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THE PLATELET AS A PERIPHERAL MARKER OF THE (RECEPTOR REGULATED CALCIUM) SECOND MESSENGER RESPONSE IN PSYCHIATRIC ILLNESS

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 1999

Declaration

I, Helene Plein declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



This 10th day of March, 1999

This work is dedicated to my mom and dad, for giving me the best years of their lives, and for giving my life the best.

Abstract

This thesis examines the use of the platelet as a peripheral marker of receptor regulated calcium second messenger responses of neuronal cells in major depression, subsyndromal depression. panic disorder and schizophrenia. These psychiatric disorders have substantial overlap in clinical symptomatology. Identification of a peripheral marker that might differentiate the disease conditions in terms of the underlying pathophysiology of the illness would greatly benefit the diagnosis and treatment of the psychiatric illnesses. Platelet intracellular calcium in response to neurotransmitter stimulation was measured using the fluorescent dye fura-2. This study has shown that in major depression, treated with electro-convulsive therapy (to control for a drug effect), the augmented intracellular calcium response to serotonin stimulation changes with treatment. The response is correlated with clinical improvement and suggests the platelet response as a state marker in major depression. This platelet response is specific for major depression, since no abnormality in second messenger transduction to serotonin stimulation was seen in patients with subsyndromal depression. Furthermore, the platelet response demonstrates selectivity; in patients with panic disorder, no difference in the calcium response is seen between patients and controls, lending further proof that major depression and panic disorder may have differences in terms of serotonergic dysregulation. The role calcium influx has in the augmented intracellular calcium response was measured by manganese influx and radiolabelled calcium uptake experiments. Calcium influx in response to serotonin stimulation is augmented in major depressive patients compared to normal controls. An interesting finding is that the uptake response is biphasic which may

substantiate the two-pool model of calcium oscillations within cells that was proposed by Berridge, 1991. Although many peripheral markers for depression have been suggested, there is a paucity of information concerning peripheral markers of schizophrenia. Serotonin and dopamine have not shown encouraging results as agonists in this disorder. Glutamate is an excitatory amino acid that has been implicated in the pathogenesis of schizophrenia. On stimulation, the platelet glutamate receptor activates divalent cation channels which cause intracellular calcium release, so in truth it is not a second messenger response. Schizophrenic patients show augmented intracellular calcium responses to glutamate stimulation in comparison to controls. This study supports the use of the platelet as a peripheral marker in schizophrenia. The last analysis looked at the intracellular calcium response to thrombin in the various psychiatric illnesses. Panic disorder and major depression have the highest intracellular calcium responses to thrombin stimulation. Electroconvulsive therapy does not alter this response, suggesting that it is a trait of the illness. Schizophrenia and subsyndromal depression have no altered intracellular response to thrombin. These results support the growing evidence suggesting that major depression and panic disorder are associated with significant cardiovascular complications, yet whether this is a question of association or causality must still be investigated. I feel that the thesis supports the use of the platelet as a peripheral marker of the receptor regulated calcium second messenger response of the neuronal cell, and the findings may provide some utility in the diagnosis and treatment of the psychiatric disorders.

Publications and Presentations arising from this thesis

Full Papers

Berk, M., Mitchell, V.S., Plein, H. (1998). The platelet intracellular calcium response to serotonin in subsyndromal depression. *International Clinical Psychopharmacology* 13:107-110

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Berk, M., Plein, H., Czismadia, T. (in press). Supersensitive platelet glutamate receptors as a possible peripheral marker in schizophrenia. *International Clinical Psychopharmacology*

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The platelet intracellular calcium response to serotonin and thrombin in depressed patients undergoing a course of electro-convulsive therapy: a pilot study. Presented at the 6th World Congress of Biological Psychiatry, Nice 1997.

The use of manganese to indirectly identify and measure (serotonin stimulated) calcium influx into platelets. Presented at the 6th World Congress of Biological Psychiatry, Nice 1997.

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Nomenclature

ACD- acid citrate dextrose
APUD- amine precursor uptake decarboxylation
Ca^{2+}- divalent calcium ion
$[Ca^{2+}_i]$- intracellular calcium concentration
$^{45}Ca^{2+}$- radiolabelled calcium
$CaCl_2$- calcium chloride
°C- degrees celsius
^{11}C- radiolabelled carbon
CBF- cerebral blood flow
CCK-4- cholecystokinin-4
CNS- central nervous system
CPM- counts per minute
m-CPP-meta-chlorophenylpiperizine
CRF- corticotropin releasing factor
CSF- cerebrospinal fluid
CT- computerized tomography
DA- dopamine
DAG- diacylglycerol
DSM-IV- diagnostic and statistical manual of mental disorders 4^{th} edition
DST- dexamethasone suppression test
DTPA- diethylenetriaminepentaacetic acid
ECG- electrocardiogram
ECS- electroconvulsive shock
ECT- electroconvulsive therapy
EEG- electroencephalogram
EGTA- *ethylene-glycol-tetrahydraacetic acid*
^{18}F- radiolabelled fluorine
FDA- federal drug administration
G_I – inhibitory G protein
G_s – stimulatory G protein
g- gravitational force
GABA- gamma-amino-benzoic acid
^{3}H- tritiated
HAMD- Hamilton Depression Rating Scale
HEPES- 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
5HIAA- 5-hydroxyindole acetic acid
HPA- hypothalamic pituitary axis
5HT- serotonin
5HTP- 5-hydroxytryptophan
HVA- homovanillic acid
^{125}I- radiolabelled iodide
IP_3- inositol-1,4,5-triphosphate
IU- international unit
KCl- potassium chloride
LSD- lysergic acid diethylamide

MAO-monoamine oxidase
MAOI- monoamine oxidase inhibitors
µCi/ml- microcuries/millilitre
MDD-major depressive disorder
$MgCl_2$- magnesium chloride
MK801- dizocilpine
ml-millilitres
µM- micromolar
mM-millimolar
Mn^{2+}- divalent manganese ion
$MnCl_2$-manganese chloride
MRI- *magnetic resonance imaging*
NA-noradrenaline
NaCl- sodium chloride
Na_2HPO_4- *disodium* hydrogen orthophosphate
NaSSA-noradrenergic specific serotonergic antidepressant
Ni^{2+}- divalent nickel ion
nm- nanometres
nM- nanomolar
NMDA- *N*-methyl-D-aspartate
NT-neurotransmitter
PCPA- para-chloro-phenylalanine
PET- positron emission tomography
PI- phosphoinositide
PIP_2- phosphotidylinositol-4,5-bisphosphate
PRP- platelet rich plasma
SPECT- single photon emission computerized tomography
SSD- subsyndromal depression
SSRI-selective serotonin reuptake inhibitors
TCA- tricyclic antidepressants
TRH- thyrotropin releasing hormone
TSH- thyroid stimulating hormone

CHAPTER ONE

1. INTRODUCTION

The diagnosis of psychiatric disorders, especially anxiety and depression, is often obscured by complicating factors, including an overlap of symptoms, discrepancies in diagnostic criteria, and the unreliability of self and observer reporting. The three major areas of psychiatric research are depression, anxiety disorders and schizophrenia, which is the most common of the "functional psychoses".

The establishment of a biological basis of psychiatry has helped to explain neurobiological abnormalities that underlie the patho-physiologies of the disease conditions. Originally, altered concentrations of neurotransmitter, such as serotonin (5HT), noradrenaline (NA) and dopamine (DA), in central synapses were hypothesized to underlie the different psychiatric conditions. Pure depletion or replenishment of neurotransmitter levels did not fully explain the complex pathology underlying the psychiatric illnesses though. Studies of receptor density have improved the understanding of the disease processes, but a review of the literature shows that the results are to a large extent inconsistent. Perhaps alterations in the function of the neurotransmitter-receptor-linked signal transduction system may explain the underlying pathophysiology of psychiatric illnesses that appear to have similar neurochemical abnormalities, and present with similar clinical symptomatology.

In this literature review, I have discussed the epidemiology of depression, panic disorder, and schizophrenia; the biological dysfunction implicated in each of these disorders; and the research tools that have been used to further the understanding of processes involved in the different psychiatric disorders. Direct access to neuronal tissue for neuroscience

research is not viable. The need for adequate peripheral markers of central nervous system structure and function to explain the processes involved in the illnesses is unequivocal. The platelet may be such a peripheral marker.

1.1 The epidemiology of major psychiatric illnesses

1.1.1 Depression

The essential feature of major depression is either a dysphoric mood, or loss of interest or pleasure in all or almost all usual activities and pastimes. This disturbance is prominent, relatively persistent, and associated with the other symptoms of the depressive episode such as appetite disturbances, changes in weight, sleep disturbances, psychomotor agitation or retardation, decreased energy, feelings of worthlessness or guilt, difficulty in concentrating or thinking, and thoughts of death or suicide or suicidal attempts.

In 1990, the World Health Organization identified this disease as the fourth ranked cause of disability and premature death worldwide (Murray *et al.*, 1996). The lifetime rates for major depression range between 0.9% in Taiwan and 17.1% in the USA, and mostly between 4 and 12% (Pelissolo and Lepine, 1998). The female: male ratio of 2:1 is the risk factor of developing major depression. The first onset of unipolar major depressive disorder initially peaks in the third decade of life (Angst and Preizig, 1995). Eight of 10 people experiencing a major depressive episode will have at least one more during their lifetime, and if episodes of minor or subsyndromal depression are included, the prevalence of recurrence would likely reach 100% (Angst and Preizig, 1995; Keller *et al.*, 1982; Keller *et al.*, 1992).

Kessler *et al.*,(1997) observed that minor depressive disorders and major depressive disorders among national comorbidity survey subjects were similar in clinical characteristics, suggesting that major depressive disorder and minor depression are on a symptomatic continuum and are expressions of the same illness. Sherbourne *et al.*, (1994) proposed that subsyndromal depression is a subthreshold variant of unipolar major depressive disorder.

There is very little research available on patients who have depressive symptoms that fall below the thresholds of current classifications, yet the frequency with which subsyndromal depression presents is very high. A secondary analysis of the epidemiological catchment area study reported that 16.9% of the general population reported one or more depressive symptom in the last month (Judd *et al.*,1994). In a primary care setting, subsyndromal depression was more frequent than syndromal depression (9.1 vs 7.3%; Olfson *et al.*,1996). Subsyndromal depression appears to have a similar rate (41%) of family history of affective disorder to patients with syndromal depression (59%), and was regarded by the authors as a variant of affective disorder (Sherbourne *et al.*,1994). The Medical Outcomes Study reported that in patients who had depressive symptoms that did not meet diagnostic thresholds, there was significant disability (Wells *et al.*,1989). Recent studies have replicated the finding that the presence of subsyndromal depression appears to be associated with significant disability and functional impairment (Jaffe *et al.*,1994; Olfson *et al.*,1996).

The question is whether major depressive disorder is a single disease or a heterogeneous cluster of distinct depressive disorders, each with different biological substrates and clinical characteristics.

1.1.1.1 Comorbidity of depression and anxiety

The comorbidity of major depression with other psychiatric disorders has been studied in seven countries by the cross-national collaborative group (Weissman *et al.*, 1996). Fifty eight percent of subjects with lifetime major depressive disorder present with at least one diagnosis of anxiety disorder. Panic disorder represents the most common anxiety disorder. Increased odds ratios for subjects with major depression have been found for lifetime comorbidity with panic disorder (4.7-19.5).

1.1.2 Panic disorder

Panic disorder (with or without) phobic avoidance is a common anxiety disorder, having a lifetime prevalence of 7.3% (Eaton *et al.*, 1994). The age at first onset of this disorder is in the second decade of life (Weissman *et al.*,1997), with females more commonly affected than males (3.6% vs 2.0%).

Panic disorder consists of a mixture of characteristic signs and symptoms that persist for at least one month. The symptoms include recurrent panic attacks and persistent concern about having another attack or worry about the implications and consequences of the attacks. Panic attacks are discrete periods of intense fear or discomfort, accompanied by at least 4 of the 13 somatic or cognitive symptoms defined by DSM-IV. An attack has an abrupt onset and reaches a peak usually within 10 minutes. It is often accompanied by a sense of imminent danger and an urge to escape.

Among individuals with panic disorder, the lifetime prevalence of major depression is 50-60% (Lesser *et al.*, 1989). There is significant overlap between panic disorder and major

depression and recurrent depression, yet the association between panic disorder and minor depression is not clearly defined. Patients with panic disorder, especially with comorbid depression are at higher risk for suicide attempts (Weissman *et al.*,1989), impaired social and marital functions, use of psychoactive medication and substance abuse (Markowitz *et al.*, 1989).

"There is relatively little information on the extent to which comorbidity affects prognosis and whether the observed high rates of comorbidity are due to chance or fundamental overlaps in psychopathology. Hence a thorough understanding of the relationship between panic disorder and other psychiatric disorders is needed"- This quote has been taken from the practice guidelines for the treatment of panic disorder in the American Journal of Psychiatry 1998. It summarizes the need for research into the biochemical basis of panic disorder.

1.1.3 Schizophrenia

Schizophrenia is the most common of the "functional psychoses". It is a severe, disabling neuropsychiatric syndrome affecting nearly 1% of most human populations (Kaplan and Sadock, 1994). Family, twin, and adoption studies of different ethnic populations have consistently implicated genetic factors as having an important pathogenic role in this disease (Kendler, 1983).

Schizophrenia is a major psychotic disorder. Its essential features consist of a mix of characteristic signs and symptoms that have been present for a significant length of time. The predominant clinical features are delusions, hallucinations, and interference with

thinking. Features of this kind are called positive symptoms. The chronic features of schizophrenia are apathy, lack of drive, slowness and social withdrawal. These features are often called negative symptoms.

Schizophrenia is equally prevalent in men and women, however the sexes show differences in the onset and course of the illness. Men have an earlier onset of schizophrenia than women. More than 50% of all male patients are under 25 years old on first hospital admission. For women, the peak age of onset is between 25 to 35 years. Men are also more likely than women to be impaired by the negative symptoms of the disease (Kaplan and Sadock, 1994).

All the psychiatric disorders are associated with significant increases in social dysfunction and disability. Therefore, the identification of a biological marker could aid in the understanding of different pathophysiologies of certain illnesses, as well as aid in the diagnosis and treatment of the disorders.

1.2 THE BIOLOGICAL APPROACH TO THE STUDY OF PSYCHIATRIC DISORDERS

Research into the biological basis of psychiatric illness involves the study of the principal neurotransmitters, serotonin (5HT), noradrenaline (NA), and dopamine (DA) in the central nervous system (CNS). It also studies the interactions between the neurotransmitters with their receptor systems and the consequent generation of second messengers that are responsible for neuronal communication.

Multiple brain mechanisms are involved and the degree to which underlying brain mechanisms differ or overlap remains unknown. The fact that to some extent similar neurotransmitter systems are involved in different illnesses does not mean that the mechanisms underlying the disorders are identical.

It must be emphasized that none of the neurotransmitter systems function in isolation. Serotonin, as the major neurotransmitter implicated in mood disorders, interacts with many neurotransmitter systems. It is a co-transmitter with noradrenaline. Noradrenergic input is necessary for post synaptic 5HT receptor sensitization by antidepressant treatments. Serotonergic neurons synapse with mesolimbic dopaminergic neurons. Dopaminergic neurons have serotonin receptors that permit the release of dopamine in certain brain regions. Serotonin therefore is closely associated with multiple transmitter systems (Benloucif and Galloway, 1991).

The neurobiological basis of psychiatric illness may be explained by extrapolating data on the mechanism of action of psychotropic drugs. Understanding neurotransmitter involvement in different disorders is usually assisted by pharmacological challenges. In this section, I explain the involvement of neurotransmitters and receptors in depression, panic disorder and schizophrenia by the effectiveness of drugs used to treat the conditions.

1.2.1 Depression- Biological Dysfunction

1.2.1.1 The original monoamine hypothesis of depression

The original theory was first proposed on the findings that reserpine, a drug used in the treatment of hypertension, produced a disorder resembling endogenous depression in 10-15% of patients. Reserpine depletes the brain of the monoamines; noradrenaline,

dopamine and serotonin. Conversely, iproniazid, used in the treatment of tuberculosis, was reported to have mood elevating properties. This drug increases the availability of these same monoamines in the central nervous system.

The theory was advanced such that reduced availability of monoamine neurotransmitters may play a role in the pathogenesis of depression (Schildkraut, 1965). In addition, based on the knowledge of the mechanism of action of antidepressant drugs, the hypothesis was put forward that alterations in receptor systems were also involved.

1.2.1.2 Monoamines in the treatment of depression

The development of the initial tricyclic antidepressants in the 1950's allowed for investigation into the role of noradrenaline and serotonin in the pathophysiology of depression. The ability of tricyclic antidepressants to raise the intrasynaptic concentrations of serotonin and noradrenaline remains a cornerstone of all monoamine theories.

1.2.1.3 Antidepressant therapies

There are different antidepressant therapies available. All involve the increase in synaptic levels of the neurotransmitters, noradrenaline and serotonin. The antidepressant therapies differ in terms of their selectivity for the neurotransmitter system.

1.2.1.3.1 Tricyclic antidepressants (TCA)

This was the first generation of antidepressants. Their function is to block the reuptake of noradrenaline and serotonin into the presynaptic neuron. Different drugs in this class

exhibit varying degrees of selectivity for the reuptake pumps for noradrenaline and serotonin. This effectively results in increased concentrations of neurotransmitter in the synaptic cleft available to interact with postsynaptic receptors. The tricyclic antidepressants also block other receptor systems, such as histamine, muscarinic and adrenergic receptors. The high risk of cardiovascular and respiratory complications associated with this group of drugs when used in overdose prompted the search for more selective agents that had fewer side effects, with the same efficacy.

1.2.1.3.2 Monoamine oxidase inhibitors (MAOI)

Monoamine oxidase A is the amine oxidase primarily responsible for noradrenaline, serotonin and tyramine breakdown. Therefore, the inhibition of this enzyme results in increased levels of serotonin and noradrenaline available presynaptically for release into the intrasynaptic cleft. The irreversible monoamine oxidase inhibitors are subject to a high risk of hypertensive reactions to tyramine ingested in food. The reversible monoamine oxidase inhibitors, such as moclobemide, are not subject to these dietary restrictions.

1.2.1.3.3 Selective serotonin reuptake inhibitors (SSRI)

Fluoxetine was the first entirely specific serotonin reuptake inhibitor. It has little or no effect on other neurotransmitter systems which affords a specificity of action that may reduce unwanted side effects. It is effective in the treatment of major depression, and so lent further proof to the growing evidence in support of serotonin as the principle neurotransmitter implicated in the pathophysiology of mood disorders. It allowed for the

testing of the relative importance of serotonin and noradrenaline in depression (Hollister and Csernansky, 1990).

1.2.1.3.4 Noradrenergic specific serotoninergic antidepressants (NaSSA)

Mirtazapine is the first noradrenergic and specific serotonergic antidepressant (NaSSA). Its pharmacological profile combines the enhancement of both noradrenergic and serotonergic transmission with specific blockade of $5HT_2$ and $5HT_3$ receptors (de Boer, 1996). Noradrenaline released directly from presynaptic noradrenergic nerve terminals interacts with specific NA receptors, and the action of noradrenergic transmission on presynaptic serotonergic nerve terminals causes an increase in serotonergic neuronal firing (de Boer, 1996).

1.2.1.3.5 Electroconvulsive therapy (ECT)

Electroconvulsive therapy is used as a treatment modality for major depression (Weiner and Krystal, 1994). Neuroendocrine studies suggest but cannot find consistent evidence that a possible mechanism of action of ECT is the elevation of serotonin levels in the brain by increasing synaptic release of this neurotransmitter (Wilkinson, 1989; de Montigny, 1984; Chaput *et al.*, 1991). In this regard, ECT has a similar mechanism of action to other different types of antidepressants which effectively enhance serotonergic transmission in the CNS, albeit all by different mechanisms (Price *et al.*, 1990; Blier *et al.*, 1990).

Several studies have compared depressed inpatients receiving ECT with those receiving antidepressant drugs. In nine comparisons with tricyclic antidepressants, ECT was

therapeutically more effective in six studies and equally effective in the remaining three. In five comparisons with monoamine oxidase inhibitors, ECT was superior in each trial. These data suggest that in severely depressed patients, ECT is probably superior to antidepressant drug therapy. Another point is that ECT may often prove effective in depressed patients who have not responded to full trials of medication.

1.2.1.3.5.1 Mode of action of electroconvulsive therapy

The specific therapeutic effects of ECT must be brought about by physiological and biochemical changes in the brain. During ECT, an electrical current is passed through the brain for 0.5 to 2 seconds. The seizure resulting from the ECT should be between 20 and 120 seconds in duration to be of therapeutic value. ECT causes some of the same biochemical changes that antidepressants cause: the $5HT_2$ receptor is affected in both ECT and TCA therapy (Brown and Mann, 1985). ECT increases the turnover and synthesis rates of 5HT in the brain. In ECT net serotonergic transmission increases (Leonard, 1992). In another study, ECT increased the responsivity of hypothalamic pituitary neurons to serotonin (Chaput *et al.*, 1991). Increased levels of 5-hydroxyindoleacetic acid (5HIAA), the principal serotonin metabolite, have been found in the serum of depressed patients following ECT (Hoffman *et al.*, 1996; Jori *et al.*, 1975).

1.2.2 Serotonin in depression

Several lines of evidence suggest that serotonin is the biogenic amine neurotransmitter that is most commonly associated with depression. Depletion of serotonin may precipitate depression. Decreased serotonin levels in the brain are documented in major depressive

illnesses (Mann *et al.*, 1996). Suicidal patients have low cerebrospinal fluid concentrations of serotonin metabolites. Serotonin is found in the limbic regions of the brain, indicating its involvement in emotional behaviour. An enhancement of serotonin neurotransmission might underlie the therapeutic response to antidepressant treatments. All selective serotonin reuptake inhibitors are effective in major depression. Since most of these drugs belong to different chemical families and the only common property they share is to inhibit the serotonin reuptake carrier, it is indisputable that they exert their therapeutic effect primarily via the serotonin system.

Although decreased serotonin neurotransmitter levels are reported in major depression, pure replenishment of the neurotransmitter does not reverse the symptoms of depression. Therefore it is not only decreased levels of the neurotransmitter itself, but also the consequent upregulation of 5HT receptor subtypes, which are reported in major depression (Hrdina *et al.*, 1997) that may be responsible for the depressive illness.

The different time courses for the effects of antidepressants can be followed in figure 1.1 below. Antidepressant therapy changes mood, levels of neurotransmitter and receptor sensitivity. The concentration of neurotransmitter changes relatively rapidly after the introduction of antidepressant therapy. However, the clinical effect is delayed, as is the down-regulation of neurotransmitter sensitivity. This temporal correlation of clinical effects with changes in receptor sensitivity has given rise to the hypothesis that changes in neurotransmitter receptor sensitivity may mediate the clinical effects of antidepressant therapy.

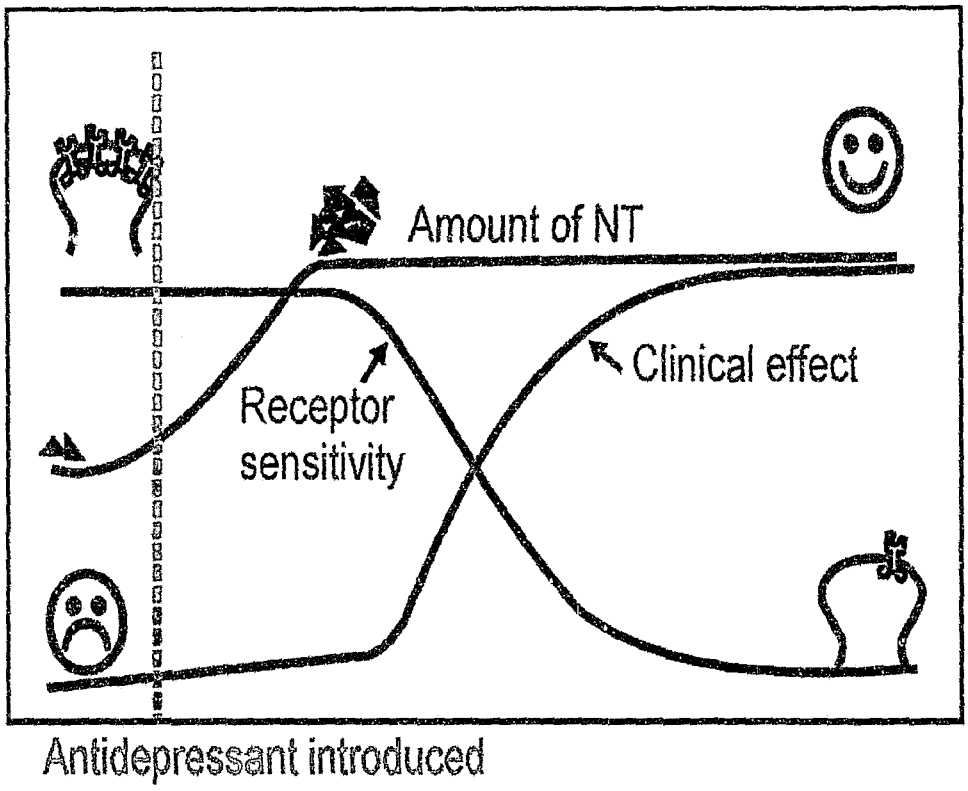


Figure 1.1 The different time courses for three effects of antidepressant drugs (Adapted from Stahl, 1997).

1.2.3 Involvement of the $5HT_2$ receptor subtype in depression

At present seventeen types of 5HT receptors have been identified in the brain; they have been classified into seven classes from $5HT_1$ to $5HT_7$ receptors, and within each of these there are subclasses. With the exception of $5HT_3$, they all belong to the family of G-protein linked receptors. These receptors transduce signals by activating G-proteins, producing responses through second messengers.

Only the $5HT_2$ receptor has been reviewed here. This is because intracellular calcium is a second messenger to $5HT_2$ receptor stimulation. Also, platelet receptor sites for serotonin are of the $5HT_2$ type (Lesch *et al.*, 1993; Mikuni *et al.*, 1992).

The density of $5HT_2$ receptors is increased in post mortem brain samples from depressed subjects and depressed suicide victims (Yates *et al.*, 1990; Hrdina *et al.*, 1993), but unchanged in other similar studies (Cheetham *et al.*, 1988). Upregulation of these receptor sites may compensate for the decreased efficacy of serotonergic transmission caused by decreased serotonin availability (Meltzer *et al.*, 1984). In experimental animals, antidepressants decrease the density of $5HT_2$ receptors in the brain (Peroutka and Snyder, 1980). On the other hand, electroconvulsive shock (ECS) and fluoxetine treatment increase the number of $5HT_2$ receptor sites (Hrdina and Vu, 1993; Klimek *et al.*, 1994). Hrdina *et al.*, (1997) and Bakish *et al.*, (1997) failed to support the notion of altered $5HT_2$ transporter receptor density in depression. Their findings showed that antidepressant therapy did not significantly alter the $5HT_2$ receptor density, with responders and non-responders showing no difference in mean receptor number.

The upregulation of receptor sites reported by Hrdina *et al.*, (1997) was not corroberated by other similar studies (Cowen *et al.*, 1987; McBride *et al.*, 1994). Regulation of the function of these receptor sites corresponds with clinical improvement in the patients and is thought to be the mechanism of action behind antidepressant drug therapy (Dubovsky, 1995).

1.2.4 Panic Disorder-Biological Dysfunction

Among the anxiety disorders, panic disorder has been the most extensively studied from a biological perspective. A number of theories are proposed to explain the etiology of panic disorder, including the dysfunction of one or more neuronal system: serotonin, noradrenaline, gamma-amino-n-butyric acid (GABA) and cholecystokinin.

The identities of the neuronal pathways involved are still being discovered, but a variety of drugs with seemingly divergent mechanisms of action are effective in the treatment of panic disorder.

Three pathways of panic induction are proposed: benzodiazepine receptor binding, noradrenergic function and serotonergic function. Evidence suggests that the serotonergic component underlies or modulates the proposed noradrenergic and benzodiazepine receptor-binding mechanisms.

1.2.4.1 Benzodiazepine (BDZ) and GABA function

Benzodiazepines have marked anxiolytic action. The benzodiazepine receptor is coupled to GABA receptors that regulate chloride ion channels. BDZ may modulate anxiety by facilitating the function of GABA as an inhibitor of neuronal excitability. In this way, they restore normal levels of receptor reactivity and modulate the onset of anxiety states. GABA and 5HT systems are anatomically and functionally linked, primarily in the median raphe sites where serotonin containing cell bodies originate. The antipanic activity of benzodiazepines decreases 5HT turnover and reduces the electrical activity of serotonergic neurons.

1.2.4.2 Noradrenergic function

Yohimbine, an alpha$_2$ adrenergic receptor antagonist induces more anxiety and panic attacks in patients with panic disorder than in healthy subjects (Charney *et al.*, 1984). Neurochemical and anatomical evidence suggests that the anxiolytic effects of noradrenergic agents are mediated through enhancements of serotonergic function. The neurotransmitter systems have complex interconnections (primarily in the locus cerruleus - the primary nucleus of noradrenergic containing neurons). Research has shown that the antipanic agents with noradrenergic activity may be related to their non-selective effect on serotonin.

1.2.4.3 Serotonergic function

Evidence in support of serotonin in panic disorder came from the findings that maprotiline, a tetracyclic antidepressant that has selective action on noradrenaline uptake sites, was ineffective in the treatment of panic disorder, whereas the TCA antidepressant imipramine blocked spontaneous panic attacks (Klein, 1964). Also, the selective serotonin reuptake inhibitor, fluvoxamine significantly reduced the mean state anxiety score (Den Boer and Westenburg, 1988). Serotonergic involvement in panic is either because of excess serotonin brought about by increased serotonin release or supersensitive post synaptic receptors. The converse theory is that of serotonin deficit. This proposes that serotonin has a restraining effect on panic behavior, and when there is a deficit in serotonin, this restraint is reduced and panic ensues (Nutt, 1998).

1.2.4.4 Drug treatment

Two drugs have FDA approval for the treatment of panic disorder: alprazolam, an atypical benzodiazepine that has anxiolytic and antidepressant properties. Alprazolam prevents the upregulation of beta adrenoreceptors produced by reserpine, although on its own it does not down-regulate these receptors (Sethy and Hodges, 1982). It also has alpha$_2$ adrenoceptor agonist activity (Eriksson *et al.*, 1986).

Paroxetine is the other drug approved by the FDA for use in panic disorder. It is an SSRI antidepressant. Traditionally other antidepressants like MAOIs and TCAs have been used in the treatment of panic disorder. The effect of these drugs is to increase the levels of serotonin in the intrasynaptic cleft, similar to the effect they have in the treatment of depression. Kahn and van Praag (in Sacchetti and Cassano, 1992) have demonstrated that these drugs cause an initial worsening of panic and take at least three to four weeks before producing any antipanic effect. The authors explained this by hypothesizing that serotonin receptors are hypersensitive in this disorder. The hypersensitive serotonin receptors to neurotransmitter stimulation are responsible for the initial deterioration, while repeated administration of the drugs leads to down-regulation of postsynaptic receptors and an amelioration of symptoms. Kahn *et al.*, (1988) studied the responsivity of 5HT receptors in panic disorder by measuring cortisol release after m-chlorophenylpiperazine (m-CPP) administration. m-CPP induced an augmented cortisol release in panic disorder patients as compared to normal controls. Based on this, the authors suggested that 5HT receptors were supersensitive in panic disorder. Ritanserin was able to abolish the m-CPP induced increase in cortisol release, suggesting that the response is mediated via 5HT$_2$ receptors (Seibyl *et al.*, 1991). There is evidence to suggest that receptor binding in panic disorder is

normal; uptake studies do not reveal abnormal ^{3}H-imipramine binding characteristics (Nutt and Fraser, 1987; Schneider, 1987; Norman *et al.*, 1989). Abnormal receptor binding is seen in major depression though (Nemeroff *et al.*, 1988). This may suggest a different pathogenesis of panic disorder than major depression.

Even though similar neurochemical changes are seen in both depression and panic disorder, it is unclear and difficult to state whether these two disorders have the same underlying pathophysiology.

1.2.5 Schizophrenia- Biological Dysfunction

An extension of the catecholamine hypothesis was that schizophrenia was associated with catecholamine elevation. The hypothesis that central biogenic amines may play a role in the pathophysiology of schizophrenia was originally based upon the fact that hallucinogenic and antipsychotic drugs have profound effects on central transmitter pathways where dopamine and serotonin are involved.

1.2.5.1 Dopaminergic function

The dopamine hypothesis of schizophrenia proposed that dopamine turnover is increased in this disease. The classical neuroleptic drugs available to treat schizophrenia (e.g. haloperidol) work on this hypothesis by blocking mainly D_2 mediated dopaminergic neurotransmission, thus decreasing the symptoms of schizophrenia. Investigation of dopaminergic function in schizophrenic patients by the measurement of cerebrospinal fluid dopamine and homovanillic acid (HVA), the principal dopamine metabolite, has

shown no differences between patients and controls (Widerlov, 1988). Direct analysis of post-mortem brain from schizophrenic patients has failed to show any evidence of increased dopamine turnover. The observation that neuroleptic medication is not able to control all the complex symptomatology of schizophrenia suggests that it is increasingly unlikely that dopamine is the only neurotransmitter that accounts for the many clinical manifestations of the illness.

Dopamine produces its physiological effects by activating postsynaptic receptors. D_2 receptors are the most important postsynaptic receptors mediating behavioural and extrapyramidal activity. Most therapeutically effective typical neuroleptics block the D_2 receptor (Leonard, 1992).

1.2.5.2 Serotonergic function

Early theories implicating serotonin in the pathogenesis of schizophrenia were based on the observation that lysergic acid diethylamide (LSD), an agent acting at serotonergic receptors, could produce a psychosis with features similar to schizophrenia. Many atypical neuroleptics (clozapine, risperidone) have serotonergic antagonist properties (Bliech *et al.*, 1988). Dopamine activity may be modulated by serotonin (Dickinson and Curzon, 1983; Korsgaard *et al.*, 1985).

There are few consistently replicated abnormalities of serotonergic neurotransmission in schizophrenia despite numerous studies. Measurements of brain and cerebrospinal fluid 5HT and 5-hydroxyindoleacetic acid (5HIAA) have produced conflicting results (Leonard, 1992; Schatzberg and Nemeroff, 1995). In the nucleus accumbens (Mackay *et al.*, 1978)

and striatum (Owen *et al.*, 1981) there is reportedly no difference between schizophrenic subjects and controls in terms of $5HT_2$ receptor binding.

1.2.5.3 Glutamatergic function

Increasing attention has been focused on the role of the excitatory amino acid neurotransmitter, glutamate and its receptors, particularly the *N*-methyl-D-aspartate (NMDA) receptor, in schizophrenia (Weinberger, 1997). Kim *et al.*, (1980) proposed a hypothesis suggesting hypofunction of the glutamatergic neuronal system in schizophrenia based on the decreased levels of CSF glutamate levels in schizophrenic patients compared to controls. This finding was replicated by Tsai *et al.*, (1995) who found decreased glutamate levels in frozen brain tissue from schizophrenic patients. Studies by Korpi *et al.*, (1987) and Altamura *et al.*, (1993) found no differences in glutamate levels between schizophrenic patients and controls though.

The ability of cortical neurons to modulate subcortical dopamine activity is probably dependent on glutamate projections, because glutamate is the dominant neurotransmitter in the cerebral cortex. Excitatory cortical efferents project heavily to the subcortical regions which contain dopamine cell bodies or terminals (Beckstead 1979, Sesack *et al.*, 1989). Hypofunction of glutamate implies decreased cortical output. Glutamate normally stimulates the inhibitory neurotransmitter GABA whereas dopamine inhibits GABA release. Dopamine seems to exert an inhibitory influence on glutamate release (Kornhuber and Kornhuber, 1986; Peris *et al.*, 1988; Maura *et al.*, 1988, 1989). Therefore similar behavioral effects can be produced with deficient glutamate activity or increased

dopamine activity (Javitt and Zukin, 1991). The data to date emphasize the possible reciprocal interactions between dopaminergic and glutamatergic systems.

Glutamate receptors can be categorized into two groups termed ionotropic and metabotropic receptors. The ionotropic receptors contain integral, cation-specific ion channels. The receptor on this ion channel is located on the protein complex of the ion channel itself, whereas the metabotropic receptors are coupled to G proteins and modulate the production of intracellular messengers. The ionotropic receptors can be subdivided into NMDA receptors, kainate receptors, and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. NMDA receptor dysfunction is hypothesized as an etiological factor in the pathogenesis of schizophrenia (Olney and Farber, 1995). NMDA receptors are involved in the regulation of intracellular free calcium concentrations and as such are linked to divalent cationic channels (Javitt and Zukin, 1990). Phencyclidine (angel dust or PCP), a NMDA antagonist is capable of producing a psychotic picture similar to schizophrenia (Javitt and Zukin, 1991). The binding site for PCP is located within the NMDA channel where it blocks this channel non-competitively.

The study of psychiatric illness has progressed since the first catecholamine theories suggested that concentrations of neurotransmitter in the synaptic cleft could explain the symptomatology of the illnesses. Pure replenishment of neurotransmitter levels did not correct the disease conditions.

Chemical neurotransmitters produce their effects as a consequence of their interactions with appropriate receptors. The study of receptor changes that occur in psychiatric illnesses managed to improve the understanding of the pathophysiologies of the disease processes, yet there is plenty of conflicting data. This inconsistency in the data raises

questions about whether down-regulation of receptors is a necessary component of antidepressant effect (Hrdina *et al.*, 1997), and by extension other psychiatric illnesses.

Receptors on neurons communicate brain signals via synaptic transmission. This is performed through the generation of intracellular second messenger signalling systems. One of the most common intracellular signalling systems in the body is calcium (Ca^{2+}). Perhaps the function of the receptor linked second messenger system is associated with the pathophysiology of the disease processes.

1.3 NEURONAL COMMUNICATION

1.3.1 Synaptic transmission

An action potential arriving at the presynaptic neuron causes a change in the membrane potential of the cell. This opens calcium channels on the cell membrane and allows extracellular calcium to enter the cell. The increase in intracellular calcium causes the release of neurotransmitter into the synaptic cleft. Once the neurotransmitter diffuses across the synaptic cleft, it binds to receptor sites on postsynaptic neurons. This has been described diagramatically in figure 1.2 below.

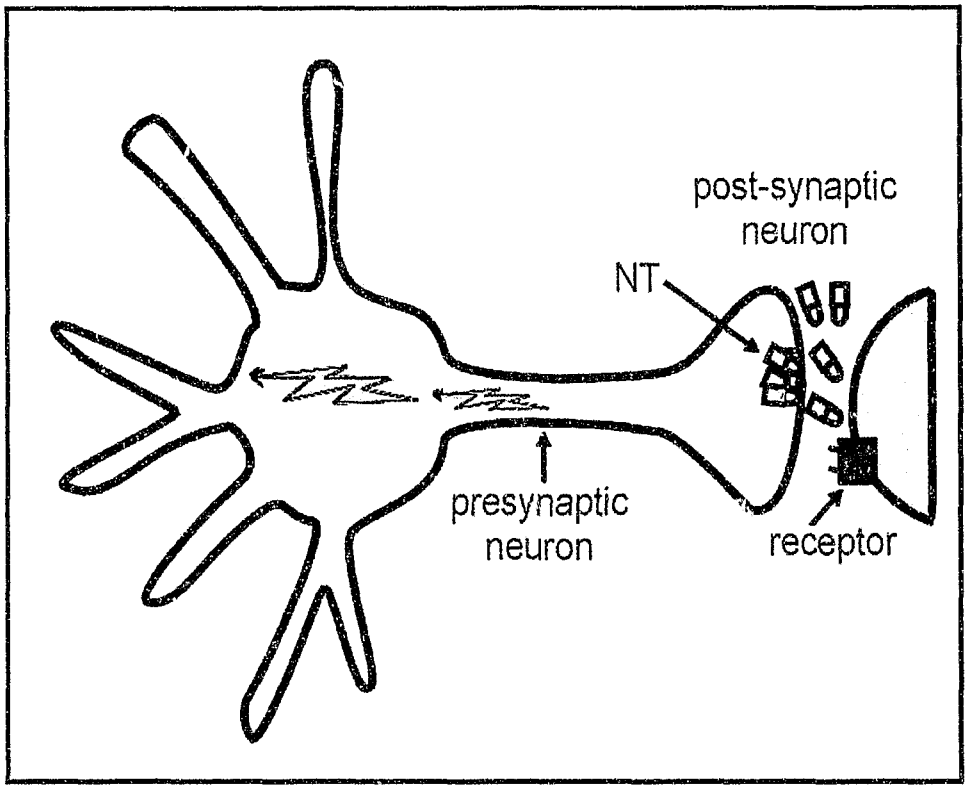


Figure 1.2 A key postsynaptic regulatory receptor (Adapted from Stahl, 1997).

Neurotransmitter-receptor complexes affect the regulation of many intracellular functions via the stimulation of intracellular second messengers. A simple diagramatic explanation for this is shown below in figure 1.3.

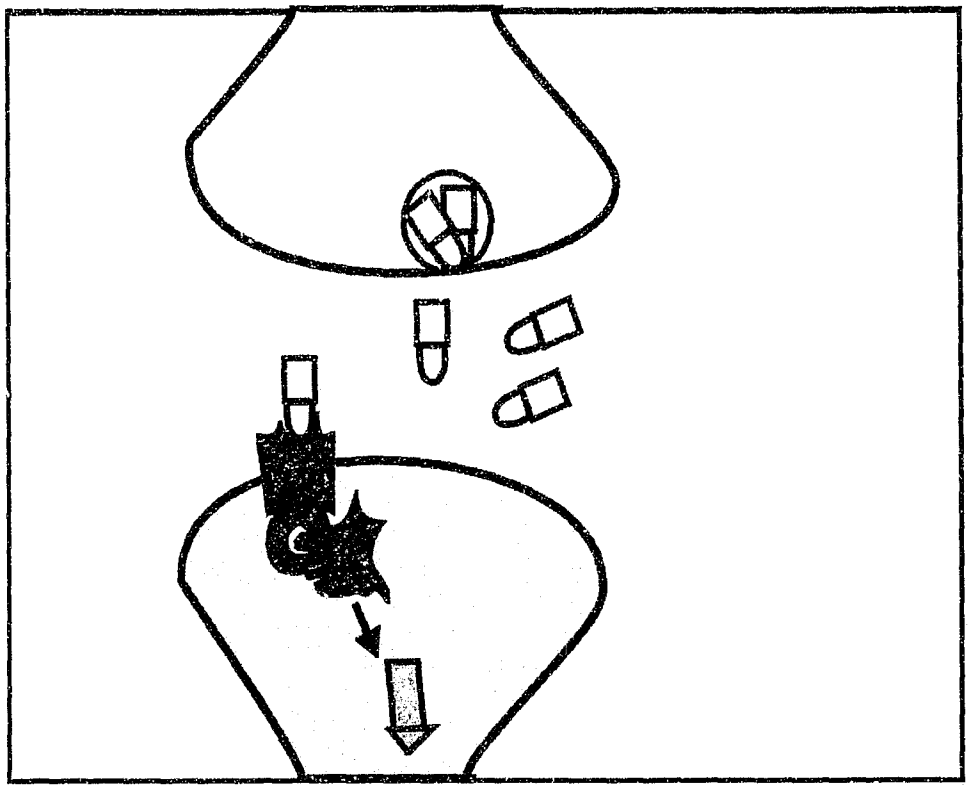


Figure 1.3 Second messenger generation from receptor activation (Adapted from Stahl, 1997).

