

**ROLE OF PROSTAGLANDINS IN NOCICEPTION
DURING ISCHAEMIA AND REPERFUSION OF THE
RAT'S TAIL**

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DECLARATION

This thesis is submitted in the optional format, approved by the faculty, of published work with encompassing introduction and conclusion.

CHAPTER 2

Gelgor, L., Butkow, N. and Mitchell, D., Effects of systemic non-steroidal anti-inflammatory drugs on nociception during tail ischaemia and on reperfusion hyperalgesia in rats, *Br. J. Pharmacol.*, 105 (1992) 412-416.

The idea for this study arose from discussion with my co-authors. The analysis and writing of the paper was done by me.

Neil Butkow assisted me in the planning and execution of the experimental work.

Duncan Mitchell, my supervisor, gave me advice with regard to statistical analysis and presentation of the data. He edited the manuscript before submission for publication.

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Steven Cartmell assisted me with the planning and execution of the experimental work. His contribution also consisted of discussions with regard to interpretation of the data.

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The original idea for this study was entirely my own. I carried out all the experiments on my own. Also, statistical analysis and the writing of the paper were done by me.

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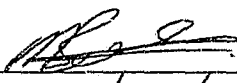
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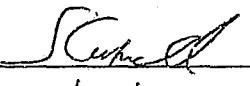
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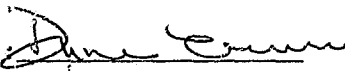
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ABSTRACT

I have investigated the effects of both systemic and intracerebroventricular administration of non-steroidal anti-inflammatory drugs (NSAIDs), of varying therapeutic potency, on i) nociception during tail ischaemia and ii) hyperalgesia to a noxious thermal stimulus, evident during reperfusion of the receptive field on the tail, in conscious Sprague-Dawley rats. NSAIDs were found to attenuate the hyperalgesia evident during reperfusion of the tail, whilst having no effect on the escape latency to a noxious ischaemic stimulus or on the tail flick latency in the absence of tail ischaemia. The intracerebroventricular doses required to attenuate reperfusion hyperalgesia were 2-3 orders of magnitude less than those required by systemic administration for the same drugs.

Using mechanical search stimuli, I located neurones in the dorsal horn of the spinal cord of rats with receptive fields in the tail. Neuronal responses to noxious and innocuous mechanical stimulation, as well as to noxious thermal stimulation before ischaemia and during reperfusion after ischaemia, were assessed. Of the population of neurones I examined, only a minority responded to thermal stimulation before ischaemia, and during reperfusion the neurones became more sensitive to mechanical stimuli, but not to noxious thermal stimuli. Furthermore, the neurones exhibited a decreased sensitivity to mechanical stimulation during ischaemia. Application of NSAIDs to the spinal cord did not alter the response properties of the neurones

during receptive field ischaemia, but decreased receptive field size and reduced spontaneous and evoked activity during reperfusion of the tail.

I have shown that the neurochemical mechanisms underlying nociception during ischaemia and reperfusion of the rat tail are different. While prostaglandins appear to play a role in mediating nociception during ischaemia, they are mediators of the hyperalgesia and neuronal hypersensitivity evident during receptive field reperfusion.

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LIST OF ABBREVIATIONS

A β	A beta fibre
A δ	A delta fibre
AMH	A-fibre mechano-heat nociceptor
AMPA	α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid
BK	bradykinin
Ca ²⁺	calcium
cAMP	cyclic adenosine monophosphate
CGRP	calcitonin gene related peptide
CMH	C-fibre mechano-heat nociceptor
CNS	central nervous system
COX	cyclooxygenase
DAG	diacylglycerol
EDRF	endothelium derived relaxing factor
EAA	excitatory amino-acid
GABA	γ -aminobutyric acid
h	hours
HTM	high threshold mechanoreceptor
5-HT	5-Hydroxytryptamine

IASP	International Association for the Study of Pain
icv	intracerebroventricular
i.d.	intradermal
IL-1 β	interleukin-1 beta
inj.	injection
IP ₃	inositol trisphosphate
i.pl.	intraplantar
LTB ₄	leukotriene B ₄
LTM	low threshold mechanoreceptor
MIA	mechanically insensitive afferent
min	minute
mech.	mechanical
Na ⁺	sodium
NGF	nerve growth factor
NK	neurokinin
NMDA	n-methyl-D-aspartate
NO-synthase	nitric oxide synthase
NS	not significant
NSAID	non-steroidal anti-inflammatory drugs

P	probability
PG	prostaglandin
PGE ₂	prostaglandin E ₂
PGI ₂	prostacyclin I ₂
PKC	protein kinase C
PLC	phospholipase C
PLA ₂	phospholipase A ₂
PMN	polymorphonuclear leukocytes
8R,15S-diHETE	dihydroxyeicosa-tetraenoic acid
s.d.	subdermal
SEM	standard error of the mean
SP	substance P
TNF	tumour necrosis factor
TXA ₂	thromboxane A ₂
TXB ₂	thromboxane B ₂
UV	ultraviolet
VIP	vasoactive intestinal peptide
WDR	wide dynamic range

PREFACE

Hyperalgesia, an enhanced sensitivity to a potentially painful stimulus, is a common feature of many clinical conditions. Following peripheral tissue injury, hyperalgesia may be evident at the site of injury as well as in non-injured tissue surrounding the injury. Many studies provide substantial evidence that hyperalgesia following peripheral tissue injury depends both on an increase in the sensitivity of primary afferent nociceptors (peripheral sensitization), as well as an increase in the excitability of central nervous system neurones (central sensitization) [Treede et al 1992]. Prostaglandins released from inflamed or damaged tissue are well known to produce hyperalgesia and sensitize primary afferent nociceptors. Prostaglandins are also released during ischaemia and excite afferent nociceptive pathways. In addition to their peripheral role, there is evidence that prostaglandins are involved in the central transmission of nociceptive information.

Together with co-workers, I have found that following a brief period of ischaemia to the rat tail, hyperalgesia to a noxious thermal stimulus is evident during subsequent reperfusion of the tail [Gelgor et al 1986a]. In this thesis, I have set out to i) examine the role of prostaglandins in nociception during ischaemia as well as reperfusion hyperalgesia, ii) evaluate the spinal neuronal substrates of reperfusion hypersensitivity and iii) assess the contribution of spinal prostaglandins to neuronal hyperexcitability during ischaemia and reperfusion of receptive fields on the rat tail.

Chapter 1 introduces the topic of hyperalgesia, emphasizing the role played by prostaglandins. Clinical phenomena, laboratory techniques used to induce hyperalgesia, somatosensory pain pathways and some of the changes in these pathways which contribute to hyperalgesia, are discussed.

In chapter 2, I have examined the effects of systemic administration of a range of non-steroidal anti-inflammatory drugs on the behavioural responses to both noxious ischaemia and noxious thermal stimulation during reperfusion. None of the agents tested had any effect on the animals response to noxious ischaemia or noxious thermal stimulation in the absence of ischaemia. They did, however, attenuate the hyperalgesia evident during reperfusion. My results indicate that the mechanisms underlying nociception during ischaemia and reperfusion are not the same.

Chapter 3 attempts to establish whether the development of reperfusion hyperalgesia depends on a release of prostaglandins in the central nervous system. Here I administered low doses of non-steroidal anti-inflammatory drugs via intracerebroventricular cannulae, directly into the central nervous system. My results show that prostaglandins are released in the central nervous system during reperfusion of the rat tail.

Chapter 4 describes experiments in which I investigated the properties of neurones in the spinal cord which might subserve reperfusion hyperalgesia. I examined the responses of convergent dorsal horn neurones, with receptive fields in the rat tail, to noxious and innocuous mechanical stimulation as well as noxious thermal stimulation before ischaemia and during reperfusion of the tail. The population of convergent neurones examined in this study exhibited hypersensitivity to mechanical stimuli during reperfusion of their receptive fields but not to thermal stimuli.

In chapter 5, I have examined whether the responses of convergent neurones to noxious and innocuous mechanical stimuli, both during ischaemia and during reperfusion of the tail, depended on local prostaglandin synthesis. Non-steroidal anti-inflammatory drugs were applied directly on to the surface of the spinal cord. The non-steroidal anti-inflammatory drugs suppressed the hypersensitivity and hyperexcitability of these neurones during reperfusion.

Chapter 6 contains a discussion on conclusions reached from the work presented in this thesis.

The experimental procedures used in this thesis were approved by the Animal Ethics Committee of the University of the Witwatersrand (Certificate numbers: 89/23/5, 89/181/2, 90/106/2) and complied with the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmerman 1983).

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CHAPTER 1

INTRODUCTION

1 Hypersensitivity in nociception

Under normal circumstances, a healthy individual can reliably discriminate between noxious and innocuous stimuli applied to the skin. In certain circumstances, however, such as following tissue or nerve injury, the normal relationship between stimulus intensity and sensation is altered. The threshold for the sensation of pain may decrease to a level where non-painful stimuli can elicit pain and the response to a normally painful stimulus is amplified. This altered state of cutaneous sensitivity has been defined as hyperalgesia [Lewis 1935/6; 1942].

Two principal theories explaining how we distinguish noxious from innocuous stimuli have been proposed. The pattern theory proposes that the perception of pain is not related to a specific receptor but to both spatial and temporal patterns of activity from multiple receptors [Nafe 1927,1929]. The specificity theory, however, maintains that there are specialized peripheral receptors and neural pathways for the transmission of noxious information [Sherrington 1906]. In recent years it has become increasingly apparent that neither of these theories alone can adequately explain the sensory changes associated with pathological pain [Laird and Cervero 1990; Melzack and Wall 1994]. Experimental evidence shows that the nervous system is not hard-wired, and is subject to plastic changes following injury [Cook et al 1987; Hylden et al 1989; Simone et al 1991c].

Changes in excitability in the peripheral and central nervous system underlie the altered state of cutaneous sensation following peripheral injury.

1.1 Hyperalgesia and allodynia

The International Association for the Study of Pain (IASP), in 1982, made a distinction in their taxonomy between an increased perception of pain induced by a normally painful stimulus, and that following non-painful stimulation, termed hyperalgesia and allodynia respectively [Merskey 1986]. These phenomena do not always occur together and their underlying mechanisms may not be the same in different clinical syndromes. Despite this differentiation in the IASP taxonomy, the term hyperalgesia is still often used to describe both phenomena, probably for consistency with earlier literature [Bonica 1992].

1.1.1 Analgesia and antihyperalgesia

In the absence of any prior injury, an animal will display escape behaviour to a given noxious thermal or mechanical stimulus with a certain latency or threshold that has a linear/exponential relationship with the intensity of the stimulus. In animal studies, hyperalgesia is manifest by either a decreased latency or threshold to a given noxious stimulus.

Antihyperalgesics, are agents which attenuate the facilitated pain state and return the response to a noxious stimulus to normal levels (Ch.1, Fig.1). Agents that either decrease the response to an acute noxious stimulus in the absence of an

abnormal pain state, or which decrease the enhanced responsiveness beyond normal if an augmented pain state is evident, by shifting the curve to the right (Ch.1, Fig.1) would be regarded as analgesics.

1.1.2 Clinical phenomena

Hyperalgesia and allodynia, which manifest as tenderness, are common symptoms of both inflammatory pain, associated with tissue damage, and of neuropathic pain arising from damage to the nervous system. Spontaneous pain is also sometimes evident following tissue damage or injury to the nervous system. Both hyperalgesia and allodynia can be elicited by mechanical, and thermal stimuli. The modality of hyperalgesia and allodynia manifested may vary with the underlying cause and is, therefore, sometimes used as a diagnostic aid [Bonica 1992].

Hyperalgesia is not always deleterious to the patient and can serve a protective function in that it leads the patient to protect the injured area. Hyperalgesia and allodynia after the initial injury has healed, however, significantly increases the suffering of the patient and appears to serve no useful purpose.

1.1.2.1 Inflammatory pain

Tissue damage usually results in the classic signs of inflammation: pain, oedema, hyperthermia, erythema and loss of function at the site of injury. These signs occur in many common clinical conditions such as traumatic injury, burns, bacterial or viral infection and following surgery. The development of

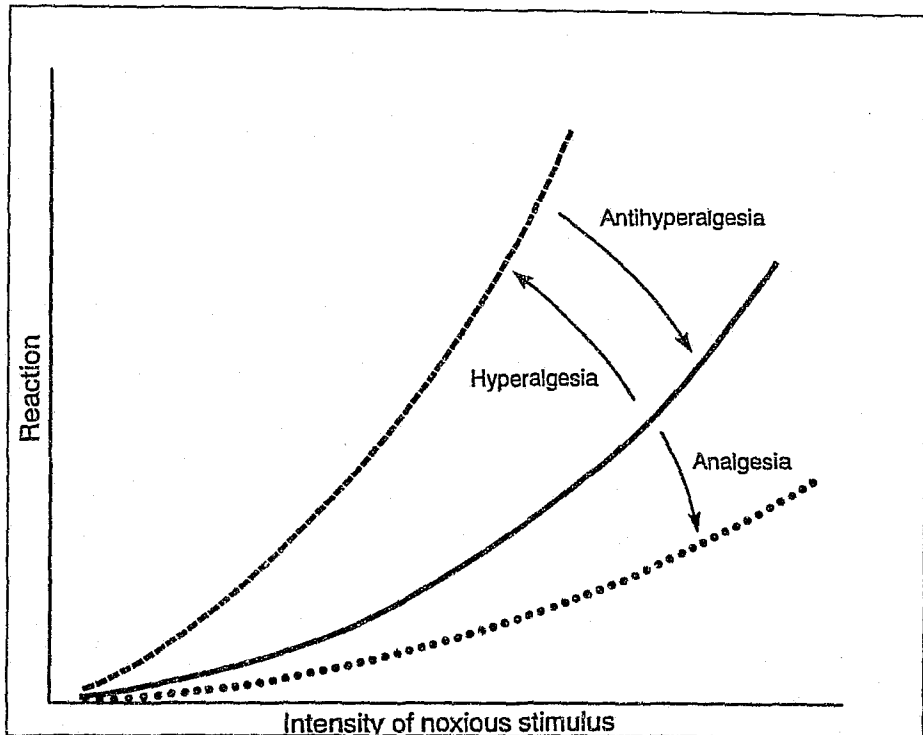


FIG. 1: Relationship between stimulus intensity and response in a conscious animal.

