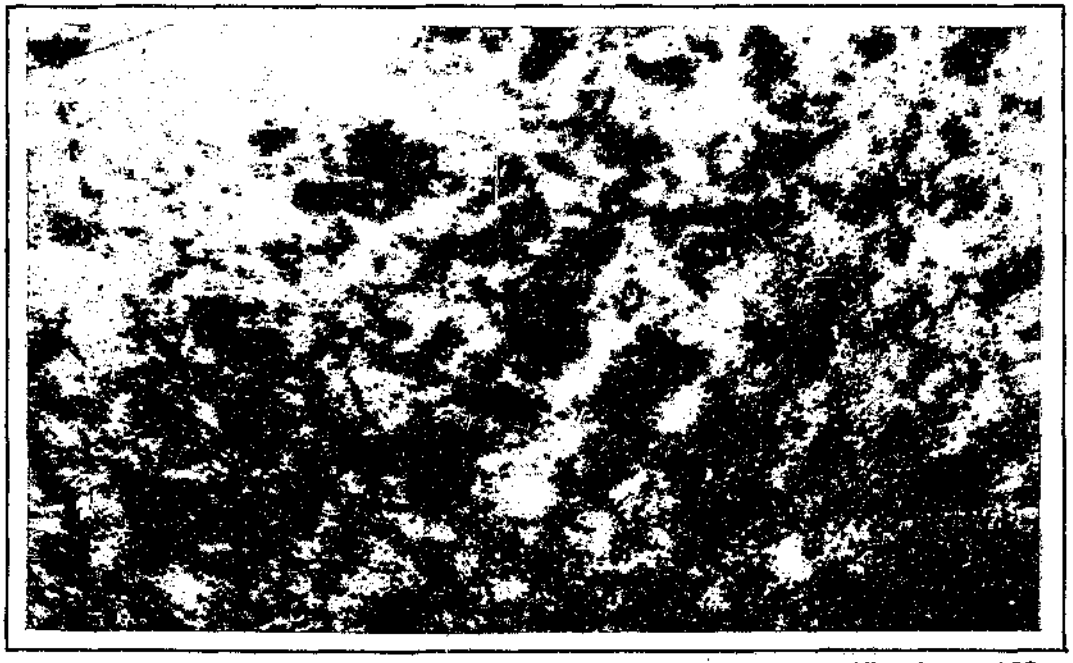

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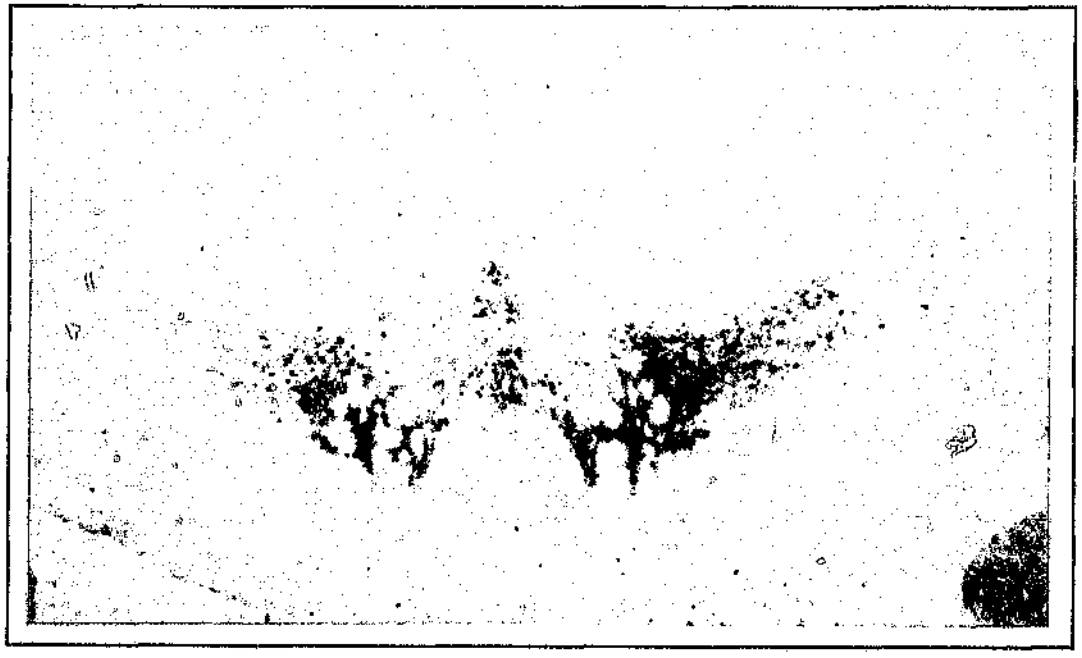
Fig 7.4 (b)

Photomicrograph of an MPTP-treated marmoset (No. 107) showing substantia nigra dopamine cell depletion particularly in the SNC anatomical region. SNr and VTA relatively spaced.