1.3.2 Calcium as a second messenger

Calcium is the most dynamic second messenger known. No other intracellular signalling pathway fluctuates so rapidly in response to receptor activation. In mammalian cells, stimulation of specific receptors by agonists is accompanied by a non-voltage-regulated mobilization of intracellular calcium stores. This results in the elevation of cytosolic calcium concentrations, which can evoke cellular changes such as, in the platelet; secretion, shape change and aggregation. The calcium supply appears to originate from

specific, non-mitochondrial sites in the cell, probably the endoplasmic reticulum or the platelet dense tubular system (Berridge, 1994).

Calcium is a second messenger of particular interest because of its involvement in the regulation of many processes implicated in the affective disorders. Synthesis, release of, and receptor responsiveness to neurotransmitter activation, maintenance, and termination of action potentials, as well as neuronal memory of previous stimulation all involve calcium (Dubovsky *et al.*, 1989).

It seems reasonable to study second messengers in an attempt to gain more insight into common cellular dysfunctions that could be associated with the manifestations of mood disorders.

1.3.2.1 Mechanism of calcium activation

The mechanism by which agonists cause a receptor-mediated Ca^{2+} mobilization has been extensively studied over recent years. A schematic representation of the second messenger pathway linked to receptor systems is shown below in figure 1.4. It appears that the transduction mechanism of occupied receptors is mainly through the phospholipase C catalysed hydrolysis of inositol phospholipid, specifically phosphatidylinositol-4-5-biphosphate (PIP_2). The immediate products of this reaction are diacylglycerol (DAG), which can activate protein kinase C, and inositol triphosphate (IP3) which will mobilize the hormone sensitive Ca^{2+} stores when applied to permeabilized cells. Therefore, the connection between receptor and calcium store mobilization has been quite convincingly established (Pacheco and Jope, 1996).

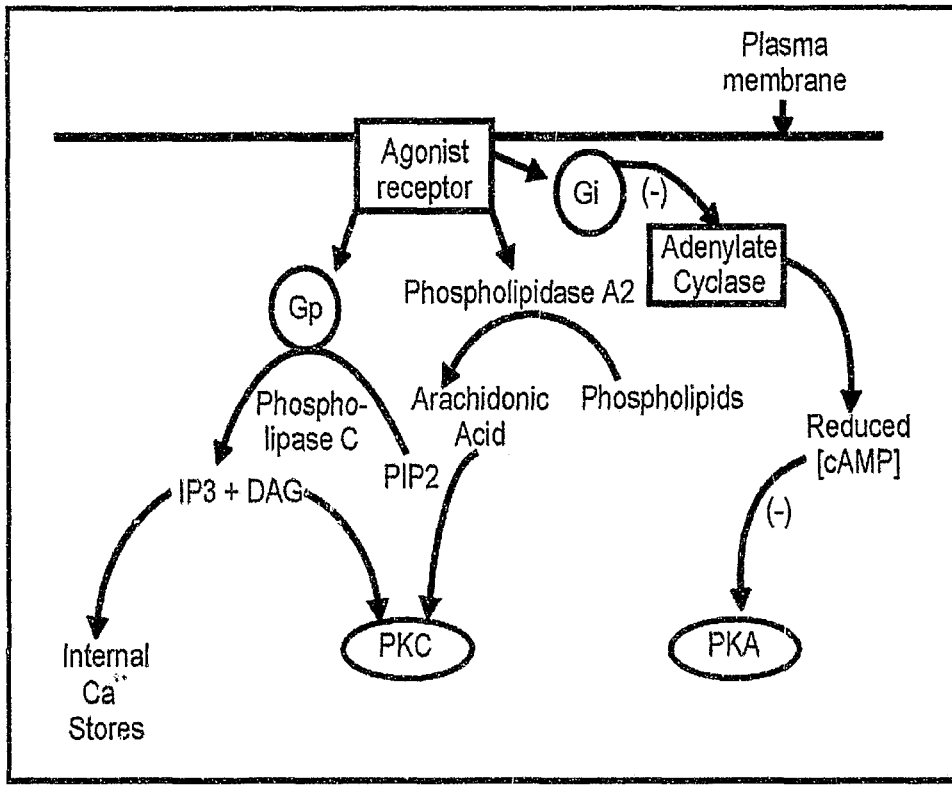


Figure 1.4 Schematic representation of second messenger pathways linked to receptors.

Serotonin and other neurotransmitters bind to cell surface receptors and result in a rise in the intracellular concentration of ionised calcium. This transient rise in calcium sets off a discrete set of cellular responses (Murray *et al.*, 1988).

Calcium is one of several intracellular signal transducers utilized by eukaryotic cells as part of their integrated response to external stimuli. Calcium is involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation.

Mobilization of calcium represents the primary mode of action of many external signals, including neurotransmitter, hormones and growth factors. The term mobilization is used loosely to imply an action of such agents on both the entry of external calcium across the plasma membrane as well as the release of this ion from intracellular stores. IP3 acts to mobilize calcium from internal stores.

Alterations in any of the individual events in the sequence of reactions responsible for generating or metabolizing the messenger IP3 are potentially capable of distorting the signalling pathway (Berridge, 1994).

Lithium, as a medication in bipolar disorder, has as one of its actions an inhibiting effect on the formation of free inositol. This in turn decreases IP3 formation and desensitizes the signalling pathway that employs this second messenger (Berridge *et al.*, 1989).

Changes in signal transduction could increase calcium mobilization or inhibit the removal of calcium. This may be because of alterations in the phosphatidyl-inositol system or other elements of the second messenger cascade (Dubovsky *et al.*, 1992). Alterations in intracellular calcium may be because of alterations in membrane function. Structural abnormalities in red blood cell and lymphocyte cell membranes have been reported in bipolar depressed patients (Pettegrew *et al.*, 1982). Alterations in either the G-protein or membrane coupling to the G-protein could also affect calcium regulation. Enhanced G-protein responses to agonists in leucocyte membranes has been found in bipolar disorder (Schreiber *et al.*,1991), as have elevated levels of G-proteins (Young *et al.*, 1994). In the brain, regionally selective deficits in G-protein function associated with phosphoinositide

signalling have been reported in subjects with major depression and with bipolar affective disorder.

Cells have access to two sources of calcium; an internal source from the endoplasmic reticulum and an external source, the extracellular environment. The mechanisms employed by the body for calcium homeostasis function in a way that results in extracellular concentrations of calcium being approximately 1 mM whereas the intracellular concentration is approximately 10^6 times lower at resting level (Hallam and Rink, 1985). Internal calcium stores are the major source of signal calcium. The initial source of calcium appears to be the intracellular organelle reservoirs, which seem to be sufficient for the early effects of the hormones. More prolonged action appears to require enhanced influx through the cytoplasmic membrane (Murray *et al.*, 1988). Calcium carries out its signalling role in very short bursts (Berridge, 1994).

Some authors suggest that the presence of extracellular calcium is essential for the increase in intracellular calcium (Rink, 1988). Other authors argue that intracellular calcium responses occur independently of extracellular calcium influx (Andersson *et al.*, 1986), although the increase in intracellular calcium is greater in the presence of external calcium. Other authors suggest that changes in intracellular calcium are the result of both intracellular calcium mobilization, and calcium entry; processes that occur independently and sequentially (Nishio *et al.*, 1993).

The brain is an area of intensive cell signalling. Because calcium has such a critical role in cell activation, alterations in this system may be the cause of a number of pathological states, such as depression, panic disorder and schizophrenia.

As already stated, receptor activation by neurotransmitters leads to the cascade of second messenger signalling. Perhaps alterations in the function of these receptor sites account for disorders seen. The density of receptor sites for neurotransmitter stimulation and the function of these neurotransmitter receptor systems may be independent entities as suggested by numerous authors (Mikuni *et al.*, 1992; Hrdina *et al.*, 1997).

1.4 THE NEED FOR PERIPHERAL MARKERS OF CENTRAL NERVOUS SYSTEM STRUCTURE AND FUNCTION

The feasibility of accessing neuronal tissue for neuroscience research into the function of neuronal cells is impractical for a variety of reasons. The availability of fresh human brain tissue is limited. Post mortem brain is useful, but constraints such as functional changes that occur after death are a concern. Therefore, the understanding of functional changes linked to receptor systems for neurotransmitters may be derived from data using peripheral tissues that have relationships with CNS function.

Peripheral markers of CNS function have evolved as the research into the biological basis of psychiatry has advanced. Initially, only peripheral neurotransmitter levels were measured. Then, challenge studies using pharmacological agents were used to elucidate the physiological responses under neurotransmitter control. With the advent of advanced computer technology it has become possible to view the structure and function of the CNS directly. This has greatly improved the understanding of psychiatric illness. The platelet has been used as a model of CNS structure and function, since $5HT_2$ receptors are present on the platelet membranes and the receptor regulated second messenger calcium response, which can be measured, may serve as an index of second messenger responses to neurotransmitter stimulation in the brain.

It is beyond the scope of this review to discuss other neurotransmitters but serotonin in terms of its involvement in peripheral markers of CNS function in relation to depression, panic disorder and schizophrenia.

1.4.1 The measurement of catecholamine levels in the body

Since the blood system throughout the body is in equilibrium with the blood circulating through the brain, changes in the blood stream should detect abnormal levels of catecholamines or metabolites that would be present in the brain.

1.4.1.1 5-Hydroxyindoleacetic acid (5HIAA) levels

5HIAA is the major serotonin metabolite found in the blood system. The measurement of 5HIAA as an index of serotonin availability in the system has been extensively used in the literature. The level of 5HIAA was shown to be significantly decreased in the CSF of depressed patients with suicidal behaviour (Asberg *et al.*, 1976). Korpi *et al.*, (1986) confirmed this by finding decreased levels of 5HT and 5HIAA in the hypothalamus of suicide victims.

There is conflicting evidence though. There is data which showed no difference in CSF concentrations of 5HIAA between depressed patients who had attempted suicide, between depressed patients with no suicide attempts and controls (Roy *et al.*, 1986). Also, no difference in the 5HT and 5HIAA concentrations were found in post mortem brain tissue of depressed suicide patients and controls (Cheetham *et al.*, 1989).

There is only one report of 5HIAA concentrations in the CSF of panic disorder patients (Eriksson *et al.*, 1991). Patients did not differ from age and sex matched controls. This does not suggest that 5HT function is abnormal in panic disorder, and may point to a neurobiological difference between panic disorder and depression.

1.4.1.2 Homovanillic acid (HVA) levels

In order to develop the catecholamine hypothesis of schizophrenia, homovanillic acid (HVA), as the major metabolite of dopamine found in the blood system has been studied. Analysis of metabolite levels in the CSF and plasma of schizophrenic patients have shown great variation in results. In general, CSF dopamine and HVA levels in schizophrenic patients have not been shown to differ from controls (Gurlov, 1988). The study of dopamine and HVA in post mortem brain tissue has also yielded inconsistent results (Davis *et al.*, 1991).

Pure replenishment or depletion of neurotransmitter levels did not completely reverse the symptoms of the illness. Human subjects receiving biogenic amine depleting drugs do not show any significant tendency to develop true depressive disorders. Similarly, decreased dopamine levels produced by neuroleptic medication does not reverse schizophrenia.

1.4.2 Neurotransmitter Challenge Studies

Neuroendocrine challenge studies are frequently used to make inferences about the pathophysiology of psychiatric illness or about the effects of drug treatment on brain monoamine function.

1.4.2.1 Tryptophan challenge studies in depression

Tryptophan is the amino acid precursor to serotonin synthesis. It is transported from the blood into the brain by carrier mediated transport, and taken up into serotonergic nerve terminals. Here it is converted by the rate limiting enzyme, tryptophan hydroxylase to 5-hydroxytryptophan (5HTP). Serotonin is produced from 5HTP by aromatic acid decarboxylase, and released from the presynaptic nerve terminal into the synaptic cleft.

In 1975, Shopsin et al. administered para-chloro-phenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, to depressed patients in clinical remission. The patients' depressive symptoms returned within one and a half days and remitted after discontinuation of PCPA. To examine the involvement of other neurotransmitters in depression, the authors administered alpha-methyl-para-tyrosine to diminish dopamine and noradrenaline levels to other patients in clinical remission. These patients did not experience any return of depressive symptoms.

The administration of a tryptophan deficient diet to rats showed that the frontal cortex tissue concentrations of tryptophan, serotonin and 5HIAA fell. The tryptophan depletion leads to a decrease in extracellular serotonin and therefore decreased concentrations of 5HT at post synaptic receptors sites (Heslop *et al.*, 1991). Tryptophan depletion in healthy subjects showed mood lowering effects (Smith *et al.*, 1987).

1.4.2.2 Prolactin Response to D-fenfluramine in Depression

Neuroendocrine challenge tests can also be used to study brain serotonin function because stimulation of brain serotonin produces increases in the secretion of certain anterior pituitary hormones, notably prolactin.

D-fenfluramine acts selectively on serotonergic pathways and increases prolactin mediated via release of serotonin in the hypothalamus. Many studies have used this response as an indirect measure of serotonergic transmission in depression, but the results are not conclusive. The prolactin response to D-fenfluramine was reduced in patients with major depression in some studies (Siever *et al.*, 1984; Coccaro *et al.*, 1989; Mitchell and Smythe 1990), unchanged in others (Asnis *et al.*,1988; Maes *et al.*, 1991; Park *et al.*, 1996) and even increased in one (Maes *et al.*, 1989). Flory *et al.*, (1998) found that the blunted prolactin response to D-fenfluramine persisted in the recovered state. The prolactin response to D-fenfluramine increased significantly in major depression treated with ECT indicating enhanced serotonergic responsivity following the therapy (Shapira *et al.*, 1992). This has not been a consistent finding in the literature since Mann *et al.*, (1995) found a blunted prolactin response to D-fenfluramine, whereas Park *et al.*, (1996) found no significant difference between patients and controls.

1.4.2.3 Challenge studies in panic disorder

Experimental evidence links changes in serotonergic transmission in panic disorder (Krystal *et al.*,1996). Three such studies compared the effect of serotonin precursors on neuroendocrine responses in healthy subjects and in patients with panic disorder. Two showed no differences in prolactin release in response to the serotonin precursor tryptophan between patients and controls (Charney and Heninger, 1988; Den Boer and Westenburg,1990). A small study of the effects of D-fenfluramine reported more

anxiogenic responses and greater elevations in plasma cortisol and prolactin among nine panic disorder patients than in a group of healthy control subjects (Targum and Marshall,1989). A study of the cortisol response to 5-hydroxytryptophan did not clearly differentiate panic patients from controls (van Vliet *et al.*,1996). Examination of postsynaptic serotonin function in panic disorder with the mixed agonist-antagonist m-CPP has shown some evidence of serotonergic dysregulation (Charney *et al.*,1987; Kahn *et al.*,1988; Kahn and Wetzler,1991). These neuroendocrine challenge studies do not robustly support the involvement of the serotonin transporter in panic disorder. Binding of ^{3}H- paroxetine to the serotonin transporter in panic disorder was not found to differ from controls or patients with social phobia (Stein *et al.*,1995).

1.4.2.4 Challenge studies in schizophrenia

The prolactin response to L-tryptophan, which is blunted in major depression compared to controls, shows no difference in response between patients with panic disorder, schizophrenia and control subjects (Price *et al.*, 1990).

Clozapine produced a significant attenuation of prolactin and cortisol response to D-fenfluramine challenge. Changes in symptom ratings correlated significantly with the decrease in prolactin response to D-fenfluramine challenge showing that functional alterations occur in the serotonin system following treatment (Curtis *et al.*, 1995).

1.4.2.5 Problems with challenge studies

The problem with such studies is often the lack of selectivity of the challenge agents used and the complexity of the mechanisms controlling the hormones under serotonergic control. They do not permit any definite conclusion about the pathogenesis of the diseases because the effects are equivocal and elicited by non-selective agents.

1.4.3 Indirect measurements of central nervous system function

1.4.3.1 The hypothalamic-pituitary-adrenal (HPA) axis

The HPA axis has been extensively studied in the mood disorders. Hypothalamic hypophysiotropic hormone secretion is controlled by many of the classical neurotransmitters, including serotonin. Components of the neuroendocrine axes, like corticotropin releasing factor (CRF) may contribute to depressive symptomatology. No significant correlations between plasma cortisol levels and platelet serotonin concentrations were found in depressed patients (Pivac *et al.*, 1997). Another study by Mokrani *et al.*, (1997) compared the relationship between the dexamethasone suppression test (DST) status and hormonal responses to D-fenfluramine to examine the interrelationship between the HPA axis and serotonergic systems. Prolactin responses were comparable in suppressors and non suppressors.

1.4.3.1.1 The dexamethasone suppression test (DST)

One method used to assess the activity of the HPA axis is the measurement of cortisol levels. In depression, elevated cortisol levels have been found (Carpenter and Bunney, 1971; Sachar *et al.*, 1970). The dexamethasone suppression test has been used for

biological studies. This test shows that the rate of cortisol nonsuppression after dexamethasone administration is correlated with the severity of the subtype of depression (Schatzberg et al., 1984). DST nonsuppressors have elevated corticotropin releasing factor concentrations in the CSF (Pitts *et al.*, 1990). The DST usually normalizes after recovery from the depression (Nemeroff and Evans, 1984).

The clinical implications of this phenomenon remain contraversial (American Psychiatric Association Task Force, 1987). In addition to depression, patients with dementia (Raskind *et al.*, 1982; Balidin *et al.*, 1983), alcoholism (Oxenkrug, 1978), schizophrenia (Castro *et al.*, 1983; Dewan *et al.*, 1982; Jakovljevic *et al.*, 1998), mania and atypical psychosis (Arana *et al.*, 1983; Coccaro *et al.*, 1984) as well as other disorders exhibit DST non suppression. Other important limitations of this test include that patients have to be hospitilized, and the diagnosis of depression is only accurate in approximately 40% of cases (Brown *et al.*, 1979; Evans *et al.*, 1983).

1.4.3.2 The hypothalamic-pituitary-thyroid axis

Patients with primary thyroid disease have high rates of depression. The use of thyroid hormone has been reported to increase the rapidity of onset of tricyclic antidepressants (Prange and Loosen, 1980), but thyroxine replacement did not affect the binding of imipramine to 5HT uptake sites in any brain region (Tejani-Butt, 1993).

The thyroid stimulating hormone (TSH) response to thyroid releasing hormone (TRH) is considered one of the most sensitive measures of hypothalamic function. 25% of depressed patients exhibit a blunted TSH response to TRH (Prange *et al.*, 1972). The

blunted response is due to chronic hypersecretion of TRH in the hypothalamus. There are reports that show that 15% of depressed patients exhibit an exaggerated TSH response to TRH (Extein *et al.*, 1981).

1.4.4 Direct Measurement of central nervous system structure and function

1.4.4.1 Brain imaging studies

The above studies indirectly assess the function of neurotransmitter systems by the measurement of neuroendocrine parameters. The characterization of neuroreceptor function in the living brain has up until now been hampered by the lack of techniques available for the measurement of physiological events within the human brain *in vivo*.

The advent of computerized tomography (CT) imaging, magnetic resonance imaging (MRI), single photon emission computerized tomography (SPECT) and positron emission tomography (PET) has made this possible.

Using PET, neuronal activity can be characterized by measurements of cerebral blood flow (CBF), oxygen uptake and utilization of glucose. Positron emitting isotopes, like ^{11}C and ^{18}F are attached to selective ligands for different receptor subtypes and quantitative measurements can be performed. Location, density and activity of receptor systems can be measured.

1.4.4.1.1 Brain imaging studies in depression

In major depression, there is decreased cerebral glucose metabolism in the basal ganglia (Baxter *et al.*, 1985). This is consistent with MRI documented morphological abnormalities of the basal ganglia. There are changes in cerebral blood flow on recovery from depression. Improvement in cerebral blood flow was state dependent on clinical remission of symptoms. Currently it is impossible to make a positive diagnosis of depression in individual patients on the basis of CT or MRI findings.

Decreased metabolism of L-tryptophan in brain regions correlated with increased depressive symptoms. Using PET, a decrease in brain metabolism was evident in the dorsolateral prefrontal cortex, thalamus and orbitofrontal cortex. Other studies also report decreased metabolism (Francois *et al.*, 1995; Sackheim *et al.*, 1990), but not all studies replicate these findings (Maes *et al.*, 1993).

Mann *et al.*, (1996) showed direct visualization of blunted regional brain responses to serotonin release in the brain of patients with major depression, a finding that supports the hypothesis of impaired serotonergic transmission in depression.

1.4.4.1.2 Brain imaging studies in panic disorder

With the use of PET, it was shown that patients with panic were vulnerable to lactate infusion, a panic inducing agent (Den Boer *et al.*, 1989; Goetz *et al.*, 1997). There was lower left to right cerebral blood flow ratios in the parahippocampal gyrus of patients susceptible to lactate than to those not vulnerable to lactate and controls. Reiman *et al.*, (1986) replicated their previous findings.

In SPECT studies of panic disorder, whole brain blood flow is increased in patients vulnerable to lactate even at rest. With panic induced by lactate infusion, cerebral blood flow changes were further observed. Patients with panic had lower increases in cerebral blood flow, especially in the left hemisphere, confirmation of the previous findings.

The use of brain imaging techniques has not been extensively used in panic disorder, and most do not appear to show anatomical abnormalities (Llepola *et al.*, 1990).

1.4.4.1.3 Brain imaging studies in schizophrenia

In order to clearly show that $5HT_2$ receptor involvement is a major mechanism of action of neuroleptic treatment in schizophrenia, PET studies have shown that clozapine and risperidone have shown high occupacy of $5HT_2$ receptor sites in regions of the brain (Sedvall *et al.*, 1995; Farde *et al.*, 1995). Proof of serotonin's influence on dopamine came from PET studies which showed that serotonin selective drugs like citalopram can produce changes in striatal dopamine (Dewey *et al.*, 1995).

Although the effect of neuroleptics on $5HT_2$ receptors is well documented in brain imaging studies, PET does not conclusively show that there are any major alterations of $5HT_2$ receptor characteristics in schizophrenia (Sedvall *et al.*, 1995). No significant difference in $5HT_2$ receptor cortical density was observed between schizophrenic patients and controls using PET (Trichard *et al.*, 1998).

This is an excellent and effective technique for measuring receptor number and receptor function together, yet the cost of the isotopes, equipment, and availability of trained technicians make PET expensive and only available in research centres.

1.4.5 The platelet as a peripheral marker of the neuronal cell

Human platelets share several characteristics with CNS neurons which make them convenient models for studying CNS receptors (Stahl, 1977; Da Prada *et al.*, 1988). They have common biochemical and morphological features, allowing for comparisons of both structure and function with CNS neurons. The similarities between the platelet and the serotonergic neuron are robust (see table 1.1). A comparison of noradrenergic and dopaminergic properties with the platelet is less compelling (table 1.2).

Table 1.1 Comparison of properties of platelets and serotonergic synaptosomes (Stahl, 1977).

	PLATELETS	SEROTONERGIC SYNAPTOSOME
Limiting Membrane	Yes	Yes
Mitochondria	Yes	Yes
Storage Granules	Yes	Yes
Active transport mechanism for tryptophan and 5HT	Yes	Yes
Synthesis of 5HT tryptophan hydroxylase	Disputed	Yes
Aromatic amino acid decarboxylase	Disputed	Yes
Catabolism of 5HT	Yes	Yes
Storage of 5HT	Yes	Yes

Table 1.2 Comparison of Properties of Platelets and Noradrenergic Synaptosomes (Stahl, 1977).

	PLATELET	NORADRENERGIC SYNAPTOSOME
Active transport mechanism for tyrosine	No	Yes
Active transport mechanism for dopamine	?	Yes
Active transport mechanism for noradrenaline	?	Yes
Active transport mechanism for adrenaline	?	Yes
Synthesis of tyrosine hydroxylase	?	Yes
Synthesis of aromatic acid decarboxylase	Disputed	Yes
Synthesis of dopamine beta hydroxylase	No	Yes
Synthesis of Phenyl-ethanolamine-N-methyl transferase	?	Yes
Catabolism of catecholamines by MAO	Yes	Yes
Catabolism of catecholamines by catechol-O-methyltransferase	No	Yes

At least three organelles in the platelet are related in function to serotonergic neurons.

a) the cytoplasmic membrane has an active transport system for serotonin with binding sites for drugs and neurotransmitters. The kinetic characteristics for this transport are similar and various drug effects on serotonin transport can be determined and extrapolated as an indication of their action in the brain (Healy and Leonard, 1987).

b) Subcellular organelles which store serotonin

c) Mitochondria with monoamine oxidase (MAO) which catabolizes serotonin

Platelets and neurons have common embryological origins, both derived from neural crest tissue. They are both part of the amine precursor uptake decarboxylation (APUD) system (Pearse, 1986). Platelet vesicles act as storage sites for serotonin that is released by a calcium dependent excitation coupling mechanism. Unlike the nerve terminal, the platelet cannot synthesize serotonin directly, and it depends on the APUD system that contains all the relevant enzymes to drive the processes.

There are major differences though between the platelet and the neuron. One main difference is that nerve terminals are part of a nerve network with more than forty neurotransmitters and neuromodulators playing an essential role whereas platelets are unconnected with other cell types (Leonard, 1992).

The most convincing evidence for the use of the platelet as a peripheral marker of serotonergic neuronal function is that the protein for the human platelet serotonin uptake site and the brain serotonin transporter are identical in structure, and are encoded by the same single-copy gene assigned to chromosome 17 (Lesch *et al.*, 1993).

1.4.5.1 Platelet receptor studies

1.4.5.1.1 Platelet receptor studies in depression

A dysregulation of platelet $5HT_2$ receptors in the affective disorders has been documented using tritiated lysergic acid diethylamide (LSD) binding (Arora and Meltzer, 1989). Platelet $5HT_2$ receptors were found to be significantly elevated in a group of young suicide attempters (Biegon *et al.*, 1990). Hrdina *et al.*, (1997) also found that the density of

platelet $5HT_2$ receptors was higher in depressed patients than in controls. As already mentioned, he suggested that the increased receptor density was a trait of the depressive illness. Treatment with antidepressant drugs of differing pharmacological profile had no significant effect on the density of the receptors and this number did not predict the response to treatment.

Major depression is associated with a reduction in the number of platelet binding sites for ^{3}H-imipramine that increases with antidepressant therapy (Nemeroff *et al.*, 1988). The tritiated imipramine binding site on the platelet is thought to be closely associated with the serotonin uptake site in the brain. In the study by Hrdina *et al.*, (1997) although $5HT_2$ receptor density was not altered with antidepressant therapy, there were changes in serotonin uptake. This was further confirmation of mounting evidence that function of receptor systems and number of receptor sites may be independent entities.

Mikuni *et al.*, (1992) suggested that it is not necessary to have an increase in the number of receptor sites to have an increased response. Serotonin-induced phospho-inositol (PI) hydrolysis is enhanced after subchronic administration of PCPA (Kusumi *et al.*, 1991), while PCPA has little effect on the density of $5HT_2$ receptors (Conn and Sanders-Bush, 1986). Thus, $5HT_2$ receptor mediated intracellular responses may be facilitated independently of the concentration of the receptor.

1.4.5.1.2 Platelet receptor studies in panic disorder

The maximal binding capacity (B_{max}) for ^{3}H-imipramine and ^{3}H-paroxetine was lower in panic patients than controls (Faludi *et al.*, 1994). ^{3}H-5HT uptake into platelets is abnormal in panic disorder (Butler *et al.*, 1992; Norman *et al.*, 1989). Butler *et al.*, (1992) suggested

that the abnormal ^{3}H-5HT uptake and abnormal receptor binding in panic disorder is a trait of the illness.

In order to identify any neurochemical link between anxiety and depression, platelet ^{3}H-imipramine and ^{3}H-paroxetine binding was measured in panic disorder and depression. No significant differences in maximal binding capacity were present between the two disorders (Iny *et al.*, 1994). The data is equivocal and does not clearly show whether abnormal serotonergic dysfunction is present in panic disorder.

1.4.5.1.3 Platelet receptor studies in schizophrenia

B_{max} values of $5HT_2$ receptors on platelets for ^{125}I-LSD showed higher levels in schizophrenic patients than controls. Platelet studies of $5HT_2$ receptors that showed increased receptor number in schizophrenics (Pandey *et al.*, 1993) were not replicated by Arora and Meltzer, (1993) once the statistical corrections for age and sex effects were made.

Comparisons of platelet ^{3}H-imipramine binding in depression, schizophrenia and panic disorder found decreased B_{max} values in all groups compared to controls (Marazziti *et al.*, 1989).

The lack of specificity in these studies is a common denominator. This points to the fact that the measurement of receptor density is not an accurate or physiological means of explaining the pathophysiologies of different psychiatric conditions.

1.4.5.2 Studies of platelet function

The study of augmented platelet intracellular calcium in response to serotonin is well documented in the affective disorders. The $5HT_2$ receptor is closely associated with the increase in intracellular calcium since blockade of $5HT_2$ receptor sites by ketanserin, abolishes the augmented intracellular response (Kusumi, 1993; Mikuni *et al.*, 1992). Noradrenaline does not cause an agonist induced rise in platelet intracellular calcium (Mikuni *et al.*, 1992; Berk *et al.*, 1995), nor does dopamine (Berk *et al.*, 1994).

The measurement of intracellular calcium as a second messenger linked to receptor systems is perhaps the most physiologically accurate means of testing the function of receptor systems as these responses mimic what occurs in the body.

Konopka *et al.*, (1996) found that exaggerated increases in intracellular calcium in response to serotonin were found in depressed patients, in both calcium free and 1 mM calcium media. This suggests that intracellular calcium is the major source of the augmented response to neurotransmitter stimulation. This increased response to serotonin was characteristic of the depressed patients and not shared with schizophrenic or substance abusers, suggesting that the response is specific to mood disorders and not found in all psychiatric conditions. A further suggestion from this study was that since an increased intracellular calcium response was found in both hypertension and depression, the association warranted further study.

1.5 MOTIVATION AND OVERVIEW OF THE THESIS

Approximately 30%-50% of patients with major depression have anxiety disorders (Pini *et al.*, 1997; Nisenson *et al.*, 1998). Patients with panic disorder have a 70% lifetime prevalence rate for major depression. Cognitive dysfunction and psychosis may occur in both depression and schizophrenia. Subsyndromal depression has a one year prevalence of 12 %, exceeding the prevalence rates for all mood disorders combined (Judd *et al.*, 1994).

These alarmingly high comorbidity statistics beg for the identification of a peripheral marker that will differentiate the disorders in terms of their underlying neurobiochemical abnormalities, so that an improved clinical diagnosis and consequently, an improved therapy may be available.

In the literature review, I have shown that the hypothesis of neurotransmitter availability in central synapses as an explanation for psychiatric illnesses was oversimplified. Receptor systems that are linked to neurotransmitter activation have explained the pathophysiology surrounding psychiatric illnesses to a greater extent, but the results are inconsistent.

Once neurotransmitters bind to receptors, they elicit a series of second messenger responses. In this way, neuronal cells communicate with one another. Serotonin is purported to be the major neurotransmitter involved in psychiatric illnesses. Calcium is the most widespread second messenger throughout the body, and there is abundant information available in support of its role in psychiatric illness.

In terms of peripheral markers, examining platelet intracellular calcium in response to neurotransmitter stimulation is probably the most physiologically accurate means of explaining what is occurring in the brain. The platelet has previously been used as a peripheral marker of the neuronal cell.

The measurement of intracellular calcium is made possible because of the fluorescent dye, fura-2. Once inside a cell, it binds to calcium. With the use of a spectrofluorometer, the measured ratios of free calcium to bound calcium are used to determine the amount of calcium released on receptor activation.

There are three sections to this thesis, all encompassing the use of the platelet as a peripheral marker in psychiatric illness. The first section deals with the use of the platelet as a peripheral marker in major depression. The next section deals with the possible use of the platelet as a peripheral marker in schizophrenia, and the final section discusses the use of the platelet to mark the predisposition of certain psychiatric illnesses to cardio-vascular disease. The investigations developed from questions concerning the information already available in the literature. These questions formed the objectives of this study.

Although intracellular calcium in response to 5HT is augmented in major depression, is this response a trait of the depressive illness, or does this response normalize after antidepressant treatment? This question formed the objective of chapter 3 where the issue of state or trait marker status of the platelet in major depression was investigated. Patients suffering from major depression and undergoing a course of ECT as a treatment modality were included in the study. ECT was selected as the treatment modality in order to control for a drug effect on the platelet response. Patients were interveiwed for each week of their

treatment and the platelet intracellular calcium response to serotonin measured. This measurement was performed at the same time of day for each week of the study. Experimentation continued until the remission of depressive symptoms as measured on Hamilton Depression Rating Scales (HAMD-see appendix). The intracellular calcium response to serotonin stimulation was correlated with the HAMD rating scales to see if the response could be used as a state marker of the depression.

The role that calcium influx plays in the augmented intracellular calcium response to serotonin stimulation reported in major depression is not widely documented, although there are conflicting reports concerning the role calcium influx plays in intracellular calcium responses. In chapter 4, calcium influx was measured using two methods, manganese influx and $^{45}Ca^{2+}$. The calcium influx response to serotonin stimulation was measured in patients with major depression and age and sex matched control subjects.

The specificity and selectivity of the augmented intracellular response to 5HT is crucial to its utility as a marker for major depression. Schizophrenics and substance abusers were not found to have any differences in response from controls (Konopka et al., 1996). Augmented responses were seen in bulimia but not in anorexia nervosa where major depression was excluded (Okamoto et al., 1995). The augmented response seen in the anorexia group was only seen if there was concommitant subsyndromal depression, and not in those patients with HAMD's less than 10 (Berk *et al.*, 1997). This perhaps suggested that the augmented response was due to subsyndromal depression.

In chapter 5 of this section of the thesis, the intracellular calcium response to serotonin was tested in a group of patients with subsyndromal depression. This would identify the specificity of the platelet response for major depression.

Another potential issue in the use of the platelet as a peripheral marker in psychiatric illness is to assess if the specificity of the response reflects the pathology of depression, or whether it is a non-selective response present in other disorders.

Panic disorder, as the most common anxiety disorder, often is comorbid with depression. As stated earlier, patients with panic disorder are more prone to develop a major depressive episode. Chapter 6 deals with the selectivity of the platelet intracellular response and was tested in a group of patients with panic disorder without major depression. This was crucial to the establishment of the platelet as a peripheral marker of major depression rather than a non-specific response.

Although a plethora of information exists concerning peripheral markers in depression, there is hardly any information concerning peripheral markers in schizophrenia. Section 2 discusses whether the platelet may be used as a peripheral marker for schizophrenia, using an agonist other than serotonin (Konopka *et al.*, 1996), noradrenaline (Mikuni *et al.*,1992) or dopamine (Berk *et al.*1994) which have not shown encouraging results in terms of their effects on platelet intracellular calcium. These studies, while demonstrating an agonist response, did not differentiate patients from controls.

Glutamate, as the most abundant excitatory amino acid in the brain has recently been implicated in the pathophysiology of schizophrenia. NMDA receptors for glutamate

activation have been identified on platelet membranes (Almazov et al., 1988). Increases in free intracellular calcium in response to agonist stimulation in non-neuronal cell lines have been linked to the NMDA receptor complex (Grant et al., 1997). Kinetic properties of glutamate uptake in platelets and brain slices revealed similar measurements and was stated as sufficiently suited for future clinical studies (Mangano and Schwarcz, 1981).

Platelet intracellular calcium responses to glutamate stimulation were measured in schizophrenic patients and compared to control responses. This would perhaps suggest the use of the platelet as a possible peripheral marker in schizophrenia. It must be stated that the glutamate receptor complex is located on the calcium ion channel itself, and so does not represent a true second messenger response.

The last section of the thesis discusses whether intracellular calcium responses to thrombin stimulation differ between psychiatric illnesses. Recent evidence shows an association between cardiovascular disease and psychiatric illnesses, notably depression. Platelets are implicated in the pathophysiology of atherosclerosis and thromboembolic disorders. An evaluation of the intracellular calcium response to thrombin stimulation in the various mood disorders may suggest the predisposition of certain mood disorders to cardiovascular complications. It is not known if it is an association or causality. This would extend the use of the platelet as a peripheral marker of both psychiatric illness and its linkage with cardiovascular disease.

CHAPTER TWO

2. MATERIALS AND METHODS

2.1 Patient selection

This differed depending on the limb of the study. Each chapter specifies exactly which criteria needed to be met for inclusion in each study. For all groups, patients and controls were between 18-70 years of age. To cancel the subjective influence that various raters may have had on the rating scores, one interviewer was used for all patients and controls within each study. Exclusion criteria included significant medical illness, the use of alcohol or social drugs, and for controls, a family history of mental illness. Raised intracellular calcium is reported in patients with hypertension (Lindner *et al.*, 1987; Sang *et al.*, 1987) and so this was another exclusion criteria.

Informed consent was obtained from all patients and controls that took part in the studies. Ethical clearance was granted by the University of the Witwatersrand Ethics committee for research on human subjects. The ethical clearance number was 29/2/92

2.2 Platelet collection

20 ml blood was drawn from an antecubital vein into 2x 10 ml silicone-coated vacutainer tubes. This blood sample was taken at approximately the same time of day (9am-11am) for all samples to control for any diurnal variation in serotonin release (Pietraszek *et al.*, 1991). Although there are reports that season may affect serotonin concentration in the body (Jakovljevic *et al.*, 1997), it was not possible to collect all the samples within one

season, but controls which were age and sex matched were collected within the same season as the study group tested.

The blood was immediately placed in a 50ml plastic NUNC® centrifuge tube containing an acid-citrate-dextrose (ACD) buffer with 100μM aspirin to prevent platelet activation.

Platelet rich plasma (PRP) was obtained by centrifuging the blood sample at 150*g* for 15 minutes. Thereafter, the PRP was recentrifuged for a further 5 minutes at 850*g* to obtain a platelet pellet.

2.3 The measurement of intracellular calcium in response to serotonin stimulation

2.3.1 Background

Small concentrations of calcium ions that are free within cells can be detected by fluorescent intracellular calcium chelators. When a lipophilic membrane permeant ester of such an indicator is incubated with cells being studied, it enters the intracellular space, where cytosolic esterases split off the ester group, leaving the hydrophilic membrane-impermeant free acid trapped within the cell. In some intracellular indicators, the lipid insoluble form shifts the peak of its excitation spectrum to a shorter wavelength when it binds calcium.

As the intracellular calcium concentration,$[Ca^{2+}_i]$, increases, the shorter wavelength of light excites more fluorescence, while the amount of fluorescence excited by the longer wavelength decreases. By comparing the intensity of fluorescence excited by the shorter

versus the longer wavelength, it is possible to determine how much dye has become bound to calcium and how much remains unbound. The ratio of calcium bound to calcium unbound dye can be used to calculate the actual $[Ca^{2+}_i]$ if the intensity of the fluorescence at the two wavelengths is known at low Ca^{2+} concentrations, when most dye molecules that emit light are calcium free, and at high concentrations when most fluorescent dye molecules are bound to calcium.

Fura-2 is a ratiometric indicator which exhibits not only intensity changes with changing calcium concentrations but the calcium free and calcium bound forms of the indicator have distinct spectra (figure 2.1). For fura-2, significant shifts are observed in the excitation spectra, but not in the emission spectra. Because calcium free and calcium bound forms are characterized by spectral peaks at different wavelengths, intensity measurements can be made at two wavelengths and a ratio can be obtained. Obtaining a ratio minimises the effect of many artifacts that are unrelated to changes in intracellular calcium.

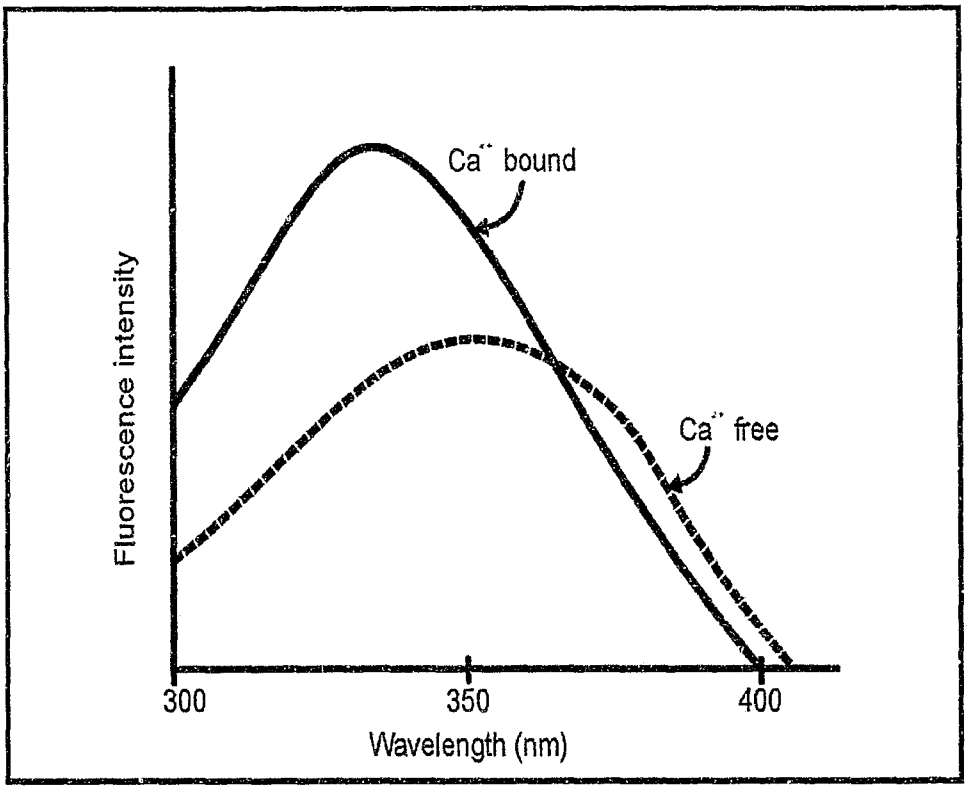


Figure 2.1 Excitation spectra of Ca^{2+} bound and Ca^{2+} free forms of fura-2 (emission wavelength = 510nm).