The solid line represents the response of an animal under normal circumstances to a given noxious stimulus. Analgesia would be reflected by a decrease in the magnitude of the response at any particular stimulus intensity (dotted line). An increase in the magnitude of the response would be indicative of hyperalgesia (dashed line).

Antihyperalgesia would be provided by agents which attenuate the facilitated pain, restoring the stimulus response curve towards normal.

inflammation is the consequence of the release and interaction of a variety of chemical mediators from the damaged tissue. Some of these mediators, for example prostaglandins, are released into the extracellular fluid and have the potential to stimulate or sensitize peripheral nociceptors to induce local pain or tenderness (primary hyperalgesia) (Ch.1, Fig.2). Nociceptors activated in this manner transmit the nociceptive message to the spinal cord, resulting in sensitization of central nervous system neurones which gives rise to pain and tenderness in an uninjured area (secondary hyperalgesia). While hyperalgesia and allodynia, arising from acute tissue damage or inflammation, usually disappear within a few hours to days following recovery of the initial injury, they can persist chronically for years in some conditions.

The clinical significance of these findings is that pain and the accompanying hyperalgesia can often be diminished, by treatment to prevent the establishment of central sensitization [Woolf and Chong 1993]. Preoperative treatment with either a non-steroidal anti-inflammatory drug (NSAID), which inhibits local prostaglandin synthesis, or a local anaesthetic, reduces the pain experienced after surgery [Campbell et al 1990; Jeebles et al 1991]. The value of pre-emptive analgesia in managing post-operative pain has recently been extensively reviewed and its clinical value questioned [McQuay 1994; Woolf and Chong 1993].

1.1.2.2 Ischaemic pain

Angina pectoris and intermittent claudication are examples of clinical ischaemic pain. Chemical mediators, similar to those released in the inflammatory process, are also released during ischaemia and excite afferent nociceptive pathways [Stasweska-Barczak et al 1976]. The pain disappears almost immediately following restoration of the blood flow, but reperfusion of previously ischaemic tissue often results in further damage to the tissue.

Primary dysmenorrhoea is a unique pain syndrome, since it is short-lived but recurs on a regular basis. It is similar to chronic pain syndromes in the sense that it will continue for a long period of time and cannot be alleviated by a single treatment [Dawood 1990]. The mechanisms underlying the pain of primary dysmenorrhoea are not clear, but there is evidence of increased endometrial production and release of the inflammatory mediator, prostaglandin [Dawood 1990; Lundstrom and Green 1978; Rosenwaks and Seegar-Jones 1980]. This process leads to abnormal uterine activity, reduced uterine blood flow (ischaemia) and hypoxia. These mechanisms are cumulatively responsible for the pain experienced. Inhibition of endometrial prostaglandin synthesis with NSAIDs is effective in relieving the pain in about 70% of the cases [Dawood 1985].

1.1.2.3 Neuropathic pain

Chronic pain arising from injury to the peripheral or central nervous system is often referred to as neuropathic pain. Spontaneous pain, mechanical (tactile) allodynia and thermal hyperalgesia are commonly associated with neuropathic pain syndromes arising from peripheral nerve lesions [Frost et al 1988; Torebjörk and Hallin 1978]. Tactile allodynia is the more common and more debilitating of the stimulus evoked responses although thermal hyperalgesia does occur in some patients [Wahren and Torebjörk 1992]. A common characteristic of many neuropathic pain syndromes, such as causalgia and reflex sympathetic dystrophy, that occurs following partial injury to the peripheral nerve, is that sympathetic ganglion blockade may relieve the spontaneous burning pain as well as the thermal hyperalgesia and mechanical allodynia and hyperalgesia associated with these conditions [Bonica 1979].

In patients suffering from central pain syndromes associated with disease or traumatic lesions of the spinal cord, brainstem or brain, one cannot distinguish between primary and secondary hyperalgesia [Boivie 1992]. Hyperalgesia to thermal stimuli may be evident, although hyperalgesia to mechanical stimuli is more often observed [Boivie 1992]. Allodynia occurs most commonly to cold, than to touch or warmth [Boivie 1992].

1.1.3 Human volunteers

Hyperalgesia and allodynia can be assessed quantitatively really only in human psychophysical studies, as an enhanced perception of painful and non-painful stimuli. In animal behavioural studies, it is generally inferred by an increased responsiveness to noxious and innocuous stimulation. Experimental studies in human volunteers have focused on cutaneous injury and are acute short-lived events. These studies do not resemble the chronic conditions of which hyperalgesia and allodynia pose such a difficult therapeutic problem. Such studies do, however, provide a valuable basis to evaluate both the sensory and affective components relating to these phenomena which cannot be achieved in an animal model. Hyperalgesia and allodynia have been observed experimentally in human volunteers following thermal [Raja et al 1984], electrical [Hardy et al 1950; Lewis 1942] and chemical insult [LaMotte et al 1991; Simone et al 1989a:b] to the skin. Particularly valuable are the studies that correlate the psychophysical responses in human volunteers with simultaneous recordings of activity in cutaneous nerve fibres [Adriaensen et al 1980; Torebjörk et al 1984; 1992; Torebjörk and Ochoa 1980]. These studies provide more insight into the peripheral mechanisms underlying the manifestation of hyperalgesia and allodynia and are a useful way to aid in the diagnosis and treatment of clinical conditions that give rise to these phenomena. Psychophysical responses in human volunteers have also been correlated with recordings of neuronal activity in the central nervous system of

primates using the same experimental paradigms [Coghill et al 1993a; LaMotte et al 1991; Simone et al 1991c].

1.1.3.1 Electrical stimulation

High intensity electrical stimulation of the skin is not widely used in human volunteers to induce hyperalgesia, as there are problems associated with the use of such an unnatural stimulus [see Handwerker and Kobal 1993]. Electrical stimulation, however, is often used as a tool once hyperalgesia is established, in order to selectively activate different axons directly, thus bypassing peripheral nociceptors [Gracely et al 1992; Torebjörk et al 1992]. Experimental studies have focused on the use of more natural stimulus modalities to induce hyperalgesia, which resemble the clinical pain state more closely.

1.1.3.2 Chemical stimulation

Intradermal or topical administration of exogenous irritants such as chloroform, mustard oil or capsaicin [Culp et al 1989; Simone et al 1989b], or administration of certain endogenous inflammatory mediators, such as prostaglandins or bradykinin [Ferreira et al 1978; Handwerker and Reeh 1991; Manning et al 1991; Whalley et al 1987] to the skin, produces hyperalgesia. Capsaicin, the pungent algescic agent of hot chilli peppers, has been used to induce hyperalgesia in human volunteers [LaMotte et al 1991; 1992; Simone et al 1991]. Capsaicin injection causes intense burning pain, accompanied by hyperalgesia, to both heat and

mechanical stimuli near the site of injection [LaMotte et al 1991; Simone et al 1989b; 1991]. The magnitude and duration of the hyperalgesia is dependent on the dose injected [Simone et al 1989b]. The hyperalgesia to heat is restricted to a local area around the injection site, whereas hyperalgesia and allodynia to mechanical stimuli are evident in a greater area of surrounding skin. Following capsaicin injection, a bleb forms, the size of which is dependent on the volume of capsaicin injected rather than the vehicle. The skin within the bleb, at the injection site, appears analgesic to pin prick and von Frey filaments [LaMotte et al 1991], with a visible area of redness ("flare") forming in the area surrounding the injection site.

1.1.3.3 Thermal stimulation

A burn injury to the skin of human volunteers produces local oedema and flare, accompanied by hyperalgesia to heat and mechanical stimulation at the site of the skin injury (primary hyperalgesia) [Hardy et al 1950; Lewis 1942; Meyer and Campbell 1981; Moiniche et al 1993; Raja et al 1984]. Further, there is evidence of mechanical hyperalgesia together with heat hypoalgesia, in the uninjured skin surrounding the injury (secondary hyperalgesia) [Raja et al 1984], indicating that different mechanisms account for the hyperalgesia to different stimulus modalities [Raja 1984].

Earlier studies examining the time course of primary and secondary hyperalgesia after a thermal injury found primary hyperalgesia to outlast secondary hyperalgesia [Hardy et al 1950]. A more recent study, however, found the

hyperalgesia within the injured area did not outlast that evident in the uninjured tissue [Moiniche et al 1993]. These differences may be due to the different methods employed: radiant heat [Hardy et al 1950] as opposed to a contact thermode [Moiniche et al 1993] to induce the heat injury.

In contrast to a thermal injury, intradermal capsaicin produces secondary hyperalgesia which outlasts the hyperalgesia evident at the injection site [LaMotte et al 1991; Simone et al 1989b]. These studies suggest differences between the neural mechanisms underlying hyperalgesia following a thermal or chemical injury.

1.1.4 Laboratory animals

Several animal models have been developed in an attempt to mimic, acutely and chronically, some of the clinical situations that give rise to both hyperalgesia and allodynia. These phenomena, in animal studies, are characterised by a decreased latency/threshold to either a noxious or innocuous stimulus.

1.1.4.1 Acute hyperalgesic assays

Inflammatory assays

Most of the experimental procedures used to induce hyperalgesia in animals involve the administration of a chemical irritant to produce inflammation. A number of substances, including phenylquinone, formalin, kaolin, capsaicin, acetic acid, carrageenan, trypsin, brewers yeast and mustard oil, are powerful

producers of pain and inflammation in animals [Hope et al 1990; Kayser et al 1988; LaMotte et al 1991; Schaible and Schmidt 1985; Woolf and Thompson 1991]. These substances have been administered either intraperitoneally, intramuscularly, subcutaneously, intraarterially or into the knee joint, to produce inflammatory pain. In addition to hyperalgesia, plasma extravasation, oedema and an increase in skin temperature may also occur [Kayser et al 1988; Willis and Cornelsen 1973].

Most inflammatory assays use the Randall and Selitto (1957) approach to assess the hyperalgesia. Randall and Sellito originally injected yeast subcutaneously into the footpad of the rat and determined pain thresholds by measuring the pressure necessary to elicit paw withdrawal. This test subsequently has been modified, using different irritants injected into the hindlimb, and applying constant pressure as opposed to increasing pressure [Ferreira 1972; Ferreira et al 1978; Vinegar et al 1976]. These hyperalgesic assays have been developed mainly to examine the effects of antinociceptives, especially non-steroidal anti-inflammatory drugs [Ferreira et al 1978].

Acute experimental arthritis can be induced by intra-articular injections of either kaolin or carrageenan, or a combination of both, into the knee joint [Schaible and Schmidt 1985;1988; Schaible et al 1991; Schepelman et al 1992]. These compounds evoke inflammation which develops within hours and produce plasma extravasation into the synovial cavity of the knee joint. Flexion and rotation of the lower leg results in vocalization responses from the animal [Coggeshall et al 1983;

Westlund et al 1992]. There is guarding of the leg and avoidance of movement persisting for at least 24 hours [Schaible and Schmidt 1985; 1988].

Carrageenan administration into the plantar hindpaw of animals produces evidence of hyperalgesia to both heat and mechanical stimuli in the injected paw only [Hargreaves et al 1988; Iadorola et al 1988; Ren et al 1992]. However, when vocalization instead of limb withdrawal was used as a measure of hyperalgesia, changes in responsiveness in both the injected and non-injected paw are evident, implying central nervous system involvement in the development of this secondary hyperalgesia [Kayser and Guilbaud 1987]. Whilst NSAIDs do not modify the threshold of uninflamed tissue but will of inflamed tissue, opiates will alter the threshold of both the inflamed and non-inflamed paws [Capetola et al 1980; Dubinsky et al 1987].

Formalin is widely used to produce inflammation in laboratory animals and the behavioural responses observed between different laboratories using this model are consistent. Subcutaneous injection of dilute formalin into the dorsal hindpaw produces a biphasic response in rats, cats, mice and primates [Alreja et al 1984; Dubuisson and Dennis 1977; Hunskaar et al 1986]. The response consists of licking or shaking the injected paw and is evident in two distinct phases: an early brief phase within five minutes of injection induced by local irritation, a period of quiescence lasting 10-15 minutes and a later tonic phase 20-60 minutes after injection, thought to reflect hyperalgesia. The effects of formalin are relatively short lived and aside from its value for the screening of putative antinociceptives,

it has proved to be useful in elucidating the neural mechanisms underlying hyperalgesia. While NSAIDs are antinociceptive only in the second phase of formalin test [Malmberg and Yaksh 1992b], opioids suppress both phases. The dissociation of the effects of anti-inflammatory agents on the first and second phase of the formalin response implies that the two phases represent two distinct processes relying on separate neural systems or chemical mediators.