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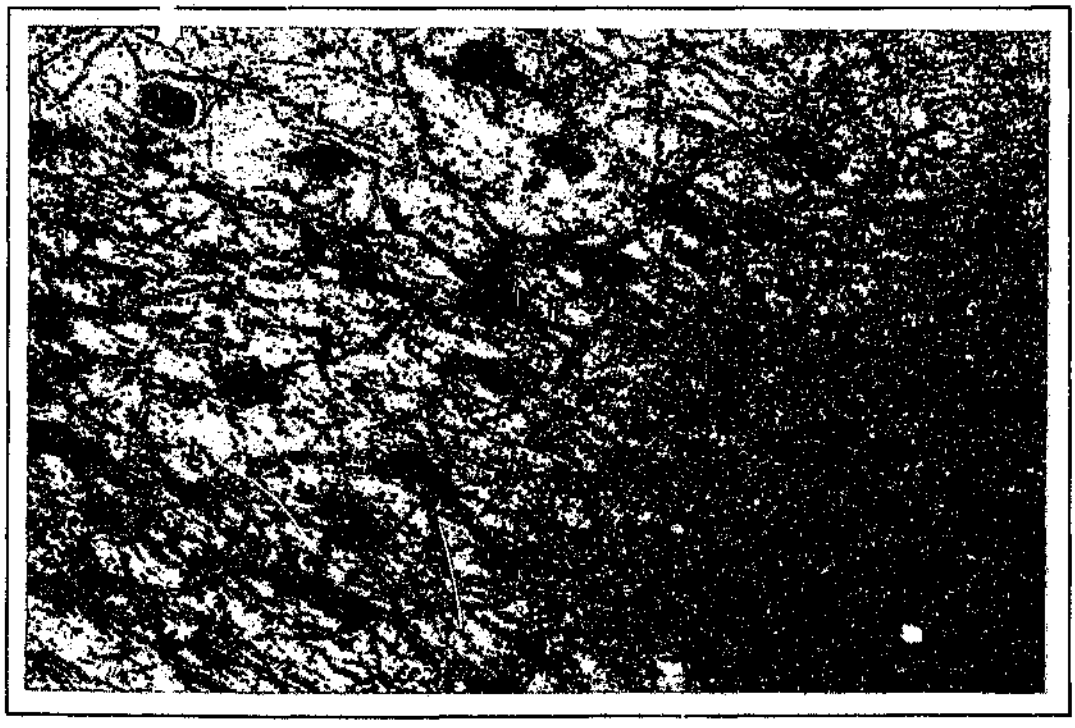
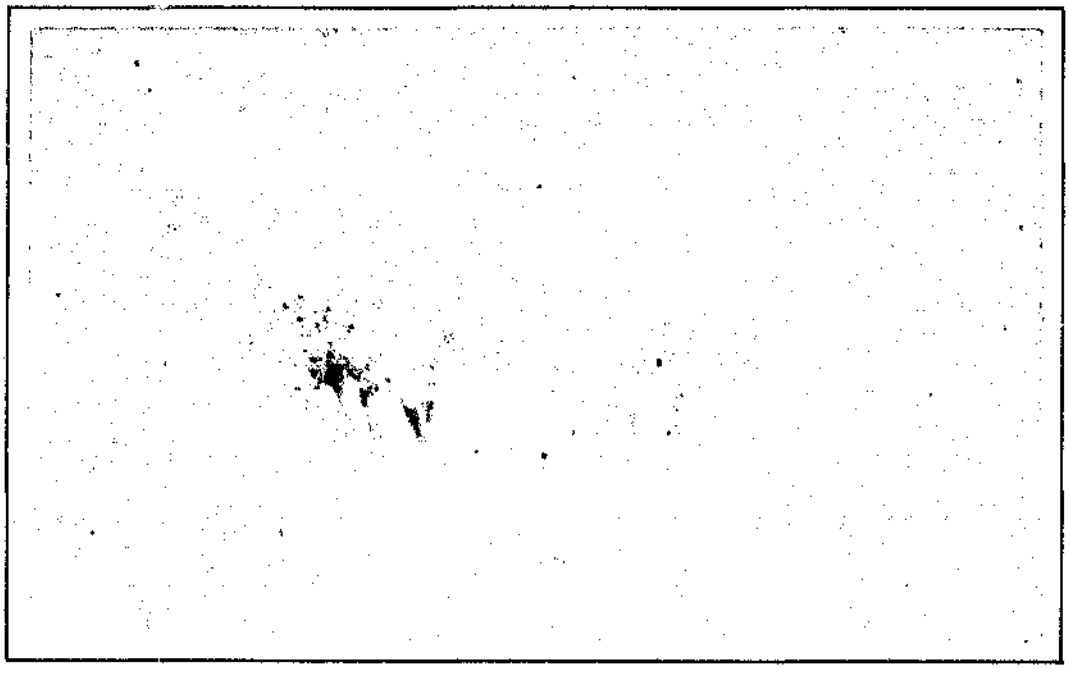