The measurement of platelet intracellular calcium was adapted from the method by Grynckiewicz *et al.*, (1985). The rationale is that the fluorescence excitation spectra shift to shorter wavelengths as intracellular calcium concentrations increase, much as the absorption spectra do (figure 2.2).

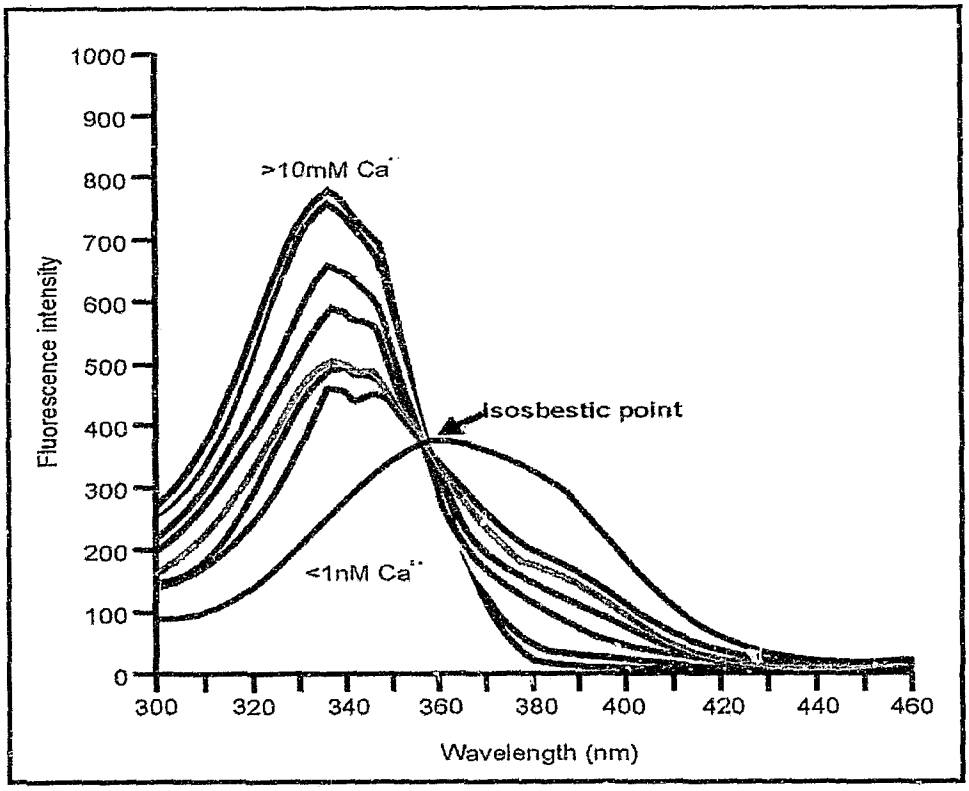


Figure 2.2 Excitation spectra for 4μM fura-2-AM at 20°C in buffer with free Ca^{2+} values ranging from <1nM to >10mM.

2.3.2 Loading of platelets with fura-2-AM

The platelets were incubated at 37°C for 45 minutes with 4 μM final concentration of fura-2 AM in an ASSAY buffer (containing 137 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM dextrose, 5 mM HEPES, pH=7,4). The membrane-permanent acetomethoxyl derivative is used to permeate the plasma bilipid membrane. Once inside the cell, this portion is hydrolysed by the cytosolic esterase leaving behind the now trapped fura-2 which then binds to the calcium inside the cell. The acetomethoxy group masks the negative charges

on the carboxyl groups present in the indicator molecule. The AM form is uncharged and can cross the lipid membrane of cells. The carboxyl groups are essential to the ability of the indicator molecule to sense calcium, so enzymatic hydrolysis to remove the AM group is essential. The negatively charged indicator dye effectively becomes trapped within the cell and binds to calcium.

After the loading period, the excess dye was washed off by centrifuging the sample at 350g for 5 minutes. The supernatant was discarded and the sedimented labelled platelets were resuspended in a HEPES buffer (containing 145 mM NaCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM glucose, 0,5 mM Na_2HPO_4, 1 mM $CaCl_2$, pH=7,55), 1 mM calcium in the buffer mimicks the physiological calcium concentration of the extracellular environment.

Before fluorescence measurements, the platelet suspension was counted in a coulter counter and the final concentration adjusted to 10^7 /ml. The cells were analysed at room temperature since leakage of dye is strongly dependent on temperature, and although 37°C would have been more physiologically suited, the loss rate of dye is maximal at this temperature (Nuccitelli, 1994).

2.3.3 Fluorescence measurements

Measurements were performed in a Perkin-Elmer LS50 spectrofluorometer. 3 ml of platelet suspension was placed in a quartz cuvette with continuous gentle stirring to prevent aggregation. Fluorescence measurements at excitation wavelengths of 340 and 380 nm were measured at an emission wavelength of 510 nm. Excitation and emission slit widths were 2.5 nm and 4.6 nm respectively. Serotonin was added sequentially in

increasing concentrations from 0-100 μM and the fluorescence measured for each concentration. Thrombin (0.1 IU/ml) was then added to measure the maximal calcium response.

To obtain fluorescence values at both wavelengths when there was either saturation or depletion of calcium, the cell suspension was first solubilised with Triton-X 100 and then an excess of 2 mM EGTA at a pH of 8.3 was added. This effectively samples free and bound forms of calcium and acts as a calibration.

The intracellular free calcium concentration was calculated from the equation:

$$[Ca^{2+}] = [(R-Rmin)/(Rmax - R)] \times 224 \times B$$

where: 224 represents the dissociation constant (Kd) ;

R = ratio of fluorescence at 340/380 nm for each experimental measurement;

Rmin = fluorescence ratio of the standard Rmin solution containing EGTA; $F_{340/380}$;

Rmax =fluorescence ratio of the standard Rmax solution containing Triton-X 100;$F_{340/380}$

B = F min 380/ F max 380

2.4 The measurement of calcium influx

The intracellular calcium response may in part rely on calcium influx from the external environment. Two methods of calcium influx measurement are described below. The one uses the divalent cation, manganese, and the other utilizes the more established method of calcium uptake, radiolabelled $^{45}Ca^{2+}$. Two methods of calcium influx were used because of reports stating that it is difficult to be sure that the cell-associated radioactivity measured with radiolabelled calcium is because of calcium influx into the cytosol, or whether the radioactivity measured is bound to the cell surface (Brass and Shattil, 1982).

2.4.1 Measurement of calcium influx using the divalent cation manganese

2.4.1.1 Background

Hallam and Rink, (1985) developed an approach using manganese entry across the platelet plasma membrane to identify calcium influx into the cell. These experiments exploited the fact that manganese binds to fura-2 AM more avidly than does calcium and quenches its fluorescence. It was postulated that any agent that promotes manganese influx into fura-2 loaded cells will cause a resultant reduction in the fluorescence signal.

In fura-2 loaded human blood platelets, agonists have been found to stimulate a quench in intracellular dye fluorescence in the presence of extracellular manganese, from which it can be concluded that agonists can stimulate manganese entry via calcium channels.

Yang *et al.*, 1994; Kass *et al.*, 1990; Serres *et al.*, 1994 decided to not only identify this quench, but to also measure the quenching rate as a measure of the magnitude of the response that different agonists make to manganese entry. The use of serotonin as the agonist to stimulate manganese entry into platelets as an indication of calcium influx was examined in this study. A Medline® search for similar experiments failed to find this application of the method.

2.4.1.2 Loading of platelets with fura-2-AM

Platelets were collected (refer 2.2) and incubated in the same manner as for the intracellular calcium measurements (refer 2.3.2). Once the incubation time was completed though, the cells were suspended in a calcium free assay buffer and the final cell suspension adjusted to 10^7/ml.

2.4.1.3 Calcium influx measurements

Calcium influx was indirectly measured using the ability of manganese (50 μM) to enter platelets and quench intracellular fura-2 fluorescence. 50 μM was chosen as the optimal concentration of manganese to use for these experiments from pilot studies. Higher concentrations of manganese produced quenchs of such magnitude that made measurements of different quenching rates with serotonin addition impossible to measure. The rate of quenching recorded after excitation at a wavelength of 360 nm reflects the manganese influx into the cells. At this excitation wavelength the intracellular calcium modifications do not influence the fluorescence level (see isosbestic point, figure 2.2).

A time drive application of the Perkin-Elmer LS50 spectrofluorometer was used for these experiments. The excitation wavelength was set at 360 nm, with emission at 510 nm. Excitation and emission slit widths were set at 2.5 and 4.6 nm, respectively. The interval and response times were set at 0.5 and 1 second, respectively. The ratio between interval and response times was calculated in pilot experiments that tested which graph had the best smoothness of fit. The scan ran for a period of 60 seconds only since the quench observed was immediate and no further quenching after the initial one was seen in pilot experiments.

The rate of fluorescence quenching is calculated as the slope of the decrease in fluorescence for the first time period where there is a consistent rate of decrease after the addition of $MnCl_2$ (50μM) and is expressed in terms of the fluorescence intensity per second (fluor.int.sec^{-1}).

The experiments initially developed by Hallam and Rink in 1985 have been replicated here, and the effects of serotonin on the manganese quench have been quantitated. Manganese inside the cell can only be present because of influx from the external environment. Figure 2.3 shows how the rate of fluorescence quenching by manganese was measured.

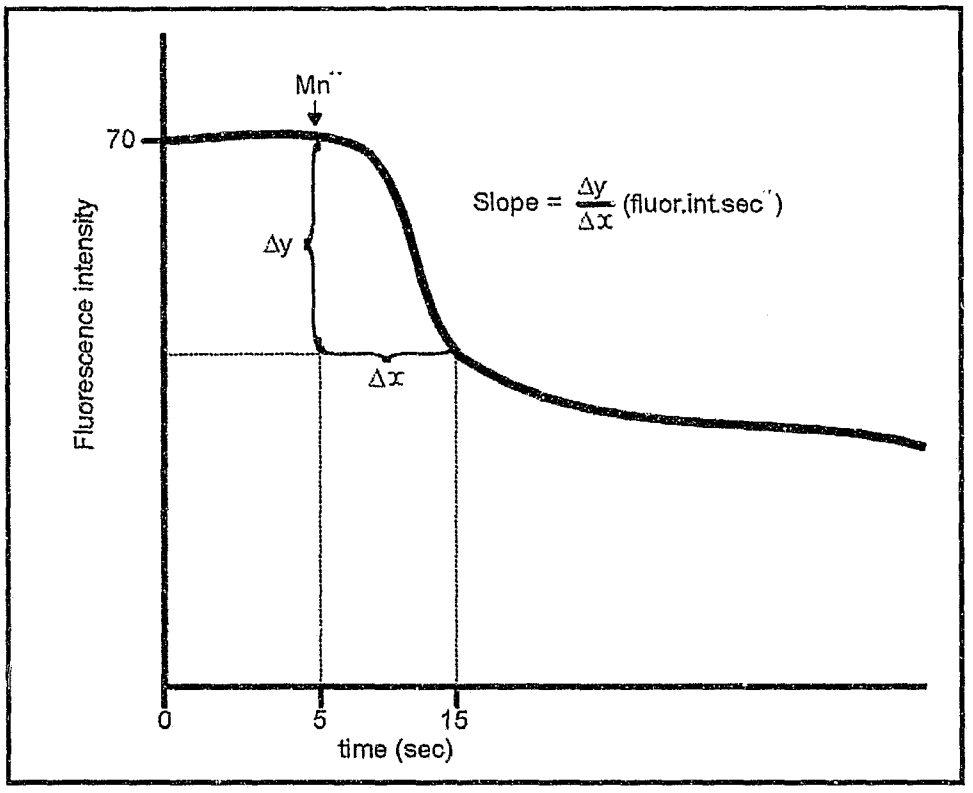


Figure 2.3 The calculation of Mn^{2+} influx into platelets by the rate of fura-2 fluorescence quenching.

The quench in fluorescence with manganese addition is because of: (a) an increase in the permeability of the plasma membrane to Mn^{2+} which enters and quenches the fluorescence of intracellular fura-2, or (b) a non-selective increase in the permeability of the plasma membrane so that there is an increased leakage of fura-2 into the extracellular medium. Such an effect would cause an overall drop in the fura-2 fluorescence because an increasing proportion of the dye would be quenched by extracellular Mn^{2+}.

Experiments like those shown in figure 2.4.1 and figure 2.4.2 suggest that quenching is largely due to Mn^{2+} entry. Following the stimulated quench, the membrane-impermeant heavy metal chelator, diethylenetriaminepentaacetic acid (DTPA) was added in excess. If the Mn^{2+}-quenched dye was extracellular, chelation of extracellular Mn^{2+} should allow the dye to bind Ca^{2+} and cause an increase in fluorescence. However, it is clear that addition of DTPA had very little effect on the fluorescence signal, confirming that most of the Mn^{2+}-quenched dye was intracellular. Addition of Rmax (figure 2.4.3), which lyses the cells, now causes a rapid increase in fura-2 fluorescence since the dye inside the cell is now exposed to excess DTPA which binds Mn^{2+} and thus relieves the quenching effect, leaving the dye to be saturated by calcium. Sufficient DTPA was added to chelate the extracellular Mn^{2+}. When Mn^{2+} was removed by addition of DTPA before stimulation with 5HT, no subsequent stimulated quench was observed (figure 2.4.4).

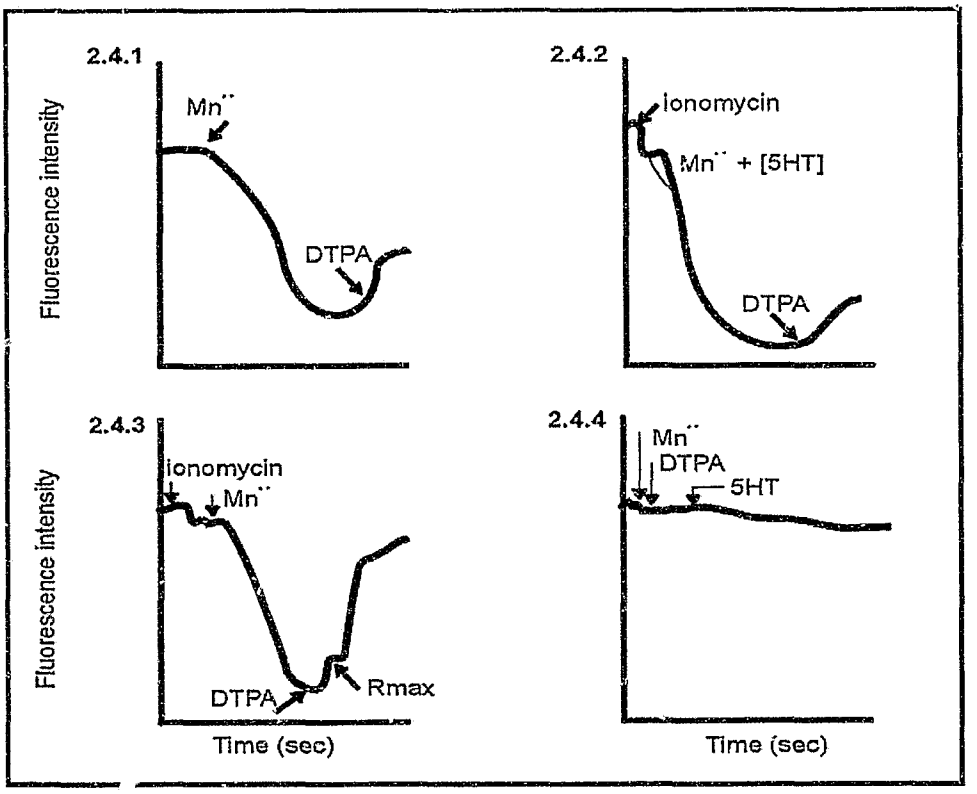


Figure 2.4 Evaluation of manganese influx into platelets.

Figure 2.5 shows the effect of Ni^{2+} on the fura-2 quench. Like Mn^{2+}, this divalent cation also strongly quenches fura-2. On addition of Ni^{2+}, there is a drop in the fluorescence signal presumably due to quenching of leaked dye. Here, DTPA reverses the effect of this quench completely, proving that Ni^{2+} does not enter the cell (figure 2.5.1). When Mn^{2+} is added after Ni^{2+}, a further decrease in fluorescent signal is observed (figure 2.5.2). This suggests that Ni^{2+} is excluded from the divalent cation channel entry system whereas Mn^{2+} enters the platelet through the divalent calcium channel.

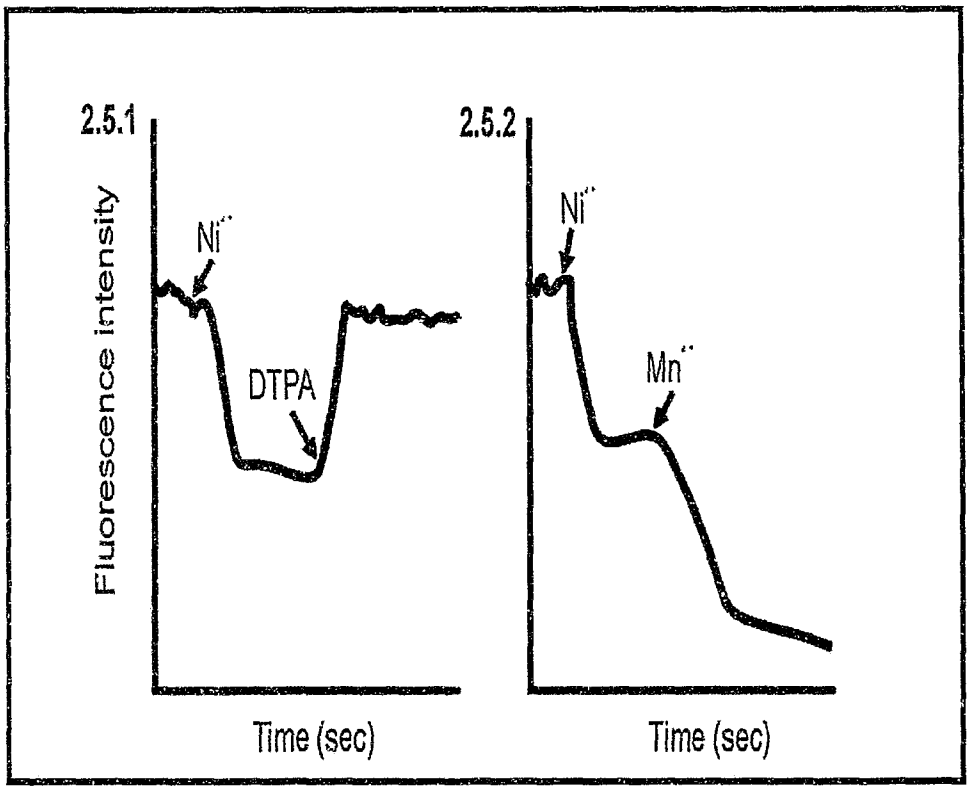


Figure 2.5 The effect of Ni^{2+} on the fura-2 fluorescence quench.

Evidence suggests that this calcium channel is receptor operated, not voltage gated (Hallam and Rink, 1985). This calcium-receptor operated channel may be selective as it does not allow Ni^{2+} to enter the platelet as it does Mn^{2+}

Differing concentrations of serotonin accelerate the rate of Mn^{2+} quenching of fura-2 (figure 2.6). Mn^{2+} (50μM) and 5HT were added to 3 ml of platelet suspension with continuous gentle stirring. Influx was measured as a percentage of the baseline response, where no serotonin was added. This was performed to cancel the effect different baseline values would have had on the significance of the results. This is an indirect method of calcium uptake, and absolute values of quenching rates do not have any meaning. Because of the limited volume of platelet suspension, only three concentrations of serotonin were used as agonists. Concentrations of 1-10μM 5HT are reported to have the most significant effect on intracellular calcium release (Mikuni *et al.*, 1992; Konopka *et al.*, 1996) and so were used in these experiments.

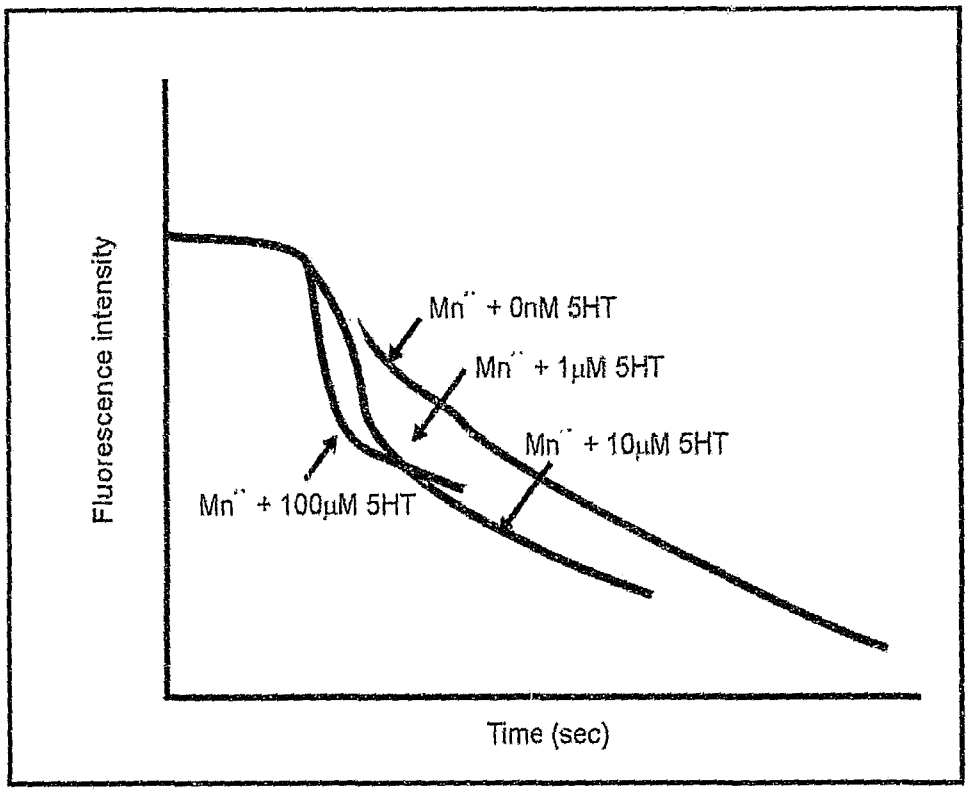


Figure 2.6 The effect of 5HT on the quenching rate of fura-2 by manganese.

In the presence of ionomycin, an ionophore which increases the permeability of the platelet, serotonin is unable to produce any measurable further increase in fura-2 fluorescence quenching, and so was not used in future experiments. (figure 2.4.2).

2.4.2 The measurement of calcium uptake into platelets using radiolabelled $^{45}Ca^{2+}$

This is a more established method of calcium uptake measurement. Calcium uptake was measured using the method established by Owen *et al.*, 1980. This method has been widely used in the literature (Nishio *et al.*, 1993; Gill *et al.*, 1992; Gulati *et al.*, 1992).

Determination of $^{45}Ca^{2+}$ transport into platelets was performed according to the method described by Owen *et al.*, (1980), but with some modification. Platelets were collected as previously described (2.2). A cell count of $50x10^6$ platelets/ml was obtained. After a 30 minute equilibration period at 37°C with 1 mM $CaCl_2$, aliquots (700µl) of platelet suspension were vortexed to ensure equal distribution of platelets in suspension. The suspension was then supplemented with 5µCi/ml $^{45}Ca^{2+}$ and 5HT ranging from 0-100µM was added as an agonist. After an uptake time of 5 minutes (optimal time of uptake- see figure 2.7), 200 µl of platelet suspension was aliquoted into 400µl phthalate oils (Dibutylphthalat: Bis(2-ethylhexyl)-phthalat) in a microfuge tube. This was performed in triplicate.

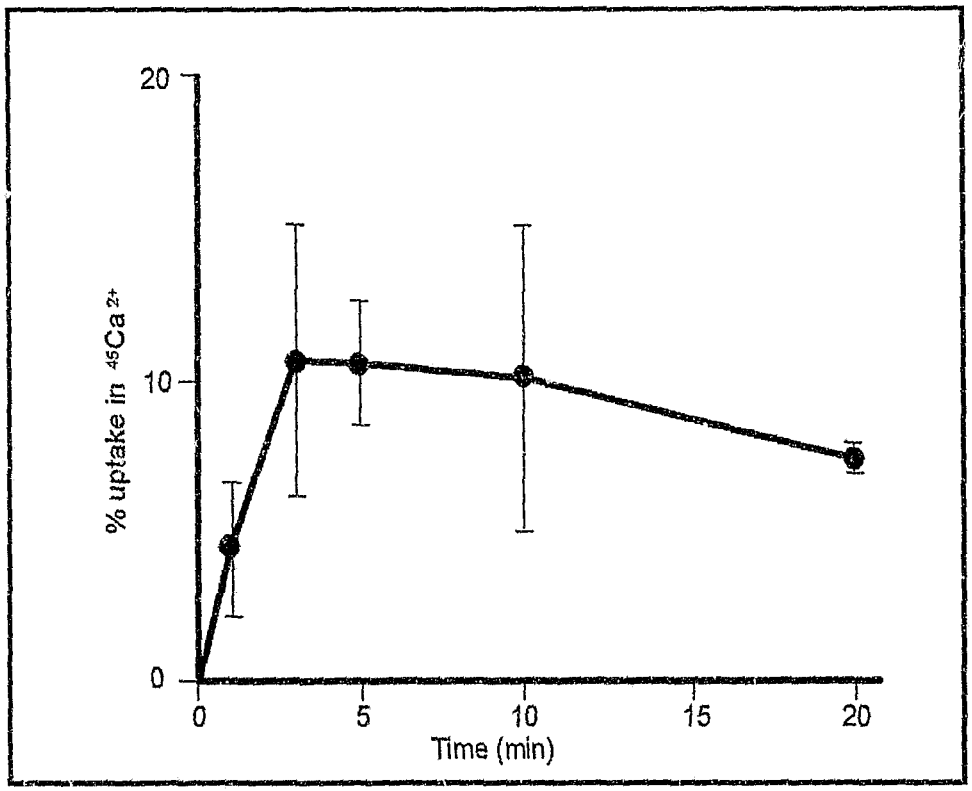


Figure 2.7 Time course of $^{45}Ca^{2+}$ influx into platelets (each point represents the mean +- SD of at least three independent control experiments).

The platelet suspension was spun at 10 000*g* for one minute to separate the platelets from the supernatant and to obtain a platelet pellet. The reaction was immediately terminated by freezing the tube containing the platelet suspension in liquid nitrogen and removing only the platelet pellet by means of an eppendorf clipper. The pellet was solubilized by adding 0.5 ml of 0.5% triton-X 100® and shaken overnight.

5 ml of aquagel® was subsequently added to determine absolute radioactivity (beta emission counts per minute, CPM). This was measured using a beta-liquid scintillation

counter over a two minute period. Uptake of $^{45}Ca^{2+}$ was expressed as a percentage of the baseline value, in which no serotonin was present. Percentage responses were used rather than absolute values in order to cancel the effect different baseline results would have made to the significance of the uptake response. All experimental counts were subtracted from a blank value, where no platelets and no serotonin was present. This cancelled the effect background radiation would have had on the experimental data.

2.5 Statistical analyses

Within each section, the analyses used are described. Non parametric methods of analysis were used throughout the study since sample sizes were less than 30 for all groups except the control subjects. Unless otherwise stated, most analyses have been made using the percentage increase in response from baseline values. This was performed so as to cancel out the effects that different baselines would have on the significance of the increased response. A 'p' value of <0.05 was considered a significant difference in response. This represents a 95% level of confidence. "I" indicates the statistical standard error bars.

RESULTS: SECTION ONE

CHAPTER THREE

3. State or trait status of the platelet as a peripheral marker in major depression

3.1 Aim

The aim of this study was to measure platelet intracellular calcium responses to serotonin stimulation in patients with major depression treated with electro convulsive therapy to assess if this marker changes with clinical improvement. ECT was selected as a treatment modality to control for a drug effect.

3.2 Methods

Patient Selection: Patients aged 18-70 years who met DSM-IV criteria for major depression and who were undergoing ECT as a treatment modality were included in the study. A Hamilton Depression Rating Scale (HAMD-see Appendix A) and a structured clinical interview (see Appendix D) to confirm the clinical diagnosis were performed. Exclusion criteria included significant medical illness, drug or alcohol abuse, and concomitant psychotropic drug therapy except benzodiazepines when necessary. The patients were all drug free with a washout period of at least two weeks (five weeks for fluoxetine). Age and sex matched control volunteers without a history of psychiatric illness and without any medical condition were included as well.

ECT administration: After induction with etomidate and muscle relaxation using suxamethonium, [illegible] were induced using a thymatron DG®, which delivers a pulsed

square wave stimulus. Electrode placement was bilateral. Seizure activity was monitored using EEG and EMG recordings. Treatments were done two mornings a week, and the number of treatments ranged from 6-12 according to response.

Platelets were collected as described in section 2.2. The platelets were suspended in an ASSAY buffer and incubated in the manner described in section 2.3.2. After the loading period the platelets were resuspended in a HEPES buffer described in section 2.3.2. and fluorescence measurements were made according to the procedure outlined in section 2.3.3.

Statistical Analysis: Statistical comparisons for responses before and after ECT treatment were made using Wilcoxon tests for paired samples. Linear regression of dose response data was measured and Pearson correlation coefficients calculated for these analyses.

3.3 Results

Over the time period of July 1996 to March 1998, patients (9 women and 4 men) who met criteria for entry into the study were recruited. The mean age was 48,5 years (range 26-70 years). The mean HAMD score on first interview was 28,3. At the final interview, which took approximately 3-4 weeks, the HAMD score had dropped to 3,0. This finding is in agreement with the study by Strober *et al.*, (1998) who reported that there is a 50% or greater reduction in the HAMD rating scales within three weeks of ECT.

Table 3.1 shows the different patients' information such as age, sex and HAMD scores over the treatment period. Some columns are empty. This is because either the patient's

HAMD score on clinical interview showed clinical remission, and so was at the end point of the study, or the patient was unavailable at the time for the blood test to be performed.

Table 3.1 Age, sex, HAMD scores for patients undergoing a course of ECT

PATIENT	AGE	SEX	HAMD PRE ECT	HAMD WEEK 1	HAMD WEEK 2	HAMD WEEK 3	HAMD WEEK 4	HAMD WEEK 5
1	34	F	32	35	21	10	5	
2	40	M	28	24	15	1		
3	40	F	32	5	10	10	8	4
4	48	M	36	29	6	3		
5	50	F	20		6	3		
6	37	F	20	21	4			
7	50	F	29	17	25	6		
8	55	F	31	12	5	5		
9	44	F	25	20	12	4		
10	67	M	25	27	8			
11	69	F	33	25	10			
12	70	F	29	19	5			
13	26	M	28	18		1	1	1

Baseline platelet intracellular calcium levels did not change significantly over the treatment period (p=0,6002). Before ECT, the mean basal intracellular calcium level was 150,5 +- 61,4 nM whereas after treatment the mean basal level of intracellular calcium was 171,4 +- 68,9 nM (figure 3.1).

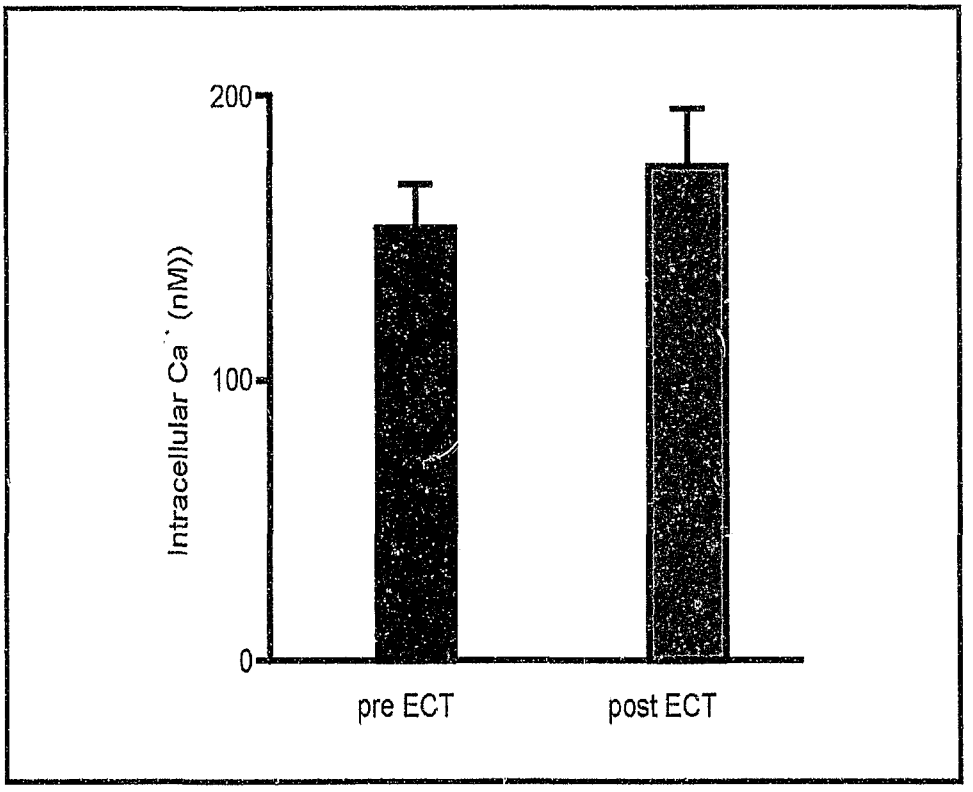


Figure 3.1 Baseline platelet intracellular calcium before and after a course of ECT.

The percentage increase in the intracellular calcium response to serotonin stimulation from baseline values before and after ECT is shown in figure 3.2. The pre-ECT data showed a significant supersensitivity to all concentrations of 5HT whereas post-ECT values showed a blunted response to all 5HT concentrations ($p=0.05$) that was similar to controls. An interesting finding is that the standard deviations were smaller in the post-ECT data compared to the pre-ECT data. A wide standard deviation in intracellular calcium data sets has previously been noted by Dubovsky *et al.*,(1989) who found larger standard deviations in affective disorders compared to control subjects.

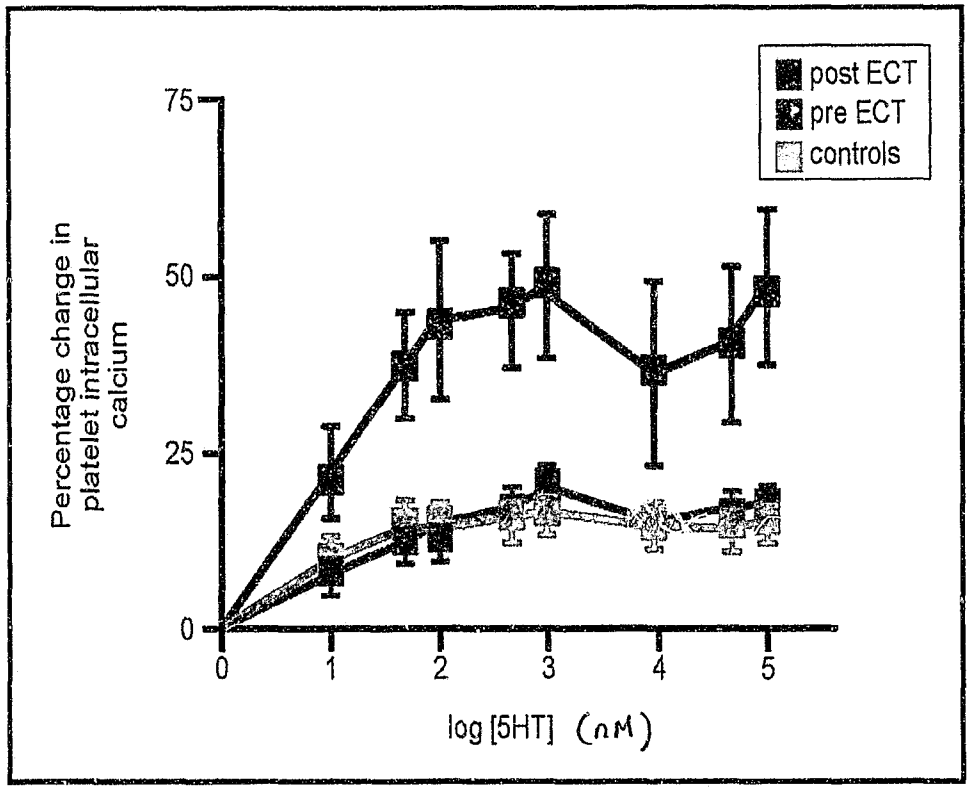


Figure 3.2 The platelet intracellular calcium response to serotonin: percentage increase from baseline values.

A graph of the individual dose response curves for each patient was created to observe whether subgroups of patients within the major depression group were present. The findings, as seen in figure 3.3 show that patient 8 has a significantly higher percentage increase in intracellular calcium in response to serotonin stimulation than all the other patients. Patients 1,2,3,4 and 5 have higher percentage responses than patients 7, 9 and 12. Correlation coefficients for the relationship between differences in intracellular calcium response are poor. The difference is not correlated with the depression score (r^2=0.014), with age (r^2=0.1034) nor with sex. Only two patients were recruited outside of July and

August. This was the winter season, which has been documented as the season in which depression is most common. The only significant difference between the groups is that patients 1 to 5 were recruited in 1996, and the other patients were recruited in 1997. Also, within the HAMD score itself, patient 8 was the only one with no loss in libido. All the other patients scored between 1 and 2 on the HAMD score for genital symptoms compared to 0 for patient 8.

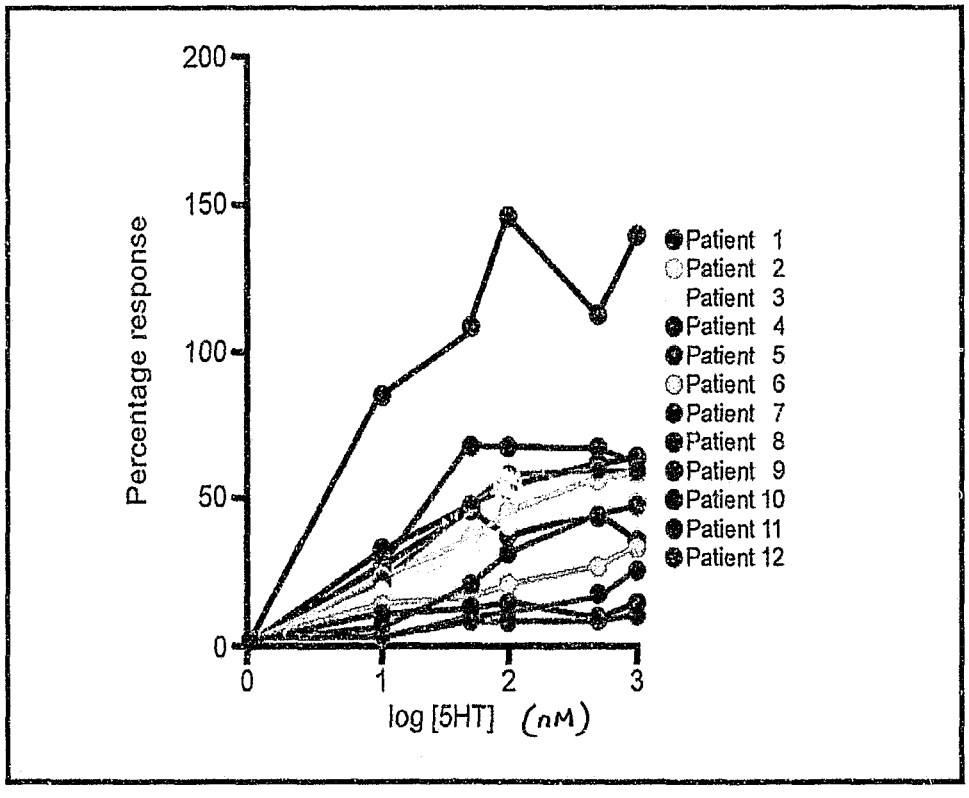


Figure 3.3 Individual patient's intracellular calcium percentage response to serotonin before ECT.

Exclusion of patient 8 from the data does not significantly affect the statistical significance of the difference between pre-ECT and post-ECT responses. Also, the standard deviation between the results is also not significantly altered.

Except for three patients, all platelet intracellular calcium responses to serotonin stimulation decreased over a course of ECT (figure 3.4).