Reperfusion Hyperalgesia

In conjunction with co-workers, I developed a novel method of inducing hyperalgesia in the rat. Ischaemia is induced by applying an inflatable cuff to the base of a restrained rat's tail and inflating the cuff to above the systolic pressure of the rat, thereby occluding the blood supply to the tail. The tourniquet is deflated the moment the rat indicates that the stimulus is noxious, either by vigorous grooming, attempting to turn around in the restrainer or jumping forward. The time between application of the tourniquet and the escape response is a measure of the sensitivity to the noxious ischaemic stimulus. To prevent tissue damage, the tourniquet is removed if the rat has not responded within 30 min.

Hyperalgesia, which occurs during reperfusion of the tail following transient ischaemia, is measured by comparing the tail flick latency using water immersion, during reperfusion, with that measured in the same animal before application of the tourniquet. Hyperalgesia results in a reduced tail flick latency during

reperfusion, and the duration of the hyperalgesia is dependent on the intensity of the subsequent noxious thermal stimulus [Gelgor et al 1986a].

Apart from inducing the hyperalgesia, the cuff gives the opportunity to assess the responses to ischaemic pain. The tail flick procedure allows the assessment of responses to thermal pain. The whole battery therefore allows the efficacy of putative antinociceptives against ischaemic pain, thermal pain and hyperalgesia to be assessed in one assay. Although the use of a thermal stimulus to assess hyperalgesia, rather than a mechanical stimulus, is usually attributed to Hargreaves et al [1988], our study was in fact the first to do so [Gelgor et al 1986a].

The hyperalgesia which occurs during tail reperfusion after transient ischaemia is dependant on the natural release of chemical mediators following ischaemia, and resolves after an hour, with no apparent sequelae. It is apparent that reperfusion hyperalgesia has some advantages over other acute hyperalgesic assays currently used [Gelgor et al 1986a]. In this assay, the noxious stimulus can be terminated as soon as the animal indicates distress, unlike those assays employing local administration of an irritant such as carrageenan, where the hyperalgesia takes a few hours to develop and the response requires pharmacological intervention to terminate it. Whilst the formalin test appears to be an extremely useful hyperalgesic assay, and the hyperalgesia develops relatively quickly compared with other inflammatory assays, oedema in the formalin test, in fact, only develops after four hours [Brown et al 1968].

These are the first studies inducing ischaemia and reperfusion hyperalgesia in the conscious rat. Previously, ischaemia had only been used in man to induce an acute noxious stimulus resembling clinical pain [Lewis et al 1931; Meyer et al 1978; Moore et al 1979; Posner 1984; Smith et al 1966; Sternbach et al 1977]. Earlier studies of ischaemic pain, relied on subjects performing exercise for the entire time the tourniquet was inflated [Harrison and Bigelow 1943; Lewis et al 1931; Williams et al 1965]. In subsequent studies the technique was modified and the duration of exercise during ischaemia varied [Moore et al 1979; Smith et al 1966; Sternbach et al 1977]. Considerable variation has been observed in human tourniquet pain scores [Sternbach et al 1977] mainly due to the different exercising techniques employed while the tourniquet was in place [Moore et al 1979]. In the rat, ischaemia is induced on non-exercising tissue. The latency of response to an ischaemic stimulus in the rat is consistent with those observed in human experiments [Woolf 1979]. Since there are species specific neuroanatomical and neurochemical substrates for pain [Boivie and Perl 1975; Webster 1977], results cannot always be extrapolated to humans and other species. The rat has, however, proved to be a useful laboratory animal for evaluating nociceptive mechanisms and for the initial assessment of putative antinociceptives. I believe, therefore, that it is useful to be able to induce ischaemic pain in the rat, since compared to other methods this type of pain resembles pathological pain more closely [Smith et al 1966], in whatever species it is used, including man.

The tail immersion test involves immersing the entire tail in a water bath controlled at 49°C. The time taken from submergence of the tail to the first coordinated motor response is measured on a stopwatch and recorded as the tail flick latency [Gelgor et al 1986 a;b]. Since the tail immersion test relies on a reflex action, changes in tail flick latency may be due to alterations in motor as well as sensory processing and cannot therefore be purely interpreted as a measure of pain sensation [Dubner 1989]. It is important to distinguish between alterations in motor function and alterations in sensation, when one is using a motor response to assess the sensation.

Motor activity in experimental animals can be assessed in many ways, including both observational and instrumental techniques [Kinnard and Watzman 1966].

The rotarod test is an instrumental technique that measures the length of time an animal can remain on a rotating rod, and was developed to measure drug effects on the motor coordination of rodents [Dunham and Miya 1957]. Most of the earlier rotarod studies used mice and employed brief trials. In our laboratory, we have modified the rotarod procedure using rats rather than mice and employing a testing time period more compatible with that of some tests of nociception [Cartmell et al 1991].

In experiments described in this thesis, I have used NSAIDs as an experimental tool to block prostaglandin synthesis. Rotarod studies have not been included in experiments presented in this thesis, since NSAIDs are not generally associated with a decrement in motor function except at doses which alter CNS function. I

have, however, subsequent to the publication of my manuscripts, confirmed that the highest doses of NSAIDs tested for antinociceptive properties, do indeed have no effect on motor coordination.

1.1.4.2 Chronic hyperalgesic assays

Inflammatory assays

The adjuvant induced arthritis test in rats [Pircio et al 1975] involves the intradermal injection of a suspension of dead *Mycobacterium butyricum* into the hindpaw. Cutaneous inflammation develops in the injected limb only, within a few hours. Hyperalgesia and oedema are present and last for approximately two weeks. There are no signs of systemic disease. This model allows direct comparisons to be made in individual rats between the inflamed and non-inflamed paws. Administration of *Mycobacterium butyricum* into the rat tail produces a polyarthritis [De Castro Costa et al 1981], resulting in a delayed hyperalgesia and inflammatory reaction of multiple joints occurring after 10 days to three weeks. The adjuvant assay, has been modified and instead of an adjuvant, sodium urate is administered into the knee joint [Coderre and Wall 1987]. Arthritis induced by the urate crystal, develops within two hours and declines after three days. The adjuvant arthritis model is used to assess the analgesic effectiveness of NSAIDs and opiates.

Recently, a model of hyperalgesia induced by ultra-violet (UV) radiation, has been described [Perkins and Kelly 1993]. Here, the hindpaw of a rat was exposed to

UV radiation twice, 18 hours apart, and hyperalgesia assessed daily using radiant heat, as well as a modified Randall Sellito test to assess mechanical hyperalgesia. The hyperalgesia is maximal at day 3 and day 5 after UV irradiation [Perkins et al 1993]. The early component, associated with inflammation, was sensitive to NSAIDs, whereas the later component was insensitive to NSAIDs. The development of hyperalgesia in the contralateral limb in this model indicates the involvement of central nervous system changes. This particular model may therefore prove extremely useful, since the hyperalgesia lasts many days better simulating the clinical situation and provides the characteristics of both primary and secondary hyperalgesia.

Neuropathic Pain

Peripheral injury

Early models of neuropathic pain, based on nerve transection, enabled the study of neuroma formation and deafferentation [Devor 1983; Devor and Janig 1981; Devor and Wall 1981; Hylden et al 1987; Wall et al 1979]. The animals, however, usually lacked signs of neuropathic pain commonly found in humans, following partial nerve injury. More recent models of peripheral neuropathy in the rat demonstrate behavioural characteristics of hyperalgesia, thermal and mechanical allodynia, spontaneous pain as well as evidence for the involvement of the sympathetic nervous system [Bennett and Xie 1988; Kim and Chung 1992; Seltzer et al 1990]. There are, however, some discrepancies in the behavioural

manifestations between different laboratories using the sciatic nerve constriction models [Attal et al 1990; Bennett and Xie 1988; Seltzer et al 1990]. The model described by Kim and Chung [1992] which involves tightly ligating the L6 and L5 spinal nerves produces more consistent behavioural changes. Most of the behavioural manifestations in these models parallel those seen in human cases of causalgia and reflex sympathetic dystrophy [Attal et al 1990; Bennett and Xie 1988; Bennett et al 1989; Kim and Chung 1992]. Whilst these models are valuable tools to investigate the mechanisms underlying peripheral nerve damage and treatment regimens, there are ethical concerns associated with using an assay of such long duration, also self mutilation has been observed in some animals.

Central nervous system injury

Hyperalgesia and allodynia arising from injury to the central nervous system has not been well studied due to the lack of a suitable animal model. Photochemically induced, spinal cord ischaemia in rats produces mechanical hyperalgesia, chronic allodynia and an increased sensitivity to cold stimuli but not to thermal stimuli [Hao et al 1991;1992]. These symptoms, observed in the rat, are similar to those described in patients following spinal cord injury [Tasker 1990].

Recently, a weight drop model of spinal cord injury in rats was used to characterize the sensory changes that occur following traumatic injury to the spinal cord [Siddall et al 1995]. The incidence of mechanical allodynia is higher in animals with partial spinal cord injury compared to animals with extensive spinal

cord damage. This finding is consistent with clinical observations of patients who have sustained partial spinal cord injury [Nathan et al 1986].

1.1.5 Neurochemistry of hyperalgesia and allodynia

Both peripheral nerve injury and tissue damage can induce hyperalgesia, allodynia and spontaneous pain. The underlying peripheral mechanisms are, however, different. Neuropathic pain, following peripheral nerve injury, is most likely due to pathological changes in peripheral nerve function, including neuroma formation, ectopic discharges and neural degeneration [Devor 1991]. A prominent feature of damaged afferent nerve fibres is their susceptibility to chemical modulation from sympathetic efferent fibres [Bonica 1979;1990]. Tissue damage, whether from disease or injury, is associated with inflammation. A multitude of endogenous chemical mediators are released during inflammation from circulating leukocytes, platelets, vascular endothelial cells, immune cells as well as cells in the peripheral nervous system. Some of these include, prostaglandins, bradykinin, adenosine, H^+ ions, K^+ ions, serotonin, lactate, interleukins, leukotrienne B_4 , histamine, endothelium derived relaxing factor (EDRF or nitric oxide), nerve growth factor (NGF) and noradrenaline [Beck and Handwerker 1984; Cotellessa et al 1984; Dray et al 1992; Guilbaud and Iggo 1984; Levine et al 1986; Lewis 1964; Lindahl 1974; Lynn 1977; Martin et al 1987; Palmer et al 1987; Parry 1979; Rocha and Silva 1964; Sicuteri et al 1979; Taiwo et al 1991].

The detection of chemical mediators, or neurotransmitters, in inflammatory exudates suggests that they may play a role in initiating or maintaining the behavioural responses associated with the injury. Administration of a particular mediator to conscious animals or human volunteers and examining its effects on responsiveness, enables one to assess whether it produces hyperalgesia (Table 1).

In most instances only one of the putative components of the inflammatory exudate is locally administered. The development of inflammation is, however, clearly dependant on a variety of chemical mediators which most likely act synergistically on the peripheral terminals of nociceptive sensory neurones [Rang et al 1991]. Furthermore, the quantities of the particular agent used to induce hyperalgesia are generally considerably higher than the concentrations found in inflammatory exudates [Lang et al 1990].

Another means of assessing the contribution of a particular mediator to the pain behaviour is to block the action of that substance with a selective receptor antagonist. The combination of agonist and antagonist manipulations provides convincing evidence that particular agents are involved in the process.

Alternatively one can use an enzyme inhibitor to block the enzyme involved in the synthesis of a particular mediator.

**Table 1: Behavioural evidence for chemical modulation of
hyperalgesia**

Chemical	Species	Stimulus	References
BK (i.d.)	human	heat	Manning et al 1991
BK (i.d.)	rat	mechanical	Taiwo & Levine 1988
PG (s.d.)	human	mechanical	Beubler and Juan 1978
PG (s.d.)	human	mechanical	Collier and Schneider 1972 Beubler and Juan 1978
PGE ₁ (i.d.)	human	mechanical	Solomon et al 1968
PGE ₂ (i.d.)	rat	mechanical	Taiwo & Levine 1988
PGE ₂ (i.pl)	rat	mechanical	Ferreira et al 1990
PGI ₂ (i.d.)	rat	mechanical	Taiwo and Levine 1988 a&b
LTB ₄ (i.d.)	rat	heat	Bisgaard and Kristensen 1985
LTB ₄ (i.d.)	rat	mechanical	Levine et al 1984
cAMP (i.d.)	rat	mechanical	Taiwo et al 1989b
NGF-OP (i.d.)	rat	mechanical	Taiwo et al 1991
NGF (i.pl)	rat	thermal	Woolf et al 1994
IL-1 β	rat	heat	Perkins and Kelly 1994
IL-1 β	rat	mechanical	Ferreira et al 1988
TNF	rat	thermal	Perkins and Kelly 1994
Adenosine	rat	mechanical	Taiwo and Levine 1990a
TNF α	rat	mechanical	Ferreira et al 1988

Systemic administration alone does not provide any indication of the site of action in the biological process. Both peripheral nerve and tissue injury induce complex changes in the neurochemistry of the central nervous system, that contribute to the hyperalgesia and allodynia arising from the injured area. To ascertain the effect of central chemical modulators in eliciting a behavioural response in conscious animals, agents are administered either directly into the lumbar intrathecal space, via chronically implanted intrathecal cannulae [Hylden and Wilcox 1981; Yaksh and Rudy 1977], or into specific brain sites via stereotactically-placed chronically-implanted microinjection guide cannulae [Gray and Gorzalka 1979]. Whilst administration of agents via intracerebroventricular cannulae allows one to assess if an agent has a central action, it does not give an indication of the precise site of action in the central nervous system.