Fig 7.4 (c)

Photomicrograph of the repeat lesioned MPTP-treated marmoset (No. 157) following the second MPTP treatment (2mg/kg ip over 5 days). Notice the severe cell depletion in all areas of the substantia nigra including the SNc, SNl, SNr and VTA.



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sultion with the veterinary surgeon, it was considered merciful, the affected animal was euthanased, usually by perfusion of cold heparinised saline, then 4% paraformaldehyde following lethal barbiturate anaesthesia.

Experimental animals were used throughout a period of 9 months (in 2 divided groups of 12 each). All animals were housed together but studied in vehicle control situations and following drug administration in special monitoring cages identical in size to their usual cage, but separated in a monitoring laboratory. Special care was taken with transport of the marmosets so as not to unduly upset them although handling primates inevitably disturbs them. Once placed in the monitoring cage the animal was allowed to settle down, the computers set to run with the video tapes and the animal was injected with the specific test compound. After a further 10-30 minutes the experiment began with data recordings divided into segments (usually 5 or 10 minutes) within each hour of each run, which lasted 4-6 hours in total. All tests took place in fluorescent light, daylight conditions, with the animals allowed free access to water throughout. They were taken back to the housing area after experiments and fed once again with "fruit rewards". In this way the animals rapidly became accustomed to individual researchers - the difference clearly manifest when another researcher attempted to handle the animals. The animals had 24 hour a day super-

vision when necessary, with a normal 12 hour day and 12 hour night cycle. When sick, the animal received continual personal attention. No animal was ever allowed to die of neglect. Ethically and practically animals were killed at the end of the experiment, but many were in such condition that they were stable and useful MPTP-treated primates for the embryonic transplantation programme that paralleled this work. Only one animal died "unexpectedly" one night and at necropsy was found to have a malignant tumour of the bowel with peritoneal spread. Only post brain removal in 4% paraformaldehyde fixation was possible and therefore immunocytochemistry avoided. The brain, however, was used for routine histology.

All animals were allowed to rest between experiments. The interval varied between 5-10 days. All animals in a specific experiment had their control run completed (with vehicle compound) the day or two preceding the experiment. No statistical difference was present between animals whether or not tested with vehicle control proceeding an experiment, even when rarely studied twice in one week. Clearly the animals were equally disturbed when given vehicle once, twice or even following an active drug. All animal alarm settled within 5 minutes, hence a period of 10 minutes was allowed following the exit of personnel from the monitoring room. The accidental entry of researchers into the laboratory was kept to a minimum, but the video

recording captured this and compensation was allowed to ensure stable and comparable environment over the complete period. All experiments took place between 7am - 2pm on chosen days.

All data was recorded, but interpreted blind to the status of the drug and where possible the video was rated blind to reduce observer bias.