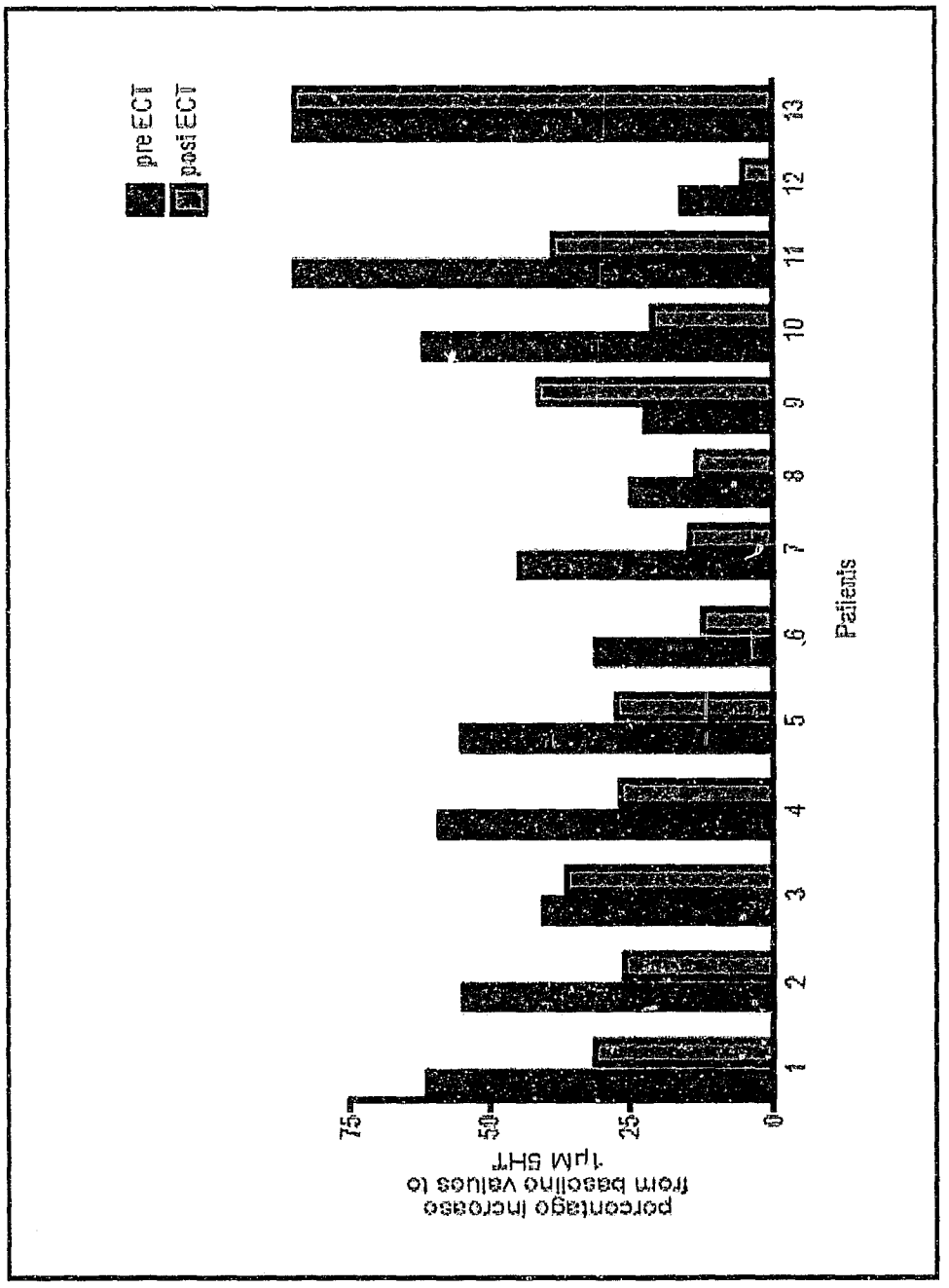


Figure 3.4 The platelet intracellular calcium response to serotonin before and after a course of ECT: percentage response.

Figure 3.5 shows the drop in percentage response to 1μM 5HT over a course of electroconvulsive therapy. This concentration was chosen since it had the smallest standard deviation in the data, and so presents the most consistent results. Pearson correlation coefficients for the percentage drop in response and HAMD scores show strong correlation (r^2=0,85 and 0,87 respectively). A scatter plot of the same data is also presented (figure 3.6). The scatter plot of the values shows that there is a random scatter. This suggests that the regression line used to fit the data is correct, and the correlation is therefore an accurate representation of the data.

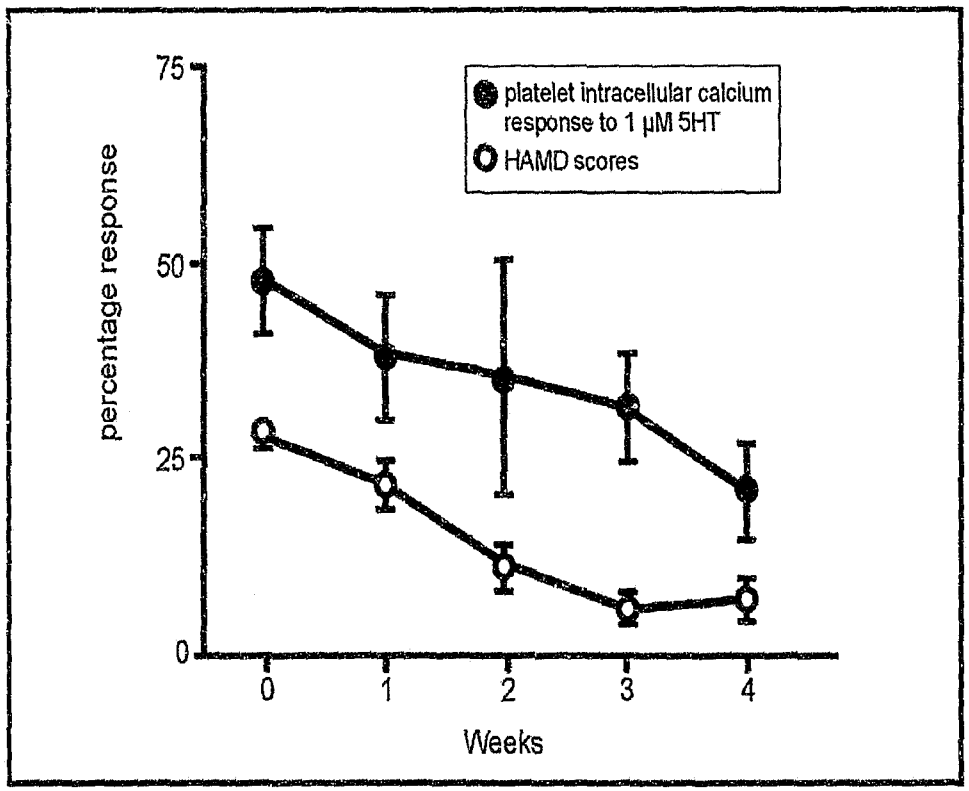


Figure 3.5 The percentage change in platelet intracellular calcium in response to 1μM 5HT over a course of ECT and its relationship with HAMD scores.

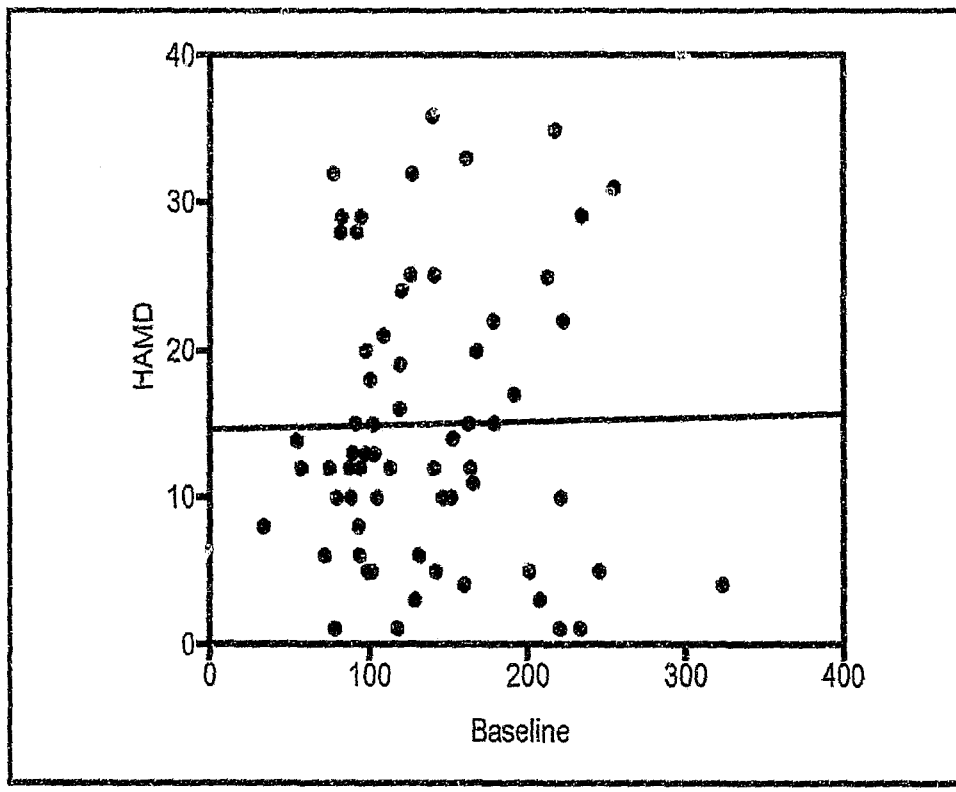


Figure 3.6.1 Scatterplot of the relationship between baseline platelet intracellular calcium response and HAMD scores.

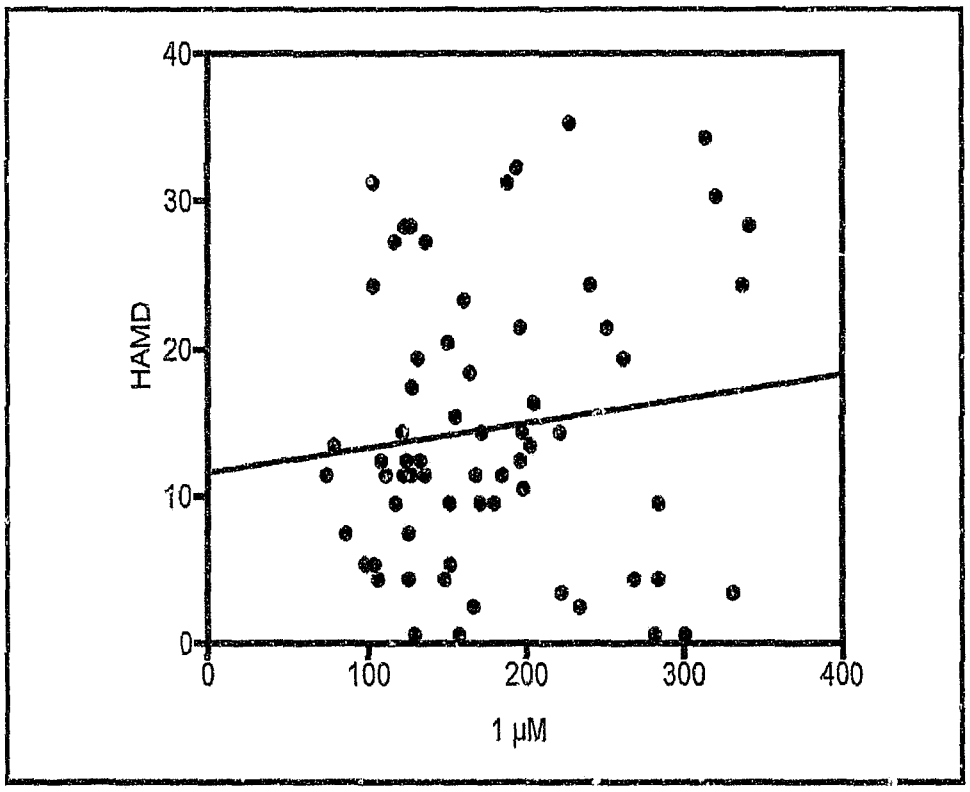


Figure 3.6.2 Scatterplot of the relationship between the platelet intracellular calcium response to 1µM 5HT and HAMD scores.

3.4 Discussion

The primary result of this study is that the platelet intracellular calcium response to serotonin stimulation decreases over a course of ECT (figure 3.2). This decreased intracellular second messenger response to neurotransmitter stimulation occurs in parallel with the decrease in the Hamilton Depression rating scale scores. The patients' intracellular calcium response to serotonin stimulation after the remission of symptoms is similar to an age and sex matched control response.

ECT causes an increase in serotonin neurotransmission, which may result in receptor down-regulation. This is the decrease in receptor number in response to a chronically high concentration of serotonin neurotransmitter. $5HT_2$ receptor down-regulation has previously been correlated with clinical improvement in patients with major depression treated with antidepressant therapy (Stahl, 1992).

The general consensus is that ECT increases $5HT_2$ receptor number (Stahl,1994; Stain-Malmgren *et al.*, 1998), but chronic administration of antidepressants and electroconvulsive shock (ECS) to rats produces a down-regulation of $5HT_2$ receptors which are accompanied by corresponding changes in associated second messenger reactions (Newman and Lerer, 1989). Pandey *et al.*, (1992) showed conflicting results though, that increased serotonin stimulated inositol-phosphate formation associated with upregulation of $5HT_2$ receptors is present after ECS administration.

One way of understanding the diversity of ECT's effects on the function of a variety of neurotransmitters and receptors is to hypothesize that like lithium, ECT stabilizes dysregulated intracellular signalling linked to multiple transmitter systems (Dubovsky and Thomas, 1995; Lerer, 1985).

The $5HT_2$ receptor is linked to the inositol-phospholipid system that is responsible for the elevation of intracellular calcium. Augmented platelet intracellular calcium responses to serotonin stimulation are reported in major depression (Mikuni *et al.*, 1992; Kusumi, 1993; Arora and Meltzer, 1989). This supersensitivity, or increased responsivity, of the platelet to serotonin may occur because of upregulation, that is increased receptor number, which

is reported in untreated major depression (Arora and Meltzer, 1989; Biegon *et al.*, 1987; Hrdina *et al.*, 1995).

Pacheco and Jope, (1996) found that not only were $5HT_2$ receptor changes involved in depression, but a decreased function rather than level of G protein stimulated phosphoinositide signalling was present in the frontal cortex of depressed patients. Hypofunctional G_s and G_i proteins on leukocytes became normalized after ECT. This normalization preceded and thus predicted clinical improvement (Avissar *et al.*, 1998). Bohus *et al.*, (1996) found that the intracellular calcium response, as a measure of the sensitivity of the phosphoinositide system, in neutrophils was significantly greater in patients with major depression than in controls. Intracellular calcium responses to SSRI treatment were measured in a study by Delisi *et al.*, (1998). Once treatment was initiated, lower intracellular calcium in response to serotonin stimulation was observed, yet this did not correlate with a decrease in the level of the patients' symptomatology.

The decreased sensitivity of the second messenger cascade to serotonin stimulation seen in this study appears to be at odds with data suggesting increased $5HT_2$ receptor binding after ECT, since upregulation of receptor sites should translate into increased responsiveness of the target cell. Stahl, (1994) suggested that the $5HT_2$ receptor may have an abnormality in its signal transduction system in major depression such that increased number of receptor sites may be unable to translate the signal of receptor occupancy into the correct physiological response.

The sensitivity of the second messenger system may or may not be linked to the density of $5HT_2$ receptor sites on the platelet membrane (Mikuni *et al.*, 1992; Arora and Meltzer,

1989). A limitation to this study is that $5HT_2$ receptor number was not measured. The decrease in sensitivity of the platelet intracellular calcium response to serotonin may be facilitated independently of the number of receptors.

The data presented in this study suggest that there is a normalization in the function of $5HT_2$ receptor coupled second messenger response over a course of ECT, which is correlated with the HAMD rating scales over the treatment period. Therefore, the platelet intracellular calcium response to serotonin stimulation may be a possible state marker for major depression treated with ECT.

CHAPTER FOUR

4. Calcium influx in response to serotonin stimulation in patients with major depression

Although there is substantial evidence concerning the effect of serotonin and other neurotransmitters on the homeostasis of intracellular calcium in platelets, as well as other cell types, data concerning the effect of agonist stimulation on calcium uptake is very scarce, especially in psychiatric disorder research.

Whether calcium uptake is important in the intracellular calcium response is a contentious issue. Also, whether calcium uptake in response to serotonin is abnormal in patients with major depression is not widely documented in the literature.

4.1 Aim

The aim of this study was based on the hypothesis that if the processes of calcium uptake and intracellular calcium release are linked, then the abnormal intracellular calcium response to serotonin observed in major depression should suggest abnormal calcium uptake in this disorder as well.

The most popular method of measuring calcium uptake is the use of radiolabelled $^{45}Ca^{2+}$. However, it has been reported that it is difficult to be sure that the cell-associated radioactivity is in the cytosol rather than bound to the cell surface (Brass and Shattil, 1982). For this reason, another method of calcium uptake measurement was employed as

well as the $^{45}Ca^{2+}$ method. This utilized the ability of manganese, a divalent cation, to enter the platelet through divalent cation channels in order to quench fura-2 fluorescence more avidly than calcium. A reduced fluorescence signal suggests the uptake of manganese into the platelet, since it is not usually present in the extracellular environment. This represents an indirect method of calcium uptake measurement.

4.2 Methods

Patient Selection: Patients aged 18-70 years who met DSM-IV criteria for major depression were included in the study. A Hamilton Depression Rating Scale (HAMD) and a structured clinical interview to confirm the clinical diagnosis were performed (see Appendix A and D). Exclusion criteria included significant medical illness, drug or alcohol abuse, and concomitant psychotropic drug therapy except benzodiazepines on a when necessary basis. The patients were all drug free with a washout period of at least two weeks (five weeks for fluoxetine). Age and sex matched control volunteers without a history of psychiatric illness and without any medical condition were included as well.

Platelets were collected as described in section 2.2. For manganese influx experiments the procedure followed that described under sections 2.4.1.2 and 2.4.1.3. For radiolabelled calcium influx experiments, the procedure follwed that described under 2.4.2.

Statistical analysis: 5HT stimulated $^{45}Ca^{2+}$ uptake and Mn^{2+} influx between patients and controls was compared using Wilcoxon two sample tests for non-parametric analysis Percentage responses from baseline values were used in all analyses.

4.3 Results

The addition of 50μM Mn^{2+} to a suspension of fura-2 loaded platelets caused a rapid quench in fluorescence due to the entry of manganese into the cells (figure 2.3). When 5HT was added together with manganese the decrease in fluorescence was accelerated in both patients and controls (figure 4.1), signifying increased calcium entry in response to serotonin. 10μM 5HT results in a significantly higher manganese influx in patients with major depression than in control subjects (p=0.04;n=15). A similar pattern of response was seen with 100μM 5HT (p=0.034;n=15). Although mean values of manganese influx at 1μM 5HT are higher in depressed patients (75,13+-79,06%) than in controls (28,48+-15,45%), the standard deviation in the patient sample was too large to show any significant difference.

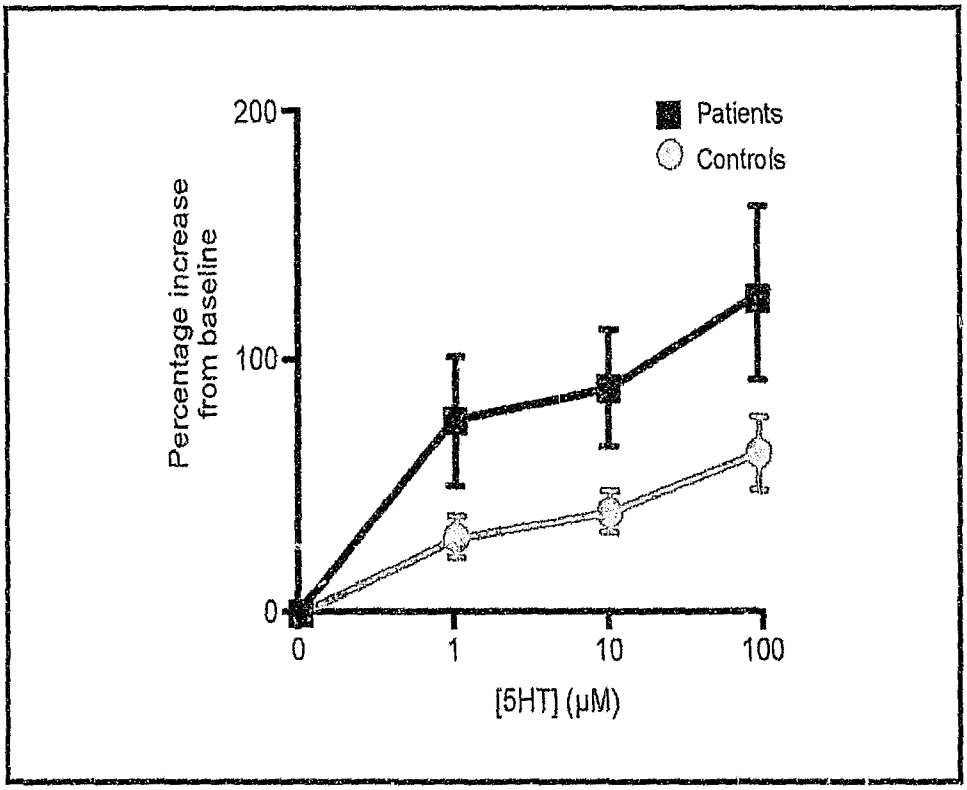


Figure 4.1 Mn^{2+} influx into platelets: effect of 5HT on influx between patients with major depression and controls.

Figure 4.2 shows that calcium uptake in response to serotonin stimulation is significantly greater in patients with major depression than in controls subjects at all 5HT concentrations ($p<0.05$; $n=15$). Control subjects show minimal calcium uptake in response to serotonin stimulation. Only at a concentration of 100μM does the percentage increase in calcium uptake from baseline reach 20,93%. At all other 5HT concentrations, the control response is approximately 3% compared to an uptake of approximately 50% in the patient group.

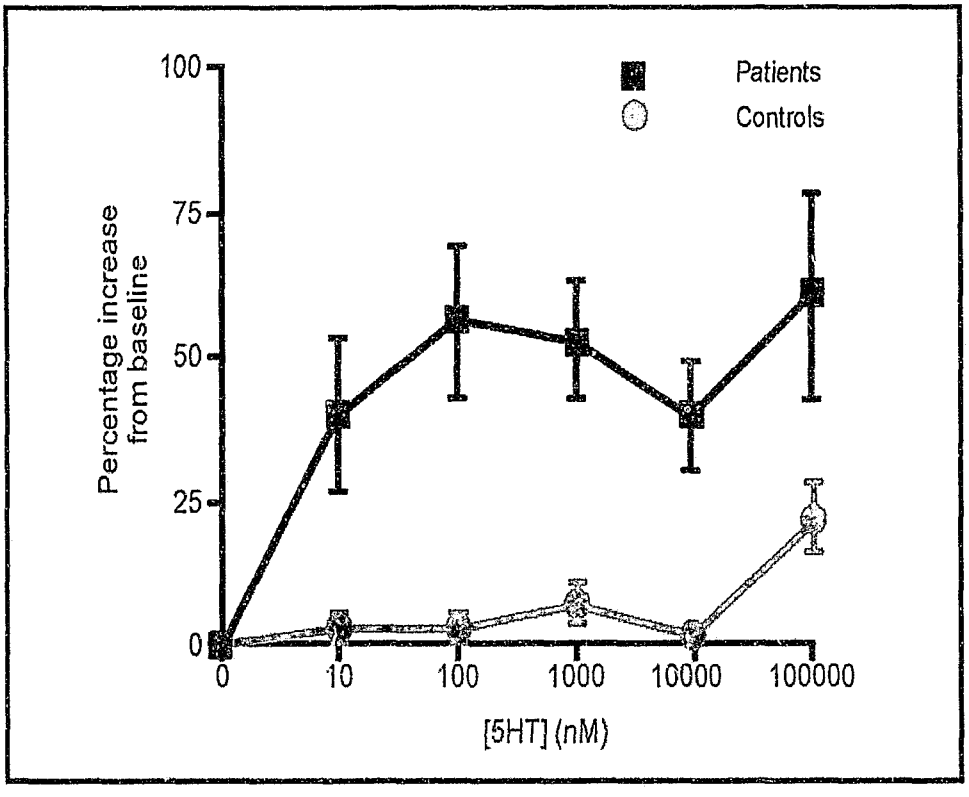


Figure 4.2 $^{45}Ca^{2+}$ uptake into platelets: effect of 5HT on uptake between patients with major depression and controls.

4.4 Discussion

This study demonstrates that 5HT stimulates platelet calcium uptake in a concentration dependent manner. Furthermore, this stimulated calcium uptake response is augmented in patients with major depression compared to controls.

The interesting and pertinent finding of this study is that two calcium influx responses were seen. The initial rapid calcium influx that occurred within the first 10 seconds of Mn^{2+} addition (refer figure 2.3) is similar to other experiments that used manganese as an

index of calcium influx (Merrit et al., 1989; Kass et al., 1990; Hallam and Rink; 1985). To the best of my knowledge, the use of serotonin as an agonist in stimulating this influx into platelets has not been done before.

Nishio *et al.*, (1993) and Gulati *et al.*, (1992) showed that maximal calcium uptake into platelets using radiolabelled $^{45}Ca^{2+}$ also only took place after 5 minutes of incubation (figure 2.7). Nishio *et al.*, (1993) further confirmed that serotonin significantly enhanced the calcium uptake into platelets and proved that this response was mediated by the $5HT_2$ receptor.

In order to explain the results presented in this study, the two pool model proposed by Berridge in 1991 has been used. The model proposes that there are two separate stores of calcium inside the platelet. Inositol triphosphate (IP3) is responsible for setting up the calcium oscillations. IP3 acts through its specific receptor to create a constant influx of primer calcium, made up of calcium from the IP3 sensitive pool inside the cell together with an influx of external calcium. Once the stores have filled, they are triggered to release their stored calcium through a process of calcium-induced calcium release to give a typical calcium spike, seen as an increase in fluorescence excitation in the intracellular calcium experiments. The initial rapid quench in fluorescence observed by Mn^{2+} may represent primer calcium influx stimulated by IP3 which is necessary for the intracellular calcium response (figure 2.3). Merrit *et al.*, (1989) showed in neutrophils that manganese entry via calcium channels correlated with the rise in intracellular calcium.

In Berridge's model, once intracellular calcium is depleted, it causes an influx of external calcium to replenish its depleted stores. The delayed uptake of calcium after 5 minutes

may represent the replenishment of depleted stores once the intracellular calcium spike has occurred.

This study suggests that in major depression there is an enhanced uptake of calcium in response to serotonin (figures 4.1 and 4.2). In major depression, it is already known that there is an augmented intracellular calcium response to serotonin compared to control subjects (refer figure 3.2). Intracellular supplies of calcium may therefore be depleted to a greater extent in the depressed group compared to the control group. A suggestion may be that if the intracellular calcium stores are restored by the influx of calcium from the external environment, the control group which has not significantly utilized its intracellular calcium stores has no need of replenishment explaining the low calcium influx response seen after five minutes. Augmented calcium uptake may be seen in the depressed group because of the need of replenishment of utilized intracellular stores. The augmented uptake response mediated by serotonin observed in the depressed patients may be linked to increased $5HT_2$ receptor activity, further proof of dysregulated serotonergic transmission in the disorder.

CHAPTER FIVE

5. *Examination of the specificity of the platelet response as a peripheral marker in major depression*

5.1 Aim

The aim of this study was to examine the platelet intracellular calcium response to serotonin in patients with subsyndromal depression. This would define the limits of the specificity of the platelet intracellular calcium response to serotonin as a marker of severity of mood disorder, and define the relationship of syndromal and subsyndromal depression in terms of this marker.

5.2 Methods

Patient selection: Patients were defined according to Hamilton Depression Rating Scale (HAMD) scores and DSM IV criteria based on a structured interview. Subsyndromally depressed patients were defined as patients who did not meet DSM IV criteria for major depression but who nevertheless had HAMD scores between 10 and 16 (see Appendix A). Controls were age and sex matched volunteers with a HAMD score less than 7 and no family or past history of depression. Control and patient subjects were drug free for at least two weeks prior to assay, with a five week exclusion period for fluoxetine.

Platelets were collected as described in section 2.2. The platelets were suspended in an ASSAY buffer and incubated in the manner described in section 2.3.2. After the loading period the platelets were resuspended in a HEPES buffer described in section 2.3.2. and

fluorescence measurements were made according to the procedure outlined in section 2.3.3. The only difference in these experiments was that the maximal serotonin concentration of stimulation was 1μM.

Statistical Analysis: Statistical comparisons for responses between patients and controls were made using Wilcoxon tests for paired samples.

5.3 Results

Levels of platelet intracellular calcium were measured, both at baseline and after stimulation with serotonin. The mean level of basal intracellular calcium was virtually identical in the subsyndromally depressed group and the controls (104,66+-28,97 nM vs 104,05+-34,03 nM- figure 5.1).

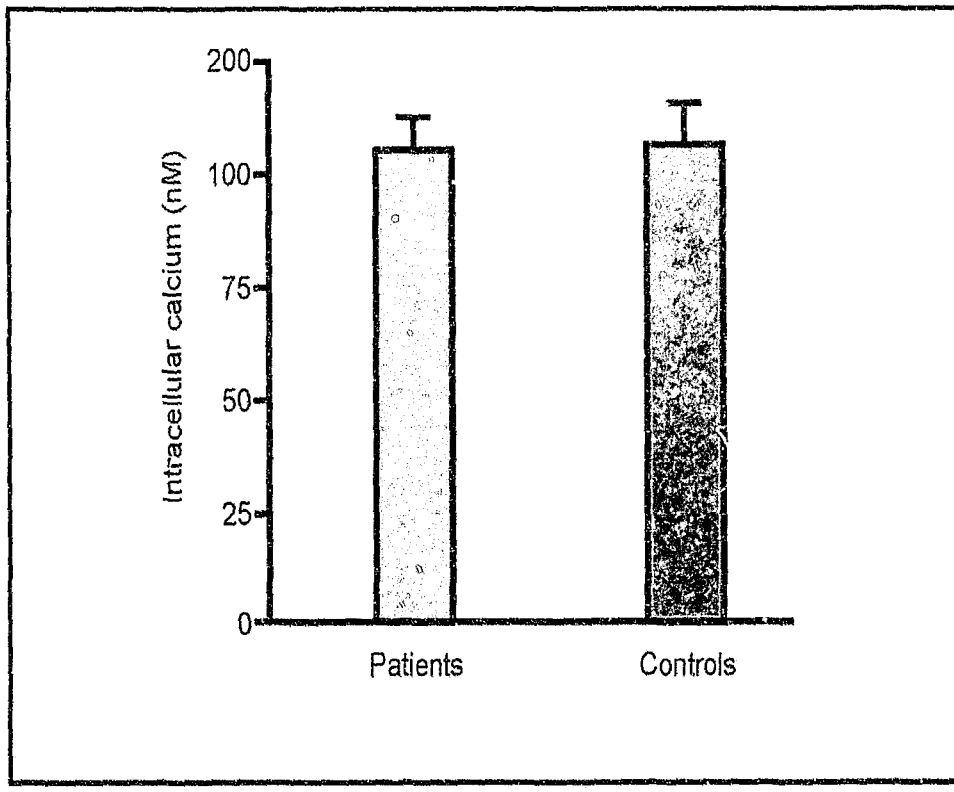


Figure 5.1 Baseline platelet intracellular calcium levels in patients with subsyndromal depression and controls.

There were higher mean levels of serotonin (1μM) stimulated (148,35+-33,14 nM vs 144,79+-42,3 nM; p=0.395) intracellular calcium in the subsyndromal group which similarly did not reach statistical significance (figure 5.2). The percentage increase from baseline to 1 μM (figure 5.3) likewise showed no significant difference in patient and control responses. The lack of statistical proof may be because of the high standard error in the data sets and this is a feature of the literature of this method (Dubovsky *et al.*, 1989). Small differences in response may have been missed because of the relatively small sample size.

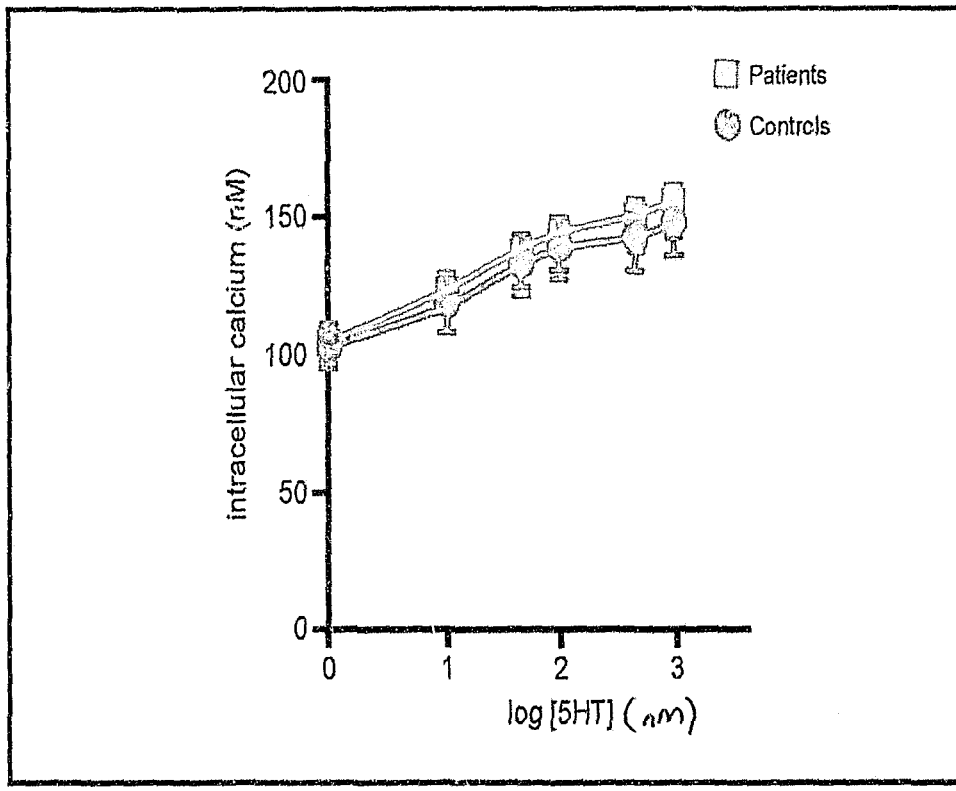


Figure 5.2 Platelet intracellular calcium in response to 5HT in patients with subsyndromal depression and controls.

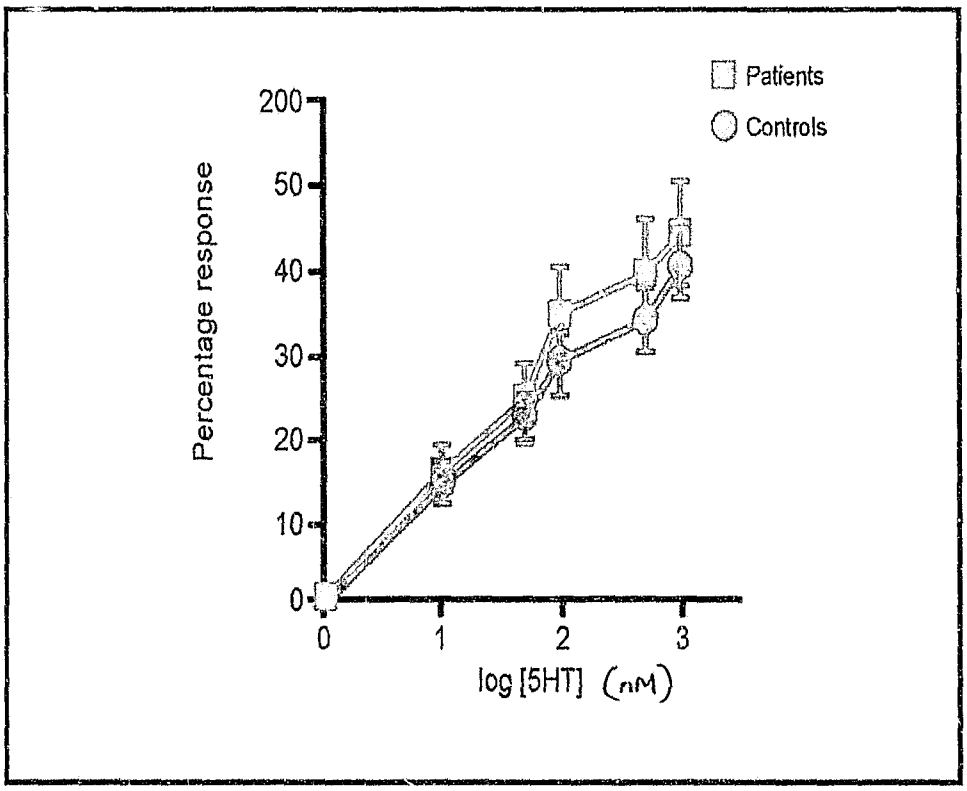


Figure 5.3 Percentage increase in platelet intracellular calcium from baseline in response to 5HT in patients with subsyndromal depression and controls.

5.4 Discussion

An augmented response of intracellular calcium to serotonin stimulation was seen in bulimia, but not anorexia nervosa in a study in which major depression was excluded (Okamoto *et al.*,1995). In a similar study (Berk *et al.*, 1997), an augmented response was seen in only those patients in the anorexia group with HAMD scores between 10 and 17, with patients in the anorexia group with scores below 10 not differing from controls. This

suggested that the augmented response in that group may be due to subsyndromal depression.

The failure of the inter-group differences in basal as well as serotonin stimulated intracellular calcium values to reach significance does not support the hypothesis that platelet serotonin type 2 receptors are supersensitive in subsyndromal depression or that intracellular calcium changes as an indicator of dysfunctional phospholipid transduction systems are in any way affected in this disorder. Although subsyndromal depression and syndromal depression may have similar clinical manifestations, these do not appear to be translated into any biochemical abnormalites of serotonergic neurotransmission.

CHAPTER SIX

6. Examination of the selectivity of the platelet response as a peripheral marker in major depression

6.1 Aim

The aim of this study was to examine the platelet intracellular calcium response to serotonin in patients with panic disorder to clarify whether the above mentioned dysfunction of the serotonergic system is found in panic disorder. This would determine whether the platelet marker was selective for major depression or a non-selective response seen in other psychiatric conditions.

6.2 Methods

Patient selection: Patients aged 18-65 who met DSM 4 criteria for panic disorder were selected for the study. A HAMD and a structured interview to confirm the clinical diagnosis were performed (see Appendix B and D). Exclusion criteria included the presence of another axis 1 diagnosis including major depression. Exclusion criteria included significant medical illness, drug or alcohol abuse, and concomitant psychotropic drug therapy except benzodiazepines when necessary. The patients were all drug free with a washout period of at least two weeks (five weeks for fluoxetine). Age and sex matched control volunteers without a history of psychiatric illness and without any medical condition were included as well.

Platelets were collected as described in section 2.2. The platelets were suspended in an AS AY buffer and incubated in the manner described in section 2.3.2. After the loading

period the platelets were resuspended in a HEPES buffer described in section 2.3.2. and fluorescence measurements were made according to the procedure outlined in section 2.3.3. The maximal concentration of serotonin stimulation was 1μM.

Statistical Analysis: Statistical comparisons between patients and controls were made using Wilcoxon tests for paired samples.

6.3 Results

Patients (n=14, mean age=37 years: 6 males; 8 females) have slightly higher basal platelet intracellular calcium levels than control subjects who were age and sex matched. This difference is not statistically significant, although it is close (p=0.089 n=14,SD=126-figure 6.1). There is a much smaller standard deviation in the control subjects than in the panic disorder patients and this is consistent with the literature of this method (Dubovsky et al., 1989).

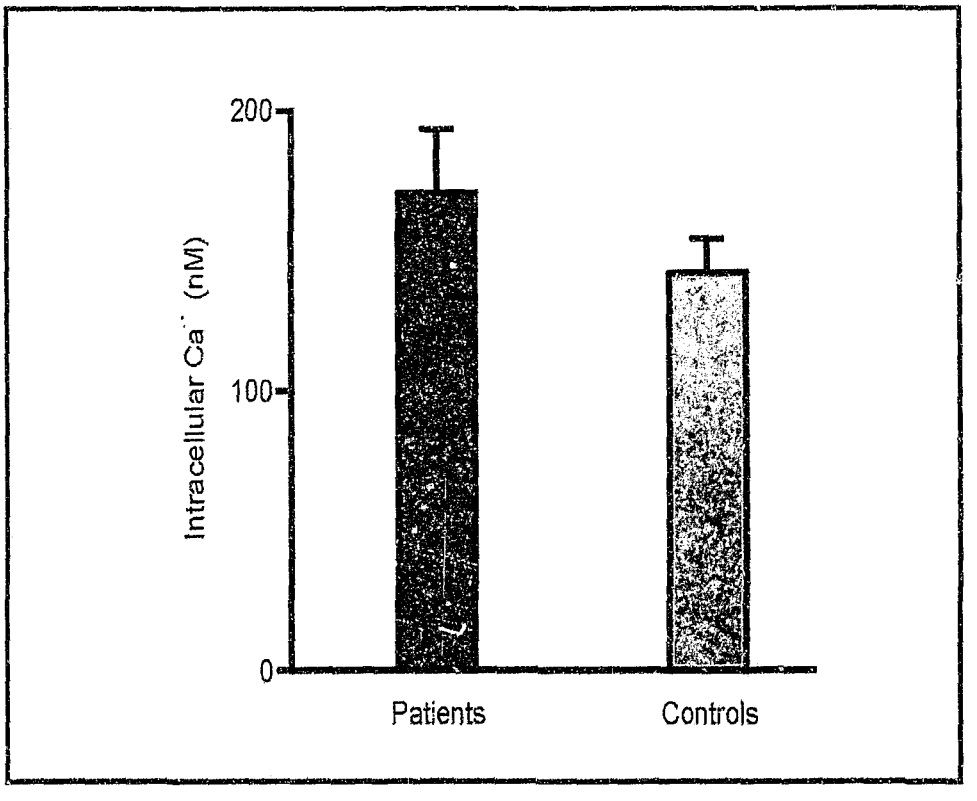


Figure 6.1 Baseline platelet intracellular calcium in response to 5HT in panic disorder patients and controls.

Figure 6.2 shows the differences in intracellular calcium responses between patients and controls at baseline platelet intracellular calcium and in response to 1 μM 5HT. Here too, as in figure 6.1, there are no statistically significant differences at any concentration range ($p=0.164$) between the samples.

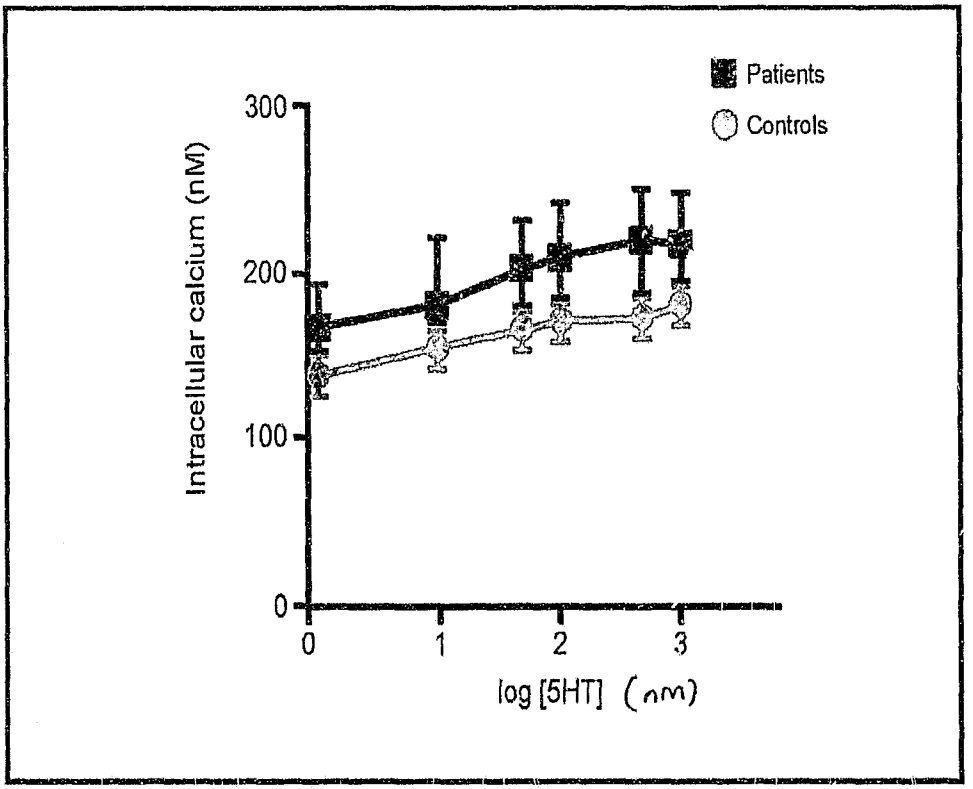


Figure 6.2 Platelet intracellular calcium in response to 5HT in panic disorder patients and controls.