Most of the putative modulators of hyperalgesia in the central nervous system have been detected in the spinal cord [Besson and Chaouch 1987; Evans 1989; see Yaksh and Malmberg 1994]. Neurotransmitters in the spinal cord are derived from afferent fibres, intrinsic spinal cord neurones and descending fibres [Table 2; extracted from Yaksh and Malmberg 1994].

Table 2: Behavioural evidence for spinal modulation of hyperalgesia

Intrathecal agent	Receptor type	Hyperalgesia	Reference
AGONIST			
Opioid	mu	attenuated	Yamamoto and Yaksh 1992
	delta	attenuated	Murray and Cowan 1991
	kappa	attenuated	Malmberg and Yaksh 1993b
Alpha Adrenergic	2A	attenuated	Malmberg and Yaksh 1993b
Adenosine	A ₁ /A ₂	attenuated	Malmberg and Yaksh 1993b
CGRP		induced	Coderre and Melzack 1991
Glutamate	NMDA	induced	Coderre and Melzack 1991 Aanonsen and Wilcox 1987
	Kainate	no effect	Coderre and Melzack 1991
	AMPA	no effect	Coderre and Melzack 1991
Tachykinins	NK-1	induced	Coderre and Melzack 1991 Malmberg and Yaksh 1994
	NK-2	induced	Coderre and Melzack 1991 Malmberg and Yaksh 1994
	NK-3	induced	Coderre and Melzack 1991
Somatostatin		no effect	Gaumann et al 1989
VIP		no effect	Seybold et al 1982
ANTAGONIST			
Glutamate	Kainate	attenuated	Yamamoto and Yaksh 1992
	AMPA	attenuated	Malmberg and Yaksh 1994
Tachykinins	NK-1	attenuated	Fleetwood-Walker et al 1990
ENZYME INHIBITOR			
Cyclooxygenase inhibitor		attenuated	Malmberg and Yaksh 1992
NO synthase inhibitor		attenuated	Keller et al 1992
			Malmberg and Yaksh 1993a

1.1.5.1 Proposed role of prostaglandins in hyperalgesia

Prostaglandins are important mediators of inflammation, and have been detected in inflammatory exudates of humans suffering from inflammatory diseases of the joints as well as in the exudates of experimentally induced injuries in animals [Brodie et al 1980; Ferreira 1972; 1983; Greaves et al 1971; Jonsson et al 1979; Juan and Lembeck 1976; Sano et al 1992].

Prostaglandins are not stored in cells and arise from fresh biosynthesis. Most cells except for erythrocytes are capable of synthesizing prostaglandins, which are released following trauma or damage to the cell membrane. Initially, prostaglandins are most likely released directly from the injured tissue, but they may then be augmented by the arrival of polymorphonuclear leukocytes (PMNs), phagocytes, macrophages and platelets, which release prostaglandins [Cronstein and Weissman 1993; Wightman and Dallob 1990]. Neural and non-neural cells can also synthesize prostaglandins in response to many of the chemical mediators released during inflammation such as bradykinin, histamine, noradrenaline, substance P, interleukin-1, and leukotrienes [Burch et al 1990; Ferreira et al 1988; Gonzales et al 1989; Juan 1977; Lembeck et al 1976; Lotz et al 1987; Pentland et al 1990].

These agents are thought to act on cell surface receptors which may be coupled to guanine nucleotide-dependent regulatory (G) proteins [Campbell 1991]. Fatty acids which are stored in membrane phospholipids, can be mobilized by

phospholipase A₂ and phospholipase C in response to noxious stimulation or to inflammatory mediators [Ferreira and Vane 1967; Irvine 1982; O'Flaherty 1987]. Arachidonic acid released by phospholipase A₂ or phospholipase C is metabolized via the lipo-oxygenase system to produce leukotrienes, or via the cyclo-oxygenase system to prostaglandins (PGE₂, PGF₂, PGD₂), prostacyclin (PGI₂) and thromboxanes (TXA₂, TXB₂) [Kuehl and Egan 1980; Samuelsson 1980; Wolfe 1982].

Cyclo-oxygenase enzymes (COX) exist in two isoforms; COX-1 is produced in normal quiescent conditions, whereas COX-2 is induced in inflammatory conditions from macrophages and other cells, possibly via other inflammatory agents like cytokines [Hla and Nielson 1992; Mitchell et al 1993]. Increased levels of COX-2 have been found in experimentally induced arthritis as well as in human arthritic conditions [Dray and Bevan 1993].

Whilst administration of low doses of prostaglandins does not cause overt pain, it does result in hyperalgesia [Collier and Schneider 1972; Crunkhorn and Willis 1971; Ferreira and Nakamura 1979; Ferreira 1983; Juan 1978]. When prostaglandins are given intradermally or intramuscularly in higher concentrations than usually occurs during inflammation, they cause long-lasting pain [Juhlin and Michaelson 1969; Karim 1971].

Prostanoids act via a number of distinct receptors, but the EP receptor for PGE₂ and the IP receptor for PGI₂ are the ones most commonly associated with

hyperalgesia [Khasar et al 1995; Matsumura et al 1995]. PGI₂-induced hyperalgesia is more potent than that produced by PGE₂, its effect, however, is of shorter duration [Ferreira et al 1978; Higgs et al 1978; Juan 1977]. Both of these prostanoids are thought to act directly on nociceptor terminals, since they induce hyperalgesia with a short onset latency, the hyperalgesia persists after blocking the indirect pathways which may contribute to hyperalgesia and they sensitize dissociated dorsal root ganglion cells [Gold et al 1996; Pitchford and Levine 1991; Taiwo and Levine 1989b; Taiwo et al 1987]. Combined administration of prostaglandins with some of the other inflammatory mediators, such as 5HT, bradykinin and substance P, shows a potentiation of the effects of these agents in the development of pain and hyperalgesia [Moncada 1982; Ferreira 1983]. Furthermore, after the induction of inflammation in the knee joint [Moncada et al 1975; 1979], skin [Ferreira et al 1972] or by the administration of bradykinin [Lembeck et al 1976; Juan 1977], the associated behavioural responses can be diminished by NSAIDs. Although aspirin and similar drugs (NSAIDs) are structurally diverse, they inhibit prostaglandin synthesis. Inhibition of prostaglandin synthesis is proposed to explain their mechanism of action [Vane 1971]. NSAIDs may also have other effects [Brune et al 1991]. However, a property shared by all NSAIDs is the inhibition of the cyclooxygenase enzymes, thereby preventing the breakdown from fatty acids to prostaglandins and thromboxanes.

Traditionally NSAIDs have been regarded as peripherally acting analgesics based on their efficacy in inflammatory conditions. It has become increasingly apparent, however, that NSAIDs can also exert an effect in the central nervous system [Yaksh 1982; Malmberg and Yaksh 1992b]. Intracerebroventricular administration of NSAIDs inhibits the hyperalgesia which follows administration of carrageenan into the rat paw [Ferreira 1983]. Furthermore, intrathecal administration of NSAIDs attenuates the behavioural responses associated with administration of formalin into the hindpaw of the rat [Malmberg and Yaksh 1992b; Yaksh 1982] and intrathecal administration of prostaglandins, PGE₂, PGD₂, and PGF₂ induces both hyperalgesia and allodynia [Ferreira 1983; Minami et al 1994; Taiwo and Levine 1986; Yaksh 1982].

1.2 Hyperexcitability

In the absence of any prior injury, impulses generated by high intensity (noxious) stimuli are conveyed by A and C primary afferent fibres to the central nervous system leading to the perception of pain. Low intensity stimuli, by activation of large diameter A β fibres, give rise to innocuous (non-painful) sensation [Treede et al 1992]. Tissue or nerve injury, however, can induce a variety of neurophysiological and neurochemical responses in both the peripheral and central nervous system. Electrophysiological recordings of changes in activity of peripheral afferent fibres and central nervous system neurones following

experimentally induced injury are interpreted on the basis of their relevance to nociception.

Primary hyperalgesia is attributed to sensitization of primary afferent A δ and C nociceptors, such that they are now activated by lower intensity stimuli [LaMotte et al 1991; Treede et al 1992]. This phenomenon of peripheral sensitization contributes directly to the enhanced responsiveness to thermal stimuli, within the injured area, and may also partially account for increased responsiveness to mechanical stimuli at the site of injury [LaMotte et al 1991; Lynn 1979; Meyer and Campbell 1981; Raja et al 1984; Treede et al 1992; Woolf and Chong 1993]. Both secondary hyperalgesia and allodynia are attributed to changes in sensory processing in the central nervous system, initiated by primary afferent nociceptive input [Cook et al 1987; Hardy et al 1952; LaMotte et al 1991; Wall and Woolf 1984; Woolf 1983; Woolf and Thompson 1991]. Central sensitization occurring in the spinal cord or at higher centres [Guilbaud 1986; Lamour 1983; Woolf and Thompson 1991] may also contribute to changes in mechanical sensitivity at the site of injury [LaMotte et al 1991; Torebjörk et al 1992]. A consequence of central sensitization is that activation of low-threshold A β afferent fibres can now evoke pain [Torebjörk et al 1992; Simone et al 1991]. Many neurophysiological studies have focused on recordings of second-order neurones in the spinal cord following injury since substantial evidence suggests that altered processing within the spinal cord contributes to the hyperalgesic state [Woolf and Thompson 1991].

1.2.1 Nociceptive pathways

1.2.1.1 Nociceptors

Nociceptors are specialized peripheral sensory units, responding preferentially to high intensity stimuli in the absence of any tissue damage [Sherrington 1906]. The physiological properties of cutaneous nociceptors have been studied extensively [for reviews see Campbell et al 1989; Raja et al 1988]. Recently, sensory changes associated with pain from the joints [Schaible and Schmidt 1988], muscle [Guilbaud 1988; Mense 1986] and viscera [Cervero 1988; Ness and Gebhart 1990] have also been discussed.

Cutaneous nociceptors belong to a subgroup of unmyelinated C-fibres, that have a conduction velocity of $< 2\text{ m/s}$ [LaMotte and Campbell 1978], or myelinated A δ -fibres that have conduction velocities of 10-25 m/s [Campbell et al 1979]. The majority of cutaneous nociceptors respond to a wide range of noxious stimuli, including mechanical, thermal and chemical stimuli and are thus referred to as polymodal nociceptors [Beck and Handwerker 1974; Beitel and Dubner 1976; Bessou and Perl 1969; Perl 1976; Szolcsanyi 1980]. Most studies use heat or mechanical stimuli to characterize nociceptors, so that terms like C-fibre mechano-heat nociceptor (CMH) or A-fibre mechano-heat nociceptor (AMH) often are used to describe the receptors [Burgess and Perl 1967; Campbell and Meyer 1983; Shea and Perl 1985].

Discrepancies between responses of nociceptors and pain reports in human volunteers imply that both spatial and temporal summation of nociceptive input is required for sensory detection of pain [Price et al 1989]. Nociceptors exhibit adaptation following prolonged application of a constant heat or mechanical stimulus [Adriaensen et al 1984; Beitel and Dubner 1976]. Furthermore, they have a limited capacity for repetitive firing, with repeated application of a noxious stimulus leading to fatigue [Handwerker et al 1987; Meyer and Campbell 1981].

C-fibre nociceptors

Polymodal C-fibre nociceptors are the most common type of nociceptor found in the skin of all mammalian species tested [Langford 1983]. They are more numerous in glabrous than hairy skin [Bessou and Perl 1969; Kumazawa and Perl 1977; Lynn and Carpenter 1982]. In non-primates, the mechanical receptive fields are usually small ($1\text{-}2\text{mm}^2$) or punctate [Bessou and Perl 1969; Lynn and Carpenter 1982], whereas in primates and man, the receptive fields tend to be larger and often composed of several discrete points [Beitel and Dubner 1976; Jorum et al 1989; Torebjörk 1974; Van Hees and Gybels 1972]. The receptive fields of polymodal C-nociceptors to mechanical and heat stimuli are largely coincident and of similar dimensions [Beitel and Dubner 1976; Thalhammer and LaMotte 1982; Treede et al 1990].

Other C-fibre nociceptors have been identified which respond preferentially to mechanical, heat, chemical or cold stimuli [Bessou and Perl 1969, Georgopoulos

1976; LaMotte and Thalhammer 1982; LaMotte et al 1988]. However, in many studies the full spectrum of stimulus modalities were not used to fully characterize a fibre [Treede et al 1992].