ANIMAL EXPERIMENTS

7.7 Experiment 1: Effects of L-dopa and (+)-PHNO on MPTP-treated marmosets

7.7.1 Method of the administration of L-DOPA and (+)-PHNO to MPTP-treated marmosets.

Twelve naive marmosets were placed in the photocell motility cages, video-taped, clinically rated undisturbed and then given vehicle subcutaneous injections (1,0 ml 0,9% sterile saline); left to acclimatise for 10 minutes and their motility monitored for 180 minutes. Six weeks following the MPTP-treatment the same animals were re-introduced into the motility cages following L-dopa or(+)-PHNO vehicle injections, allowed to settle and similarly rated,

video-taped and their locomotor activity monitored for 180 minutes. All vehicle controls comprised the exact drug injection mixing compounds except the active drug ingredient, at the same volume, pH and route to be administered. All animals were acclimatised to the test cage environment for at least 30 minutes prior to injection treatments.

Eight of the most parkinsonian animals were selected to receive L-dopa (3,125; 6,25 or 12.5 mg/kg ip.) and after a week's rest (+)-PHNO (2-4 ug/kg sc.). Animals were treated with carbidopa (12,5 mg/kg po.; alphamethyldopa hydrazine) suspended in 1% methylcellulose, 60 minutes before the L-dopa administration. Locomotor activity was monitored in 10 minute segments and computer stored over 180 minutes. Rating and scoring the animals took place from a distance and directly from the video-taped at a later stage, 40-60 minutes following drug administration. Video-taped rating thereafter was also completed and the direct assessment plus video-taped rating score was used to calculate the degree of parkinsonism.

7.7.2

Results of the administration of L-dopa and (+)-PHNO to MPTP-treated marmosets. (Table 7.1 and Fig. 7.5).

Locomotor activity was significantly reduced in the photocell cages following MPTP-treatment (Fig.7.5) and animals exhibited obvious akinesia, poor balance, flexed posture and diminished vocalisation (Table 7.1). Immediately prior to the administration of any drugs all 12 animals were video-taped, scored and placed in the photocell recording cages and these assessments were similar to recordings at the completion of the experiment. Administration of L-dopa vehicle and (+)-PHNO vehicle had no significant effect on motor activity or disability scores. Administration of serially increasing doses of L-dopa (3,125; 6,25 or 12,5 mg/kg ip.) plus oral carbidopa (12,5 mg/kg) rapidly reversed motor deficit, induced locomotor hyperactivity and improved parkinsonian scores, up to 4 hours. Similarly, administration of (+)-PHNO (1,2 or 4 ug/kg sc.) reversed motor deficits in the MPTP-treated animals, which lasted up to 2 hours, showing dose dependant increased mobility (Fig. 7.5 and Table 7.1). Behavioural changes following L-dopa and (+)- PHNO were identical but nausea and retching followed only the (+)-PHNO administration.

Table 7.1

The effect of prior MPTP-treatment and the subsequent administration of levodopa plus carbidopa, (+)-PHNO or SKF 38390 on the motor disability scores of common marmosets.

Treatment status	Score	Range
Normal (n = 8)	0±0	0
MPTP (n = 8)	12.8±0.2*	11-13
+ L-dopa (n = 8)	3.6±0.6**	2-6.5
+ (+)-PHNO (n = 8)	5.6±1.3**	2-10.5
+ SKF 38393 (n = 6)	11.0±0.9	8-13

* $p < 0.05$ compared to before MPTP.
** $p < 0.05$ compared to MPTP treatment alone.