The percentage change in platelet intracellular calcium response from baseline to 1 μM 5HT shows that patients and controls display similar patterns (figure 6.3), but like the graph of absolute values (figure 6.2), no significant difference exists (p=0.304).

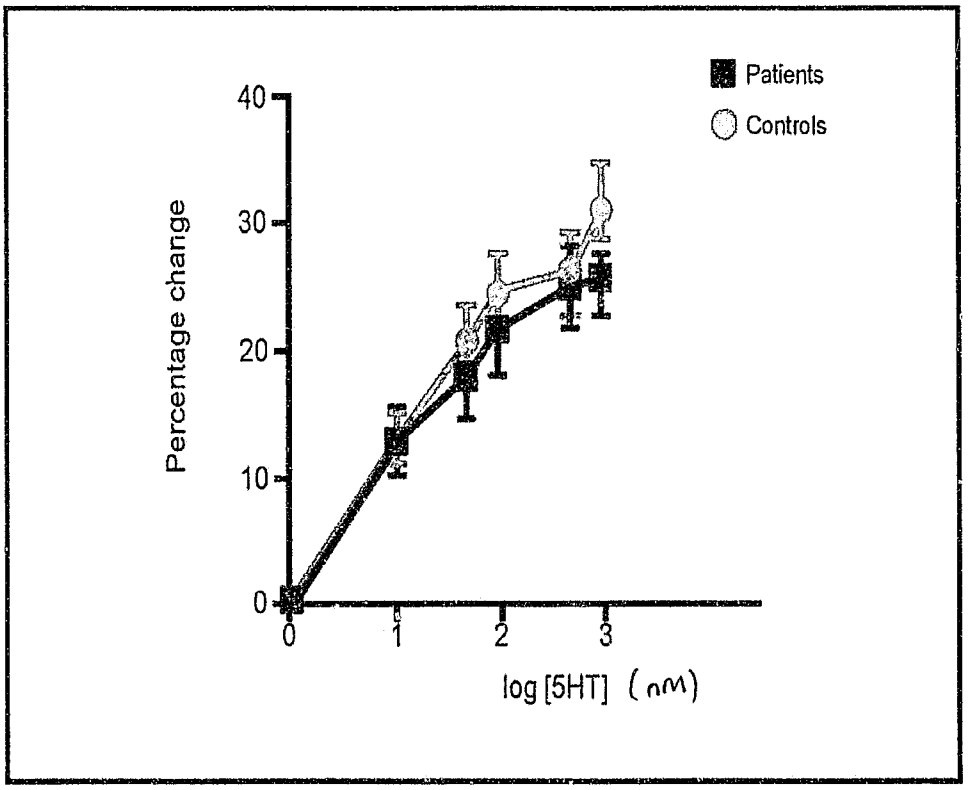


Figure 6.3 The percentage change in platelet intracellular calcium from baseline in response to 5HT in panic disorder patients and controls.

6.4 Discussion

Second messenger involvement in panic disorder is suggested by an enhanced T cell cholecystokinin (CCK) -4 stimulated intracellular calcium response (Akiyoshi *et al.*,1996). In this study there is a trend showing higher intracellular calcium levels in response to serotonin stimulation in panic patients compared to controls, although these levels did not reach statistical significance (figure 6.2 and 6.3). The lack of statistical significance seen

after stimulation with serotonin in this study may be due to the large standard deviations in the patient sample.

Comorbidity of depression and panic disorder is common (Gorman and Coplan, 1996). Although there are differences in second messenger responses seen in major depressive episodes , these patients had HAMD scores which fell below the thresholds of syndromal major depression.. This highlighted the presence of either comorbid subsyndromal depression, or the absence of depressive symptoms. The previous chapter has shown that the platelet intracellular calcium response as a second messenger to serotonin stimulation in subsyndromal depression is not significantly different from control patients (figure 5.2 and 5.3). The lack of statistical significance observed in this study concurs with those findings.

The failure of this study to find enhanced sensitivity of $5HT_2$ receptors in panic disorder and/or altered intracellular phosphoinositide function as measured by the intracellular calcium response is compatible with the findings of previous challenge studies that found no consistent dysregulation of serotonin in this disorder (Charney and Heninger,1988; Den Boer and Westenberg, 1990; van Vliet *et al.*,1996). There is substantial overlap in the literature with respect to the incidence of panic disorder in conjunction with major depression, and the debate continues as to whether they represent different manifestations of one disorder or two separate disorders which tend to overlap. This study suggests that the pathophysiology of panic disorder and depression differ in terms of the involvement of serotonin.

RESULTS: SECTION TWO

CHAPTER SEVEN

7. The platelet as a possible peripheral marker in schizophrenia

7.1 Aim

The aim of this study was to measure the platelet intracellular calcium response to glutamate stimulation in order to assess if the platelet could be used as a marker of schizophrenia. Glutamate was selected as the agonist rather than serotonin and dopamine because these have not shown significant promise in previous studies (Berk *et al.*, 1994; Koniopka *et al.*, 1996).

7.2 Methods

Patient selection: The study group consisted of 16 patients diagnosed on a structural clinical interview (Mini International Neuropsychiatric Interview- see Appendix D) as suffering from schizophrenia, and 16 age and sex matched controls. They were all inpatients at Chris Hani Baragwanath hospital. All patients were drug free for a minimum of two weeks (four weeks for depot preparations). The Brief Psychiatric Rating Scale (BPRS-see Appendix C) was performed on all patients, the mean score was 16.8. The mean age of the sample was 25.4 years of age, there were 13 males and 3 females. Patients with a history of substance abuse or positive urine cannabis assays were excluded, as were patients with any significant history of medical illness. After complete description of the

study to the subjects, all patients gave informed consent. Control subjects were subject to the exclusion criteria as before.

Platelets were collected as described in section 2.2. The platelets were suspended in an ASSAY buffer and incubated in the manner described in section 2.3.2. In experiments designed to block the intracellular calcium response, platelets were incubated with 100μM dizocilpine (MK801). Dizocilpine, which is a non-competitive NMDA receptor antagonist that cannot be displaced by glutamate, has been used in other studies (Grant et al., 1997; Javitt et al., 1996).

After the loading period the platelets were resuspended in a HEPES buffer described in section 2.3.2. Flourescence measurements differed in this study.

Fluorescence Measurements: Glutamate concentrations of 0-100μM were added sequentially. Thrombin (0.1 IU/ml) was then added to measure the maximum calcium response. The cytoplasmic free calcium concentration was measured by the method of Grynckiewicz *et al.*,(1985), that is by lysing the cells with Triton-X-100 in order to obtain maximum fluorescence and then quenching the dye with 2 mM EGTA.

The measurements were initially performed in triplicate, but because of the dye leakage over time, only the initial measurements were used in analysis.

Statistical Analysis: Statistical comparisons for responses between patients and controls were made using Wilcoxon tests for paired samples.

7.3 Results

There was a significant difference between the baseline (unstimulated) levels of intracellular calcium between patients and controls (p=0.03-figure 7.1). The schizophrenic patients had significantly lower mean basal intracellular calcium levels (92,319+- 47,795 nM) than control subjects (148,297+-46,756 nM). Pearson correlations of baseline intracellular calcium in schizophrenic patients with BPRS scores yielded a coefficient of 0,069 which suggests that there is no significant correlation between BPRS values and baseline intracellular calcium. Age and sex were not included in correlation analyses since the sample was too homologous to note any differences in terms of these two variables.

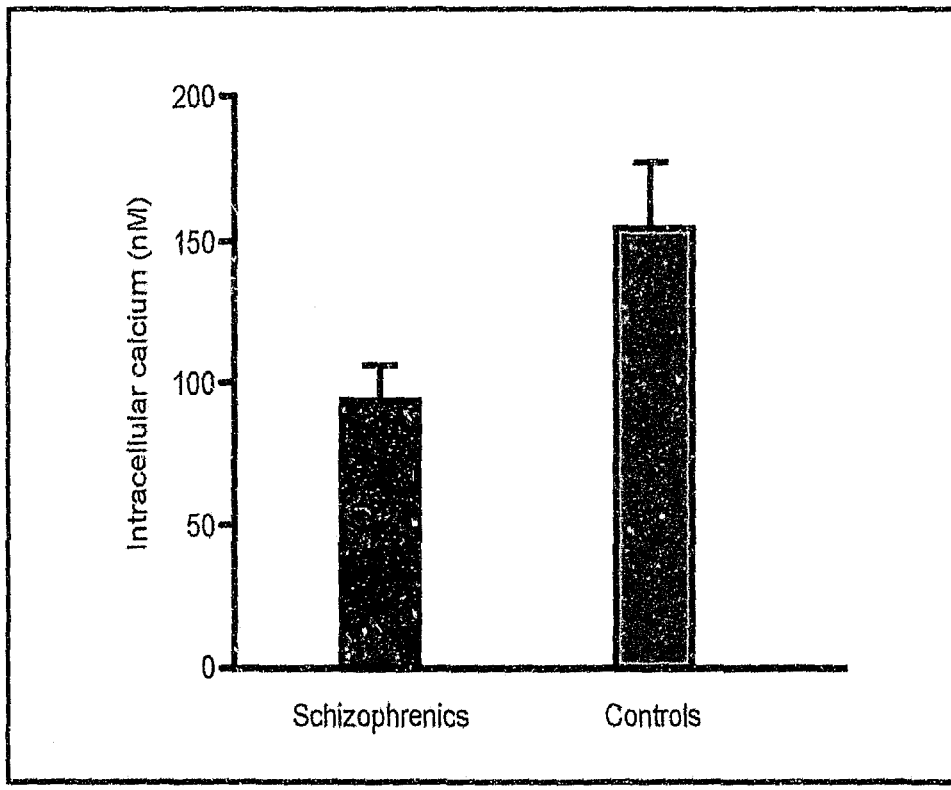


Figure 7.1 Baseline platelet intracellular calcium in schizophrenics and controls.

The actual levels of intracellular calcium did not differ significantly between patients and controls at all concentrations of glutamate stimulation (figure 7.2).

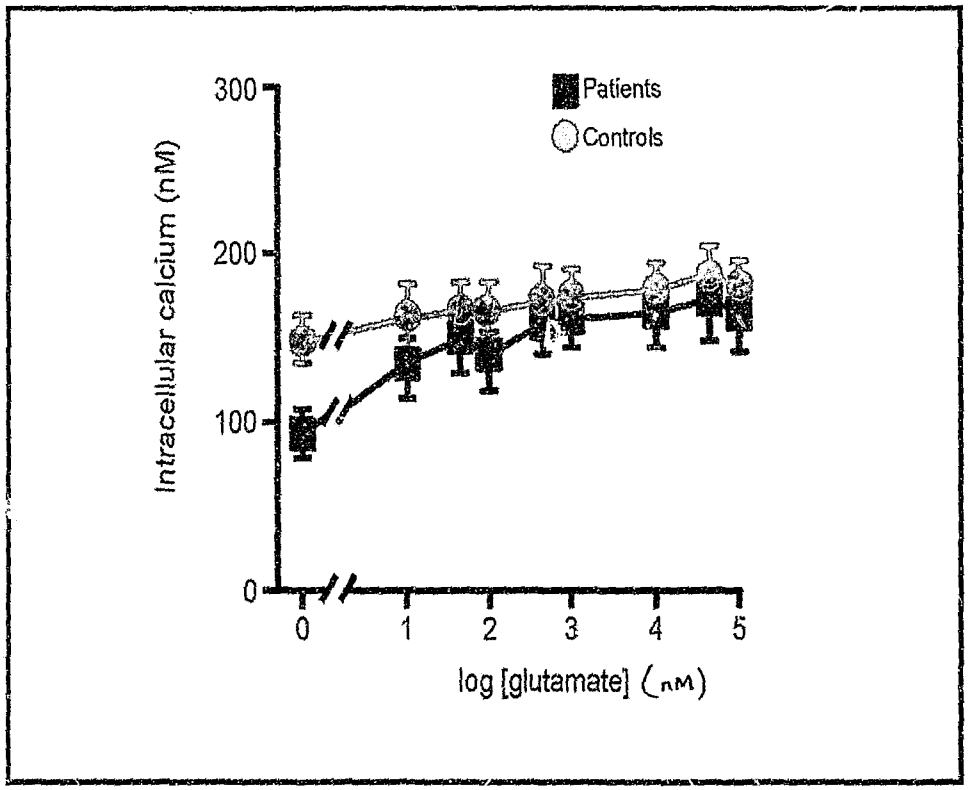


Figure 7.2 The platelet intracellular calcium response to glutamate stimulation in schizophrenic patients and controls.

On the other hand, the percentage response to stimulation with glutamate produced highly significant differences ($p<0.001$) between patients and controls (figure 7.3).

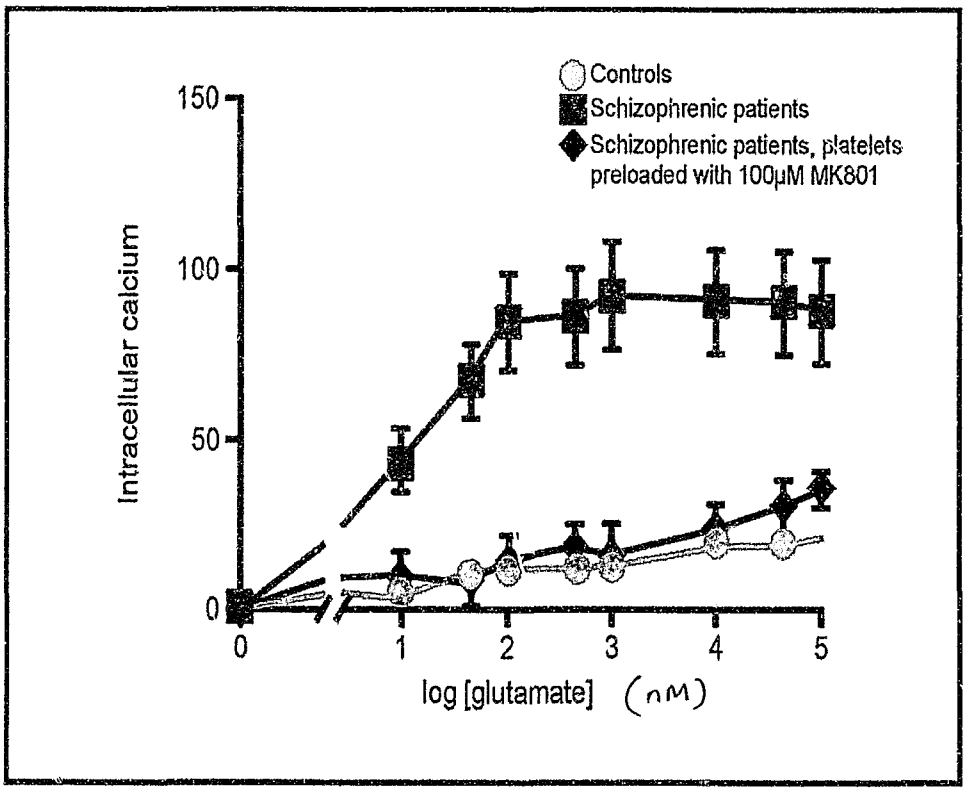


Figure 7.3 The percentage change in platelet intracellular calcium in response to glutamate stimulation between schizophrenic patients and controls.

This may have been because of the much lower level of baseline calcium in the schizophrenic sample (figure 7.1). The maximal percentage response to stimulation is seen at a concentration of 1μM glutamate where the schizophrenic patients have a mean intracellular calcium percentage response of 89,908 +- 57,085% compared to controls who only responded by 11+-5,005%. The NMDA antagonist, dizocilpine (MK801) blocked the increased response in the schizophrenic patients, suggesting that this effect is mediated via the NMDA receptor.

7.4 Discussion

The dopamine theory of schizophrenia is the dominant biochemical theory, but many limitations exist, mostly because of the lack of direct evidence of increased dopamine neuronal activity in clinical studies. Glutamatergic and dopaminergic pathways are thought to be linked, in that they exert mutually antagonistic effects (Lieberman and Koreen, 1993). The hypofunction of glutamatergic neurotransmission in schizophrenia was first proposed in 1980 by Kim *et al.*, who found decreased CSF levels of glutamate in schizophrenic patients. Tsai *et al.*, (1995) supported this hypothesis by demonstrating decreased glutamate levels in the schizophrenic brain. Postmortem studies have also shown increased binding of glutamate recognition sites in frontal cortex tissues. Moreover there is an increased number of NMDA receptors in the basal ganglia of schizophrenic patients (Lieberman and Koreen, 1993).

This study shows lower basal intracellular calcium levels in schizophrenic patients compared to controls (figure 7.1). This has not been found in other studies (Tan *et al.*, 1995) A speculative hypothesis for this finding is that, if excitatory neurotransmission is hypofunctional, and intracellular calcium levels are elevated by glutamate stimulation, then a hypoglutamatergic state may be a factor in the lower basal intracellular calcium levels. The study suggests that there may be increased inositol phospholipid turnover in platelets of schizophrenic patients compared to controls. This concurs with the finding of Gattaz *et al.*, (1995) and Das *et al.*, (1994) who found increased platelet phospholipase A2 in schizophrenia. Ross *et al.*, (1997) showed increased calcium-independent phospholipase A2 activity in serum of patients with schizophrenia. Phospholipase A2 plays an important role in cell signalling, and the observed abnormality may be reflected by impairment in

signal transduction via the inositol phospholipid pathway which may ultimately lead to the behavioural abnormalities observed in this disorder. The mechanism by which calcium is released intracellularly in response to glutamate activation may differ from the other studies. It is not a second messenger response. NMDA receptors for glutamate activation are located on the ionic calcium channel itself.

This study further suggests that the receptor sites for glutamate on platelets of schizophrenic patients may be supersensitive. This supports evidence of decreased glutamate function in schizophrenia, as hypofunction may cause secondary upregulation of receptor sites. The lack of overlap between the experimental and the control curves (figure 7.3) suggests significant sensitivity of this marker, although the specificity of the marker needs to be established by further research. In addition, novel therapeutic approaches involving modulation of NMDA receptors may offer promise in the therapy of schizophrenia.

CHAPTER EIGHT

8. An analysis of the platelet intracellular calcium response to thrombin stimulation in psychiatric illness

8.1 Introduction

Several recent studies have suggested that the presence of depression substantially increases the morbidity associated with cardiovascular disease (Carney *et al.*, 1988; Barefoot and Schroll, 1996; Pratt *et al.*, 1996). Patients with depression have an increased frequency of vascular disease than non-depressed subjects. Depression is highly prevalent in patients with hypertension (Rabkin *et al.*, 1983), coronary artery disease (Carney *et al.*, 1987) and vascular dementia (Sulzer *et al.*, 1993). Depression is a frequent complication of stroke (Mendez *et al.*, 1989). Krishnan *et al.*, (1988) and Coffey *et al.*, (1988, 1989, 1990) noted that elderly patients with depression have white brain matter hyperintensities more frequently than non depressed subjects. These white matter hyperintensities correspond with arteriosclerotic changes of perforating arteries (Braffman *et al.*, 1988).

Although anxiety in general has not been associated with death, there are reports that panic states are associated especially with sudden death over a long term (Haines *et al.*, 1987; Kawachi *et al.*, 1994). Follow-up studies of psychiatric patients with panic disorder have shown an abnormally high mortality rate in men due to cardiovascular and cerebrovascular events (Weissman *et al.*, 1990). There is a paucity of data regarding any associations between schizophrenia and cardiovascular disease.

Thrombin is a potent agonist that elicits platelet physiological responses such as shape change, granular content secretion and aggregation. Activation includes the stimulation of phospholipase C (PLC) and phospholipase A2 once thrombin-receptor associated G-proteins are activated. PLC mobilizes inositol 1,4,5 triphosphate (IP3) which in turn mobilizes the second messenger, Ca^{2+} from the endoplasmic reticulum to the cytosol. The stimulation of phospholipase A2 results also in the production of active molecules. These include the endoperoxidases and thromboxanes that effectively induce further platelet activation. Although the exact regulation of phospholipase A2 is not yet known, Ca^{2+} mobilization is a prerequisite for the activation of phospholipase A2. Increased platelet Ca^{2+} is seen in major depression (Mikuni *et al.*, 1992; Kisumi, 1991).

8.2 Aim

The association between depression and cardiovascular disease is documented, but the selectivity of the increased platelet response in other psychiatric illnesses is not as clearly elucidated. This analysis studied the platelet intracellular calcium response to thrombin stimulation in subsyndromal depression, major depression treated with ECT, panic disorder and schizophrenia to determine the selectivity of the thrombin response in these disorders. This was undertaken to determine an association between psychiatric illness and cardiovascular disease, as well as for the purpose of using the platelet thrombin response as a possible peripheral marker in psychiatric illness.

8.3 Methods

Patient Selection: Patients aged 18-70 years who met the study criteria for major depression, subsyndromal depression, schizophrenia and panic disorder were selected.

Either BPRS scores for schizophrenic patients (Appendix C) or HAMD rating scales for depression (Appendix A) and panic disorder (Appendix B) as well as a structured clinical interview (Appendix D) to confirm the clinical diagnosis were performed. Exclusion criteria included significant medical illness, drug or alcohol abuse, and concomitant psychotropic drug therapy except benzodiazepines when necessary. The patients were all drug free with a washout period of at least two weeks (five weeks for fluoxetine). Those patients on depot neuroleptic medication had a washout period of two weeks. Age and sex matched control volunteers without a history of psychiatric illness and without any medical condition were included as well.

Platelets were collected as described in section 2.2. The platelets were suspended in an ASSAY buffer and incubated in the manner described in section 2.3.2. After the loading period the platelets were resuspended in a HEPES buffer described in section 2.3.2. and fluorescence measurements were made according to the procedure outlined in section 2.3.3.

Statistical Analysis: Kruskal-Wallis tests for overall differences between the groups were performed. Pairwise comparisons between the groups, using Wilcoxon sign tests, were then performed to test where the differences were.

8.4 Results

At baseline, there is no significant difference in the intracellular calcium response between all groups of psychiatric patients and controls ($p>0.05$), except for the schizophrenic patients who had lower baseline levels ($p=0,03$) (figure 8.1, table 8.1).

Table 8.1 The platelet intracellular calcium response to thrombin stimulation in different psychiatric illnesses.

Patient Group	*Patients (n)*	*Mean Age (years)*	*Mean [Ca2+]i At baseline (nM)*	*Mean [Ca2+]i In response to thrombin stimulation (nM)*
CONTROLS	65	27,9	102,05	310,16
SUBSYNDROMAL	16	28,3	113,18	250,74
MDD	13	46,7	110,93	498,04
MDD AFTER ECT	13	46,7	110,93	456,50
PANIC DISORDER	14	37	139,42	621,86
SCHIZOPHRENIA	15	30,5	81,51	329,30

Where MDD= major depressive disorder

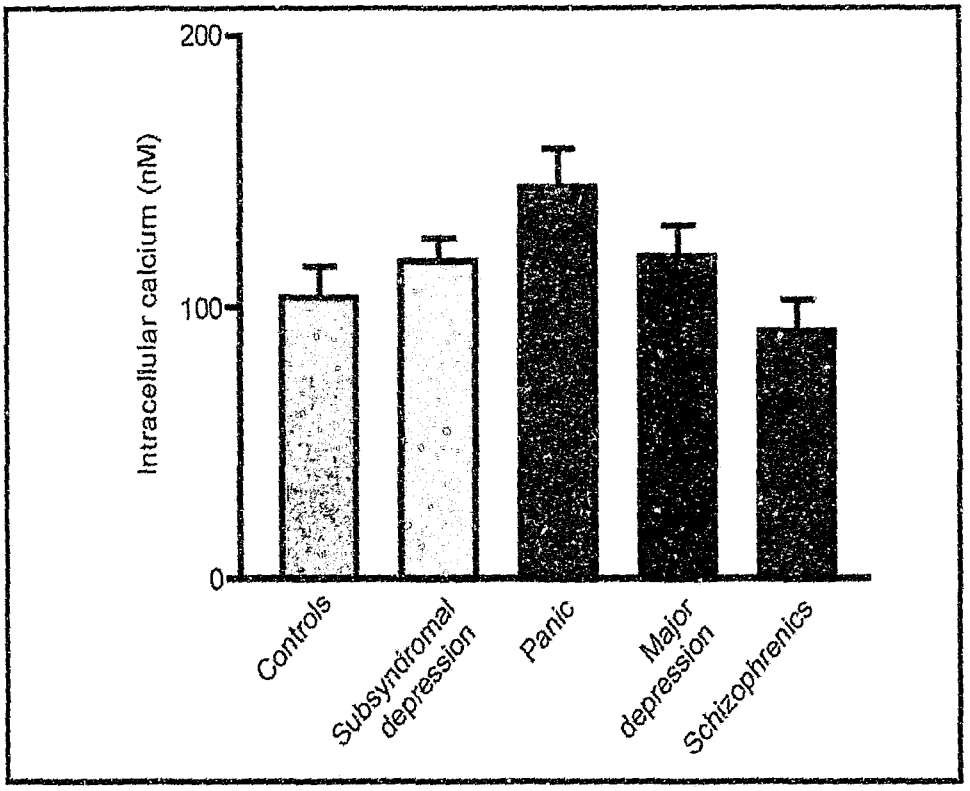


Figure 8.1 Baseline platelet intracellular calcium levels in different psychiatric illnesses.

The intracellular calcium response to thrombin stimulation showed an overall difference between the five groups on the Kruskal-Wallis test (p=0,0002) (figure 8.2). Pairwise comparisons, using Wilcoxon sign tests between the groups showed that the panic disorder group (n=14) presented with the highest intracellular calcium response to thrombin stimulation compared to controls (p=0.000). The mean intracellular calcium level secondary to thrombin in the panic disorder group was 621,86+-136,94nM) which was not significantly different (p=0,148) to the intracellular calcium response in the major depression group ($[Ca^{2+}_i]$= 498,04+-181,62 nM). This suggests that panic disorder has the

highest intracellular calcium response to thrombin stimulation in the disorders tested, followed by major depression.

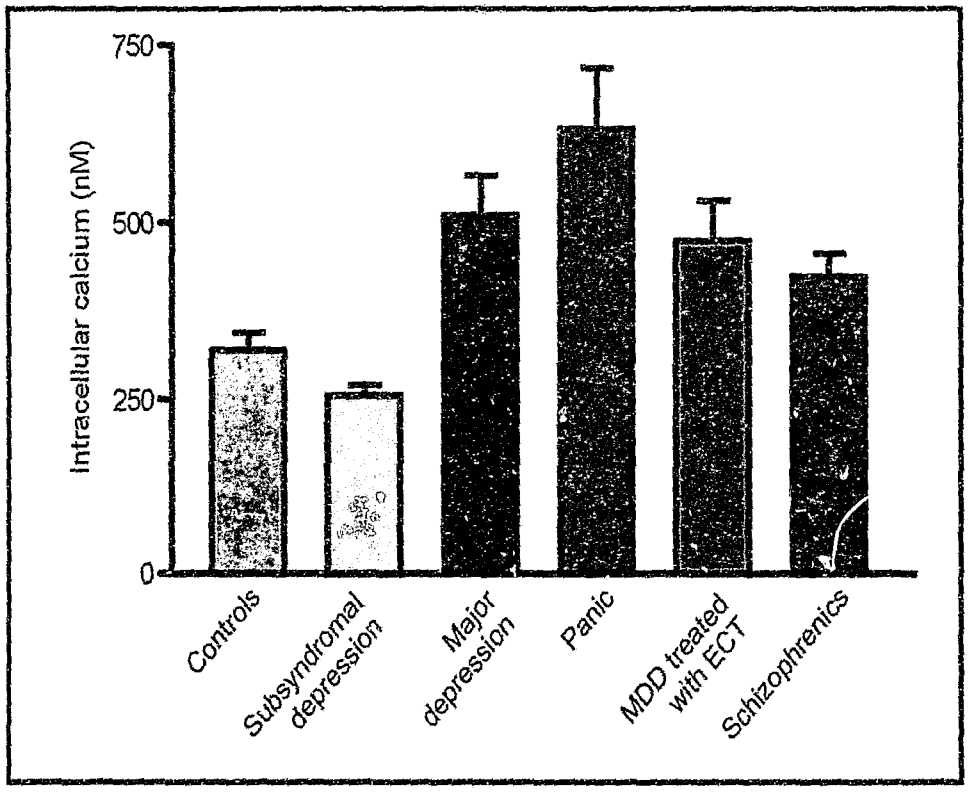


Figure 8.2 Platelet intracellular calcium levels in response to thrombin stimulation in different psychiatric illnesses.

The patients with schizophrenia (n=15) did not have significantly different thrombin responses than control subjects (p=0,51) and neither did the patients with subsyndromal depression (p=0,670;n=16). Mann-Whitney comparisons between males and females showed no statistically significant difference between intracellular calcium levels (p=0,612) across all groups of psychiatric patients and controls.

Although the numeric value for platelet intracellular calcium was lower in the major depression group after treatment with ECT, there was no significant change in the intracellular calcium response to thrombin stimulation (p=0.534).

8.5 Discussion

This analysis suggests that the platelet intracellular calcium response to thrombin stimulation may be used as a peripheral marker in psychiatric illness (fig 8.1 and 8.2). It also supports the suggested association between cardiovascular disease and certain affective disorders, namely major depression and panic disorder.

Panic disorder and depression have significantly increased thrombin responses compared to controls (fig 8.2). In support of this finding, Musselman *et al.*, (1996) and Laghrissi-Thode *et al.*, (1997) also found abnormal platelet function in drug free depressed patients. These studies showed an increased propensity for platelets to aggregate. Noting that platelets are studied as markers for depression, Anda *et al.*, (1993) hypothesized that platelets could represent a mechanism explaining the association between depression and ischaemic disease.

There appears to be a differential thrombin response dependent on the severity of the depressive state. There is no apparent difference in the thrombin response between control subjects and the patients that have depressive symptoms below the threshold for major depressive disorder whereas the major depression group has significantly higher thrombin responses. Delisi *et al.*, (1998) found that increases in platelet intracellular calcium were correlated with symptom levels in depressed patients, especially in those patients that had

high anxiety levels. The authors suggested that since elevations in intracellular calcium mediate platelet aggregation and secretion cascades, the enhanced response observed in depressed patients may contribute to their increased risk of vascular disease. In the major depression group the increased intracellular calcium concentration in response to thrombin did not decrease after a course of ECT, suggesting that this augmented response may be a trait marker of the depressive illness.

Patients with schizophrenia do not differ from control subjects in terms of their intracellular calcium response to thrombin stimulation (table 8.1; figure 8.2).

This study suggests that the platelet intracellular calcium response to thrombin stimulation may be used as a peripheral marker in psychiatric illnesses. This study also lends support to the hypothesis that panic states and major depressive disorders may predispose individuals to cardiovascular disease, although it is not known if this is an association or causality.

CHAPTER NINE

9. Final Discussion and Conclusion

I have attempted to explain certain aspects of neuroscience research that were of interest to me, and were up until the present time unknown. It in no way answers all the outstanding questions and certainly raises many more.

A review of the literature highlighted the progression of research into the biological basis of certain psychiatric disorders such as major depression, panic disorder and schizophrenia. It explained how the mere availability of neurotransmitter in central nervous system synapses was an oversimplified explanation of the pathophysiology involved in the disorders. Receptor studies improved the understanding of the biochemical pathology associated with psychiatric illnesses, but the results of these studies remain equivocal.

The measurement of neurotransmitter levels in the body, or the measurement of receptor density on peripheral markers of neuronal cells does not really explain the complex physiology of neuronal communication that occurs when neurotransmitters activate receptor systems. The examination of second messenger responses may represent a more accurate physiological approach to neuroscience investigation. Intracellular calcium, as a second messenger response serves as an index of the functional capability of the receptor-regulated neurotransmitter response, and may differentiate the psychiatric illnesses to a significant degree, allowing for effective diagnosis and treatment of conditions that have a significant overlap in symptomatology.

As outlined in the literature review, the platelet is justified as a peripheral marker of the neuronal cell. The focus of this thesis was to use the platelet second messenger calcium response to neurotransmitter stimulation as a peripheral marker in psychiatric illness, in order to examine functional abnormalities in receptor regulated second messenger responses specifically in major depression, panic disorder and schizophrenia. Generally, serotonin was used as the agonist. This is because of its close association with the pathophysiology of psychiatric illnesses, and because the platelet and the serotonergic neuron have documented similarities.

The thesis was split into three sections. The first section deals with whether the augmented intracellular calcium response to serotonin stimulation already documented in major depression is a trait of the depressive illness, or whether this response changes with successful treatment. I have shown that this response may represent a state of the depression, because the augmented response normalizes after a course of ECT (figure 3.2). Also, the decrease in responsivity of the intracellular response to serotonin correlates with the decrease in Hamilton Depression Rating Scales (figure 3.5) which measures clinical improvement. ECT was chosen as a treatment modality to control for a drug effect. As one of the reported mechanisms of action of ECT is to increase serotonin levels in the CNS, any difference in the intracellular calcium response to serotonin stimulation may reflect the biochemical changes brought about by ECT as a result of enhanced serotonergic transmission.

Chapter 3 has extensively discussed what may be the explanation behind the decreased sensitivity of the second messenger response after a course of ECT in view of the fact that

second messenger responses are linked to receptor systems, and $5HT_2$ receptor density is reported to increase with ECT. Without measuring the density of $5HT_2$ receptors, which was not performed in this thesis, I believe that the best explanation may be that either receptor density and receptor function are independent entities and increased receptor density may be a homeostatic correction secondary to dysfunctional second messenger transduction.

The issue of whether calcium uptake is involved to some extent in the augmented intracellular calcium response was examined in the next chapter of section one. The hypothesis was that if calcium uptake is involved in intracellular calcium release, then calcium uptake in response to serotonin should be augmented in major depression as well.

The hypothesis was correct. Augmented calcium uptake in response to serotonin was observed in patients with major depression (figure 4.1 and 4.2). With the use of two methods of calcium uptake, it was noted that there is an immediate influx of calcium within the first ten seconds of manganese addition into the medium (2.3). Also, there was another calcium uptake process occurring after five minutes (figure 2.7). This evidence may support the two pool model for calcium oscillations inside cells (Berridge, 1991). The initial calcium influx may represent primer calcium influx and may be partly necessary for intracellular calcium release. Once intracellular stores are depleted of calcium, there is another uptake process to replenish the depleted stores. The uptake is greater in depressed patients because of the increased utilization of intracellular calcium seen by the augmented intracellular calcium response in major depression.

Chapter 5 of section one dealt with establishing whether or not the intracellular calcium response to serotonin was specific for major depression or seen in subsyndromal depression as well. The introductory review mentioned that there is the belief that the two disorders are on a symptomatic continuum and may be expressions of the same disease. The issue was whether the clinical similarities were expressed in a similar neurobiological dysfunction as well. The evidence suggests that the underlying dysregulation of second messenger responses to serotonin seen in major depression are not present in subsyndromal depression (figure 5.2 and 5.3). No dysregulation of intracellular calcium homeostasis was seen in these patients. The intracellular calcium response to serotonin was similar to a control group that was age and sex matched. This suggests that although the clinical characteristics of major depression and subsyndromal depression may be similar in clinical presentation, they are distinctly different in terms of biological dysfunction. Therefore the platelet intracellular calcium response to serotonin may be a specific peripheral marker in major depression.

A crucial issue in the establishment of the platelet response as a peripheral marker was to examine its selectivity. Panic disorder often has comorbidity with major depression. The literature review showed that similar drugs treat both conditions. Serotonin dysfunction, among other biological substrates, has been implicated in the pathophysiology of this disorder. Whether serotonin dysfunction in both disorders leads to the high incidence of comorbidity is still unknown. The literature review also cautioned that although similar neurotransmitter systems may be involved in different illnesses, this does not necessarily mean that the mechanisms underlying the disorders are identical. The platelet intracellular calcium response to serotonin was measured in patients with panic disorder without major depression to test whether there were any similar alterations in functional capacity in terms

of serotonin. This would determine the selectivity of the response. The study failed to show any dysregulation of platelet intracellular calcium to serotonin in patients with panic disorder. The dysregulation of serotonin which is stated in this disorder is not seen in this peripheral marker (figure 6.2 and 6.3). The study suggested that the platelet intracellular response to serotonin may be specific and selective for major depression.

Calcium uptake in response to serotonin was not measured in the subsyndromal group or the panic disorder group. The justification behind this was that since there were no intracellular calcium response differences between these disorders and control groups, no alteration in calcium uptake would be present either. This is perhaps a limitation of the study and future research might look at these aspects.

Section two deals with the use of the platelet as a peripheral marker in schizophrenia. There is very little encouraging research available on effective peripheral markers in schizophrenia. Dopamine and serotonin as the classical neurotransmitters involved in schizophrenia do not show any significant effect on the platelet intracellular calcium response in this disorder. New evidence suggests that hypofunctional glutamatergic neurotransmission may play a role in the pathogenesis of schizophrenia. Glutamate receptors have been found on platelet membranes. The NMDA receptor is found on the protein complex of the calcium ion channel. These factors prompted the investigation of the intracellular calcium response to glutamate stimulation as a possible peripheral marker in schizophrenia. It must be emphasized that this does not represent the classical second messenger response seen in the other studies.

The intracellular calcium response to glutamate stimulation was significantly augmented in schizophrenic patients (figure 7.3). If the increased sensitivity of the intracellular calcium response is linked to upregulation of NMDA receptors on the calcium ion channel, then the evidence supports the hypoglutamatergic theory of schizophrenia, and suggests the use of the platelet as a possible peripheral marker in this disorder. The specificity of this response in other psychotic disorders would be the next step into the research of this platelet marker.

In the next section, intracellular calcium responses to thrombin in the different disorders were analysed. Thrombin was used in all the experiments to elicit maximal calcium release before the cell was solubilized with Triton-X 100. On analysis, patients with major depression and panic disorder had significantly higher sensitivity to thrombin stimulation than patients with schizophrenia and subsyndromal depression whose responses were not significantly different to controls (figure 8.2).

The serendipitous findings support the growing hypotheses of associations of certain psychiatric disorders with cardiovascular disease. Patients with anxiety and depression have a high incidence of cardiac disease. Thrombin plays a crucial role in platelet activation and is therefore associated with cardiovascular disease. Intracellular calcium is raised in essential hypertensive patients and patients with major depression. Although the clinical relevance of this association can be appreciated, it remains unknown which disorder may cause the other. Future research needs to be done in this field.

The evidence that there is an augmented intracellular calcium response to thrombin stimulation in panic disorder suggests that some abnormality in signal transduction exists

in the disorder which may be unrelated to serotonin dysfunction. Also, the persistently high intracellular calcium response to thrombin seen after a course of ECT suggests that this response may represent a trait of the depressive illness.

These results suggest that thrombin and serotonin exert their effects on second messenger signal transduction via different mechanisms. Suffice it is to propose that the intracellular calcium response to thrombin may be used as a peripheral marker which shows the predisposition of certain psychiatric illnesses to cardiovascular disease.

Calcium changes inside the platelet are a reflection of the activity of second messenger responses to receptor stimulation. Using the platelet as a marker, examination of intracellular second messengers allows for the understanding of the functional capability of the receptor regulated signal transduction mechanism employed by cells for communication of neuronal information. In this way it represents the most physiological approach to the study of biological dysfunction in psychiatric disorders.

This thesis cannot possibly have studied every psychiatric disorder in terms of second messenger response to neurotransmitter stimulation of receptors. By choosing the disorders I did, I hope I have justified the platelet as a peripheral marker which has specificity and selectivity in its response. Future research should be aimed at discovering the specificity of the platelet response to glutamate in other psychotic disorders. Also, the association of cardiovascular disease and psychiatric illnesses warrants further investigation.

I do believe that it is reasonable to suggest that intracellular second messenger responses in psychiatric illnesses may underlie the pathogenesis of the disease processes and

modification at this level will allow for better understanding and treatment of the conditions.

Appendix A- Rating Scale for Depression

Patient..... Age.... Sex

HAMD Grading Scores:
(0-4) 0: absent 1: mild 2: moderate 3: severe 4: very severe
(0-2) 0: absent 1: slight or doubtful 2: clearly present

Depressed mood (0-4):
Feelings of guilt (0-4):
Suicidal impulses (0-4):
Insomnia early (0-2):
Insomnia middle (0-2):
Insomnia late (0-2):
Work and Activities (0-4):
Retardation (0-4):
Agitation (0-4):
Anxiety psychic (0-4):
Anxiety somatic (0-4):
Somatic symptoms gastrointestinal (0-2):
Somatic symptoms general (0-2):
Genital symptoms (0-2):
Hypochondriasis (0-4):
Loss of weight (0-2):
Insight (0-2):
Diurnal variation (0-2):
Depersonalization (0-4):
Paranoid symptoms (0-4):
Obsessive and compulsive symptoms (0-2):

Total score: 0-7 = no depression
8-15 = subsyndromal depression
16 or more = major depression

DSM IV (Yes/No)
Depressed mood
Loss of interest
Weight/appetite
Sleep change
Agitation/retardation
Fatigue
worthlessness/guilt
concentration
death/suicide thoughts
major depression = >5 yes
SSD = 2-5

Exclusion Criteria (Yes/No)
Drug free (1 week)
Fluoxetine (5 weeks)
no other medicines
no medical illness
no hypertension
no drug abuse
Family/past history of
mental illness (controls)

Melancholia (Yes/No)

Anhedonia
Loss of reactivity
Distinct quality
Diurnal
Terminal insomnia
Retardation/ Agitation
Anorexia/ weight loss
Guilt

Appendix B- Rating scales for panic disorder

Patient..... Age.... Sex

HAMD Grading Scores:
(0-4) 0: absent 1: mild 2: moderate 3: severe 4: very severe
(0-2) 0: absent 1: slight or doubtful 2: clearly present

Depressed mood (0-4):
Feelings of guilt (0-4):
Suicidal impulses (0-4):
Insomnia early (0-2):
Insomnia middle (0-2):
Insomnia late (0-2):
Work and Activities (0-4):
Retardation (0-4):
Agitation (0-4):
Anxiety psychic (0-4):
Anxiety somatic (0-4):
Somatic symptoms gastrointestinal (0-2):
Somatic symptoms general (0-2):
Genital symptoms (0-2):
Hypochondriasis (0-4):
Loss of weight (0-2):
Insight (0-2):
Diurnal variation (0-2):
Depersonalization (0-4):
Paranoid symptoms (0-4):
Obsessive and compulsive symptoms (0-2):

Total score: 0-7 = no depression
8-15 = subsyndromal depression
16 or more = major depression

DSM IV (Yes/No)
Depressed mood
Loss of interest
Weight/appetite
Sleep change
Agitation/retardation
Fatigue
worthlessness/guilt
concentration
death/suicide thoughts
major depression = >5 yes
SSD = 2-5

Exclusion Criteria (Yes/No)
Drug free (1 week)
Fluoxetine (5 weeks)
no other medicines
no medical illness
no hypertension
no drug abuse
Family/past history of
mental illness (controls)

Melancholia (Yes/No)
Anhedonia
Loss of reactivity
Distinct quality
Diurnal
Terminal insomnia
Retardation/ Agitation
Anorexia/ weight loss
Guilt

Panic Attack (4 or more)
Palpitations Nausea
Sweating Dizzy
Trembling chills
Choking paraesthesias
Shortness of Breath loss of control

Recurrent panic attacks (Yes/No)
(one of the following for one month)
Fear of another attack
Fear of the implications of the attack
Behaviour change
Agoraphobia

Appendix C- Rating Scale for Schizophrenia

Patient.... Age..... Sex.....