A-fibre nociceptors

Two distinct classes of AMH nociceptors with different heat response characteristics have been observed [Meyer et al 1985]. Type 1 AMH nociceptors respond preferentially to high intensity mechanical stimuli (HTMs) [Adriaensen et al 1983], are generally insensitive to chemical stimulation and usually respond to heat stimuli at temperatures only exceeding 50°C or following injury to their receptive field [Szolcsanyi et al 1988]. Their receptive fields are generally complex with multiple sensitive spots [Burgess and Perl 1967; Campbell et al 1979; Fitzgerald and Lynn 1977]. These nociceptors have been found in both glabrous and hairy skin. Type 2 AMH nociceptors respond rapidly to the onset of a noxious heat stimulus [Adriaensen et al 1983; Dubner and Bennett 1983] and can also be activated by mechanical and chemical stimuli [Adriaensen et al 1983]. They are found less frequently than type 1 AMHs and have been identified in the hairy skin of monkey and humans [Meyer et al 1985; Treede et al 1990].

Mechanically insensitive nociceptors

It has become apparent that not all cutaneous nociceptors respond to mechanical stimulation [Meyer and Campbell 1988]. Recently, a population of A δ and C afferent fibres that display mechanical sensitivity only following chemical

stimulation has been identified in the skin of monkeys and rats [Handwerker et al 1991; Meyer and Campbell 1988; Meyer et al 1991]. This novel class of chemosensitive nociceptors are called mechanically insensitive afferents (MIAs) or silent fibres [Davis et al 1990; 1993; LaMotte et al 1988; Meyer et al 1991]. The recruitment of these previously unresponsive primary afferent fibres most probably contributes to the hyperalgesia in inflammatory conditions [Handwerker et al 1991; Meyer et al 1991]. There are also reports of other cutaneous afferents responding exclusively to either heat or cold stimuli applied to their receptive fields [Baumann et al 1991; Beck et al 1974 b; Georgopoulos 1976; Kress et al 1992; LaMotte and Thalhammer 1982; Meyer et al 1991; Schmidt et al 1994].

Response of nociceptors to noxious stimuli

Thermal stimulation

Application of a brief, noxious heat stimulus to human hairy skin elicits a double pain sensation. This pain is perceived first as a pricking pain, followed by burning pain [Bromm and Treede 1987; Campbell and LaMotte 1983]. Latency measurements of the first pain sensation indicate that the afferent fibres responsible are in the A-fibre range [Campbell and LaMotte 1983]. The response properties of Type 2 AMHs indicate that they subserve first pain sensations [Adriaensen et al 1983; Dubner and Bennett 1983; Price and Dubner 1977]. Second pain sensation is attributed to the slower conducting C nociceptors [Price and Dubner 1977]. Parallel electrophysiological and psychophysical studies provide substantial evidence that activity in CMH nociceptors in glabrous skin

accounts for the response to heat (second) pain near threshold [for reviews see Handwerker and Kobal 1993; Raja et al 1988; Meyer et al 1994; Treede et al 1992]. C-fibre mechano-heat (CMH) nociceptors exhibit adaptation following prolonged application of a heat stimulus to the glabrous skin of the human hand whereas type 1 AMHs, despite a slightly delayed onset latency to heat stimuli, respond throughout the prolonged heat stimulus [Meyer and Campbell 1981]. Therefore, it is likely that type 1 AMHs play a role in signaling the sustained pain during a prolonged heat stimulus [Meyer and Campbell 1981; Meyer et al 1985].

Mechanical stimulation

While there is a reasonably good correlation between nociceptor discharges to heat stimulation and human pain ratings, this does not appear to be the case for mechanical stimulation [Adriaensen et al 1984; Koltzenburg and Handwerker 1994; Van Hees and Gybels 1981]. Mechanical stimuli eliciting a higher discharge in CMHs than heat stimuli do, are not perceived as painful, whereas the heat stimulus is perceived as painful [Torebjörk et al 1984]. The lack of correlation between a mechanical stimulus and nociceptor activity has been attributed partially to the heat stimulus eliciting a response in more nociceptors than a punctate mechanical stimulus, which excites fewer nociceptors [Raja et al 1988]. Alternatively, a mechanical stimulus, in addition to eliciting a response in nociceptors, may also excite low threshold mechanoreceptors (LTMs) which modulate nociceptive input in the spinal cord [Adriaensen et al 1984]. Thus, although the receptive fields of cutaneous nociceptors to heat and mechanical

stimuli are largely coincident, the transducer elements for mechanical and thermal stimuli appear to differ [Davis et al 1992; 1993; Simone and Ochoa 1991; Treede et al 1990].

Chemical stimulation

Chemical irritants and endogenous mediators of inflammation such as bradykinin, serotonin, K^+ and H^+ , can activate Type 2 AMHs and C fibre nociceptors [Cohen and Perl 1988; Khan et al 1992; LaMotte et al 1988; Lang et al 1990; Manning et al 1991; Simone et al 1989b]. Following administration of a chemical irritant, however, there is a lack of correlation between psychophysical pain ratings, in human volunteers, and discharges in mechanically sensitive polymodal nociceptors [Adriaensen et al 1980; LaMotte et al 1988], implying that there may be specific chemonociceptors in the skin [LaMotte et al 1988].

1.2.1.2 Spinal cord neurones

Two principal classes of spinal cord neurones have been implicated in the encoding of nociceptive information: nociceptive- specific neurones (class 3 neurones), which respond exclusively to noxious stimuli [Christensen and Perl 1970] and wide dynamic range (WDR) neurones (convergent or class 2 neurones), which respond maximally to noxious stimulation of their peripheral receptive fields as well as to innocuous stimulation [Wall 1960: for reviews Besson and Chaouch 1987; Willis 1991; Willis and Westlund 1997].

Nociceptive-specific and wide dynamic range neurones are found in spinal cord pathways considered most important in nociceptive processing [Simone et al 1991; Willis and Westlund 1997]. The spinothalamic tract is the major ascending pathway relaying nociceptive information to the brain [Dubner et al 1989; Price 1988; Willis 1985; Simone et al 1991]. The ventral posterior lateral nucleus of the thalamus is a major area of termination of the spinothalamic tract neurones [for review see Willis and Westlund 1997]. Nociceptive information from the spinal cord is ultimately relayed, via the thalamus and brainstem, to the cortex where it is presumed that activity occurs which underlies the conscious appreciation of pain.

Nociceptive specific neurones

Nociceptive specific neurones are found primarily in the superficial layers of the spinal cord (lamina I-II), [Christensen and Perl 1970; Price et al 1978; Willis 1985; 1991]. Some do, however, exist in deeper layers (lamina IV and V). They receive excitatory inputs from C polymodal and A δ HTM. A significant population of nociceptive specific neurones receives input exclusively from A δ HTMs [Price et al 1976] and, therefore, responds exclusively to mechanical stimulation, it is likely that they encode the distinction between noxious mechanical and noxious thermal stimuli [Price et al 1976]. Nociceptive-specific neurones appear to play a prominent role in the encoding of a reasonably protracted mechanical stimulus [Cervero 1988]. Since nociceptive specific neurones have small receptive fields, they are thought to convey precise

information about the peripheral location of a noxious stimulus [Laird and Cervero 1990].

WDR neurones

Wide dynamic range neurones are located predominantly in lamina V and VI of the dorsal horn [Christensen and Perl 1970; Price et al 1978; Willis 1985; 1991]. These neurones receive inputs from large diameter A β LTMs and from small diameter (A and C) nociceptive afferents. Convergence of cutaneous and visceral input [Ness and Gebhart 1990], and cutaneous and muscular, input on to WDR neurones, has been found [Mense 1986], indicating that these neurones may play a role in referred pain.

The receptive fields of WDR neurones are usually much larger than those of primary afferent neurones and nociceptive specific neurones [Bushnell et al 1984; Price and Dubner 1977; Willis 1985]. They exhibit gradients of sensitivity to peripheral stimulation [Price 1988], noxious stimuli, applied to the centre of their peripheral receptive field, produces a greater response than innocuous stimuli [Price et al 1979]. They are inhibited by low-threshold mechanical stimuli applied to the receptive field [for review see Dubner and Bennett 1983; Wall 1984] and excited only by noxious stimuli [Willis 1985; Price et al 1978]. On the basis of their large receptive fields, the radiation of pain is attributed to WDR neurones [Price 1988; Willis 1985].

Recently a study using both metabolic imaging in rats and stimulation of axons in the anterolateral quadrant of the spinal cord in conscious humans, indicates that the combination of spatial and temporal summation of somatosensory input by WDR neurones enables them to encode 'pain', even though they respond to both noxious and innocuous input of their receptive fields [Coghill et al 1993b]. It has been suggested that WDR neurones may also contribute to the encoding of stimulus location, depending on which part of their receptive field is stimulated [Coghill et al 1993a]. Based on the electrophysiological and psychophysical evidence, it seems as though activity in WDR neurones contributes substantially to the sensory discriminative aspects of prolonged pain.

Response of spinal cord neurones to noxious stimuli

Most earlier studies used acute stimuli to characterise the response properties of dorsal horn neurones [Coghill et al 1993a]. Recent studies, using prolonged thermal stimulation or subcutaneous administration of a chemical irritant, showed the responses of nociceptive-specific neurones to decline more rapidly than those of WDR neurones [Banna et al 1986; Coghill et al 1993a; Simone et al 1991]. So WDR neurones seem better suited to encode changes in intensity of noxious stimuli than nociceptive specific neurones do.

WDR neurones also seem more appropriate for encoding noxious thermal stimuli [Coghill et al 1992; Cervero et al 1988; Chung et al 1986; Dubner 1989; Maixner et al 1986; 1989; Surmeier et al 1986]. Furthermore, the responses of WDR

correlate better with psychophysical ratings of pain to chemical and thermal stimuli, than with those of nociceptive specific neurones [Coghill et al 1993b; Simone et al 1991].

1.2.2 Peripheral hyperexcitability

Following peripheral tissue damage, or injury to their receptive fields, nociceptors can be activated by stimuli which are not normally of sufficient intensity to activate them [Beitel and Dubner 1976; Bessou and Perl 1969]. This neurophysiological phenomenon of sensitization is characterized by a decrease in threshold to noxious thermal and mechanical stimulation, an augmented response to suprathreshold stimuli and sometimes, spontaneous activity [Raja et al 1988]. This sensitization following tissue injury is thought to underlie the peripheral contribution to hyperalgesia at the site of injury [Treede et al 1992]. The phenomenon has been observed in both AMHs and CMHs following heat injury [LaMotte et al 1983; Lynn and Carpenter 1982; Meyer and Campbell 1981], mechanical injury [Reeh et al 1987], antidromic electrical stimulation [Fitzgerald et al 1979; Meyer et al 1988; Reeh et al 1986], inflammation [Hylden et al 1989; Kocher et al 1987], application of exogenous chemicals such as mustard oil and capsaicin [Reeh et al 1986], and application of various endogenous mediators such as PGE₂, LTB₄ and bradykinin [Taiwo and Levine 1988b; Martin et al 1988].

1.2.2.1 Thermal stimuli

Sensitization of peripheral nociceptors to heat stimuli, within the receptive field, has been studied extensively [Beitel and Dubner 1976; Lynn 1979; Raja et al 1984; Thalhammer and LaMotte 1982; Torebjörk et al 1984; for review see Treede et al 1992]. The proportion of myelinated and unmyelinated nociceptors which exhibit hypersensitivity to heat stimulation depends on skin type. A dominant role for CMHs has been demonstrated in hairy skin [LaMotte et al 1982; for reviews see Raja et al 1988; Treede et al 1992]. Type 1 AMHs, known to respond to heat only following injury to their receptive fields, are believed to code for thermal hyperalgesia in glabrous skin [LaMotte et al 1982; Meyer and Campbell 1981]. Substantial evidence from many studies indicate that sensitization of primary afferent fibres can account for the hyperalgesia to heat stimuli occurring only in the injured area [Meyer et al 1994; Treede et al 1992].

1.2.2.2 Mechanical stimuli

Following heat injury [Bessou and Perl [1969] or administration of endogenous chemical mediators [Davis et al 1993; Martin et al 1988; Steen et al 1990; 1992], decreased mechanical thresholds in some CMHs have been observed. Lowered mechanical thresholds in AMH nociceptors following mechanical injury have also been noted, with no evidence of sensitization in C-fibre nociceptors to mechanical stimuli [Reeh et al 1987].

There is increasing evidence that a population of afferent fibres exist which are unresponsive in normal tissue but become mechanically sensitive in inflammatory conditions. Mechanically insensitive afferent (MIA) fibres in the skin, which exhibit marked chemosensitivity, may play an important role in mechanical hyperalgesia and chemogenic pain [Davis et al 1990; 1993; Handwerker et al 1991; Meyer et al 1991]. A high incidence of MIAs which became mechanically sensitive has also been found following inflammation of non-cutaneous tissue such as the knee joint [Neugebauer et al 1989; Schaible and Schmidt 1988], cornea [Tanelian 1991] and urinary bladder [Koltzenburg and McMahon 1986; Habler et al 1991]. The extent to which MIAs contribute to mechanical hyperalgesia, both at the site of injury and in uninjured tissue, needs further elucidation.