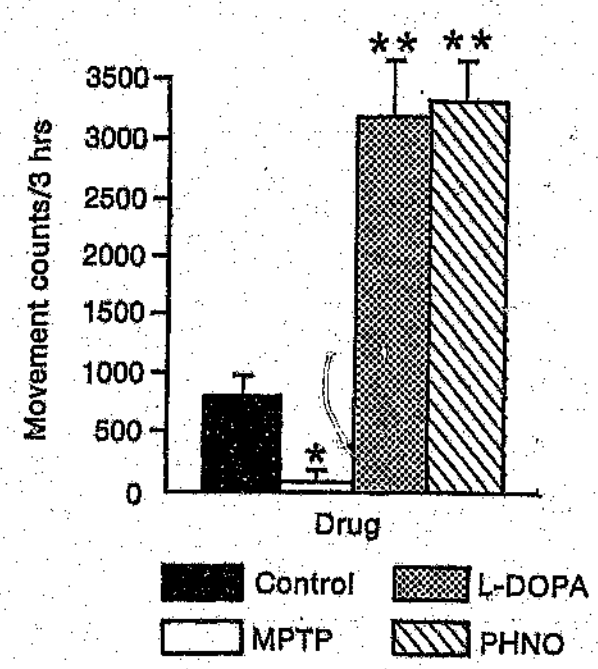

Fig 7.5

Histogram showing the effect of levodopa(12.5 mg/kg ip with carbidopa 12.5 mg/kg po) or (+)-PHNO (4 ug/kg sc) compared with spontaneous movement and vehicle control injections. Statistical significance was *$p<0.05$ compared to activity in animals prior to MPTP treatment **$p<0.05$ compared to activity in MPTP-treated animals receiving drug vehicle.

7.8 Experiment 2: Effects of CY208-243 or SKF38393

7.8.1 Method of administration of CY 208-243 or SKF 38393 to MPTP-treated marmosets.

CY 208-243 was administered (0,5, 0,75, 1,0, 1,25, 1,5 mg/kg sc.) after 30 minutes' acclimatisation in the motility cages. Ten minutes after injections, motility was then recorded for 180 minutes. Similarly SKF 38393 (10 mg/kg ip.) was administered allowing animals a 2 week drug free interval between each experiment. All animals received vehicle control injections the day before the drug injections. Allocation of random doses of CY 208-243, between 0,5 and 1,5 mg/kg sc. were given a minimum of 48 hours apart. No other drugs were given during this phase of the experiment and special caution in avoiding D-2 agonist drugs was exercised. All animals were video-taped but the rating took place only at the higher drug doses, 40 minutes after receiving the drug.

7.8.2 Results of the administration of CY 208-243 or SKF 38393 to MPTP-treated marmosets. (Table 7.1 and Fig. 7.5, 7.6 and 7.7).

Administration of SKF 38393 (10 mg/kg ip.) or its vehicle

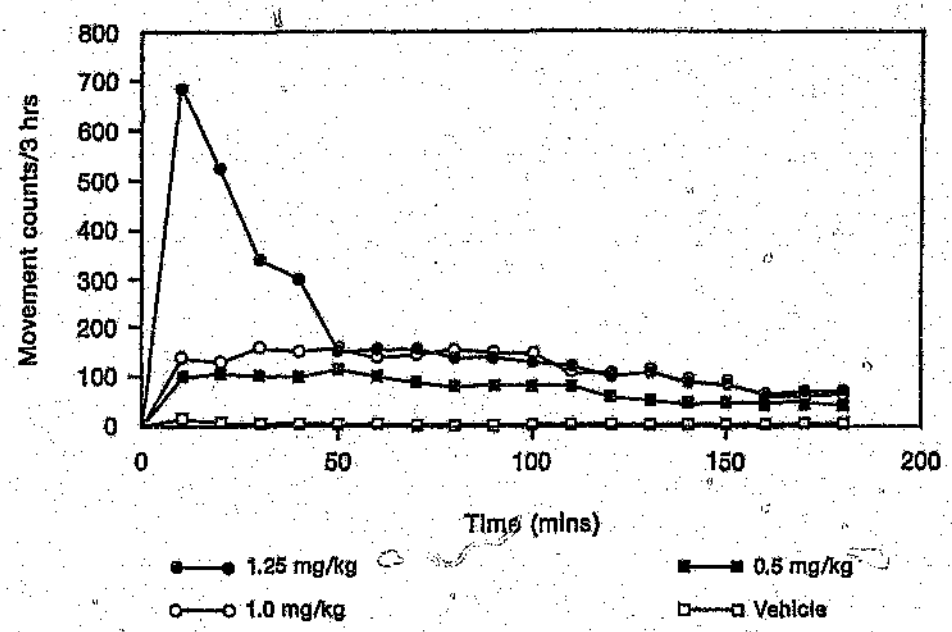

Fig 7.6

Movement counts recorded following the administration of CY 208-243 (0.5 to 1.25 mg/kg sc) in MPTP-treated marmosets. Counts were accumulated over 10 minute periods, SEM's varied from 18 - 26% of the mean shown for each dose. The solid line with open squares represents the movements recorded following vehicle injections.