Brief Psychiatric Rating Scale (BPRS)

D= depressive symptoms
S= schizophrenic symptoms
The DSM IV criteria for schizophrenia include the following symptoms:
I Bizarre delusions
II somatic or grandiose illusions
III delusions with persecutory ideas
IV auditory hallucinations
V incoherence associated with at least one of the following
a) blunted affect
b) delusions or hallucinations
c) catatonic behaviour

When using BPRS for DSM IV schizophrenia a score of 3 or more on one of the 5 DSM IV symptoms is needed.

Somatic concerns (0-4)	D
Anxiety (psychic) (0-4)	D
Emotional withdrawal (0-4)	S
Conceptual disorganization (0-4)	S
Self depreciation and guilt feelings	D
Anxiety (somatic) (0-4)	D
Specific motor disturbances (0-4)	S
Exaggerated self esteem (0-4)	S
Lowered mood (0-4)	D
Hostility (0-4)	S
Suspiciousness (0-4)	S
Hallucinatory behaviour (0-4)	S
Decreased psychomotor activity	D
Uncooperativenesss	S
Unusual thought content	S
Blunted or inappropriate affect	S
Increased psychomotor activity	S
Disorientation and confusion	S

Exclusion Criteria
Cannabis in the urine
Hypertension
Depot neuroleptic (1 month)
Substance abuse
No medical illness

Appendix D

M.I.N.I.

Mini-International Neuropsychiatric Interview, Clinician Rated (version 4.4)

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PATIENT NAME: _____ *PROTOCOL NUMBER:* _____
DATE OF INTERVIEW: _____ *Time Interview Began:* _____
DATE OF BIRTH: _____ *Time Interview Ended:* _____
INTERVIEWER'S NAME: _____ Total Time: _____

☞ means: Go to end of disorder, circle NO and move to next disorder.

A. MAJOR DEPRESSIVE EPISODE

A 1	Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	NO	YES	1
A 2	In the past two weeks, have you been less interested in most things or less able to enjoy the things you used to enjoy most of the time?	NO	YES	2

If Both A1 & A2 = NO, Circle NO in A4b and skip to B1

A 3	In the past two weeks, when you felt depressed or uninterested, most of the time:			
a	Did your appetite change significantly or did your weight increase or decrease ± 8 lbs. (i.e., ±5% of body weight) without trying intentionally?	NO	YES	3
b	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES	4
c	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still?	NO	YES	5
d	Did you feel tired or without energy most of the time?	NO	YES	6
e	Did you feel worthless or guilty (most of the time)?	NO	YES	7
f	Did you have difficulty concentrating or making decisions?	NO	YES	8
g	Did you consider hurting yourself, feel suicidal, or wish that you were dead?	NO	YES	9
A 4 a	**ARE 3 OR MORE ITEMS FROM A3 CODED YES - OR 4 ITEMS FROM A3 IF A1 OR A2 ARE CODED NO?**	NO	YES	
b	**CODES POSITIVE FOR CURRENT MDE (A1 & A4a = YES) and/or (A2 & A4a = YES)?**	NO	YES	Major Depressive Episode Current

IF PATIENT CODES POSIT[illegible]OR MAJOR DEPRESSION (A4b = YES), SKIP TO BIPOLAR DISORDERS

☞

B. DYSTHYMIA

If patient currently meets criteria for major depressive episode, do not explore this section.

B 1	Have you felt sad, low or depressed most of the time for the last two years?	NO ☞	YES	10
B 2	Was this period interrupted by your feeling OK for two months or more?	NO	YES ☞	11
B 3	During this period of feeling depressed most of the time:			
a	Did your appetite change significantly?	NO	YES	12
b	Did you have trouble sleeping or sleep excessively?	NO	YES	13
c	Did you feel tired or without energy?	NO	YES	14
d	Did you lose your self-confidence?	NO	YES	15
e	Did you have trouble concentrating or making decisions?	NO	YES	16
f	Did you feel hopeless?	NO	YES ☞	17
B 4	Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially, or in your other daily activities?	NO	YES	18

ARE 2 OR MORE ITEMS FROM B3 & B4 CODED YES?

NO	YES
Dysthymia Current	

NOTE: The diagnosis of double depression and major depression in partial remission are not explored in the MINI but can be explored in detail with additional questions in the MINI Plus.

C. BIPOLAR DISORDERS

C 1 a	Have you ever had a period of time when you were feeling 'up' or 'high' or so full of energy or full of yourself that you got into trouble, or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.) If patient is puzzled or unclear about what you mean by 'up' or 'high', clarify as follows: By 'up' or 'high' I mean: •having elated mood, •increased energy, •needing less sleep, •having rapid thoughts, •being full of ideas, • having an increase in productivity, creativity, motivation or impulsive behavior?	NO	YES	1
b	Have you ever been persistently irritable, so that you shouted or started fights or arguments with people outside your family?	NO	YES	2

If NO to all of C1a-b, Circle NO in C5 and skip to D1

C 2	Have you been feeling 'up' or 'high', full of energy or irritable in the past month (manic symptoms)? NOTE: If currently manic (C2 = YES), explore only current episode. If no current mania, explore most symptomatic past episode.	NO	YES	3
C 3	During the times when you felt high, full of energy, or irritable did you:			

☞ means: Go to end of disorder, circle NO and move to next disorder

a	Feel that you could do things others couldn't do, or that you were an especially important person?	NO	YES	4
b	Need less sleep (e.g., feel rested after only a few hours sleep)?	NO	YES	5
c	Talk too much without stopping, or so fast that people had difficulty understanding?	NO	YES	6
d	Have thoughts racing through your head so fast that you had difficulty keeping track of them?	NO	YES	7
e	Become easily distracted so that any little interruption could distract you?	NO	YES	8
f	Become so active or physically restless that others were worried about you?	NO	YES	9
g	Want so much to engage in pleasurable activities that you ignored the risks or consequences? (e.g., spending sprees, reckless driving, or sexual indiscretions)?	NO	YES	10
		☞		
	Summary of C3: Are 3 of the C3 answers coded YES (or 4 if C1a is NO)? RULE: Elation/Expansiveness requires only three C3 symptoms while the other criteria require four of the C3 symptoms.	NO	YES	
C 4	Did these symptoms last at least a week and cause problems beyond your control at home, work, school, or were you hospitalized for these problems?	NO → HYPOMANIC EPISODE	YES → MANIC EPISODE	11

C 5 a	**CODES POSITIVE FOR CURRENT MANIC EPISODE? HYPOMANIC EPISODE (C2 = YES and C3(summary)=YES)**	NO	Current Bipolar Disorder YES
b	**Prior to the last month, has the patient ever had a manic (hypomanic) episode? (C3 = YES and C1a-b=YES)**	NO	Past Bipolar Disorder YES

NOTE: The diagnosis of past major depression is not explored in the MINI but can be explored in detail with additional questions in the MINI Plus.

D. PANIC DISORDER

D 1	**PANIC DISORDER**	☞		
a	Have you, on more than one occasion, had spells or attacks when you suddenly felt anxious, frightened, uncomfortable or uneasy in a situation where most people would not feel that way?	NO	YES	1
		☞		
b	At any time in the past, did any of those spells or attacks come on unexpectedly or occur in an unpredictable or unprovoked manner?	NO	YES	2
c	Have you ever had one such attack followed by a month or more of persistent fear of having another attack, or worries about the consequences of the attack?	NO	YES	3

☞ means: Go to end of disorder, circle NO and move to next disorder

d During the worst spell that you can remember:

1	Did you have skipping, racing or pounding of your heart?	NO	YES	4
2	Did you have sweating or clammy hands?	NO	YES	5
3	Were you trembling or shaking?	NO	YES	6
4	Did you have shortness of breath or difficulty breathing?	NO	YES	7
5	Did you have a choking sensation or a lump in your throat?	NO	YES	8
6	Did you have chest pain, pressure or discomfort?	NO	YES	9
7	Did you have nausea, stomach problems or sudden diarrhea?	NO	YES	10
8	Did you feel dizzy, unsteady, lightheaded or faint?	NO	YES	11
9	Did you feel detached from things around you or detached from part of your body?	NO	YES	12
10	Did you fear that you were losing control or going crazy?	NO	YES	13
11	Did you fear that you were dying?	NO	YES	14
12	Did you have tingling or numbness in parts of your body?	NO	YES	15
13	Did you have hot flushes or chills?	NO	YES	16

Summary D1d: Are at least 4 of the above D1d symptoms coded YES? NO YES

e **CODES POSITIVE FOR LIFETIME PANIC DISORDER?**
D1a and D1b and D1c and Summary of D1d = YES
IF e IS CODED NO, SKIP TO D1g.

NO YES
Panic Disorder Lifetime

f In the past month, did you have such attacks repeatedly, or did you have one attack followed by persistent fear of having another attack? (If this is denied by the patient - challenge by reviewing the symptoms endorsed in D1d) NO YES 17
Panic Disorder Current

IF D1f IS CODED YES SKIP TO E1.

g Apart from the panic attacks with 4 or more symptoms that we just discussed, in the past month, did you have sudden attacks of only 3, 2 or 1 of the above symptoms. NO YES 18
CODES POSITIVE FOR CURRENT LIMITED SYMPTOM ATTACKS.
Current Limited Symptom Attacks

E. AGORAPHOBIA

E 1 a Do you feel particularly uneasy in places or situations from which escape might be difficult or embarrassing, or help might not be available: like being in a crowd, standing in a line, being alone away from home, crossing a bridge, or traveling in a bus, train or car? ☞ NO YES 19

b Do you fear these situations so much that you avoid them, suffer through them, or need a companion to face them? ☞ NO YES 20
Agoraphobia Current

c Patient codes positive for Panic Disorder, Current. Patient does not code positive for Agoraphobia, Current

NO Current YES
PANIC DISORDER without Agoraphobia

☞ means: Go to end of disorder, circle NO and move to next disorder

d Patient codes positive for Panic Disorder (current) with Agoraphobia, Current.

NO Current YES
PANIC DISORDER with Agoraphobia

e Patient codes positive for Current Agoraphobia without history of Panic Disorder, Lifetime.

NO Current YES
AGORAPHOBIA without history of Panic Disorder

F. SOCIAL PHOBIA

F 1 a In the past month, were you fearful or embarrassed being the focus of attention or fearful of being humiliated? This includes things like speaking in public, using public toilets, writing while someone watches, or being in social situations.

☞ NO YES 1

b Is this fear excessive or unreasonable?

☞ NO YES 2

c Do you fear these situations so much that you avoid them or suffer through them?

☞ NO YES 3

d Does this fear disrupt normal work or social functioning or cause marked distress?

NO YES 4
Social Phobia Current

G. SPECIFIC PHOBIA

G 1 a In the past month, have you been excessively afraid of things like: flying, driving, heights, storms, animals, insects, or seeing blood or needles?

☞ NO YES 1

b Is this fear excessive or unreasonable?

☞ NO YES 2

c Do you fear these situations so much that you avoid them or suffer through them?

☞ NO YES 3

d Does this disrupt normal work or social functioning or cause marked distress?

NO YES 4
Specific Phobia Current

H. OBSESSIVE COMPULSIVE DISORDER

*Skip to Question H4

H 1 In the past month, have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? (e.g., the idea that you were dirty or had germs, or of hurting someone even though you didn't want to)
(DO NOT INCLUDE SIMPLY EXCESSIVE WORRIES ABOUT REAL LIFE PROBLEMS. DO NOT INCLUDE OBSESSIONS DIRECTLY RELATED TO EATING DISORDERS, SEXUAL BEHAVIOR, PATHOLOGICAL GAMBLING, OR ALCOHOL OR DRUG ABUSE BECAUSE THE PATIENT MAY DERIVE PLEASURE FROM THE ACTIVITY AND MAY WANT TO RESIST IT ONLY BECAUSE OF ITS NEGATIVE CONSEQUENCES.)

NO* YES 1

H 2 Did they keep coming back into your mind even when you tried to ignore or get rid of them?

NO* YES 2

☞ means: Go to end of disorder, circle NO and move to next disorder

H 3	Do you think that these obsessions are the product of your own mind and that they are not imposed from the outside?	NO	YES	3
H 4	In the past month, did you do something repeatedly without being able to resist doing it, like washing excessively, counting or checking things over and over?	NO**	YES	4
H 5	Did you recognize that either these obsessional thoughts or compulsive behaviors were excessive or unreasonable?	NO	YES	5
H 6	Did these obsessions or compulsions significantly interfere with your normal routine, occupational functioning, usual social activities, or relationships, or did they take more than one hour a day?	NO	YES	6

****IF NO TO H4 AND TO H1 OR H2: GO TO END OF DISORDER, CIRCLE NO AND MOVE TO NEXT DISORDER.**

CODES POSITIVE FOR CURRENT OCD IF EITHER (H1 & H2 & H3 & H5 & H6) OR (H4 & H5 & H6) IS YES.

NO Current O.C.D. YES

I. GENERALIZED ANXIETY DISORDER

Skip this disorder if the patient's anxiety is restricted exclusively to or better explained by any disorder prior to this point.

I a 1	Have you worried excessively or been anxious about 2 or more things (e.g., finances, children's health, misfortune) over the past 6 months? More than most others would? Are these worries present most days? Have several people told you that you worry too much?	NO	YES	1
2	Do you find it difficult to control the worries or do they interfere with your abiltiy to focus on what you are doing?	NO	YES	2
I b	*During these worried periods when you are anxious, do you:* (DO NOT CODE SYMPTOMS OCCURRING ONLY DURING PANIC ATTACKS)			
1	Feel restless, keyed up or on edge?	NO	YES	3
2	Feel tense?	NO	YES	4
3	Feel tired, weak or exhausted easily?	NO	YES	5
4	Have difficulty concentrating or find your mind going blank?	NO	YES	6
5	Feel irritable?	NO	YES	7
6	Have difficulty sleeping?	NO	YES	8
	Summary of Ib: Are at least 3 of Ib answers YES?	NO	YES	

CODES POSITIVE FOR CURRENT GAD (Ib (summary) = YES)?

NO Current G.A.D. YES

J. ALCOHOL ABUSE AND DEPENDENCE

			☞		
J 1		IN THE PAST 12 MONTHS, have you had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions?	NO	YES	1
J 2		IN THE PAST 12 MONTHS:			
	a	Did you need to drink more in order to get the same effect that you did when you first started drinking?	NO	YES	2
	b	When you cut down on drinking did your hands shake, did you sweat or feel agitated? Did you drink to avoid these symptoms or to avoid being hungover, e.g., "the shakes", sweating or agitation?	NO	YES	3
	c	During the times when you drank alcohol, did you end up drinking more than you planned when you started?	NO	YES	4
	d	Have you tried to reduce or stop drinking alcohol?	NO	YES	5
	e	On the days that you drank, did you spend more than two hours per day in obtaining alcohol, drinking, and in recovering from the effects of alcohol?	NO	YES	6
	f	Did you spend less time working, enjoying hobbies, or being with others because of your drinking?	NO	YES	7
	g	Have you continued to drink even though you knew that the drinking caused health or mental problems?	NO	YES	8
				☞	
	h	**CODES POSITIVE FOR CURRENT ALCOHOL DEPENDENCE? (At least 3 of J2 are coded YES)**	NO	YES Alcohol Dependence Current	
J 3	a	In the PAST 12 MONTHS, have you been intoxicated, high, or hungover more than once when you had other responsibilities at school, at work, or at home? Did this cause any problems? (Code YES only if this caused problems.)	NO	YES	9
	b	In the PAST 12 MONTHS, were you intoxicated in any situation where you were physically at risk, e.g., driving a car, boating, using machinery, etc.?	NO	YES	10
	c	In the PAST 12 MONTHS, have you had any legal problems because of your drinking, e.g., an arrest or disorderly conduct?	NO	YES	11
	d	In the PAST 12 MONTHS, have you continued to drink even though your drinking caused problems with family or other people?	NO	YES	12
	e	**CODES POSITIVE FOR CURRENT ALCOHOL ABUSE? (J3a or b or c or d = YES)**	NO	YES Alcohol Abuse Current	

K. NON-ALCOHOL PSYCHOACTIVE SUBSTANCE USE DISORDERS

			☞		
K 1	a	Now I am going to read to you a list of street drugs or medicines. Stop me if, IN THE PAST 12 MONTHS, you have taken more than once, any of them to get high, to feel better, or to change your mood. **CIRCLE EACH DRUG TAKEN:** Quaalude, Seconal ("reds"), Valium, Xanax, Librium, Ativan, Dalmane, Halcion, barbiturates, Miltown or tranquilizers. Marijuana: hashish ("hash"), THC, "pot", "grass", "weed", "reefer". Amphetamine:	NO	YES	

☞ means: Go to end of disorder, circle NO and move to next disorder

"speed", crystal meth, "rush", dexadrine, Ritalin, diet pills. Cocaine: snorting, IV, freebase, crack, "speedball". Narcotics: heroin, morphine, dilaudid, opium, demerol, methadone, codeine, percodan, darvon. LSD ("acid"), mescaline, peyote, PCP ("angel dust", "peace pill"), psilocybin, STP, or "mushrooms". Steroids, "glue", ethyl chloride, nitrous oxide, ("laughing gas"), amyl or butyl nitrate ("poppers"), Ecstasy, MDA, MDMA, nonprescription sleep or diet pills. Any others?
Specify MOST USED Drug(s): _____

b **SPECIFY WHICH WILL BE EXPLORED IN CRITERIA BELOW:**

If concurrent or sequential polysubstance use:

Each drug class used individually. ☐

Most used drug class only. ☐

If one drug used:

Single drug class only. ☐

K 2 **Considering the drug class selected, IN THE PAST 12 MONTHS:**

a	Have you found that you needed to use more of the drug to get the same effect that you did when you first started taking it?	NO	YES	1
b	When you reduced or stopped using drugs did you have withdrawal symptoms? (Aches, shaking, fever, weakness, diarrhea, nausea, sweating, heart pounding, difficulties sleeping, or feeling agitated, anxious, irritable, or depressed.) Did you use any drug(s) to keep yourself from getting sick (WITHDRAWAL SYMPTOMS) or so that you would feel better?	NO	YES	2
c	Have you often found that when you used drug(s), you ended up taking more than you thought you would?	NO	YES	3
d	Have you tried to reduce or stop taking these drug(s)?	NO	YES	4
e	On the days that you used drug(s), did you spend more than 2 hours per day obtaining, using and recovering from drug(s), or thinking about drug(s)?	NO	YES	5
f	Did you spend less time working, enjoying hobbies, or being with family or friends because of your drug use?	NO	YES	6
g	Have you continued to use drug(s) even though it caused health or mental problems?	NO	YES	7
h	**CODES POSITIVE FOR CURRENT PSYCHOACTIVE SUBSTANCE DEPENDENCE (At least three K 2's are coded YES)? specify drug(s):** _____	NO	YES Current Drug Dependence	

Considering the drug class selected:

K 3 a	In the PAST 12 MONTHS, have you been intoxicated, high, or hungover from drug(s), more than once, when you had other responsibilities at school, at work, or at home? Did this cause any problem? (Code YES only if this caused problems.)	NO	YES	8
b	In the PAST 12 MONTHS, have you been high or intoxicated from drug(s) in any situation where you were physically at risk (e.g., driving a car, boating, using machinery, etc.)?	NO	YES	9

☞ means: Go to end of disorder, circle NO and move to next disorder

c	In the PAST 12 MONTHS, have you had any legal problems because of your drug use, e.g., an arrest or disorderly conduct?	NO	YES	10
d	In the PAST 12 MONTHS, have you continued to use drug(s) even though it caused problems with your family or other people?	NO	YES	11
e	CODES POSITIVE FOR CURRENT PSYCHOACTIVE SUBSTANCE ABUSE (K3a or b or c or d = YES)?	NO	YES Drug Abuse Current	

L. PSYCHOTIC SYNDROMES

Ask for an example of each question answered positively.
Code Yes only if the examples clearly show a distortion of thought or of perception.
Before coding, investigate whether delusions qualify as "bizarre".

Delusions are "bizarre" if: clearly implausible, absurd, not understandable, and cannot derive from ordinary life experience.

Hallucinations are scored "bizarre" if: a voice comments on the person's thoughts or behavior, or when two or more voices are conversing with each other.

Now I am going to ask you about usual experiences that some individuals may experience.

				Bizarre	
L 1 a	Have your relatives or friends ever considered any of your beliefs strange or unusual? Please give me an example. Interviewer: Only Code Yes if the examples are CLEARLY delusional ideas of GRANDIOSITY, HYPOCHONDRIASIS, RUIN, GUILT, etc.)	NO	YES	YES	1
b	IF YES: do they currently consider your beliefs strange?	NO	YES *Skip to L6	YES*	2
L 2 a	Have you ever believed that people were spying on you, or that someone was plotting against you, or trying to hurt you?	NO	YES	YES	3
b	IF YES: do you currently believe these things? NOTE: Ask for examples, to rule out actual stalking.	NO	YES *Skip to L6	YES*	4
L 3 a	Have you ever believed that someone was reading your mind or could hear your thoughts or that you could actually read or hear what another person was thinking?	NO		YES	5
b	IF YES: do you currently believe these things?	NO	 *Skip to L6	YES*	6
L 4 a	Have you ever believed that someone or some force outside of yourself put thoughts in your mind that were not your own, or made you act in a way that was not your usual self? CLINICIAN: Ask for examples and discount any that are not psychotic.	NO		YES	7
b	IF YES: do you currently believe these things?	NO	 *Skip to L6	YES*	8
L 5 a	Have you ever believed that you were being sent special messages through the TV, radio, or newspaper, or that a person you did not personally know was particularly interested in you?	NO	YES	YES	9

☞ means: Go to end of disorder, circle NO and move to next disorder

	b	IF YES: do you currently believe these things?	NO	YES	YES* *Skip to L6	10
L 6	a	Have you ever heard things other people couldn't hear, such as voices? Hallucinations are scored "bizarre" only if patient answers YES to the following: Did you hear a voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other?	NO	YES	YES*	11
	b	If YES, have you heard these things in the past month? Score as "YES Bizarre" if patient heard a voice commenting on their thoughts or behavior or heard two or more voices talking to each other.	NO	YES	YES* *Skip to L10	12
L 7	a	Have you ever had visions or have you ever seen things other people couldn't see?	NO	YES		13
	b	IF YES, have you seen these things in the past month?	NO	YES		14
		CLINICIAN'S JUDGMENT				
L 8		Is the patient currently exhibiting incoherence, disorganized speech, or marked loosening of associations?	NO	YES		15
L 9		Is the patient currently exhibiting disorganized or catatonic behavior?	NO	YES		16

L 10 **ARE 1 OR MORE ITEMS FROM L1b, L2b, L3b, L4b, L5b, L6b, CODED YES BIZARRE?**

OR

ARE 2 OR MORE ITEMS FROM L1b, L2b, L5b, L6b, L7b, L8, L9 CODED YES (RATHER THAN YES BIZARRE)?

NO / YES — Current Psychotic Syndrome

L 11 **ARE 1 OR MORE ITEMS FROM L1a, L2a, L3a, L4a, L5a, L6a, CODED YES BIZARRE?**

OR

ARE 2 OR MORE ITEMS FROM L1a, L2a, L5a, L6a, L7a, L8, L9 CODED YES (RATHER THAN YES BIZARRE)?

NO / YES — Lifetime Psychotic Syndrome

L 12 **IF L11 CODED YES: DOES THE PATIENT CODE POSITIVE FOR CURRENT MAJOR DEPRESSION OR CURRENT OR PAST BIPOLAR DISORDER?** NO YES

L 13 **IF L11 CODED YES:**
Were the beliefs and experiences you just described (give examples to patient) restricted exclusively to times when you were feeling depressed/high/very irritable? NO / YES — Mood Disorder with Psychotic Features 17

M. ANOREXIA NERVOSA CRITERIA (optional)

M 1	How tall are you?				Ft/In
M 2	What was your lowest weight in the past 3 months?				Lbs
M 3	IS PATIENT'S WEIGHT LOWER THAN THE THRESHOLD CORRESPONDING TO HIS/HER WEIGHT? (See table on opposite page.)	☞ NO	YES		1
	IN THE PAST 3 MONTHS:				
M 4	In spite of this low weight, have you tried not to gain weight?	☞ NO	YES		2
M 5	Have you feared gaining weight or becoming fat, even though you were underweight?	☞ NO	YES		3
M 6 a	Have you considered yourself fat or that part of your body was too fat?	NO	YES		4
b	Has your body weight or shape greatly influenced how you felt about yourself?	NO	YES		5
c	Have you thought that your current low body weight was normal or excessive?	NO	YES		6
M 7	**ARE 1 OR MORE ITEMS FROM M6 CODED YES?**	☞ NO	YES		
M 8	FOR WOMEN ONLY: During the last 3 months, did you miss all your menstrual periods when they were expected to occur (when you were not pregnant)?	NO	YES		7
	CODES POSITIVE FOR ANOREXIA NERVOSA CURRENT? (Patient codes yes to M3-M8, if male, only M3-M7.)	☞ NO	YES Anorexia Nervosa Current		

N. BULIMIA NERVOSA CRITERIA (optional)

N 1	In the past three months, did you have eating binges or times when you ate a very large amount of food within a 2-hour period?	☞ NO	YES	8
N 2	In the last 3 months, did you have eating binges as often as twice a week?	☞ NO	YES	9
N 3	During these binges, did you feel that your eating was out of control?	☞ NO	YES	10
N 4	Did you do anything to compensate for, or to prevent a weight gain from these binges, like vomiting, fasting, exercising or taking laxatives, enemas, diuretics (fluid pills), or other medications?	☞ NO	YES	11
N 5	Does your body weight or shape greatly influence how you feel about yourself?	☞ NO	YES	12
	IF PATIENT'S SYMPTOMS MEET CRITERIA FOR ANOREXIA NERVOSA, ASK:			
N 6	Do these binges occur only when you are underweight for your height? (INTERVIEWER: Underweight is a weight below threshhold according to the height/weight table.)	NO	YES	13

☞ means: Go to end of disorder, circle NO and move to next disorder

CODES POSITIVE FOR BULIMIA NERVOSA?
(Patient codes YES to N1, 2, 3, 4 and 5 and NO to N6)

☞ NO YES
Bulimia Nervosa Current

CODES POSITIVE FOR ANOREXIA NERVOSA, BINGE EATING TYPE?
(Patient codes YES to N6.)

NO YES
Anorexia Nervosa Binge eating type Current

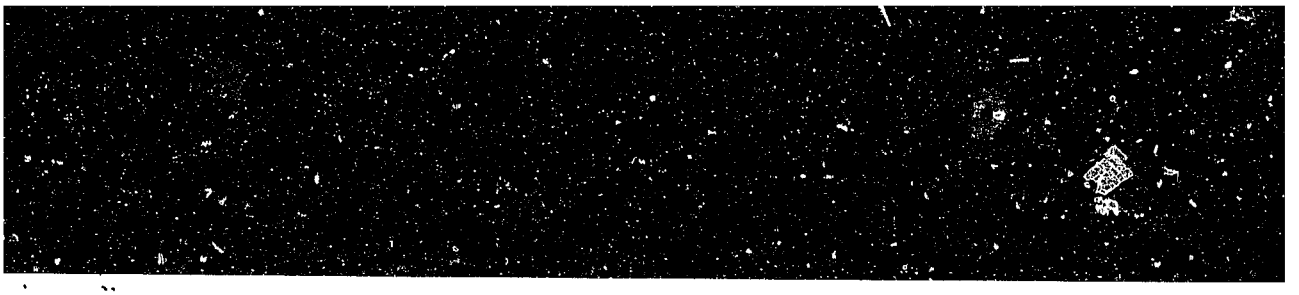
O. POST-TRAUMATIC STRESS DISORDER (optional)

			☞		
O 1		Have you ever experienced an unusually traumatic or stressful event (e.g., earthquakes, floods, physical assault or rape, being in war or combat, killing someone, seeing people killed, fires, serious accidents)?	NO	YES	1
			☞		
O 2		During the past month, have you re-experienced the event in a distressing way (i.e., dreams, intense recollections, flashbacks or physical reactions)?	NO	YES	2
O 3		IN THE PAST MONTH:			
	a	Have you avoided thinking about the event, or have you avoided things that remind you of the event?	NO	YES	3
	b	Have you had trouble recalling some important part of what happened?	NO	YES	4
	c	Have you become less interested in hobbies or social activities?	NO	YES	5
	d	Have you felt detached?	NO	YES	6
	e	Have you noticed that your feelings are numbed?	NO	YES	7
	f	Have you felt that your life would be shortened because of this trauma?	NO	YES	8
			☞		
		Summary of O3: Are at least 3 of O3 responses coded YES?	NO	YES	
O 4		IN THE PAST MONTH:			
	a	Have you had difficulty sleeping?	NO	YES	9
	b	Were you especially irritable or did you have outbursts of anger?	NO	YES	10
	c	Have you had difficulty concentrating?	NO	YES	11
	d	Were you nervous or constantly on your guard?	NO	YES	12
	e	Were you easily startled?	NO	YES	13
			☞		
		Summary of O4: Are at least 2 O4 responses coded YES?	NO	YES	
O 5		During the past month, have these problems significantly interferred with your work or social activities, or caused marked distress?	NO	YES	14

CODES POSITIVE FOR POST-TRAUMATIC STRESS DISORDER (O1, O2, O3 (Summary), O4 (Summary), & O5 = YES)?

NO YES
P.T.S.D. Current

☞ means: Go to end of disorder, circle NO and move to next disorder



P. SUICIDALITY

P 1	Did you ever make a suicide attempt?	NO	YES	1
	IN THE PAST MONTH DID YOU:			
P 2	Wish you were dead?	NO	YES	2
P 3	Want to harm yourself?	NO	YES	3
P 4	Think about suicide?	NO	YES	4
P 5	Have a suicide plan?	NO	YES	5
P 6	Attempt suicide?	NO	YES	6

If YES to one or more of the above then "patient is at
P1 or P2 or P3 YES = LOW
P4 or (P1 + P3) YES = MODERATE
P5 OR P6 or (P1 + P4) YES = HIGH
risk for suicide."

NO YES
Suicide Risk

ADDENDUM

Q. ANTISOCIAL DISORDER (optional)

Q 1	Before you were 15 years old, did you:			
	• repeatedly skip school or run away from home overnight?	NO	YES	1
	• repeatedly lie, cheat, "con" others, or steal?	NO	YES	2
	• start fights or bully, threaten, or intimidate others?	NO	YES	3
	• deliberately destroy things or start fires?	NO	YES	4
	• deliberately hurt animals or people?	NO	YES	5
	• force someone to have sex with you?	NO	YES	6

If at least 2 Q1 = YES continue with Q2

Q 2	Since you were 15 years old, have you:			
a	repeatedly behaved in a way that others would consider irresponsible, like failing to pay for things you owed, deliberately being impulsive or deliberately not working to support yourself? (DO NOT CODE YES if the behavior is ONLY politically or religiously different.)	NO	YES	7
b	done things that are illegal even if you didn't get caught (i.e., destroying property, shoplifting, stealing, selling drugs, or committing a felony)?	NO	YES	8
c	been in physical fights repeatedly (including physical fights with your spouse or children)?	NO	YES	9
d	often lied or "conned" other people to get money or pleasure, or lied just for fun?	NO	YES	10
e	exposed others to danger without caring?	NO	YES	11

☞ means: Go to end of disorder, circle NO and move to next disorder

f felt no guilt after hurting, mistreating, lying to, or stealing from others, or after damaging property? NO YES 12

Q 3 CODES POSITIVE FOR ANTISOCIAL DISORDER (AT LEAST 3 Q2a-f = YES)

NO	YES
Antisocial Disorder Lifetime	

☞ means: Go to end of disorder, circle NO and move to next disorder

TIME FRAMES

	DISORDER	INTERVIEW TIME FRAME
A.	MAJOR DEPRESSIVE EPISODE	PAST 2 WEEKS
B.	DYSTHYMIA	PAST 2 WEEKS
C.	MANIA	LIFETIME + CURRENT
D.	PANIC DISORDER	PAST MONTH
E.	AGORAPHOBIA	PAST MONTH
F.	SOCIAL PHOBIA	PAST MONTH
G.	SPECIFIC PHOBIA	PAST MONTH
H.	OBSESSIVE COMPULSIVE DISORDER	PAST MONTH
I.	GENERALIZED ANXIETY DISORDER	PAST 6 MONTHS
J.	ALCOHOL DEPENDENCE/ABUSE	PAST 12 MONTHS
K.	DRUG DEPENDENCE/ABUSE (Non-alcohol)	PAST 12 MONTHS
L.	PSYCHOTIC DISORDER	LIFETIME + CURRENT
M.	ANOREXIA NERVOSA	PAST 6 MONTHS
N.	BULIMIA	PAST 3 MONTHS
O.	POST TRAUMATIC STRESS DISORDER	PAST MONTH
P.	SUICIDALITY	PAST MONTH
Q.	ANTISOCIAL DISORDER	LIFETIME

WARNING

Even if a patient has a clear life stress aggravating their symptoms first explore the other diagnosis above. Never use an adjustment disorder diagnoses if the disturbance meets criteria for any of the above disorders.

☞ means: Go to end of disorder, circle NO and move to next disorder

International Clinical Psychopharmacology 1998, 13:107–110

The platelet intracellular calcium response to serotonin in subsyndromal depression

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The relationship of subsyndromal depression to syndromal depression is unclear. Increased sensitivity of platelet serotonin type 2 receptor has been reported in depression using the Fura-2 technique. The sensitivity of the platelet serotonin type 2 receptor was assessed using the Furn-2 method in 16 patients with subsyndromal depression and 14 controls. The patient group had higher numerical values of both basal and serotonin-stimulated levels of platelet intracellular calcium that did not however reach statistical significance. Int Clin Psychopharmacol 13:107–110 © 1998 Lippincott-Raven Publishers

Keywords: platelet, depression, subsyndromal, serotonin, calcium, intracellular

INTRODUCTION

While extensive data on depression exist, there is a paucity of research on patients who have depressive symptoms that fall below the thresholds of current classifications. This is despite the frequency with which subsyndromal depression is present in epidemiological studies. A secondary analysis of the epidemiological catchment area study reported that 16.9% of the general population reported one or more depressive symptom in the past month (Judd *et al.*, 1994). In a primary care setting, subsyndromal depression was more frequent than syndromal depression (9.1 vs 7.3%; Olfson *et al.*, 1996). Subsyndromal depression appears to have a similar rate (41%) of family history of affective disorder to patients with syndromal depression (59%), and was regarded by the authors as a variant of affective disorder (Sherbourne *et al.*, 1994). The Medical Outcomes Study reported that in patients who had depressive symptoms that did not meet diagnostic thresholds, there was significant disability (Wells *et al.*, 1989). More recent studies have replicated the finding that the presence of subsyndromal depression appears to be associated with significant disability and functional impairment (Jaffe *et al.*, 1994; Olfson *et al.*, 1996).

Supersensitivity of the 5HT2 receptor in unipolar depression has been described using the Fura-2 technique in a number of studies (Mikuni *et al.*, 1992; Eckert *et al.*, 1993; Kusumi, 1993). In a study of patients with bulimia and anorexia nervosa, only those patients with anorexia with subsyndromal depression (defined as a Hamilton Depression score between 10 and 16) manifested supersensitivity of the 5HT2 receptor (Berk *et al.*, 1997).

The aim of this study was to examine the platelet intracellular calcium response to serotonin in patients with subsyndromal depression in a prospective design. This would define the limits of the specificity of the platelet intracellular calcium response to serotonin as a marker of mood disorder, and define the relationship of syndromal and subsyndromal depression in terms of this marker.

METHODS

Patient recruitment

Patients were defined according to Hamilton Depression rating scale (HAMD) scores and DSM 4 criteria based on a structured interview. Subsyndromally depressed patients were defined as patients who do not meet DSM 4 criteria for major depression but who nevertheless have HAMD scores between 10 and 16. Controls were age- and sex-matched

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volunteers with a HAMD less than 7 and no family or past history of depression. Control and patient subjects were drug free for at least 2 weeks prior to assay, with a 5 week exclusion period for fluoxetine.

Exclusion criteria included patients under the age of 18 or over the age of 65, and patients with significant medical illnesses. Patients with significant hypertension were excluded, since elevated platelet intracellular calcium has been described in hypertension (Lindner *et al.*, 1987; Sang *et al.*, 1987). Ethical approval from the Committee for Research on Human Subjects was obtained, and informed consent was obtained from all subjects.

Experimental procedure

A 20-ml sample of blood was drawn by venepuncture and decanted into a plastic tube containing 100 μM aspirin (to prevent platelet aggregation) and 6 ml ACD buffer (85 mM sodium citrate, 6.25 mM citric acid, 110 mM dextrose in water at a pH of 4.9). This mixture was centrifuged at 150 *g* for 15 min. Platelet-rich plasma was removed by pipette and centrifuged at 850 *g* to form a platelet pellet. The platelets were then re-suspended in assay buffer (137 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM dextrose, 5 mM HEPES, pH 7.4 (Rao *et al.*, 1985). The platelet count was adjusted to approximately 75×10^6 platelets/ml with assay buffer (Pollock *et al.*, 1987).

A stock solution of Fura-2-acetoxy methyl ester (Fura-2-AM) was obtained by dissolving 1 mg of Fura-2-AM in 1 ml DMSO. The platelets were loaded with 20 μl (4 μM) Fura-2-AM per 5 ml sample. This was done in duplicate. The loaded platelets were incubated for 45 min at 37°C. The cells were spun at 350 *g* for 5 min. The supernatant was discarded and the cells were re-suspended in calcium-free or calcium-containing HEPES buffer: 145 mM NaCl, 1 mM $MgCl_2$, 10 mM Hepes, 5 mM glucose, 0.5 mM Na_2HPO_4; calcium in the calcium-containing buffer was 1 mM $CaCl_2$ (buffer pH 7.55) (Sage and Rink, 1986). The cell count was adjusted to 10×10^5 platelets/ml. Fura-2 fluorescence was measured at room temperature in a Perkin Elmer LS 50 spectrophotometer with excitation wavelengths of 340 and 380 nm and emission wavelength of 510 nm. Intracellular calcium concentration ($[Ca^{2+}]_i$) was calculated using a calcium–dye dissociation coefficient (Kd) for Fura-2 of 224 nm, and using the ratio (R) of fluorescence at the two excitation wavelengths, 340 and 380 nm, which correspond to sample-free and bound forms of the dye respectively. The calculations were done according to the method of Grynckiewicz *et al.*, (1985).

Serotonin was added to obtain a dose-response curve for intracellular calcium. The concentrations of neurotransmitter added in 50 μl aliquots were: 0, 10 nM, 50 nM, 100 nM, 500 nM and 1 μM sequentially. Thrombin (0.1 IU) was added to obtain a maximal response to stimulation. To measure R_{max} (maximum Fura-2-bound calcium), 50 μl of 10% Triton X-100 in 250 nm Tris was added to the sample. To measure R_{min} (unbound free Fura-2), 2 mM EGTA (in sufficient 250 mM Tris buffer to ensure a pH of > 8.3) was added to the sample. The primary statistical tools utilized were the analysis of variance (ANOVA) and analysis of covariance (ANCOVA), with post-hoc testing using Bonferroni's multiple comparison tests.

RESULTS

Levels of platelet intracellular calcium were measured, both at baseline and after stimulation with serotonin and thrombin. Assays were done in duplicate, in both calcium-free and calcium-containing HEPES buffer. While the mean level of basal intracellular calcium was numerically higher in the subsyndromally depressed group than the controls (102.5, SD = 31.1 vs 85.7, SD = 23.3; calcium-free HEPES buffer), this did not reach statistical significance (DF = 1, $F = 2.66$, $p = 0.115$; ANCOVA; Fig. 1). There were higher mean levels of both thrombin (240, SD = 87.1 vs 205, SD = 70.8; DF = 1, $F = 0.91$, $p = 0.670$; Fig. 2) and serotonin (1 μM) – stimulated (137.9, SD = 39.5 vs 122.7, SD = 40.3; DF = 1, $F = 0.91$, $p = 0.395$, Fig. 1) intracellular calcium in the subsyndromal depression group which similarly did not reach statistical significance. An AUC of the serotonin dose-response data again showed a numerically higher result in the subsyndromally depressed group (607 vs 518) which was not statistically significant (DF = 1, $F = 2.16$, $p = 0.153$; data not illustrated). This was probably due

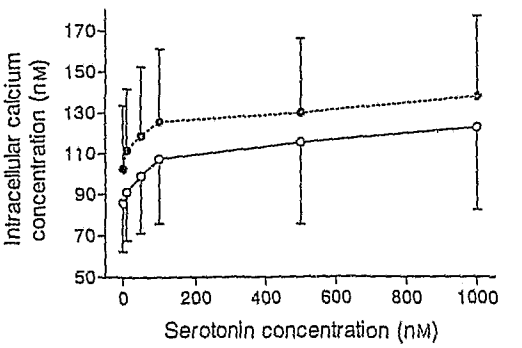


Figure 1. Effect of serotonin concentration on platelet intracellular calcium response. Closed circles, patients with subsyndromal depression; open circles, controls.