Expansion of the receptive field of nociceptors into an adjacent area of injury may also contribute to mechanical hyperalgesia [Thalhammer and LaMotte 1982]. Following cutaneous injury, some enlargement in the mechanical receptive fields of predominantly AMHs has been observed [Reeh et al 1987; Thalhammer and LaMotte 1982]. Thus, a stimulus applied to the injured region elicits a response in more nociceptors than before the injury. Studies in monkeys [Baumann et al 1991; Campbell et al 1988] and humans [LaMotte et al 1992], however, show cutaneous hyperalgesia to spread extensively beyond the area of nociceptor sensitization. While spatial summation may contribute to mechanical hyperalgesia at the site of injury, it is unlikely to account for the striking mechanical hyperalgesia that

occurs in the uninjured tissue. Several studies show the expansion of the peripheral receptive field, in part, to be due to changes in sensory processing in the dorsal horn of the spinal cord [Cervero et al 1988; Cook et al 1987; H. Heisel and Mense 1989; Woolf and King 1990]. Mechanical hyperalgesia, even within the injured area, cannot be attributed entirely to primary afferent nociceptor sensitization, further necessitating central nervous system involvement in the phenomenon [Handwerker 1991].

1.2.3 Central hyperexcitability

Based on psychophysical studies, Hardy et al [1950] proposed that hyperalgesia, following peripheral tissue injury, in part, is due to changes in the central nervous system. Woolf [1983] demonstrated changes in spinal cord excitability following a peripheral injury. He showed that brief conditioning stimuli applied to activate C-fibres result in a prolonged increase in the flexion withdrawal reflex in the spinal-decerebrate rat. More direct evidence of spinal cord excitability has been observed in studies where repetitive C-fibre stimulation induces an increase in the response of WDR dorsal horn neurones even after termination of the peripheral stimulus [Chung et al 1979; Cook et al 1987; Davies and Lodge 1987; Dickenson and Sullivan 1987b; 1990; Mendell 1966; Schouenborg and Dickenson 1985; Wall and Woolf 1984; Woolf and King 1987].

Increases in excitability of dorsal horn neurones have been repeatedly demonstrated following various forms of experimentally induced peripheral injury

including, thermal injury [Kenshalo et al 1982; Price et al 1978], chemical injury [Dougherty and Willis 1992; Haley et al 1990; Simone et al 1991], acute joint inflammation [Dougherty et al 1992c; Schaible et al 1987; 1991], polyarthritis [Calvino et al 1987; Menetrey and Besson 1982], ischaemia of the hindlimb [Sher and Mitchell 1990a], unilateral hindpaw inflammation [Hylden et al 1989] and peripheral nerve injury [Laird and Bennett 1993; Palecek et al 1992]. Sensitization of WDR neurones following injury is characterized by: 1) a reduction in threshold to noxious stimuli 2) an increase in responsiveness to both noxious and innocuous peripheral stimulation of the receptive field, 3) an increase in spontaneous firing rate 4) an increase in the size of the peripheral receptive field and 5) recruitment of novel inputs from A β fibres [Cook et al 1987; Hoheisel and Mense 1989; Hylden et al 1989; Laird and Cervero 1989; Woolf and King 1990; Neugebauer and Schaible 1990; Simone et al 1991].

The changes in receptive field properties of spinal cord neurones are due to the recruitment of previously subthreshold components of the receptive field, most likely as a result of increased synaptic output or increased excitability of the post-synaptic cell [Woolf and King 1990; Woolf 1991]. Synaptic connections between WDR neurones and low threshold A β fibres exist anatomically. There is also evidence that nociceptive-specific neurones develop a response to low-threshold input following C-fibre activation [Cook et al 1987; Neugebauer and Schaible 1990; Woolf and King 1990]. The capacity of nociceptive-specific neurones effectively to change into WDR neurones following peripheral injury or

inflammation, however, has been disputed [Hylden et al 1989; Laird and Cervero 1989; Dougherty and Willis 1992].

The spatial, temporal and threshold changes in the receptive fields of spinal cord neurones, particularly of WDR neurones, following injury, closely parallels the changes in sensation observed in humans and animals following experimentally induced injury [Coderre and Melzack 1985; Dickenson and Sullivan 1987 b; Koltzenburg et al 1992; LaMotte et al 1992; Raja et al 1984; Simone et al 1991; Torebjörk et al 1992]. These changes in sensation do not parallel changes of primary afferent nociceptors following peripheral injury. It has not been established yet whether ongoing peripheral input is required to maintain central hyperexcitability. A relatively brief activation of peripheral C-fibre nociceptors, with chemical irritants, produces changes in the spinal cord lasting up to 180 min [Cook et al 1987; Torebjörk et al 1992; Woolf and King 1990; Simone et al 1991]. Furthermore, a 20s electrical stimulus can produce changes in the flexor reflex which persists from a few minutes to an hour [Wall and Woolf 1984; 1986]. There, however, is evidence that continued low-level C-fibre activity is required to maintain central hyperexcitability [Koltzenburg et al 1992]. Interestingly, electrical stimulation of C-afferent fibres innervating muscle and joints produces longer lasting central hyperexcitability than does stimulation of C-afferents innervating cutaneous tissue [Wall and Woolf 1986]. These data indicate that the pain and hyperalgesia associated with non-cutaneous tissue is more severe and longer lasting.

1.2.4 Neurochemistry of peripheral hyperexcitability

Single fibre recordings of nociceptors show that administration of inflammatory mediators produces changes in the transduction properties of nociceptors (Table 3). These correlate with behavioural evidence of hyperalgesia, namely sensitization to mechanical and thermal stimuli (Table 1). The agents such as PGE_2 , PGI_2 , adenosine and serotonin, which sensitize the nociceptor directly, are all thought to exert their action via the cAMP second messenger system which modulates sodium channels [Duarte et al 1992; Gold et al 1996; Levine and Taiwo 1989; Taiwo and Levine 1989; 1991; Pitchford and Levine 1991].

Polymodal nociceptors, the most abundant nociceptors found in the skin, are not a homogeneous population [Handwerker 1991]. Examination of the effects of some individual mediators, such as bradykinin and PGE_2 , on cutaneous afferents, show them to excite only certain AMH and CMH afferents [Handwerker et al 1976; 1991; Martin 1987]. Similar differential effects on nociceptors have been observed following administration of capsaicin [Lamotte et al 1988]. Furthermore, analysis of individual C-polymodal nociceptors, following heat-induced sensitization, shows only some units to be suppressed by NSAIDs [Cohen and Perl 1990; King et al 1976]. These observations imply different signaling characteristics of polymodal nociceptors. Since a single mediator excites only a subset of nociceptors it is likely that a mixture of chemical mediators is required to sensitize a larger population of nociceptors (Ch1. Fig.2) [Burgess and Perl 1973;

**TABLE 3: Evidence of chemically-induced sensitization of
nociceptors**

Chemical	Species	Response Modality	References
Acid (in vitro superfusion)	rat	CMH (mech. stim.)	Steen et al 1990
BK (intradermal inj.)	monkey	CMH/AMH (heat stim.)	Khan et al 1992
BK (intraarterial inj.)	cat	CMH (heat stim.)	Beck and Handwerker 1974
BK (in vitro superfusion)	rat	CMH (heat stim.)	Lang et al 1990
BK (intraarterial inj.)	rabbit	CMH (heat stim.)	Szolcsanyi 1980
PGE ₁ (intradermal inj.)	monkey	CMH/AMH (heat stim.)	Raja et al 1990
PGE ₂ (intraarterial inj.)	cat	CMH (heat stim.)	Handwerker 1976
PGE ₂ (intradermal inj.)	rat	CMH /AMH (heat & mech. stim.)	Martin et al 1987 Martin et al 1988
PGE ₂ (intradermal inj.)	rat	CMH (mech. stim.)	White et al 1991
8R,15S-diHETE (intradermal inj.)	rat	CMH (heat & mech. stim.)	White et al 1990
LTB ₄ (intradermal inj.)	rat	CMH /AMH (heat & mech. stim.)	Martin et al 1987 Martin et al 1988
5HT (in vitro superfusion)	rat	CMH (to BK)	Handwerker 1991
PGI ₂ (intraarterially)	cat	CMH (BK & mech. stim.)	Schepelman et al 1992
Adenosine (intraarterially)	rat	CMH (mech. stim.)	Monteiro and Ribiero 1987

Szolcsanyi 1987]. Studies examining the effects of a combination of inflammatory mediators on cutaneous afferent units, in a skin-nerve preparation in the rat [Handwerker et al 1991; Kessler et al 1992; Reeh 1986], show application of a mixture of inflammatory mediators to induce a greater discharge in AMH and CMH units to heat stimulation than application of an individual mediator, however, no significant sensitization of the fibres was observed in these studies. Intradermal injection of an inflammatory soup containing bradykinin, histamine, serotonin and PGE_1 induced a response in 60% of the MIAs [Davis et al 1990].

Injection of an inflammatory mixture induces sensitization of certain peripheral afferent fibres to mechanical stimuli but not to heat stimuli, and vice versa.

Furthermore, sensitization of nociceptors to mechanical stimuli but not to thermal stimuli is dependent on the pH of the inflammatory mixture [Steen et al 1990; Handwerker and Reeh 1991]. These data indicate that the membrane mechanisms of sensitization to heat and mechanical stimuli differ [Handwerker and Reeh 1991; Davis et al 1993].

1.2.4.1 Efferent properties of C-fibre nociceptors

C-fibre nociceptors, in addition to their role in the afferent transmission of nociceptive information, have efferent functions. Depolarization of the peripheral terminals of C fibres by antidromic stimulation, inflammatory mediators [Chahl et al 1984], or high concentrations of capsaicin [Williams and Zieglsberger 1982;

PERIPHERAL SENSITIZATION

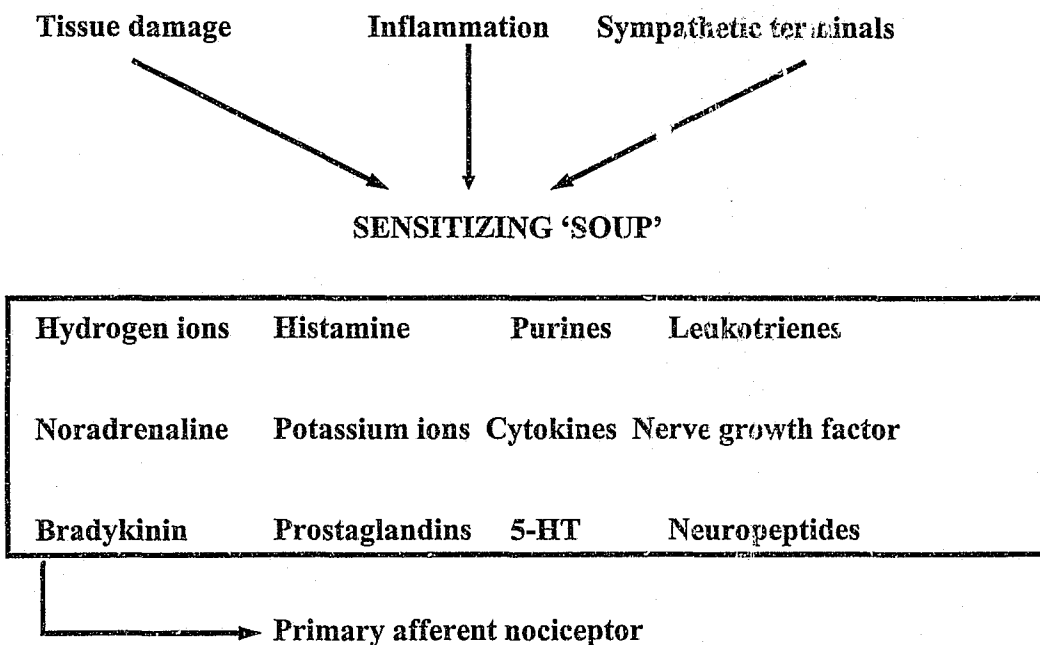


FIG.2: The chemical mediators produced by injury and inflammation either act directly on the primary afferent nociceptor or stimulate induce the release of other mediators from immune cells, blood cells and sympathetic neurones, including the primary afferent nociceptor [Lembeck et al 1976; Levine et al 1984; 1986 a;b; Taiwo et al 1987]. These mediators act synergistically as a “sensitizing soup” to modify the sensitivity of the primary afferent nociceptors [From Woolf and Chong 1993]. For detailed reviews see Rang et al [1991] and Levine et al [1993].

Fitzgerald 1983], induces the release of neuropeptides such as substance P (SP), neurokinin A (NKA) and calcitonin gene related peptide (CGRP) [for review see Levine et al 1993]. While C-fibre neuropeptides themselves do not appear to have a significant direct sensitizing effect on polymodal nociceptors [Cohen and Perl 1990; Kumazawa et al 1988; Reeh et al 1988], they stimulate the release of other chemical mediators such as interleukins, tumour necrosis factor [Lotz et al 1988], prostaglandins [Lotz et al 1987] and histamine [Johnson and Erdos 1973], from inflammatory cells. These mediators, in turn, act on nociceptors inducing the further release of neuropeptides, thereby mediating neurogenic inflammation. The addition of SP to an inflammatory soup enhances its excitatory effect on nociceptors [Kessler et al 1989].