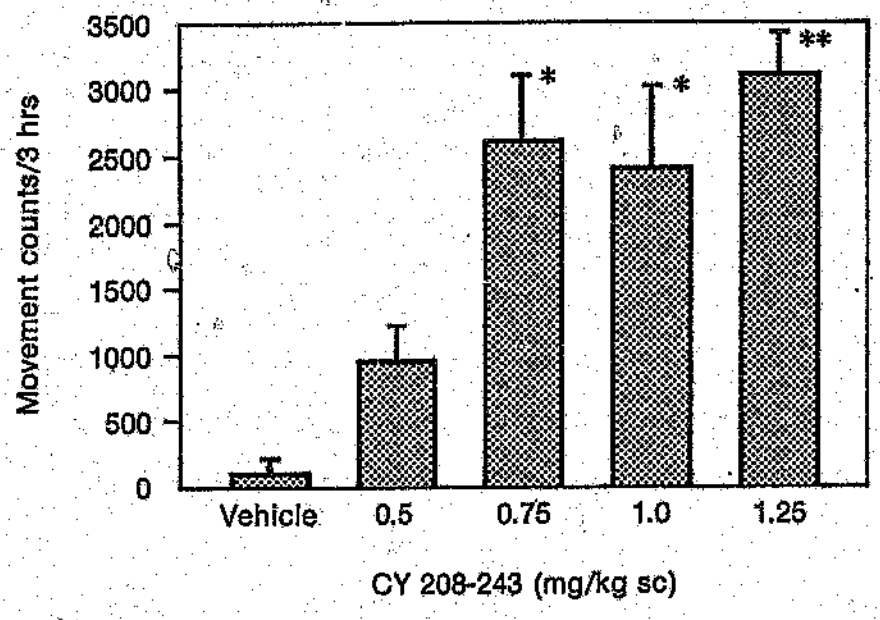

Fig 7.7

The effect of administration of CY 208-243 (0.5 to 1.25mg/kg sc) compared to vehicle. The increased mobility recorded in 8 animals had a significant statistical difference ($p<0.05$) in doses >0.5 mg/kg sc compared to vehicle control injections.

had no appreciable effect on locomotor motility or parkinsonian scores. All animals remained akinetic and no other behavioural changes were noted in the MPTP-treated animals over the 3 hour period of observation. No nausea or vomiting was observed on review of the video-tapes. Administration of CY 208-243 (0,25-1,5 mg/kg sc.) to MPTP-treated marmosets produced a reversal of motor deficits within a few minutes and reached a peak at 30 minutes (Fig. 7.6). Locomotor hyperactivity and reversal of motor deficits lasted 2 hours and was in all ways identical to those produced by L-dopa or (+)- PHNO (Fig. 7.5 , 7.6 and Table 7.2). The effects of CY 208-243 were dose related throughout the range employed but a statistically significant increase in motility as recorded by the photocell motility cages only occurred when doses greater than 0,5 mg/kg sc. were administered (Fig. 7.7). CY 208-243 did not cause nausea or vomiting in any animal. Higher doses of CY 208-243 than 1,5 mg/kg sc., produced intense hyperactivity, self inflicted injury and cataplexy in the animals. A few animals who received 2 mg/kg sc. developed a flurry of seizures lasting up to 30 minutes.

7.9 Experiment 3: Effects of pre-treatment with sulpiride or SCH23390 on the response to CY208-243

7.9.1 Method of pre-treatment with sulpiride or SCH 23390 on the response to CY 208-243

CY 208-243 (1,0 mg/kg sc.) was administered to eight animals. Five days later this effect was compared to the same dose but following pre-treatment with sulpiride (20 mg/kg ip.) 60 minutes beforehand, to completely inhibit D-2 dopamine receptors. Similarly the D-1 receptor antagonist SCH 23390 (2 mg/kg ip.) was administered 60 minutes prior to CY 208-243 (1,0 mg/kg sc.) to inhibit the D-1 dopamine receptor. Since 2 injections were required at this stage vehicle control injections were given, 1 hour apart and motility recorded the day before, for 180 minutes, following the second vehicle injection. At least 5 animals received either the D-1 or the D-2 dopamine receptor antagonist drugs on different occasions, allowing a drug free interval of more than 6 days. Rating of the animals from video-taped segments was noted 60 minutes after CY 208-243 was given.