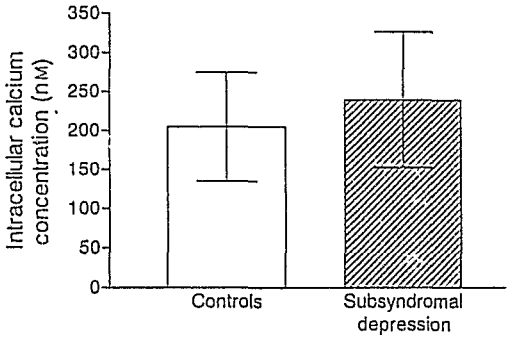


Figure 2. Effect of thrombin on platelet intracellular calcium.

to a very high standard error in the data sets (Fig. 1). A high standard deviation in intracellular calcium readings is a feature of the literature of this method (Dubovsky *et al.* 1989). This suggests that a type 2 statistical error may be a factor in these negative results.

DISCUSSION

Elevated intracellular calcium in bipolar disorder has been described by Dubovsky *et al.*, (1989), and has been replicated in platelet studies (Tan *et al.*, 1990; Dubovsky *et al.*, 1991; Berk *et al.*, 1995) and in lymphocytes (Dubovsky *et al.*, 1992). Negative studies however have been reported (Bothwell *et al.*, 1994). In contrast to the findings in bipolar disorder, elevation of basal intracellular calcium has not been found in unipolar disorder (Dubovsky *et al.*, 1989; Mikuni *et al.*, 1992). This is an interesting finding, as it suggests that unipolar and bipolar disorders may differ with respect to calcium pathophysiology.

Serotonin type 2 receptor hypersensitivity has been described in depression using tritiated lysergic acid diethylamide (LSD) binding to platelet membranes (Arora & Meltzer, 1989; Pandey *et al.*, 1990; Mikuni *et al.*, 1992) and tritiated ketanserin binding (Biegon, 1987). Platelet conformational change was found to be caused by lower serotonin concentrations in bipolar and unipolar depression than controls, suggesting supersensitivity of the 5HT2 receptor (Brusov *et al.*, 1989).

Supersensitivity of the 5HT2 receptor in unipolar depression has been described using the Fura-2 technique. While the basal levels did not differ from controls, there was significantly greater calcium mobilization from platelets in response to serotonin at a concentration of 10 μM in the depressed group (Mikuni *et al.*, 1992). This result was replicated in a mixed depressive group by Eckert *et al.*, (1993) and in bipolar and unipolar depression by Kusumi (1995), who found that serotonin-mediated intracellular calcium concentrations were significantly higher in patients with bipolar as well as melancholic major depression than controls or euthymic treated patients. The serotonin response was found to be inhibited by $5HT_2$ antagonists, confirming this receptor's role in the response.

The specificity of the augmented intracellular response to serotonin is crucial to its utility as a marker. Few reports using this marker in psychiatric disorders other than depression exist. Schizophrenic and substance abuse patients were not found to differ from controls, while depressed patients showed an augmented response in a study by Konopka *et al.* (1996). An augmented response was seen in bulimia but not Anorexia Nervosa in a study in which major depression was excluded (Okamoto *et al.*, 1995). In a similar study by our group (Berk *et al.*, 1997), an augmented response was seen in only those patients in the anorexia group with Hamilton Depression scores between 10 and 17, with patients in the anorexia group with scores below 10 not differing from controls. This suggested that the augmented response in that group may be due to subsyndromal depression.

The failure of the inter-group differences in basal as well as serotonin-stimulated intracellular calcium values to reach significance does not support the hypothesis that platelet serotonin type 2 receptors are supersensitive in subsyndromal depression. This finding may however be an artefact of a type 2 statistical error. The importance of subsyndromal depression from a clinical and conceptual perspective suggests that further biological markers should be studied in this disorder.

REFERENCES

Arora RC and Meltzer HY (1989). Increased serotonin 2 receptor binding as measured by ^{3}H-LSD binding in the blood platelets of depressed patients. *Life Sci* 44:725–734. 259–67.

Berk M, Bodemer W, Van Oudenhove T and Butkow N (1995). The platelet intracellular response to serotonin is augmented in bipolar manic and depressed patients. *Human Psychopharmacol* 10:189–193.

Berk M, Kessa K, Szabo CP and Butkow N (1997). The augmented platelet intracellular calcium response to serotonin but not bulimia may be due to subsyndromal depression. *Int J Eat Disord* 22:57–63.

Biegon A, Weizman A, Karp L, Ram A, Tiano S and Wolff MI (1987). Increased serotonin 5-HT_2 receptor binding on blood platelets-a peripheral marker for depression? *Life Sci* 41:2485–2492.

Bothwell RA, Eccleston D and Marshall E (1994). Platelet intracellular calcium in patients with recurrent affective disorders. *Psychopharmacol* 114:375–381.

Brusov OS, Beliaev BS, Katsonov AB, Zlobina GP. Factor MI and Lideman RR (1989). Does serotonin receptor supersensitivity accompany endogenous depression? *Biol Psychiatry* 25:375–381.

Dubovsky SL, Christiano J, Daniell LC, Franks RD, Murphy J, Adler L *et al.* (1989). Increased platelet intracellular calcium concentration in patients with bipolar affective disorders. *Arch Gen Psychiatry* 46:632–638.

Dubovsky SL, Lee C, Christiano J and Murphy J (1991). Elevated platelet intracellular calcium concentration in bipolar depression. *Biol Psychiatry* 29:441–450.

Dubovsky SL, Murphy J, Thomas M and Rademacher J (1992). Abnormal intracellular calcium ion concentration in platelets and lymphocytes of bipolar patients. *Am J Psychiatry* 149:118–120.

Eckert A, Gann H, Rieman D, Aldenhoff J and Müller WE (1993). Elevated intracellular calcium after 5-HT2 receptor stimulation in platelets of depressed patients. *Biol Psychiatry* 34:565–568.

Grynckiewicz G, Poenie M and Tsien RY (1985). A new generation of calcium indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450.

Jaffe A, Froom J and Galambos N (1994). Minor depression and functional impairment. *Archives of Family Medicine* 3:1081–1086.

Judd LL, Rapaport MH, Paulus MP and Brown JL (1994). Subsyndromal symptomatic depression: A new mood disorder? *J Clin Psychiatry* 55(4 suppl), 18–28.

Konopka LM, Cooper R, Crayton JW (1996). Serotonin-induced increases in platelet cytosolic calcium concentration in depressed schizophrenic and substance abuse patients. *Biol Psychiatry* 39:706–713.

Kusumi I (1993). Serotonin-2 receptor-mediated intraplatelet calcium mobilisation in affective disorders: relevance to the pathophysiology of depression. *Hokkaido Igaku Zasshi* 68:325–336.

Lindner A, Kenny M and Meacham AJ (1987). Effects of a circulating factor in patients with essential hypertension on intracellular free calcium in normal platelets. *NEJM* 316:509–513.

Mikuni M, Kagaya A, Takahashi K and Meltzer HY (1992). Serotonin but not norepinephrine-induced calcium mobilisation of platelets is enhanced in affective disorders. *Psychopharmacol* 106:311–314.

Okamoto Y, Okamoto Y, Kagaya A, Tamiya S, Fujita Y, Tohoda Y *et al.* (1995). Serotonin-induced platelet calcium mobilisation is enhanced in bulimia nervosa but not anorexia nervosa. *Biol Psychiatry* 38:274–276.

Olfson M, Broadhead WE, Weissman MM, Leon AC, Farber L, Hoven C *et al.* (1996). Subthreshold psychiatric symptoms in a primary care practice. *Arch Gen Psychiatry* 53:880–886.

Pandey GN, Pandey SC, Janiack PG, Marks RC and Davis JM (1990). Platelet serotonin 2 receptor binding sites in depression and suicide. *Biol Psychiatry* 28:215–222.

Pollock WK, Sage SO and Rink TJ (1987). Stimulation of calcium efflux from Fura 2 loaded platelets activated by thrombin or phorbol myristate acetate. *Febs Letters* 210:132–136.

Rao GHR, Peller JD and White JG (1985). Measurement of ionised calcium in blood platelets with a new generation calcium indicator. *Biochem Biophys Res Commun* 123:652–657.

Resnik LM (1992). Cellular ions in hypertension, insulin resistance, obesity and diabetes: A unifying theme. *J Am Soc Neph* 3 (suppl 1), S78–S85.

Sage SO and Rink TJ (1986). Kinetic differences between thrombin induced and ADP induced calcium influx and release from internal stores in Fura 2 loaded human platelets. *Biochem Biophys Res Commun* 136:1124–1129.

Sang KHLQ, Laude D and Devynck M-A (1987). Platelet cytosolic free Ca2+ and serotonin content in essential hypertension. *J Hypertens* 5:237–240.

Sherbourne CD, Wells KB, Hays RD, Rogers W, Burnam MA and Judd LL (1994). Subthreshold depression and depressive disorder: Clinical characteristics of general medical and mental health speciality outpatients. *Am J Psychiatry* 151:1777–1784.

Tan CH, Javors MA, Seleshi E, Lowrimore PA and Bowden CL (1990). Effects of Lithium on platelet intracellular calcium concentration in patients with Bipolar (Manic Depressive) disorder and healthy controls. *Life Sci* 46:1175–1180.

Wells KB, Stewart A, Hays RD, Burnham MA, Rodgers W, Daniels M *et al.* (1989). The functioning and wellbeing of depressed patients: results from the medical outcomes study. *JAMA* 262:914–919.

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EUROPEAN NEURO-PSYCHOPHARMACOLOGY

The platelet intracellular calcium response to serotonin and thrombin in patients with panic disorder

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Abstract

Serotonin is implicated in both the biology of depression and anxiety. The aim of this study was to examine the platelet intracellular calcium response to serotonin and thrombin using spectrofluorometry in 14 patients with DSM-4 panic disorder compared to 14 matched controls. Patients did not show significantly higher baseline platelet intracellular calcium levels and serotonin stimulated levels of intracellular calcium than control subjects. There was a much smaller standard deviation in the control subjects than in the panic patients. The intracellular calcium response to thrombin activation was however greater in panic patients than in control subjects ($P<0.001$). The failure of this study to find enhanced sensitivity of 5-HT2 receptors in panic disorder is compatible with the findings of previous challenge studies that found no consistent dysregulation of serotonin in panic disorder. The enhanced thrombin sensitivity, nevertheless suggests some receptor mediated second messenger changes independent of serotonin in the disorder. © 1998 Elsevier Science B.V./ECNP

Keywords: Panic disorder; Platelet intracellular calcium response; Serotonin; Thrombin

1. Introduction

Panic disorder (with or without phobic avoidance) is a common anxiety disorder, occurring in 2%–6% of the population (Myers et al., 1984). Panic disorder is a distinct syndrome characterised by the recurrence of spontaneous panic attacks that arise from a dysfunctional biological substrate and elicit additional symptomatic reactions.

Alterations in several measures of serotonergic function, including levels of serotonin metabolites, receptor binding, and uptake in platelets and endocrine responses have been linked to depression (Sheehan and Harnett-Sheehan, 1996). Serotonin is implicated in the biology of anxiety, and similar experimental evidence also links changes in serotonergic neurotransmission in panic disorder (Krystal et al., 1996). Three such studies compared the effect of serotonin precursors on neuroendocrine responses in healthy subjects and in patients with panic disorder. Two showed no differences in the prolactin release in response to the serotonin precursor tryptophan between patients and controls (Charney et al., 1988; Den Boer and Westenberg, 1990). A small study of the effects of fenfluramine, a serotonin-releasing agent, reported more anxiogenic responses and greater elevations in plasma cortisol and prolactin among nine panic disorder patients than in a group of healthy control subjects (Targum and Marshall, 1989). A study of the cortisol response to 5-hydroxytryptophan did not clearly differentiate panic patients from controls (van Vliet et al., 1996). Examination of postsynaptic serotonin receptor function in panic disorder with the mixed agonist–antagonist *m*-chlorophenylpiperizine has shown some evidence of serotinergic dysregulation (Charney et al., 1987; Khan et al., 1988; Khan and Wetzler, 1991). These neuroendocrine challenge studies do not robustly support the involvement of serotonin in panic disorder. Binding of [3-H] paroxetine to the serotonin transporter in panic disorder was not found to differ from controls or patients with social phobia (Stein et al., 1995).

Binding sites for serotonin (5HT) are present on platelet membranes. Serotonin binds to cell surface receptors and

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results directly or indirectly in a rise in the intracellular concentration of ionised calcium (Murray and Harper, 1988). The platelet intracellular calcium response to serotonin has been found to be abnormal in depression (Mikuni et al., 1991; Eckert et al., 1993), suggesting increased sensitivity of the 5-HT2 receptor. The aim of this study was to examine the platelet intracellular calcium response to serotonin and thrombin in patients with panic disorder to clarify whether the above mentioned dysfunction of the serotinergic system is found in panic disorder.

2. Method

Patients aged ... 65 who met DSM-IV criteria for panic disorder were selected for the study. A Hamilton Depression Rating Scale (HAMD) and a structured interview to confirm the clinical diagnosis were done. Exclusion criteria included the presence of another axis I diagnosis including major depression, a HAMD score of over 16, significant medical illness, concomitant psychotropic drug therapy except PRN benzodiazepines with a washout period of 2 weeks (5 weeks for fluoxetine); hypertension and drug or alcohol abuse. Control subjects were age and sex matched and had HAMD scores between 0 and 7.

Twenty ml of blood was freshly drawn by venipuncture into an acid–citrate–dextrose buffer containing 100 μM aspirin from patients and controls who met the criteria for the study selection. Platelet-rich-plasma was obtained by 15 min centrifugation at 150g. This suspension was then centrifuged for 5 min at 850 g. The pelleted platelets were resuspended in an assay buffer (containing 137 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM dextrose, 5 mM HEPES, pH 7.4).

2.1. Loading of platelets with fura-2-AM

The platelets were incubated at 37°C for 45 min with 4 μM final concentration of fura-2-AM. After the loading period the platelets were kept at room temperature. Before the fluorescence measurements, the platelets were spun down at 350 g for 5 min. The supernatant was discarded and the pellet was resuspended in a HEPES buffer (containing 145 mM NaCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM glucose, 0.5 mM Na_2HPO_4, 1 mM $CaCl_2$, pH 7.55) and the cell count adjusted to 10×10^6 platelets/ml.

2.2. Fluorescence measurements

Fluorescence was measured in a Perkin–Elmer LS50 spectrophotometer with continuous gentle stirring. The excitation wavelengths were 340 nm and 380 nm with an emission wavelength of 410 nm. Serotonin concentrations of 0–1 μM were added sequentially. Thrombin (0.1 IU/ml) was then added to measure maximum calcium response. The cytoplasmic free calcium concentration was measured by the method of Grynkiewicz et al., 1985, that is by lysing the cells with Triton-X-100 in order to obtain maximum fluorescence, and then quenching the dye with 2 mM EGTA.

Data was analysed using the Systat® statistical program and Graph Pad Prism®.

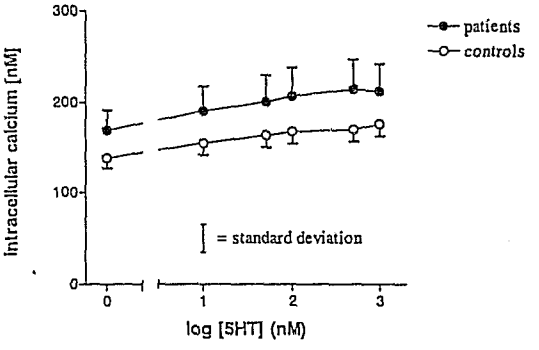


Fig. 1. Platelet intracellular calcium response to serotonin.

3. Results

Patients have slightly higher platelet intracellular calcium levels than control subjects (Fig. 1). This difference is not statistically significant at any of the 5HT concentrations. Only at 50 nM and at 500 nM does the confidence interval approach significance: (P=0.089; n=14; S.D.=126) There is a much smaller standard deviation in the control subjects than in the panic patients. A wide standard deviation of intracellular calcium results in mood disorders is a consistent finding in the literature (Dubovsky et al., 1989).

The percentage change in platelet intracellular calcium response from baseline to 1 μM 5HT shows that patients and controls display similar patterns (Fig. 2). There are

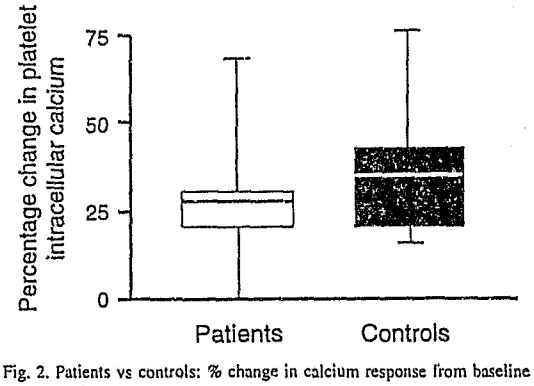


Fig. 2. Patients vs controls: % change in calcium response from baseline to 1 μM 5HT.

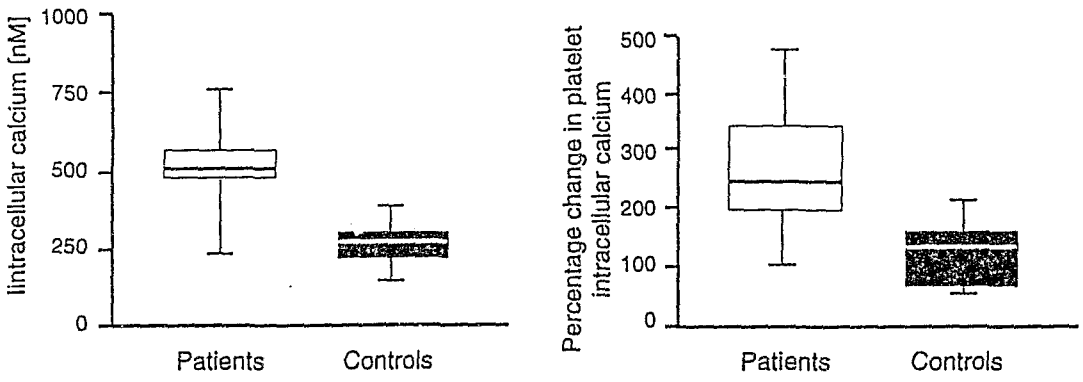


Fig. 3. Platelet intracellular calcium response to thrombim stimulation.

large standard deviations in both the patient and the control groups, which could account for the lack of statistical significance ($P=0,304$; $n=14$).

The intracellular calcium response to thrombin activation is greater in panic patients than in control subjects ($P<0.001$; $n=14$), even though the standard deviations in the patient sample is large (S.D.=126; Fig. 3).

4. Discussion

Second messenger involvement in panic disorder is suggested by an enhanced T cell CCK-4 stimulated intracellular calcium response (Akiyoshi et al., 1996). In this study there is a trend showing higher intracellular calcium levels in response to serotonin stimulation in panic patients compared to controls, although these levels do not reach statistical significance. There nevertheless is a significant statistical difference in the intracellular calcium response to thrombin activation. This suggests that there are differences in the receptor mediated second messenger responses of patients with panic disorder compared to control subjects and that the lack of statistical significance seen after stimulation with serotonin in this study may be due to the relatively small sample size, the large standard deviations in the patient sample or it may reflect no real inter-group differences in terms of serotonin receptor sensitivity.

Comorbidity of depression and panic disorder is common (Gorman and Coplan, 1996). Changes in the second messenger response to serotonin have been demonstrated in patients suffering from major depression (Van Praag et al., 1987). Greater calcium mobilisation from platelets in response to serotonin in depression has been described (Mikuni et al., 1992). This was replicated in a mixed depressive group by Eckert et al. (1993) and in bipolar and unipolar depression by Kusumi (1993), who found that serotonin mediated intracellular calcium concentrations were significantly higher in patients with bipolar as well as melancholic major depression than controls or euthymic treated patients. The serotonin response was found to be inhibited by $5HT_2$ antagonists, confirming that receptors role in this response. A similar pattern, with augmented levels in bipolar depressed and manic groups compared to controls and euthymic bipolar groups has been described (Berk et al., 1995). Okamoto et al. (1994) found enhanced serotonin induced calcium mobilisation in mania.

The failure of this study to find enhanced sensitivity of 5-HT2 receptors in panic disorder is compatible with the findings of previous challenge studies that found no consistent dysregulation of serotonin in this disorder (Charney et al., 1988; Den Boer and Westenberg, 1990; van Vliet et al., 1996). This suggests that the pathophysiology of panic disorder and depression differ in terms of the involvement of serotonin. The enhanced thrombin sensitivity, which has also been described in depression (Eckert et al., 1993), nevertheless suggests some receptor mediated second messenger changes independent of serotonin in the disorder.

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References

Akiyoshi, J., Moriyama, T., Isogawa, K. et al., 1996. CCK-4 induced calcium mobilisation in T cells is enhanced in panic disorder. J. Neurochem. 66, 1610–1616.

Berk, M., Bodemer, W., Van Oudenhove, T., Bukow, N., 1995. The platelet intracellular response to serotonin is augmented in bipolar manic and depressed patients. Hum. Psychopharmacology 10, 189–193.

Charney, D.S., Heninger, S.R., 1988. Serotonin function in panic disorders: The effect of intravenous tryptophan in healthy subjects and patients with panic disorder before and during alprazolam treatment. Arch. Gen. Psychiatry 43, 1059–1065.

Charney, D.S., Woods, S.W., Goodman, W.K., Heninger, G.R., 1987. Serotonin function in anxiety, II: Effects of the serotonin agonist *m*-CPP in panic disorder patients and healthy subjects. Psychopharmacology (Berl) 92, 14–24.

Den Boer, J.A., Westenberg, H.G.M., 1990. Behavioral, neuroendocrine and biochemical effects of 5-HTP administration in panic disorder. Psychiatry Res. 31, 267–278.

Dubovsky, S.L., Christiano, M.A., Daniell, L.C. et al., 1989. Increased platelet intracellular calcium in patients with bipolar affective disorders. Arch. Gen. Psychiatry 46, 632–638.

Eckert, A., Gann, H., Riemann, D., Aldenhoff, J., Muller, W.E., 1993. Elevated intracellular calcium levels after 5-HT2 receptor stimulation in platelets of depressed patients. Biol. Psychiatry 34, 565–568.

Gorman, J.M., Coplan, J.D., 1996. Comorbidity of depression and panic disorder. J. Clin. Psychiatry 57 (10), 34–41.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improves fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Khan, R.S., Asnis, C., Wetzler, S., Van Praag, H.M., 1988. Neuroendocrine evidence for a serotonin receptor supersensitivity in panic disorder. Psychopharmacology (Berl) 96, 360–364.

Khan, R.S., Wetzler, S., 1991. *m*-CPP as a probe of serotonin function. Biol. Psychiatry 30, 1139–1166.

Krystal, J.H., Deutsch, D.N., Charney, D.S., 1996. Biological Basis of panic disorder. J. Clin. Psychiatry 57 (10), 23–31.

Kusumi, I., 1993. Serotonin-2 receptor-mediated intraplatelet calcium mobilisation in affective disorders: relevance to the pathophysiology of depression. Hokkaido Igaku Zasshi 68, 325–336.

Mikuni, M., Kagaya, A., Takahashi, K., Meltzer, H.Y., 1992. Serotonin but not norepinephrine induced calcium mobilization of platelets is enhanced in affective disorders. *Psychopharmacology 106, 311–314.*

Murray, R.K., Harper, H.A., 1988. Harpers Biochemistry. Appleton and Lange, Norwalk, Conn.

Myers, J.K., Weissman, M.M., Tischler, G.L. et al., 1984. Six-month prevalence of psychiatric disorders in three communities: 1980–1982. Arch. Gen. Psychiatry 41, 959–967.

Okamoto, Y., Kagaya, A., Shinno, H., Motohashi, N., Yamawaki, S., 1994. Serotonin–induced platelet calcium mobilisation is enhanced in mania. Life Sci 56, 327–332.

Sheehan, D.V., Harnett-Sheehan, K., 1996. The role of SSRI's in panic disorder. J. Clin. Psychiatry 57 (10), 51–58.

Stein, M.B., Delaney, S.M., Chartier, M.J., Croft, C.D., Hazen, A.L., 1995. [3H] paroxetine binding to platelets of patients with social phobia: Comparison to patients with panic disorder and healthy volunteers. Biol. Psychiatry 37, 224–228.

Targum, S.D., Marshall, L.E., 1989. Fenfluramine provocation of anxxety in patients with panic disorder. Psychiatry Res. 28, 295–306.

Van Praag, H.M., Kahn, R.S., Asnis, G.M., Wetzler, S., Brown, S.L., Bleich, A., Korn, M.L., 1987. Denosologization of biological psychiatry or the specificity of 5-HT disturbances in psychiatric disorders. J. Affect. Disord. 13, 1–8.

van Vliet, I.M., Westenberg, H.G., Den Boer, J.A., 1996. *Behavioral, neuroendocrine and biochemical effects of different doses* of 5-HTP in panic disorder. Eur. Neuropsychopharmacology, 6, 103–110.

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Supersensitive platelet glutamate receptors as a possible peripheral marker in schizophrenia

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Hypoglutamatergic function is implicated in the pathogenesis of schizophrenia. The aim of this study was to examine the platelet intracellular calcium response to glutamate using spectrofluorometry in 15 schizophrenic patients and 15 matched control individuals as an index of platelet glutamate receptor sensitivity. Patients with schizophrenia had significantly lower baseline intracellular calcium levels than matched control individuals ($P = 0.03$). The percentage response of the schizophrenic individuals to glutamate stimulation was significantly greater than control individuals ($P < 0.001$). These data suggest that platelet glutamate receptors may be supersensitive in schizophrenia. Furthermore, the platelet may be a possible peripheral marker of glutamate function in schizophrenia. Int Clin Psychopharmacol 14:000–000 © 1999 Lippincott Williams & Wilkins

Key words: glutamate, platelet intracellular calcium response, receptor supersensitivity, schizophrenia

INTRODUCTION

Increasing attention has been focused on the role of the neurotransmitter, glutamate and its receptors, particularly the *N*-methyl-D-aspartate (NMDA) receptor, in schizophrenia (Weinberger, 1997). Kim *et al.* (1980) proposed a hypothesis that suggested hypofunction of the glutamatergic neuronal system in schizophrenia, which was based on the decreased levels of cerebrospinal fluid glutamate levels in schizophrenic patients compared with control individuals. This finding was replicated by Tsai *et al.* (1995) who found decreased glutamate levels in frozen brain tissue from schizophrenic patients. However, studies by Korpi *et al.* (1987) and Altamura *et al.* (1993) found no differences in glutamate levels between schizophrenic patients and control individuals.

Glutamate receptors consist of different subtypes. NMDA receptor dysfunction is hypothesized as an etiological factor in the pathogenesis of schizophrenia (Olney and Farber, 1995). NMDA receptors are involved in the regulation of intracellular free calcium concentrations and as such are linked to divalent cationic channels (Javitt and Zukin, 1990). Phencyclidine (PCP, angel dust), a NMDA antagonist is capable of producing a psychotic picture similar to schizophrenia (Javitt and Zukin, 1991). The binding site for PCP is located within the NMDA channel where it blocks this channel non-competitively.

NMDA receptors have been identified on platelet membranes (Almazov *et al.*, 1988). Increases in free intracellular calcium in response to agonist stimulation in non-neuronal cell lines have been linked to the NMDA receptor complex (Grant *et al.*, 1997). Kinetic properties of glutamate uptake in platelets and brain slices revealed similar measurents and were proposed as being sufficiently suited for future clinical studies (Mangano and Schwarcz, 1981). These studies justify further investigation into the disease using the platelet as a possible peripheral marker. Platelet intracellular

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calcium as a second messenger response to neurotransmitter stimulation is widely used as an indirect marker of receptor alterations in psychiatric illnesses (Dubovsky *et al.* 1989; Mikuni *et al.*, 1992; Berk *et al.*, 1995). Accessible peripheral markers remain an elusive goal in psychiatry in general, and schizophrenia in particular. The aim of the present study was to examine the platelet intracellular calcium response to glutamate stimulation in patients with schizophrenia in order to explore glutamate receptor changes in this disorder.

PATIENTS AND METHODS

The study group comprised 15 patients diagnosed by a structured clinical interview (Mini International Neuropsychiatric Interview) as suffering from schizophrenia, and 15 age and sex matched control individuals. All patients were drug free for a minimum of 2 weeks (4 weeks for depot preparations). The Brief Psychiatric Rating Scale was carried out on all patients, producing a mean score of 16.8. The mean age of the sample was 25.4 years (13 men and three women). Patients with a history of substance abuse or positive urine cannabis assays were excluded, as were patients with any significant history of medical illness. After complete description of the study to the participants, all patients gave written informed consent, and the project was approved by the University of the Witwatersrand Committee for Research on Human Subjects.

Blood (20 ml) was freshly drawn by venipuncture into an acid-citrate-dextrose buffer containing 100 μM aspirin from schizophrenic patients who met the criteria for study selection. Platelet-rich-plasma was obtained by 15 min centrifugation at 150 *g*. This suspension was then centrifuged for 5 min at 850 *g*. The pelleted platelets were resuspended in an assay buffer (containing 137 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM dextrose, 5 mM HEPES, pH 7.4).

Loading of platelets with fura-2-AM

The platelets were incubated at 37°C for 45 min with 4 μM final concentration of fura-2-AM. After the loading period, the platelets were maintained at room temperature. Before the fluorescence measurements, the platelets were spun down at 350 *g* for 5 min. The supernatant was discarded and the pellet was resuspended in a HEPES buffer (containing 145 mM NaCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM glucose, 0.5 mM Na_2HPO_4, 1 mM $CaCl_2$, pH 7.55) and the cell count adjusted to 10×10^8 platelets/ml. In experiments designed to block the intracellular calcium response, platelets were incubated with 100 μM dizocilpine (MK-801). Dizocilpine, which is a non-competitive NMDA receptor antagonist that cannot be displaced by glutamate, has been used in other studies (Javitt *et al.*, 1996; Grant *et al.*, 1997).

Fluorescence measurements

Fluorescence was measured in a Perkin-Elmer (Norwalk, Connecticut) LS50 spectrophotometer with continuous gentle stirring. The excitation wavelengths were 340 nm and 380 nm with an emission wavelength of 510 nm. Glutamate concentrations of 0–100 μM were added sequentially. Thrombin (0.1 IU/ml) was subsequently added to measure maximum calcium response. The cytoplasmic free calcium concentration was measured by the method of Grynkiewicz *et al.* (1985), i.e. lysing the cells with Triton-X-100 in order to obtain maximum fluorescence, and subsequently quenching the dye with 2 mM EGTA (ethylene glycol tetra acetic acid).

RESULTS

There was a significant difference between the baseline (unstimulated) levels of intracellular calcium between patients and control individuals ($P = 0.03$). The schizophrenic patients had significantly lower mean basal intracellular calcium levels (92 319 nM ± 47 795) than control individuals (148 297 nM ± 46 756). The actual levels of intracellular calcium did not differ significantly between patients and control individuals at all concentrations of glutamate stimulation (Fig. 1). On the other hand, the percentage response to stimulation with glutamate (Fig. 2) produced highly significant differences ($P < 0.001$) between patients and control individuals. This may have been because of the much lower level of baseline calcium in the schizophrenic sample. The maximal percentage response to

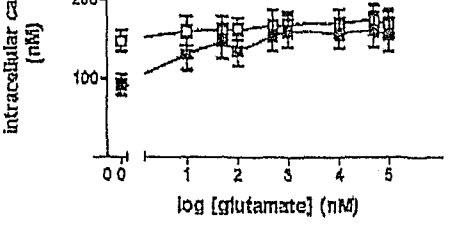


Figure 1. The platelet intracellular calcium response to glutamate. ■, schizophrenic patients; □ controls.

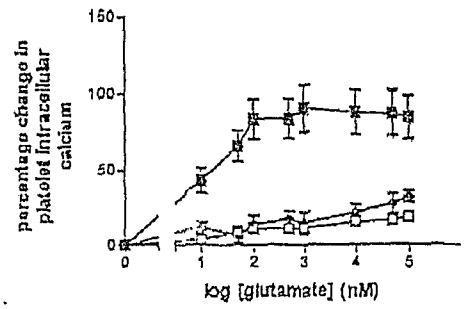


Figure 2. The percentage response of platelet intracellular calcium to glutatmate. ■, schizophrenic patients; □ controls; ♦, schizophrenic patients platelets preloaded with 100 μm MK801.

stimulation is seen at a concentration of 1 μM glutamate where the schizophrenic patients have a mean intracellular calcium percentage response of 89 908% ± 57 085 compared with control individuals who only respond by 11% ± 5005. The NMDA antagonist, dizocilpine (MK-801), blocked the increased response in the schizophrenic patients, suggesting that this effect is mediated via the NMDA receptor.

DISCUSSION

The dopamine theory of schizophrenia is the dominant biochemical theory of schizophrenia, but many limitations exist, mostly because of the lack of direct evidence of increased dopamine neuronal activity in clinical studies. Glutamatergic and dopaminergic pathways are thought to be linked in that they exert mutually antagonistic effects (Lieberman and Koreen, 1993). The hypofunction of glutamatergic neurotransmission in schizophrenia was first proposed by Kim *et al.* (1980) who found decreased cerebrospinal fluid levels of glutamate in schizophrenic patients. Tsai *et al.* (1995) supported this hypothesis by demonstrating decreased glutamate levels in the schizophrenic brain. Post-mortem studies have also shown increased binding of glutamate recognition sites in frontal cortex tissues. Moreover there is an increased number of NMDA receptors in the basal ganglia of schizophrenic patients (Lieberman and Koreen, 1993).

This study shows lower basal intracellular calcium levels in schizophrenic patients compared with control individuals. A speculative hypothesis for this finding is that, if excitatory neurotransmission is hypofunctional and intracellular calcium levels are elevated by glutamate stimulation, then a hypoglutamatergic state may be a factor in the lower basal intracellular calcium levels. This study further suggests that the receptor sites for glutamate on platelets of schizophrenic patients may be supersensitive. This supports evidence of decreased glutamate function in schizophrenia because hypofunction may cause a secondary upregulation of receptor sites. The lack of overlap between the experimental and control curves suggests significant sensitivity of this marker, although the specificity of the marker needs to be established by further research. In addition, novel therapeutic approaches involving modulation of NMDA receptors may offer promise in the therapy of schizophrenia.

REFERENCES

Almazov VA, Popov YG, Gorodinskii AI, Mikhailova OA, Dambinova SA, Gurevich VS (1988): Sites of high affinity binding of L-[^{3}H]-glutamic acid in human platelets. New type of platelet receptors? *Biokhimiya* 53:848–852.

Altamura CA, Mauri MC, Ferrara A, Moro AR, D'Andrea C, Zamberlan F (1993): Plasma and platelet excitatory amino acids in psychiatric disorders. *Am J Psychiatry* 150:1731–1733.

Berk M, Bodemer W, Van Oudenhove T, Butkow N (1995): The platelet intracellular calcium response to serotonin is augmented in bipolar manic and depressed patients. *Hum Psychopharmacol Clin Exp* 10:189–193.

Dubovsky SL, Christiano MA, Daniell LC, Franks RD, Murphy J, Adler L, *et al.* (1989): Increased platelet intracellular calcium in patients with bipolar affective disorders. *Arch Gen Psychiatry* 46:632–638.

Grant ER, Bacskai BJ, Pleasure DE, Pritchett DB, Gallagher MJ, Kendrick SJ, *et al.* (1997): N-methyl-D-aspartate receptors expressed in a non-neuronal cell line mediate subunit-specific increases in free intracellular calcium. *J Biol Chem* 272:647–656.

Grynckiewicz G, Poenie M, Tsien RY (1985): A new generation of Ca^{2+} indicators with greatly improved fluorescences properties. *J Biol Chem* 260:3440–3450.

Javitt DC, Zukin SR (1990): The role of excitatory amino acids in neuropsychiatric illness. *J Neuropsychiatry Clin Neurosci* 2:44–52.

Javitt DC, Zukin SR (1991): Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* 148:1301–1308.

Javitt DC, Steinschneider M, Schroeder CE, Arezzo JC (1996): Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: Implications for schizophrenia. *Proc Natl Acad Sci U S A* 93:11962–11967.

Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmuller B (1980): Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neurosci Lett* 20:379–382.

Korpi ER, Kaufmann CA, Marnela K, Weinberger DR (1987): Cerebrospinal fluid amino acid concentrations in chronic schizophrenia. *Psychiatry Res* 20:337–345.

Lieberman JA, Koreen AR (1993): Neurochemistry and neuroendocrinology of schizophrenia: a selective review. *Schizophrenia Bull* 19:371–429.

Mangano RM, Schwarcz R (1981): The human platelet as a model for the glutamatergic neuron: platelet uptake of L-glutamate. *J Neurochem* 36:1067–1076.

Mikuni M, Kagaya A, Takahashi K, Meltzer HY (1992): Serotonin but not norepinephrine induced calcium mobilization of platelets is enhanced in affective disorders. *Psychopharmacology* 106:311–314.

Olney JW, Farber NB (1995): Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 52:998–1007.

Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE, *et al*, (1995): Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Arch Gen Psychiatry* 52:829–836.

Weinberger DR (1997): The biological basis of schizophrenia: new directions. *J Clin Psychiatry* 58 (Suppl. 10):22–27.

REFERENCES

Akiyoshi, J., Moriyama, T., Isogawa, K., *et al.*1996. CCK-induced calcium mobilization in T cells is enhanced in panic disorder. *Journal of Neurochemistry* 66: 1610-1616.

Almazov, V.A., Popov, Y.G., Gorodinskii, A.I., *et al.* 1988. Sites of high affinity binding of L-[3H]-glutamic acid in human platelets.New type of platelet receptors? *Biokimiya* 53: 848-852.

Altamura, C.A., Mauri, M.C., Ferrara, A., *et al.* 1993. Plasma and platelet excitatory amino acids in psychiatric disorders. *American Journal of Psychiatry* 150: 1731-1733.

American Journal of Psychiatry 1998. Practice guidelines for the treatment of patients with panic disorder. 155, suppl 5: 26.

American Psychiatric Association Task Force on Laboratory Tests in Psychiatry 1987. The dexamethasone suppression test: an overview of its current status in psychiatry *American Journal of Psychiatry* 144: 1253-1262.

Anda, R.F., Williamson, D.F., Jones, D., *et al.* 1993. Depressed affect, hopelessness, and the risk of ischaemic heart disease in a cohort of U.S. adults. *Epidemiology* 4: 285-294.

Andersson, T., Dahlgren, C., Pozzan, T., *et al.* 1986. *Molecular Pharmacology* 30: 437-443.

Angst, J. & Preizig, M. 1995. Course of a clinical cohort of unipolar, bipolar, and schizoaffective patients: results of a prospective study from 1959-1985. *Schweiz Archives of Neurology and Psychiatry* 146: 1-16.

Arana, W., Barreira, P.J., Cohen, B.M., *et al.* 1983. The dexamethasone suppression test in psychotic disorders. *American Journal of Psychiatry* 140(11): 1521-1523.

Arora, R.C. & Meltzer, H.Y. 1989. Increased serotonin 2 receptor binding as measured by 3H-LSD binding in blood platelets of depressed patients. *Life Sciences* 44: 725-734.

Arora, R.C. & Meltzer, H.Y. 1993. Serotonin 2 receptor binding in blood platelets of schizophrenic patients. *Psychiatry Research* 47: 111-119.

Asberg, M., Traskman, L., Thoren, P. 1976. 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? *Archives of General Psychiatry* 33(10): 1193-1197.

Asnis, G.M., Eisenberg, J., van Praag, H.M., *et al.* 1988. The neuroendocrine response to fenfluramine in depressives and normal controls. *Biological Psychiatry* 24 (1): 117-120.

Avissar, S., Nechamkin, Y., Roitman, G., *et al.* 1998. Dynamics of ECT normalization of low G protein function and immunoreactivity in mononuclear leukocytes of patients with major depression. *American Journal of Psychiatry* 155: 666-672.

Bakish, D., Cavazzoni, P., Chudzik, J., *et al.* 1997. Effects of serotonin reuptake inhibitors on platelet serotonin parameters in major depressive disorder. *Biological Psychiatry* 41: 184-190.

Balidin, J., Gottfries, C.G., Karlsson, I., *et al.* 1983. Dexamethasone suppression test and serum prolactin in dementia disorders. *British Journal of Psychiatry* 143: 277-281.

Barefoot, J.C. & Schroll, M. 1996. Symptoms of depression, acute myocardial infarction and total mortality in a community sample. *Circulation* 93: 1976-1980.

Baxter, L.R., Phelps, M.C., Mazziotta, J.C. *et al.* 1985. Cerebral metabolic rates for glucose in modd disorders. Studies with positron emission tomography (PET) and (F-18)-fluoro-2-deoxyglucose (FDG). *Archives of General Psychiatry* 42: 441-447

Beckstead, R.M. 1979. Convergent preforontal and nigral projections to the striatum of the rat. *Neuroscience Letters* 12: 59-64.

Benloucif, S & Galloway, M.P. 1991. Facilitation of dopamine release in vivo by serotonin agonists: studies with microdialysis. *European Journal of Pharmacology* 200 (1): 1-8.

Berk, M., Bodemer, W., Van Oudenhove, T. *et al.* 1994. Dopamine increases platelet intracellular calcium in bipolar disorder and controls. *International Clinical Pharmacology* 9: 291-293.

Berk, M., Bodemer, W., Van Oudenhove, T. *et al.* 1995. The platelet intracellular calcium response to serotonin is augmented in bipolar manic and depressed patients. *Human Psychopharmacology Clinical and Experimental* 10: 189-193.

Berk, M., Kessa, K., Szabo, C.P. *et al.* 1997. The augmented platelet intracellular calcium response to serotonin in anorexia nervosa but not bulimia may be due to subsyndromal depression. *International Journal of Eating Disorders* 22: 57-63.

Berridge, M.J. & Irvine, R.F. 1989. Inositol phosphates in cell signalling. *Nature* 341: 197-205.

Berridge, M.J. 1991. Cytoplasmic calcium oscillations: A two pool model. *Cell Calcium* 12: 63-72.

Berridge, M.J. 1994. The biology and medicine of calcium signalling. *Molecular Cell Endocrinology* 98 (2): 119-124.

Biegon, A., Weizman, A., Karp, L. *et al.* 1987. Serotonin 5HT2 receptor binding on blood platelets- a peripheral marker for depression? *Life sciences* 41 (22): 2485-2492.