Neuropeptide release produces local cutaneous vasodilation and plasma extravasation in the region of tissue innervated by the stimulated sensory nerve [Holzer 1988; White and Helme 1985]. This axon reflex mechanism is believed to account for the flare surrounding a cutaneous injury [Holzer 1988]. Over fifty years ago, it was proposed that hyperalgesia, both at the site of injury and in the uninjured tissue, was due to an axon reflex mechanism [Lewis 1942]. There is, however, little evidence to date to support the theory that an axon reflex mechanism underlies the phenomenon of secondary hyperalgesia.

1.2.4.2 Role of the sympathetic nervous system.

Evidence for the role of the sympathetic nervous system in neuropathic conditions is provided by the effectiveness of a sympathetic blockade in relieving the symptoms associated with those conditions. Similarly, the pain and hyperalgesia in experimental animals associated with a peripheral nerve lesion can be attenuated by sympathectomy [Kim and Chung 1991; Neil et al 1991; Shir and Seltzer 1991]. Interestingly, some inflammatory mediators, such as noradrenaline and bradykinin, produce mechanical hyperalgesia by an action at the sympathetic postganglionic neurone [Taiwo and Levine 1988b; Taiwo et al 1986; 1990]. Under normal circumstances, however, nociceptors do not respond to sympathetic activity [Barassi and Lynn 1986; Roberts and Elardo 1985 a;b; Shea and Perl 1985].

While, the precise mechanism by which the sympathetic nervous system influences nociceptor activity, following tissue or nerve injury, is as yet unknown, it has been suggested that noradrenaline produces hyperalgesia via the upregulation of α -adrenergic receptors on the injured primary afferent nociceptors [Blumberg and Janig 1984; Devor 1983; Devor and Janig 1981; Wall and Gutnick 1974]. Undamaged C-fibre nociceptors also exhibit noradrenergic sensitivity following partial nerve injury [Sato and Perl 1991]. Alternatively it has been proposed that inflammatory mediators, such as bradykinin, noradrenaline and possibly interleukin-1, produce hyperalgesia by an action on the sympathetic

postganglionic neurone which stimulates the synthesis of prostaglandins capable of sensitizing the nociceptor [Levine et al 1986 b; 1990]. A study by Tracey et al [1995] using a model of peripheral neuropathy supports the hypothesis of Levine et al [1986]. They found that hyperalgesia to both heat and mechanical stimuli were dependent on the action of noradrenaline on α_2 -adrenoreceptors located on the post-ganglionic sympathetic terminals which was eliminated by subcutaneous indomethacin. However, Koltzenburg et al [1992] report that the sensitization of nociceptors to heat by bradykinin is not dependant on sympathetic neurones and interleukin-8 produces a sympathetic-dependant hyperalgesia which is not mediated by prostaglandins [Cunha et al 1991].

1.2.4.3 Role of prostaglandins

Prostaglandins, particularly PGI_2 and PGE_2 [Taiwo and Levine 1990], play a prominent role in hyperalgesia (section 1.1.1.5). Electrophysiological studies provide direct evidence that prostaglandins sensitize C-fibre nociceptive afferents, as well as A δ mechanoreceptors. Prostaglandins also potentiate the sensitizing effects of other inflammatory mediators such as vasoactive amines and kinins on nociceptors. The view that prostaglandins have a role in sensitization is further substantiated by experiments showing that the administration of NSAIDs attenuates sensitization and hyperalgesia [Chahl and Iggo 1977; Cohen and Perl 1988; Dray et al 1992; Handwerker 1976; Pateromickelakis and Rood 1982; Perl 1976; Schaible and Schmidt 1988]. NSAIDs are thought to exert their antinociceptive action peripherally by inhibiting the production of prostaglandins [Guzman et al 1964; Lim et al 1964; Lim 1970], as well as by minimizing the effects of the other inflammatory mediators potentiated by prostaglandins [Chahl and Iggo 1977; Fock and Mense 1976; Handwerker 1976].

While prostaglandins E_2 and I_2 are known to produce hyperalgesia by a direct action on the peripheral terminals of primary afferent nociceptors (Table 2) [Paterimickelakis and Rood 1982; Pitchford and Levine 1991; Taiwo and Levine 1989 b; 1990; 1991], the precise mechanism by which prostanoids sensitize primary afferent nociceptors remains unclear. They are generally thought to result in an increase in the intracellular concentration of the second messengers cAMP, Ca^{2+} or both [Ferreira and Nakamura 1979; Taiwo et al 1989b; Taiwo and Levine 1989 b; 1990; 1992]. However, a recent study by Gold et al [1996] suggests that PGE_2 modulates Na^+ channels via cAMP.

The receptors of agents that regulate cAMP are coupled to G proteins [Taiwo and Levine 1989 a]. The effects of prostanoids are mediated by a number of distinct prostaglandin receptors. The receptors have been divided into five types named after the prostaglandin for which they have the greatest affinity (DP, FP, IP, EP, and TP). The PGE_2 (EP) receptors have been subdivided further into at least three subgroups [Thierauch et al 1994]. They couple to different G proteins to cause activation of adenylate cyclase, the enzyme modulating cAMP production [Sonnenburg and Smith 1988], and to increase Ca^{2+} concentrations [Negishi et al 1989]. A recent study, however, shows that an EP_3 agonist which decreases cAMP accumulation [Sugimoto et al 1993] enhances the effects of bradykinin on the polymodal nociceptor [Kumazawa et al 1993]. This study indicates that cAMP may not be the common second messenger through which PGE exerts its effects at each of its different receptors sites.

1.2.5 Neurochemistry of central hyperexcitability

As discussed previously, various forms of peripheral injury sensitize spinal cord neurones. A common feature of these diverse peripheral injuries is that discharges from primary afferent C-fibres, occurs and it is the so-called "C-fibre barrage" which sensitizes dorsal horn neurones. Since neuropeptides and excitatory amino-acids are co-localized in the central terminals of primary afferent neurones [DeBiasi and Rustioni 1988], they are, prime candidates for the transmission of nociceptive information from the primary afferent fibres to neurones in the dorsal horn.

Activation of C-fibre afferents following noxious stimulation or peripheral inflammation results in the co-release of many neuropeptides such as SP [Duggan et al 1988; Go and Yaksh 1987], neurokinin A (NKA) [Duggan et al 1990; Hua et al 1986], somatostatin [Kuraishi et al 1985; Morton et al 1988], calcitonin gene-related peptide (CGRP) [Saria et al 1986], galanin [Morton and Hutchison 1990] and the excitatory amino-acids transmitters (EAAs), glutamate and aspartate [Skilling et al 1988; Sorkin et al 1992] in the spinal cord dorsal horn. The prominent role played by EAAs and SP in the excitation of dorsal horn neurones has been demonstrated in many studies by intrathecal administration of either receptor agonists [Aanonsen et al 1990; Dougherty and Willis 1990; 1991; Dougherty and Willis 1991a; Dougherty et al 1992; Salter and Henry 1991] or antagonists [Davies and Watkins 1983; Dickenson and Sullivan 1990; Dougherty

and Willis 1991b; Dougherty et al 1992; Fleetwood-Walker et al 1990; Salt and Hill 1983; Schouenborg and Sjolund 1986; Urban and Randic 1984]. Evidence of their role in hyperalgesia, in conscious animals, is provided in Table 2. It is likely that EAAs and SP are co-released following C-fibre activation, and combined microiontophoretic treatment with SP and the EAA receptor agonist NMDA produces a significantly greater enhancement of the responses of dorsal horn neurones to noxious and innocuous mechanical stimulation [Dougherty and Willis 1991a], as well as behavioural responses to noxious chemical stimulation following intrathecal treatment [Mjellem-Joly et al 1992], than with either individually.

The precise mechanisms underlying the differential contribution of neuropeptides and EAAs to dorsal horn excitability are unclear [Dougherty et al 1993]. The transmission of nociceptive information at spinal synapses is modulated by numerous neurotransmitters and neuromodulators, see Table 4 for effects of some these transmitters on the excitability of WDR neurones) [for review see Dickenson 1995; Randic et al 1995]. Neuronal EAAs, including glutamate produce their effects through either ionotropic or metabotropic receptors [Watkins et al 1990]. The EAA ionotropic receptor subtypes are classified on the basis of selectivity to such synthetic agonists as N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl- 4 - isoxazolepropionic acid (AMPA), and kainate (KA) receptors

TABLE 4: Spinal modulators of activity in wide dynamic range**neurones**

Iontophoretic agent	Receptor type	WDR Neurones	Reference
AGONIST			
Opioid	mu delta kappa	decrease decrease decrease	Hope et al 1990 Hope et al 1990 Hope et al 1990
Alpha Adrenergic	2A	decrease	Fleetwood-Walker et al 1985
Adenosine	A ₁ /A ₂	decrease	Salter and Henry 1987
GABA	A/benzodiazepine B	decrease decrease	Cartmell and Mitchell 1994 Dickenson et al 1985
Cholinergic	M ₁ /M ₂	decrease	Dickenson et al 1985
Neurotensin		increase	Miletić and Randić 1979
Serotonin	5-HT 5-HT _{1A} 5-HT _{1B}	decrease decrease decrease	El-Yassir et al 1988 El-Yassir et al 1988 El-Yassir et al 1988
Dopamine	D ₂	decrease	Fleetwood-Walker et al 1988
CGRP		increase	Miletić and Tan 1988
Glutamate	NMDA non-NMDA	increase increase	Dougherty and Willis 1991b Dougherty and Willis 1991b
Tachykinins	NK-1 NK-2	increase increase	Fleetwood-Walker et al 1993 Fleetwood-Walker et al 1993
Somatostatin		decrease	Randić and Miletić 1978
VIP		increase	Dickinson et al 1997
ANTAGONIST			
Glutamate	NMDA non-NMDA	decrease	Sher and Mitchell 1990 Haley et al 1990
Serotonin	5-HT ₂ 5-HT ₃	no effect decrease	El-Yassir et al 1988 Alhaider et al 1991
Tachykinins	NK-1 NK-2	decrease decrease	Dougherty et al 1994 Dougherty et al 1994
ENZYME INHIBITOR			
Cyclooxygenase inhibitor		decrease	Chapman and Dickenson 1992
NO synthase inhibitor		decrease	Haley et al 1992

[Watkins et al 1990]. The contribution to polysynaptic responses of the NMDA or non-NMDA receptor subtypes through which glutamate exerts its effects are complicated [Lodge and Johnson 1990; Nakanishi 1992; Zeman and Lodge 1992]. Non-NMDA receptor activation is required for the fast depolarization of dorsal horn neurones. Under normal conditions the ion channel linked to the NMDA receptor is blocked by a magnesium ion. When the cell is depolarized, the magnesium ion is removed resulting in an influx of Ca^{2+} and Na^{+} into the cell, leading to further depolarization. The metabotropic glutamate receptors appear to be coupled to phospholipase C through G-proteins. Activation of metabotropic receptors results in an increase in polyphosphoinositides and release of Ca^{2+} from intracellular stores [Schoepp et al 1991]. Substance P, a member of the tachykinin family, which includes NKA and NKB, preferentially binds to the NK-1 receptor, while NKA binds to the NK-2 receptor [Yashphal et al 1991]. Activity at either NK-1 receptors induced by SP [Mantyh et al 1984], or at NMDA and non-NMDA receptors induced by glutamate and aspartate [Sugiyama et al 1987], results in an influx of calcium [MacDermott et al 1986] which is responsible for significant changes in the excitability of the cell, involving complex enzymatic changes including PKC activation, nitric oxide release, and the induction of various genes such as c-fos [for a detailed review see Coderre et al 1993].

1.2.5.1 Role of prostaglandins

Influx of Ca^{2+} into the cell activates phospholipase A_2 within the cell, leading to increases in intracellular arachidonic acid. In the presence of cyclo-oxygenase arachidonic acid is broken down to diffusable prostaglandins which then: 1) augment the release of afferent neurotransmitters [Yaksh and Malmberg 1994], 2) enhances the release of substance P induced by another stimulus [Geppetti et al 1991; Miller et al 1992; Nicol et al 1992], 3) augment a voltage-sensitive Ca^{2+} current in dorsal root ganglion cell cultures (Ch.1, Fig. 3).

Arachidonic acid released by activation of NMDA and other receptors potentiates NMDA receptor currents resulting in a further increase in the intracellular Ca^{2+} caused by glutamate [Miller et al 1992]. Additionally arachidonic acid has been shown to evoke the release of CGRP from capsaicin stimulated primary afferents. This release is inhibited by indomethacin [Geppetti et al 1991].

It seems, therefore, that the initial C-fibre barrage produces an increase in cytosolic AA available for conversion by cyclo-oxygenase to prostaglandins, which then facilitates neuronal excitability within the spinal cord by a feedback mechanism and thus may not require continued afferent input to maintain a hyperexcitable state. Although it is most likely, following C-afferent fibre stimulation, that some release of prostaglandins will derive from afferent neurones [Gonzales et al 1989], low concentrations of SP can induce the release of prostaglandins from rat spinal cord astrocyte cultures [Marriott et al 1990].