Biegon, A., Grinspoon, A., Blumenfeld, B. *et al.* 1990. Increased serotonin 5HT2 receptor binding on blood platelets of suicidal men. *Psychopharmacology (Berl)* 100 (2): 165-167.

Bleich, A., Brown, S.L., Kahn, R. *et al.* 1988. The role of serotonin in schizophrenia. *Schizophrenia Bulletin* 14: 297-315.

Blier, P., de Montigny, C., Chaput, Y. 1990. A role for the serotonin system in the mechanism of action of antidepressant treatments: Pre-clinical evidence. *Journal of Clinical Psychiatry* 51: 14-20.

Bohus, M., Forstner, U., Kiefer, C. *et al.* 1996. Increased sensitivity of the inositol-phospholipid system in neutrophils from patients with acute major depressive episodes. *Psychiatry Research* 65: 45-51.

Braffman, B.H., Zimmerman, R.A., Trojanowski, J.Q. *et al.* 1988. Brain MRI: pathologic correlation with gross and histopathology, 2: hyperintense white-matter foci in the elderley. *American Journal of Neuroradiology* 151: 559-566.

Brass, L.F. & Shattil, S.J. 1982. Changes in surface-bound and exchangeable calcium during platelet activation. *Journal of Biological Chemistry* 257 (23): 14000-14005.

Brown, R.P. & Mann, J.J. 1985. A clinical perspective on the role of neurotransmitters in mental disorders. *Hospital Communications in Psychiatry* 36: 141.

Brown, W.A., Johnston, R., Mayfield, D. 1979. The 24 hour dexamethadone suppression test in a clinical setting: relationship to diagnosis, symptoms, and response to treatment. *American Journal of Psychiatry* 136 (4B): 543-547.

Butler, J., O'Halloran, A., Leonard, B.E. 1992. The Galway study of panic disorder II Changes in some peripheral markers of noradrenergic and serotonergic function in DSM III-R panic disorder. *Journal of Affective Disorders* 26 (2): 89-99.

Carney, R.M., Rich, M.W., Tevelde, A. *et al.* 1987. Major depressive disorder in coronary artery disease. *American Journal of Cardiology* 60: 1273-1275.

Carney, R.M., Rich, M.W., Friedland, K.E. *et al.* 1988. Major depressive disorder predicts cardiac events in patients with coronary artery disease. *Psychosomatic medicine* 50: 627-633.

Carpenter, W.T. Jr. & Bunney, W.E. Jr. 1971. Adrenal cortical activity in depressive illness. *American Journal of Psychiatry* 128 (1): 31-40.

Castro, P., Le Maire, M., Toscano-Aguilar, M. 1983. Abnormal DST results in patients with chronic schizophrenia (letter). *American Journal of Psychiatry* 140: 1261.

Chaput, Y., de Montigny, C., Blier, P. 1991. Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments: an in-vivo electrophysiologic study in the rat. *Neuropsychopharmacology* 5: 219-229.

Charney, D.S., Heninger, G.R., Breier, A. 1984. Noradrenergic function in panic anxiety: effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. *Archives of General Psychiatry* 41: 751-763.

Charney, D.S., Woods, S.W., Goodman, W.K. *et al.* 1987. Serotonin function in anxiety, II: Effects of the serotonin agonist m-CPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)* 92: 14-24.

Charney, D.S. & Heninger, S.R. 1988. Serotonin function in panic disorders: the effect of intravenous tryptophan in healthy subjects and patients with panic disorder before and during alprazolam treatment. *Archives of General Psychiatry* 43: 1059-1065.

Cheetham, S.C., Crompton, M.R., Katona, C.L. *et al.* 1988. Brain 5HT2 receptor binding sites in depressed suicide victims. *Brain Research* 443: 272-280.

Cheetham, S.C., Crompton, M.R., Czudek, C. *et al.* 1989. Serotonin concentrations and turnover in brains of depressed suicides. *Brain Research* 502 (2): 332-340.

Coccaro, E.F., Prudic, J., Rothpearl, A. *et al.* 1984. Clinical utilization of the dexamethasone suppression test. *Journal of Clinical Psychiatry* 45: 382-384.

Coccaro, E.F., Siever, L.J., Klar, H.M. *et al.* 1989. Serotonergic studies in patients with affective and personality disorders. *Archives of General Psychiatry* 46: 587-599.

Coffey, C.E., Figiel, G.S., Djang, W.T. *et al.* 1988. Leukoencephalopathy in elderley depressed patients referred for ECT. *American Journal of Psychiatry* 145 (6): 701-706

Coffey, C.E., Figiel, G.S., Djang W.T. *et al.* 1989. White matter hyperintensity on MRI clinical and neuroanatomic correlates in the depressed elderley. *Journal of Neuropsychiatry Clinical Neuroscience* 1: 135-144.

Coffey, C.E., Figiel, F.S., Djang W.T. *et al.* 1990. Subcortical hyperintensity on MRI: a comparison of normal and depressed elderley subjects. *American Journal of Psychiatry* 147: 187-189.

Conn, P.J. & Sanders-Bush, E. 1986. Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5HT-2 binding site. *Journal of Neuroscience* 6 (12): 3669-3675.

Cowen, P.J., Charig, E.M., Fraser, S. *et al.* 1987. Platelet 5HT receptor binding during depressive illness and tricyclic antidepressant treatment. *Journal of Affective Disorders* 13: 45-50.

Curtis, V.A., Wright, P., Reveley, A. *et al.* 1995. Effect of clozapine on D-fenfluramine evoked neuroendocrine responses in shizophrenia and its relationship to clinical improvement. *British Journal of Psychiatry* 166 (5): 642-646.

Da Prada, M., Cesura, A.M., Launay, J.M. *et al.* 1988. Platelets as a model for neurones? *Experentia* 44: 115-126.

Das, I., Essali, M.A., de Belleroche, J. *et al.* 1994. Elevated platelet phosphatidylinositol bisphosphate in mediated schizophrenics. *Schizophrenia Research* 12: 265-268.

Davis, K.L., Kahn, R.S., Ko, G. *et al.* 1991. Dopamine in schizophrenia: a review and reconceptualization. *American Journal of Psychiatry* 148 (11): 1474-1486.

De Boer, T. 1996. The pharmacologic profile of mirtazapine. *Journal of Clinical Psychiatry* 57 ,Suppl 4: 19-25.

Delisi, S.M., Konopka, L.M., O'Connor, F L. *et al.* 1998. Platelet cytosolic calcium responses to serotonin in depressed patients and controls: Relationship to symptomatology and medication. *Biological Psychiatry* 43: 327-334.

De Montigny, C. 1984. Electroconvulsive shock treatments enhance responsiveness of forebrain neurons to serotonin. *Journal of Pharmacology and Experimental Therapeutics* 228: 230-234.

Den Boer, J.A. & Westenberg, H.G.M. 1988. Effect of a serotonin and noradrenaline uptake inhibitor in panic disorders: a double blind comparative study with fluvoxamine and maprotiline. *International Clinical Psychopharmacology* 3: 59-74.

Den Boer, J.A., Westenberg, H.G.M., Klompmakers, A.A. *et al.* 1989. Behavioral biochemical and neuroendocrine concommitants of lactate-induced panic anxiety. *Biological Psychiatry* 26: 612-622.

Den Boer, J.A. & Westenberg, H.G.M. 1990. Behavioral, neuroendocrine and biochemical effects of 5-HTP administration in panic disorder. *Psychiatry Research* 31: 267-278.

Dewan, M.J., Pandurangi, A.K., Boucher, M.L. *et al.* 1982. Abnormal dexamethasone suppression test results in chronic schizophrenic patients. *American Journal of Psychiatry* 139: 1501-1503.

Dewey, S.L., Smith, G.S., Logan, J. *et al.* 1995. Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and in vivo microdialysis. *Journal of Neuroscience* 15 (1): 821-829.

Dickinson, S.L. & Curzon, G. 1983. Roles of dopamine and 5HT in stereo-typed behavior. *Neuropharmacology* 22: 805-812.

Dubovsky, S.L., Christiano, J., Daniell, L.C. *et al.* 1989. Increased platelet intracellular calcium concentration in patients with bipolar affective disorders. *Archives of General Psychiatry* 46: 632-638.

Dubovsky, S.L., Murphy, J., Christiano, J. *et al.* 1992. The calcium second messenger system in bipolar disorders: data supporting new research directions. *Journal of Neuropsychiatry and Clinical Neuroscience* 4: 3-14.

Dubovsky, S.L. & Thomas, M. 1995. Beyond specificity: Effects of serotonin and serotonergic treatments on psychobiological dysfunction. *Journal of Psychosomatic Research* 39: 429-444.

Eaton, W.W., Kessler, R.C., Wittchen, H.U. *et al.* 1994. Panic and panic disorder in the United States. *American Journal of Psychiatry* 151: 413-420.

Eriksson, E., Carlsson, M., Nilsson, C. *et al.* 1986. Does alprazolam, in contrast to diazepam, activate alpha-2-adrenoceptors involved in the regulation of rat growth hormone secretion? *Life Sciences* 38: 1491-1498.

Eriksson, E., Westberg, P., Alling, C. *et al.* 1991. Cerebrospinal fluid levels of monoamine metabolites in panic disorder. *Psychiatry Research* 36 (3): 243-251.

Evans, D.L., Burnett, G.B., Nemeroff, C.B. 1983. The dexamethasone suppression test in the clinical setting. *American Journal of Psychiatry* 140: 586-589.

Extein, I., Pottash, A.L., Gold, M.S. 1981. The thyrotropin-releasing hormone test in the diagnosis of unipolar depression. *Psychiatry Research* 5 (3): 311-316.

Faludi, G., Tekes, K., Tothfalusi, L. 1994. Comparative study of platelet 3H-paroxetine and 3H-imipramine binding in panic disorder patients and healthy controls. *Journal of Psychiatry Neuroscience* 19 (2): 109-113.

Farde, L., Nyberg, S., Oxenstierna, G. *et al.* 1995. Positron emission tomography studies on D2 and 5HT2 receptor binding in risperidone-treated schizophrenic patients. *Journal of Clinical Psychopharmacology* 15 (1), Suppl 1: 19S-23S.

Flory, J.D., Mann, J.J., Manuck, S.B. *et al.* 1998. Recovery from major depression is not associated with normalization of serotonergic function. *Biological Psychiatry* 43 (5): 320-326.

Francois, A., Biver, F., Goldman, S. *et al.* 1995. Decrease in the frontal-superobasal metabolic ratio in unipolar depression. (Reduction du rapport metabolique frontal supero-basal dans la depression unipolaire) *Acta Psychiatrica Belgica* 95: 234-245.

Gattaz, W.F., Schmitt, A., Maras-Athanasios, M. 1995. Increased platelet phospholipase A2 activity in schizophrenia. *Schizophrenia Research* 16: 1-6.

Gill, J., Desouza, V., Wakeling, A. *et al.* 1992. Differential changes in alpha- and beta-adrenoceptor linked [45Ca2+] uptake in platelets from patients with anorexia nervosa. *Journal of Clinical and Endocrinological Metabolism* 74 (2): 441-446

Goetz, R.R., Klein, D.F., Gorman, J.M. 1997. Symptoms essential to the experience of sodium lactate-induced panic. *Neuropsychopharmacology* 14: 355-366.

Gorman, J.M. & Coplan, J.D. 1996. Comorbidity of depression and panic disorder. *Journal of Clinical Psychiatry* 57 (10): 34-41.

Grant, E.R., Bacskai, B.J., Pleasure, D.E. *et al.* 1997. N-methyl-D-aspartate receptors expressed in a non-neuronal cell line mediate subunit-specific increases in free intracellular calcium. *Journal of Biological Chemistry* 272: 647-656.

Grynkyewicz, G., Poenie, M., Tsien, R.Y. 1985. A new generation of Ca2+ indicators with greatly improved fluorescence properties. *Journal of Biological Chemistry* 260: 3440-3450.

Gulati, S., Khullar, M., Sagar, S. *et al.* 1992. Ca2+ transport in unstimulated platelets of essential hypertensives and their blood relatives. *Journal of Hypertension* 10: 1213-1219.

Haines, A.P., Imeson, J.D., Meade, T.W. 1987. Phobic anxiety and ischaemic heart disease. *British Medical Journal* 295: 297-299.

Hallam, T.J. & Rink, T.J. 1985. Agonists stimulate divalent cation channels in the plasma membrane of human platelets. *Febs Letters* 186: 175-179.

Healy, D. & Leonard, B.E. 1987. Monoamine transport in depression: kinetics and dynamics. *Journal of Affective Disorders* 12 (2): 91-103.

Hoffmann, P., Loimer, N., Chaundhry, H.R. *et al.* 1996. 5-hydroxy-indole-acetic acid (5HIAA) serum levels in depressed patients and ECT. *Journal of Psychiatry Research* 30: 209-216.

Hollister, L.E. & Csernansky, J.G. 1990. *Clinical Pharmacology of psychotherapeutic drugs*; Churchill Livingstone 194p First Edition

Hrdina, P.D. & Vu, T.B. 1993. Chronic fluoxetine treatment upregulates 5HT uptake sites and 5HT2 receptors in rat brain: an autoradiographic study. *Synapse* 14: 324-331.

Hrdina, P.D., Demeter, E., Vu, T.B. *et al.* 1993. 5HT uptake sites and 5HT2 receptors in brain of antidepressant-free suicide victims/depressives: increase in 5HT2 sites in cortex and amygdala. *Brain Research* 614: 37-44.

Hrdina, P.D., Bakish, D., Chudzik, J. *et al.* 1995. Serotonergic markers in platelets of patients with major depression: upregulation of 5HT2 receptors. *Journal of Psychiatry Neuroscience* 20 (1): 11-19.

Hrdina, P.D., Bakish, D., Ravindran, A. *et al.* 1997. Platelet serotonergic indices in major depression: up-regulation of 5HT2A receptors unchanged by antidepressant treatment. *Psychiatry Research* 66: 73-85.

Iny, L.J., Pecknold, J., Suranyi Cadotte, B.E. *et al.* 1994. Studies of a neurochemical link between depression, anxiety, and stress from [3H] imipramine and [3H] paroxetine binding on human platelets. *Biological Psychiatry* 36 (5): 281-291.

Jaffe, A., Froom, J., Galambos, N. 1994. Minor depression and functional impairment. *Archives of Family Medicine* 3: 1081-1086.

Jakovljevic, M., Muck-Seler, D., Pivac, N. *et al.* 1997. Seasonal influence on platelet 5-HT levels in patients with recurrent major depression and schizophrenia. *Biological Psychiatry* 41 (10): 1028-1034.

Jakovljevic, M., Muck-Seler, D., Pivac, N. *et al.* 1998. Platelet 5HT and plasma cortisol concentrations after dexamethasone suppression test in patients with different time course of schizophrenia. *Neuropsychobiology* 37 (3): 142-145.

Javitt, D.C. & Zukin, S.R. 1990. The role of excitatory amino acids in neuropsychiatric illness. *Journal of Neuropsychiatry Clinical Neuroscience* 2: 44-52.

Javitt, D.C. & Zukin, S.R. 1991. Recent advances in the phencyclidine model of schizophrenia. *American Journal of Psychiatry* 148: 1301-1308.

Javitt, D.C., Steinschneider, M., Schroeder, C.E. *et al.* 1996. Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: Implications for schizophrenia. *Proceedings of the National Academy of Sciences U.S.A.* 93: 11962-11967.

Jori, A., Dofini, E., Casati, C. *et al.* 1975. Effect of ECT and imipramine treatment on the concentration of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) in the cerebrospinal fluid of depressed patients. *Psychopharmacologia* 44: 87-90.

Judd, L.L., Rapaport, M.H., Paulus, M.P. *et al.* 1994. Subsyndromal symptomatic depression: A new mood disorder? *Journal of Clinical Psychiatry* 55, Suppl 4: 18-28.

Kahn, R.S., Asnis, G.M., Wetzler, S. *et al.* 1988. Neuroendocrine evidence for serotonin hypersensitivity in panic disorder. *Psychopharmacology* 96: 360-364.

Kahn, R.S., Asnis, C., Wetzler, S. *et al.* 1988. Neuroendocrine evidence for a serotonin receptor supersensitivity in panic disorder. *Psychopharmacology (Berl)* 96: 360-364.

Kahn, R.S. & Wetzler, S. 1991. m-CPP as a probe of serotonin function. *Biological Psychiatry* 30: 1139-1166.

Kaplan and Sadock Ed. 1994. *Synopsis of Psychiatry 7th Edition* (Publishers: Williams and Wilkins)

Kass, G.E.N., Llopis, J., Chow, S.C. *et al.* 1990. Receptor-operated calcium influx in rat hepatocytes. *Biological Psychiatry* 265 (29): 17486-17492.

Kawachi, I., Colditz, G.A., Ascherio, A. *et al.* 1994. Prospective study of phobic anxiety and risk of coronary heart disease in men. *Circulation* 89: 1992-1997.

Keller, M.B., Shapiro, R.W., Lavori, P.W. *et al.* 1982. Relapse in MDD: analysis with the life table. *Archives of General Psychiatry* 39: 911-915.

Keller, M.B., Lavori, P.W., Mueller, T.I. *et al.* 1992. Time to recovery, chronicity and levels of psychopathology in major depression. *Archives of General Psychiatry* 49: 809-816.

Kendler, K.S. 1983. Overview: A current perspective on twin studies of schizophrenia. *American Journal of Psychiatry* 140: 1413-1425

Kessler, R.C., Zhao, S., Blazer, D.G. *et al.* 1997. Prevalence, correlates, and course of minor depression and major depression in the National Comorbidity Survey. *Journal of Affective disorders* 45 (1-2): 19-30.

Kim, J.S., Kornhuber, H.H., Schmid-Burgk, W. *et al.* 1980. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neuroscience Letters* 20: 379-382.

Klein, D.F. 1964. Delineation of two drugs responsive anxiety syndromes. *Psychopharmacologia* 5: 397-408.

Klimek, V., Zak Knapik, J., Mackowiak, M. 1994. Effects of repeated treatment with fluoxetine and citalopram, 5-HT uptake inhibitors, on 5-HT1A and 5-HT2 receptors in the rat brain. *Journal of Psychiatry and Neuroscience* 19 (1): 63-67.

Konopka, L.M., Cooper, R., Crayton, J.W. 1996. Serotonin-induced increases in platelet cytosolic calcium in depressives, schizophrenic, and substance abuse patients. *Biological Psychiatry* 39: 708-713.

Kornhuber, J. & Kornhuber, M.E. 1986. Presynaptic dopaminergic modulation of cortical input to the striatum. *Life Sciences* 39: 669-674.

Korpi, E.R., Kleinman, J.E., Goodman, S.I. *et al.* 1986. Serotonin and 5-hydroxyindoleacetic acid in brains of suicide victms. Comparison in chronic schizophrenic patients with suicide as cause of death. *Archives of General Psychiatry* 43 (6): 594-600.

Korpi, E.R., Kaufmann, C.A., Marnela, K. *et al.* 1987. Cerebrospinal fluid amino acid concentrations in chronic schizophrenia. *Psychiatry Research* 20: 337-345.

Korsgaard, S., Gerlach, J., Christensson, E. 1985. Behavioral aspects of serotonin-dopamine interaction in the monkey. *European Journal of Pharmacology* 118: 245-252.

Krishnan, K.R.R., Goli, V., Ellinwood, E.H. *et al.* 1988. Leukoencephalopathy in patients diagnosed as major depressive. *Biological Psychiatry* 23: 519-522.

Krystal, J.H., Deutsch, D.N., Charney, D.S. 1996. Biological basis of panic disorder. *Journal of Clinical Psychiatry* 57(10): 23-31.

Kusumi, I., Koyama, T., Yamashita, I. 1991. Serotonin stimulated calcium response is increased in the blood platelets of depressed patients. *Biological Psychiatry* 30:310-312.

Kusumi, I. 1993. Serotonin-2 receptor-mediated intraplatelet calcium mobilization in affective disorders: relevance to the pathophysiology of depression. *Hokkaido Igaku Zasshi* 68 (3): 325-336.

Laghrissi-Thode, F., Wagner, W.R., Pollock, B.G. *et al.* 1997. Elevated platelet factor 4 and beta-thromboglobulin plasma levels in depressed patients with ischaemic heart disease. *Biological Psychiatry* 42: 290-295.

Le[illegible]nard, B.E. 1992. *Fundamentals of Psychopharmacology*. John Wiley Publishers Chichester

Lerer, B. 1985. Studies on the role of brain cholinergic systems in the therapeutic mechanisms and adverse effects of ECT and lithium. *Biological Psychiatry* 20 (1): 20-40.

Lesch, K.P., Wolozin, B.L., Murphy, D.L. *et al.* 1993. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. *Journal of Neurochemistry* 60 (6): 2319-2322.

Lesser, I.M., Rubin, R.T., Rifkin, R.P. *et al.* 1989. Secondary depression in panic disorder and agoraphobia,II: dimensions of depression symptomatology and their response to treatment. *Journal of Affective Disorders* 16: 49-58.

Lieberman, J.A., & Koreen, A.R. 1993. Neurochemistry and neuroendocrinology of schizophrenia: A selective review. *Schizophrenia Bulletin* 19: 371-429.

Lindner, A., Kenny, M., Meacham, A.J. 1987. Effects of a circulating factor in patients with essential hypertension on intracellular free calcium in normal platelets. *New England Journal of Medicine* 316: 509-513.

Llepola, U., Nousiainen, U., Puranen, M. *et al.* 1990. EEG and CT findings in patients with panic disorder. *Biological Psychiatry* 28: 721-727.

Mackay, A.V.P., Dobler, A., Birch, E.D. *et al.* 1978. 3H-spiperone binding in normal and schizophrenic post mortem brain. *Life Sciences* 23: 527-532.

Maes, M., Jacobs, M.P., Suy, E. *et al.* 1989. Cortisol, ACTH, prolactin and beta-endorphin responses to fenfluramine administration in major depressed patients. *Neuropsychobiology* 21 (4): 192-196.

Maes, M., D'Hondt, P., Suy, E. *et al.* 1991. HPA-axis hormones and prolactin responses to dextro-fenfluramine in depressed patients and healthy controls. *Progress in Neuropsychopharmacology and Biological Psychiatry* 15 (6): 781-790.

Maes, M., Meltzer, H.Y., Suy, E. *et al.* 1993. Seasonality in severity of depression: relationships to suicide and homicide occurrence. *Acta Psychiatrica Scandinavica* 88 (3): 156-161.

Maes, M., Dierckx, R., Meltzer, H.Y. *et al.* 1993. Regional cerebral blood flow in unipolar depression measured with Tc-99m-HMPAO single photon emission computed tomography:negative findings. *Psychiatry Research* 50 (2): 77-88.

Mangano, R.M. & Schwarcz, R. 1981. The human platelet as a model for the glutamatergic neuron: Platelet uptake of L-glutamate. *Journal of Neurochemistry* 36: 1067-1076.

Mann, J.J., McBride, A., Malone, K.M. *et al.* 1995. Blunted serotonergic responsivity in depressed inpatients. *Neuropsychopharmacology* 13: 53-64.

Mann, J.J., Malone, K.M., Diehl, D.J. *et al.* 1996. Demonstration in vivo of reduced serotonin responsivity in the brain of untreated depressed patients. *American Journal of Psychiatry* 153: 174-182.

Marazziti, D., Placidi, G.F., Cassano, G.B. *et al.* 1989. Lack of specificity of reduced platelet imipramine binding in different psychiatric conditions. *Psychiatry Research* 30 (1): 21-29.

Markowitz, J.S., Weissman, M.M., Ouellette, R. *et al.* 1989. Quality of life in panic disorder. *Archives of General Psychiatry* 46: 984-992.

Maura, G., Giardi, A., Raiteri, M. 1988. Release-regulating D-2 dopamine receptors are located on striatal glutamatergic nerve terminals. *Journal of Pharmacology and Experimental Therapeutics* 247 (2): 680-684.

Maura, G., Carbone, R., Raiteri, M. 1989. Aspartate-releasing nerve terminals in rat striatum possess D-2 dopamine receptors mediating inhibition of release. *Journal of Pharmacology and Experimental Therapeutics* 251 (3): 1142-1146.

McBride, P.A., Brown, R.P., DeMeo, M. *et al.* 1994. The relationship of platelet 5HT2 receptor indices to major depressive disorder, personality traits, and suicidal behavior. *Biological Psychiatry* 35: 295-308.

Meltzer, H.Y., Umberkoman-Wiita, B., Robertson, A. *et al.* 1984. Effect of 5-hydroxytryptophan on serum cortisol levels in major affective disorder. Enhanced response in depression and mania. *Archives of General Psychiatry* 41: 366-374.

Mendez, M.F., Adams, N.L., Lewandoski, K.S. 1989. Neurobehavioral changes associated with caudate lesions. *Neurology* 39: 349-354.

Merritt, J.E., Jacob, R., Hallam, T.J. 1989. Use of manganese to discriminate between calcium influx and mobilization from internal stores in stimulated human neutrophils. *Journal of Biological Chemistry* 264 (3): 1522-1527.

Mikuni, M., Kagaya, A., Takahashi, K. *et al.* 1992. Serotonin but not norepinephrine induced calcium mobilization of platelets is enhanced in affective disorders. *Psychopharmacology* 106: 311-314.

Mitchell, P. & Smythe, G. 1990. Hormonal responses to fenfluramine in depressed and control subjects. *Journal of Affective Disorders* 19 (1): 43-51.

Mokrani, M.C., Duval, F., Crocq, M.A. *et al.* 1997. HPA axis dysfunction in depression: correlation with monoamine system abnormalities. *Psychoneuroendocrinology* 22, Suppl 1: S63-S68.

Montgomery, S.A. 1990. *Anxiety and depression.* Wrightson Biomedical Publishing Ltd.

Murray, C.J.L. & Lopez, A.D.S. Eds 1996. *The global burden of disease*. Geneva, Switzerland. *The herald school of public health on behalf of the WHO and the World Bank.*

Murray, R.K., Granner, D.K., Mayer, P.A. *et al* 1988. *Harpers Biochemistry,* 21st edition: Appleton and Lange 700p.

Musselman, D.L., Tomer, A., Manatunga, A.K. *et al.* 1996. Exaggerated platelet reactivity in major depression. *American Journal of Psychiatry* 153: 1313-1317.

Nemeroff, C.B. & Evans, D.L. 1984. Correlation between the dexamethasone suppression test in depressed patients and clinical response. *American Journal of Psychiatry* 141:247-249.

Nemeroff, C.B., Knight, D.L., Krishnan, R.R. *et al.* 1988. Marked reduction in the number of platelet tritiated imipramine binding sites in geriatric depression. *Archives of General Psychiatry* 45 (10): 919-923.

Newman, M.E. & Lerer, B. 1989. Modulation of second messenger function in rat brain by in-vivo alteration of receptor sensitivity: relevance to the mechanism of action of electro-convulsive therapy and antidepressants. *Progress in Neuropsychopharmacology and Biological Psychiatry* 13 (1-20: 1-30.

Nisenson, L.G., Pepper, C.M., Schwenk, T.L. *et al.* 1998. The nature and prevalence of anxiety disorders in primary care. *General Hospital Psychiatry* 20 (1): 21-28.

Nishio, H., Ikegami, Y., Nakata, Y. *et al.* 1993. Relationships between serotonin induced elevation of intracellular Ca2+ concentration and stimulation of Ca2+ influx in blood platelets. *Neurochemistry International* 22 (2): 205-210.

Norman, T.R., Sartor, D.M., Judd, F.K. *et al.* 1989. Platelet serotonin uptake and 3H-imipramine binding in panic disorder. *Journal of Affective Disorders* 17 (1): 77-81.

Nuccitelli, R. Ed. 1994. *A practical guide to the study of calcium in living cells.* Academic Press, Inc. pg 368.

Nutt, D.J. & Fraser, S. 1987. Platelet binding studies in panic disorder. *Journal of Affective Disorders* 12: 7-11.

Nutt, D.J. 1998. Antidepressants in Panic disorder: clinical and pre-clinical mechanisms. *Journal of Clinical Psychiatry* 59, suppl 8: 24-28.

Okamoto, Y., Kagaya, A., Tamiya, S. *et al.* 1995. Serotonin-induced platelet calcium mobilisation is enhanced in bulimia nervosa but not anorexia nervosa. *Biological Psychiatry* 38: 274-276.

Olfson, M., Broadhead, W.E., Weissman, M.M. *et al.* 1996. Subthreshold psychiatric symptoms in a primary care practice. *Archives of General Psychiatry* 53: 880-886.

Olney, J.W. & Farber, N.B. 1995. Glutamate receptor dysfunction and schizophrenia. *Archives of General Psychiatry* 52: 998-1007.

Owen, F., Cross, A.J., Crow, T.J. *et al.* 1981. Neurotransmitter receptors in brain and schizophrenia. *Acta Psychiatria Scandinavia* 63: 20-28.

Owen, N.E., Feinberg, H., Le Breton, G.C. 1980. Epinephrine induces Ca2+ uptake in human blood platelets. *American Journal of Physiology* 239: H483-H488.

Oxenkrug, G.F. 1978. Dexamethasone test in alcoholics (letter). *Lancet* 2:795.

Pacheco, M.A. &; Jope, R.S. 1996. Phosphoinositide signalling in human brain. *Progress in Neurobiology* 50: 255-273.

Pandey, G.N., Pandey, S.C., Isaac, L. *et al.* 1992. Effect of electroconvulsive shock on 5HT2 and alpha 1-adrenoceptors and phosphoinositide signalling in rat brain. *European Journal of Pharmacology* 226: 303-10.

Pandey, S.C., Sharma, R.P., Janicak, P.G. *et al.* 1993. Platelet serotonin-2 receptors in schizophrenia: effects of illness and neuroleptic treatment. *Psychiatry Research* 48: 57-68.

Park, S.B.G., Williamson, D.J., Cowen, P.J. 1996. 5HT neuroendocrine function in major depression: prolactin and cortisol responses to D-fenfluramine. *Psychological Medicine* 26: 1191-1196.

Pearse, A.G.E. 1986. The diffuse neuroendocrine system:Peptides,amines,placodes and the APUD theory. *Progress in Brain Research* 68:25-31.

Pellissolo, A. & Lepine, P. 1998. Epidemiology of depression and anxiety disorders. *In SSRI's in depression and Anxiety.* Ed. S.A. Montgomery and J.A. den Boer (John Wiley and sons Ltd)

Peris, J., Dwoskin, L.P., Zahniser, N.R. 1988. Biphasic modulation of evoked 3H-D-aspartate release by D-2 dopamine receptors in rat striatal slices. *Synapse* 2: 450-456.

Peroutka, S.J. & Synder, S.H. 1980. Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potency. *American Journal of Psychiatry* 137 (12): 1518-1522.

Pettegrew, J.W., Nichols, J.S., Minshew, N.J. *et al.* 1982. Membrane physiological studies of lymphocytes and erythrocytes in manic depressive illness. *Journal of Affective Disorders* 4: 237-247.

Pietraszek, M.H., Takahashi, S., Takada, Y. *et al.* 1991. Diurnal patterns of serotonin,5-hydroxyindoleacetic acid,tryptophan and fibrinolytic activity in blood of depressive patients and healthy volunteers. *Thrombosis Research* 64 (2): 243-252.

Pini, S., Cassano, G.B., Simonini, E. *et al.* 1997. Prevalence of anxiety disorders comorbidity in bipolar depression, unipolar depression and dysthymia. *Journal of Affective Disorders* 42 (2-3): 145-153.

Pitts, A.F., Kathol, R.G., Gehris, T.L. *et al.* 1990. Elevated cerebrospinal fluid corticotropin-releasing hormone and arginine vasopressin in depressed patients with dexamethasone nonsuppression. *Society for Neuroscience Abstracts* 16: 454.

Pivac, N., Jakovljevic, M., Muck Seler, D. *et al.* 1997. Hypothalamic-pituitary-adrenal axis function and platelet serotonin concentrations in depressed patients. *Psychiatry Research* 73 (3): 123-132.

Prange, A.J. Jr., Lara, P.P., Wilson, I.C. *et al.* 1972. Effects of thyrotropin-releasing hormone in depression. *Lancet* 2 (785): 999-1002.

Prange, A.J. Jr. & Loosen, P.T. 1980. Some endocrine aspects of affective disorders. *Journal of Clinical Psychiatry* 41 (12): 29-34.

Pratt, L.A., Ford, D.E., Crum, R.M. *et al.* 1996. Depression, psychotropic medication, and risk of myocardial infarction. *Circulation* 94: 3123-3129.

Price, L.H., Charney, D.S., Delgado, P.L. *et al.* 1990. Clinical data on the role of serotonin in the mechanism(s) of action of antidepressant drugs. *Journal of Clinical Psychiatry* 51: 44-50.

Rabkin, J.G., Charles, E., Kass, F. 1983. Hypertension and DSM-III depression in psychiatric outpatients. *American Journal of Psychiatry* 140: 1072-1074.

Raskind, M., Peskind, E., Rivard, M.F. *et al.* 1982. Dexamethasone suppression test and cortisol circadian rhythm in primary degenerative dementia. *American Journal of Psychiatry* 139: 1468-1471.

Reiman, E.M., Raichle, M.E., Robins, E. *et al.* 1986. The application of positron emission tomography to the study of panic disorder. *American Journal of Psychiatry* 143: 469-477.

Rink, T.J. 1988. A real receptor operated calcium channel? *Nature* 334: 649-650.

Ross, B.M., Hudson, C., Erlich, J. *et al.* 1997. Increased phospholipid breakdown in schizophrenia. Evidence for the involvement of a calcium independent PLA2. *Archives of General Psychiatry* 54: 487-494.

Roy, A., Agren, H., Picker, D. *et al.* 1986. Reduced CSF concentrations of homovanillic acid and homovanillic acid to 5-hydroxyindoleacetic acid ratios in depressed patients: relationship to suicidal behaviour and dexamethasone nonsuppression. *American Journal of Psychiatry* 143: 1539-1545.

Sacchetti, E. & Cassano, G.B. Ed. 1992. *Panic and related disorders: current knowledge and perspectives.* Giardini Editori SA Lugani Switzerland.

Sachar, E.J., Hellman, L., Fukushima, D.K. *et al.* 1970. Cortisol production in depressive illness. A clinical and biochemical clarification. *Archives of General Psychiatry* 23 (4): 289-298.

Sackeim, H.A., Prohovnik, I., Moeller, J.R. *et al.* 1990. Regional cerebral blood flow in mood disorders.1. Comparison of major depressives and normal controls at rest. *Archives of General Psychiatry* 47: 60-70.

Sang, K.H.L.Q., Laude, D., Devynck, M.A. 1987. Platelet cytosolic free Ca2+ concentration and serotonin (5HT) content in essential hypertension. *Journal of Hypertension* 5: 237-240.

Schatzberg, A.F., Rothschild, A.J., Bond, T.C. *et al.* 1984. The DST in psychotic depression: diagnostic and pathophysiologic implications. *Psychopharmacology Bulletin* 20: 362-364.

Schatzberg, A.F. & Nemeroff C.B. Ed 1995. *The American Psychiatric Press textbook of psychopharmacology* 1st edition pg 896.

Schneider, L.S., Munjack, D., Severson, J.A. *et al.* 1987. Platelet 3H-imipramine binding in generalized anxiety disorder, panic disorder and agoraphobia with panic attacks. *Biological Psychiatry* 22:59-66.

Schreiber, G., Avissar, S., Danon, A. *et al.* 1991. Hyperfunctional G proteins in mononuclear leucocytes of patients with mania. *Biological Psychiatry* 29: 273-280.

Schildkraut, J.J. 1965. The catecholamine hypthesis of affective disorders: a review of supporting evidence. *American Journal of Psychiatry* 122: 509-522.

Sedvall, G., Pauli, S., Farde, L. *et al.* 1995. Recent developments in PET scan imaging of neuroreceptors in schizophrenia. *Israel Journal of Psychiatry and Related Sciences* 32 (1): 22-29.

Seibyl, J.P., Krystal, J.H., Price, L.H. *et al.* 1991. Effects of ritanserin on the behavioral, neuroendocrine and cardiovascular responses to m-chlorphenylpiperazine in healthy subjects. *Psychiatry Research* 38: 227-236.

Serres, C., Yang, J., Jouannet, P. 1994. RU486 and calcium fluxes in human spermatozoa. *Biochemical and Biophysical Research Communications* 204 (3): 1009-1015.

Sesack, S.R., Deutch, A.Y., Roth, R.H. *et al.* 1989. Topographic organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study using *Phaseolus vulgaris* leucoagglutinin. *Journal of Comparative Neurology* 290: 213-242.

Sethy, V.H. & Hodges, D.H. 1982. Role of beta-adrenergic receptors in the anti-depressant activity of alprazolam. *Research Communications in Chemical Pathology and Pharmacology* 36: 329-332.

Shapira, B., Lerer, B., Kindler, S. *et al.* 1992. Enhanced serotonergic responsivity following electro-convulsive therapy in patients with major depression. *British Journal of Psychiatry* 160: 223-229.

Sherbourne, C.D., Wells, K.B., Hays, R.D. *et al.* 1994. Subthreshold depression and depressive disorder: Clinical characteristics of general medical and mental health speciality outpatients. *American Journal of Psychiatry* 151: 1777-1784.

Shopsin, B., Gershon, S., Goldstein, M. *et al.* 1975. Use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. *Psychopharmacology Communications* 1 (2): 239-249.

Siever, L.J., Murphy, D.L., Slater, S. *et al.* 1984. Plasma prolactin changes following fenfluramine in depressed patients compared to controls: an evaluation of central serotonergic responsivity in depression. *Life Sciences* 34 (11): 1029-1039.

Smith, S.E., Pihl, R.O., Young, S.N. *et al.* 1987. A test of possible cognitive and environmental influences on the mood lowering effect of tryptophan depletion in normal males. *Psychopharmacology (Berl)* 91 (4): 451-457.

Stahl, S.M. 1977. The human platelet. A disgnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Archives of General Psychiatry* 34: 509-516.

Stahl, S.M. 1992. Serotonin neuroscience discoveries usher in a new era of novel drug therapies for psychiatry. *Psychopharmacology Bulletin* 28: 3-9.

Stahl, S.M. 1994. 5HT1A receptors and pharmacotherapy. *Psychopharmacology Bulletin* 30 (1): 39-43.

Stahl, S.M. 1997. *Psychopharmacology of antidepressants* Ed. Martin Dunitz Ltd

Stain-Malmgren, R., Tham, A., Aberg-Wistedt, A. 1998. Increased platelet 5HT2 receptor binding after electroconvulsive therapy in depression. *The Journal of ECT* 14: 15-24.

Stein, M.B., Delaney, S.M., Chartier, M.J. *et al.* 1995. [3H] paroxetine binding to platelets of patients with social phobia: Comparison to patients with panic disorder and healthy volunteers. *Biological Psychiatry* 37: 224-228.

Strober, M., Rao, U., DeAntonio, M. *et al.* 1998. Effects of electroconvulsive therapy in adolescents with severe endogenous depression resistant to pharmacotherapy. *Biological Psychiatry* 43: 335-338.

Sulzer, D.L., Levin, H.S., Mahler, M.E. *et al.* 1993. A comparison of psychiatric symptoms in vascular dementia and Altzheimers disease. *American Journal of Psychiatry* 150: 1806-1812.

Tan, C.H., Lee, H.S., Kua, E.H. *et al.* 1995. Resting and thrombin stimulated cytosolic calcium in platelets of patients with alcoholic withdrawal, bipolar manic disorder and chronic schizophrenia. *Life sciences* 56 (21): 1817-1823.

Targum, S.D. & Marshall, L.E. 1989. Fenfluramine provocation of anxiety in patients with panic disorder. *Psychiatry Research* 28: 295-306.

Tejani-Butt, S.M., Yang, J., Kaviani, A. 1993. Time course of altered thyroid states on 5HT1A receptors and 5HT uptake sites in rat brain: an autoradiographic analysis. *Neuroendocrinology* 57 (6): 1011-1018.

Trichard, C., Paillere –Martinot, M.L., Attar-Levy, D. *et al.* 1998. No serotonin 5HT2 receptor density abnormality in the cortex of schizophrenic patients studied with PET. *Schizophrenia Research* 31 (1): 13-17.

Tsai, G., Passani, L.A., Slusher, B.S. *et al.* 1995. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Archives of General Psychiatry* 52: 829-836.

Van Vliet, I.M., Slaap, B.R., Westenberg, H.G. *et al.* 1996. Behavioral, neuroendocrine and biochemical effects of different doses of 5-HTP in panic disorder. *European Neuropsychopharmacology* 6: 103-110.

Weinberger, D.R. 1997. The biological basis of schizophrenia: New directions. *Journal of Clinical Psychiatry* 58, Suppl 10: 22-27.

Weiner, R.D. & Krystal, A.D. 1994. The present use of electro-convulsive therapy. *Annual Reveiws of Medicine* 45: 273-281.

Weissman, M.M., Klerman, G.L., Markowitz, J.S. *et al.* 1989. Suicidal ideation and attempts in panic disorder and attacks. *New England Journal of Medicine* 321: 1209-1214.

Weissman, M.M., Markowitz, J.S., Ouellette, R. *et al.* 1990. Panic disorder and cardiovascular/cerebrovascular problems: Results from a community survey. *American Journal of Psychiatry* 147: 1504-1508.

Weissman, M.M., Bland, R.C., Canino, G.J. *et al.* 1996. Cross national epidemiology of major depression and bipolar disorder. *Journal of the American Medical Association* 276: 293-299.

Weissman, M.M., Bland, R.C., Canino, G.J. *et al.* 1997. The cross national epidemiology of panic disorder. *Archives of General Psychiatry* 54: 305-309.

Wells, K.B., Stewart, A., Hays, R.D. *et al.* 1989. The functioning and wellbeing of depressed patients: results from the medical outcomes study. *Journal of the American Medical Association* 262: 914-919.

Widerlov, E. 1988. A critical appraisal of CSF monoamine metabolite studies in schizophrenia. *Ann NY Acad Sci* 537: 309-323.

Wilkinson, D.G. 1989. *Depression, recognition and treatment in general practice.* Oxford Radcliffe Medical Press.

Yang, J., Serres, C., Philbert, D. *et al.* 1994. Progesterone and RU486: Opposing effects on human sperm. *Proceedings of the National Academy of Sciences USA* 91: 529-533.

Yates, M., Leake, A., Candy, J.M. *et al.* 1990. 5HT2 receptor changes in major depression. *Biological Psychiatry* 27 (5): 489-496.

Young, L.T., Li, P.P., Kamble, A. *et al.* 1994. Mononuclear leucocyte levels of G proteins in depressed patients with bipolar disorder or major depressive disorder. *American Journal of Psychiatry* 151: 594-596.

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