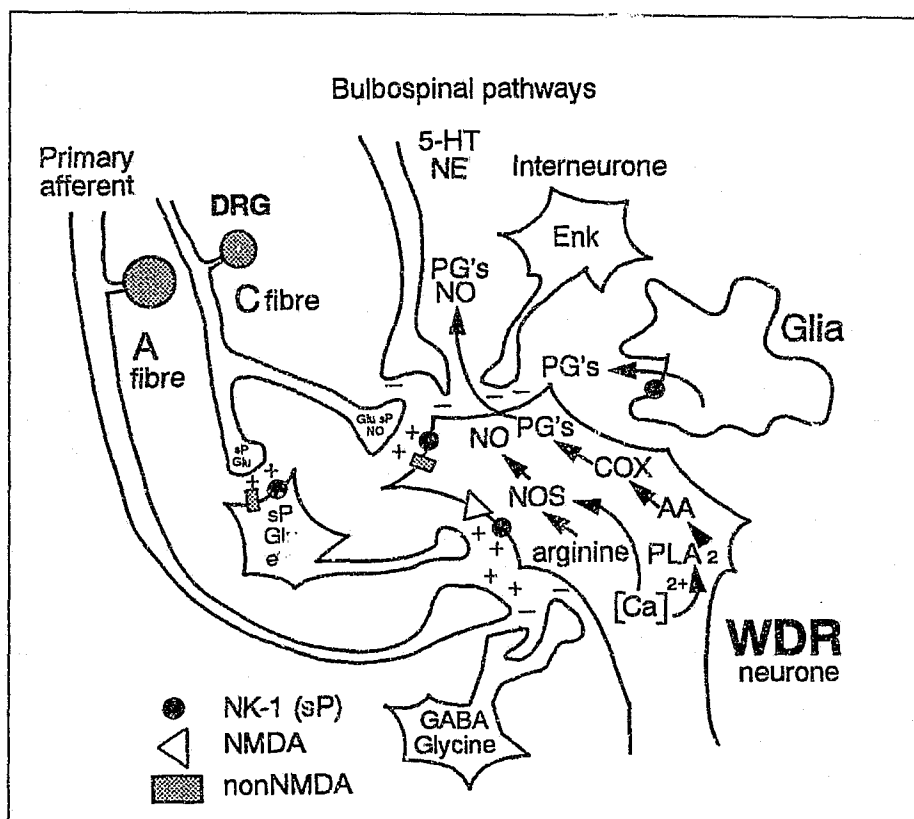


Figure 3: Schematic diagram of the cellular organisation and neuromodulators in the dorsal horn which affect the processing of afferent input, as discussed in the text.

Reproduced from Yaksh, T.L., and Malmberg, A.B., Central Pharmacology of nociceptive transmission. In: Textbook of Pain, Third Edition; Eds: P.D. Wall and R. Melzack, 1994, pp 165-200.

The transmission of nociception in the spinal cord is subject to powerful inhibitory control, both by segmental and by descending systems of supraspinal origin [Besson and Chaouch 1987]. Yaksh and Malmberg [1994] provides an excellent overview of intrinsic spinal and supraspinal receptor systems which can influence spinal cord excitability. Loss of both intraspinal and supraspinal inhibitory mechanisms results in an expansion of receptive fields and enhanced excitability of dorsal horn neurones [Noble and Riddle 1989; Wall 1967]. Prostaglandins, in addition to facilitating afferent transmission could diminish inhibition [Yaksh 1982]. Prostaglandins of the E type presynaptically inhibit noradrenergic transmission from post-ganglionic sympathetic neurones [Bergstrom et al 1973; Hedqvist 1973]. It is therefore possible that prostaglandins in the spinal cord could reduce the release of noradrenaline from the terminals of descending noradrenergic fibres and diminish descending inhibitory control of nociceptive inputs [Vasko 1995]. The afferent evoked release of prostaglandins can block endogenous opioid mediated analgesia systems by inhibition of the bulbospinal noradrenergic component of this pathway [Taiwo and Levine 1988a].

1.3 Aims of my study

1.3.1 The role of prostanoids in nociception during ischaemia and reperfusion hyperalgesia

Given the important peripheral and central role which prostaglandins play in mediating hyperalgesia associated with tissue injury, I have used a range of

NSAIDs to: 1) assess the role of prostaglandins in nociception during ischaemia and in reperfusion of the rat's tail following relief of ischaemia, 2) assess whether prostaglandins are released into the central nervous system during ischaemia and reperfusion of the tail, 3) compare the effects of central and peripheral prostaglandin synthesis in modifying nociception during tail ischaemia and reperfusion.

1.3.2 Neuronal substrates of reperfusion hypersensitivity

Recent studies indicate that activity in WDR neurones, in particular the encoding of noxious thermal stimuli and changes in intensity of mechanical stimuli, corresponds closely with the behavioural manifestations of hyperalgesia in many experimental models. I have, therefore, examined the responses of WDR neurones to both noxious and innocuous mechanical stimulation as well as noxious thermal stimuli during reperfusion of their receptive fields on the rat tail after a period of ischaemia.

1.3.3 The role of prostanoids in reperfusion hyperexcitability and hypersensitivity

I administered NSAIDs directly onto the surface of the spinal cord of anaesthetized animals in order to evaluate the effects of prostaglandins on responsiveness of WDR neurones during ischaemia and reperfusion. Most previous studies with similar aims have administered prostaglandins or NSAIDs systemically.

CHAPTER 2

**EFFECTS OF SYSTEMIC ADMINISTRATION OF NON-
STEROIDAL ANTI-INFLAMMATORY DRUGS ON
NOCICEPTION DURING TAIL ISCHAEMIA AND
REPERFUSION HYPERALGESIA IN RATS,
BR. J. PHARMACOL., 105 (1992) 412-416.**

Effects of systemic non-steroidal anti-inflammatory drugs on nociception during tail ischaemia and on reperfusion hyperalgesia in rats

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- 1 We have investigated the effects of five non-steroidal anti-inflammatory drugs (NSAIDs) on nociception during ischaemia and on reperfusion hyperalgesia in rats.
- 2 We induced tail ischaemia in conscious rats by applying a tourniquet at the base of the tail until the rats exhibited co-ordinated escape behaviour when we released the tourniquet.
- 3 We assessed hyperalgesia by measuring the tail flick latency following tail immersion in water at 49°C, before applying and immediately after releasing the tourniquet, and then at 30 min intervals for 2 h.
- 4 Intraperitoneal injection of NSAIDs prior to applying the tourniquet had no effect on the co-ordinated escape behaviour during ischaemia, nor on tail flick latency in the absence of prior ischaemia. However all the drugs attenuated reperfusion hyperalgesia in a log dose-dependent manner. Doses required to abolish hyperalgesia, were indomethacin 5 mg kg⁻¹, diclofenac sodium 42 mg kg⁻¹, ibuprofen 54 mg kg⁻¹, dipyrrone 168 mg kg⁻¹ and paracetamol 170 mg kg⁻¹.
- 5 We conclude that the mechanisms underlying nociception during ischaemia are not the same as those underlying reperfusion hyperalgesia. Moreover our procedure provides a rapid and more humane method for measuring the antinociceptive potency of NSAIDs.

Keywords: Reperfusion hyperalgesia; ischaemia; tail flick test; prostaglandin; non-steroidal anti-inflammatory drugs

Introduction

Inflamed or damaged tissue, including previously ischaemic tissue, becomes more sensitive to subsequent noxious or previously innocuous stimulation. This hyperalgesia could result from changes in excitability of central nervous system neurones (Woolf, 1983; Wall & Woolf, 1984) or from the local release of metabolites, such as prostaglandins, which have the potential to sensitize nociceptors (Ferreira & Vane, 1974; Sicuteri *et al.*, 1974; Juan, 1978; Lynn, 1987). Prostaglandins also are believed to be released during ischaemia and to increase activity in afferent nociceptive pathways (Sztaszewicz-Barczak *et al.*, 1976; Sacchetti *et al.*, 1980; Stebbins *et al.*, 1985; Longhurst & Dittman, 1987; Pal *et al.*, 1989). Thus the non-steroidal anti-inflammatory drugs, which all inhibit prostaglandin synthesis, might be expected to attenuate hyperalgesia and to be antinociceptive during ischaemia.

We have shown that lysine acetylsalicylate, a prostaglandin synthesis inhibitor, did indeed abolish the hyperalgesia to a noxious thermal stimulus during reperfusion of the rat's tail following ischaemia, but, contrary to expectations, did not affect the latency to co-ordinated escape behaviour following application of a tourniquet to the tail (Gelgor *et al.*, 1986b). Our observations indicate either that prostaglandins did not contribute to nociception during ischaemia itself, or that the particular drug and dose we used did not inhibit their synthesis.

It is important to resolve what role prostaglandin synthesis plays in nociception during ischaemia and in reperfusion, and other types of hyperalgesia. Non-steroidal anti-inflammatory drugs have potential clinical use as analgesics during ischaemic events. Also, several assays developed to assess the efficacy of non-steroidal anti-inflammatory drugs depend on their action during hyperalgesia (Randall & Selitto, 1957; Weichman, 1989).

We have examined the effect of a range of non-steroidal anti-inflammatory drugs of varying therapeutic potency on nociception during ischaemia induced by applying a tourniquet to the tail of the rat, and on the resultant hyperalgesia following reperfusion of the tail. Our results imply that prostaglandins or related substances do indeed contribute to nociception during hyperalgesia but not during ischaemia. They also provide the basis for a new assay of the antinociceptive potency of non-steroidal anti-inflammatory drugs which is free of the ethical problems which beset many existing assays, in which experimental animals are exposed to inescapable, and often chronic, noxious stimuli.

Some of the results have been reported at the Second International Pain Symposium, Jerusalem, and the Physiological Society of Southern Africa (Gelgor *et al.*, 1990).

Methods

Animals

Male Sprague-Dawley rats weighing 250–300 g were used. The animals were housed in groups of five per cage at an ambient temperature of 21–23°C on a 12 h dark/12 h light cycle, and were allowed free access to standard rat chow and tap water. Different groups of 10 rats were used for each of the different drugs.

Ischaemia

Ischaemia was induced by applying an inflatable cuff to the base of the rat's tail, as previously described (Gelgor *et al.*, 1986a,b). The cuff was connected to a sphygmomanometer, and was inflated to a pressure of 200 mmHg, well above systolic pressure of the rat. The moment the rat exhibited an escape response the cuff was deflated. The time between application of the tourniquet and the escape response, that is the escape latency, is a measure of the noxious effect of the ischaemia. If the rat had not responded within 30 min, the cuff was deflated, to avoid excessive tissue damage.

Application of a similar tourniquet to the arms of the authors and other human subjects caused an intense, aching, poorly localized pain. Following removal of the tourniquet

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there was hyperaemia, temporary paraesthesia, and then resolution of all pain within a few minutes. There were no sequelae.

Tail flick test

Nociception before ischaemia and during reperfusion was tested by the modified tail flick test which we have employed previously (Gelgor *et al.*, 1986a,b). The rat's tail was submerged in a water bath controlled at 49°C, and tail flick latency measured on a stop watch, as the time from submergence to the first coordinated motor response, indicated by a flicking of the tail. A mean of three measurements 1 min apart was recorded as the latency. To avoid thermally induced tissue damage, animals which failed to respond within 20 s had their tails removed from the water.

Experimental procedure

The rats were placed in clear perspex restrainers which allowed free movement of the tail and slightly restricted movement of the rest of the body. The rats were placed in these restrainers for 2–3 h per day on two consecutive days before any experimentation to allow them to habituate to these conditions. On experimental days the animals were placed in the restrainers for 15 min before any testing. At least 48 h were allowed between successive measurements on individual animals. Experiments were carried out between 09 h 00 min and 13 h 00 min at an ambient temperature of 24°C. Tail flick latencies were measured before any treatment, immediately after cuff deflation, and then at 0.5 h intervals for 2 h. Control experiments were performed by placing an uninflated (sham) cuff on the tail for 12 min; this time is equal to the mean escape latency measured in previous experiments in our laboratory.

Because tail flick latency varies with tail temperature, in a pilot experiment, we measured tail skin temperature, using copper-constantan thermocouples taped to the dorsal surface of the tail 30 mm from the base, before, during and after ischaemia. Temperatures were stored on a data logger (MCS 120; MC Systems). Temperatures were recorded for 30 min before any testing and at 10 min intervals throughout the experiment. Specific temperatures were recorded during ischaemia and immediately before tail flick latencies were measured.

All agents as well as their vehicles were administered in 0.5 ml boluses intraperitoneally 30 min before application of tourniquet (test groups) or sham tourniquet (control groups). The vehicle was normal saline except for ibuprofen where it was polyethylene glycol, and paracetamol for which we used corn oil. The drugs and dosages used were: indomethacin (Merck, Sharp and Dohme Research Laboratories) 1, 2, 5 mg kg⁻¹; diclofenac sodium (Ciba Geigy) 5, 20, 50 mg kg⁻¹; ibuprofen (Lennon) 10, 30, 100 mg kg⁻¹; paracetamol = 4-acetamidophenol (Lennon) 10, 50, 200 mg kg⁻¹; dipyron (Hoechst AG) 50, 100, 200 mg kg⁻¹.

Student's *t* test with Bonferroni correction for multiple comparisons and a one-way analysis of variance were used for data analysis.

The experimental procedures were approved by the Animal Ethics Committee of the University of the Witwatersrand (Certificate number 89/23/3) and complied with the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmerman, 1980).

Results

Tail temperature decreased significantly during ischaemia ($P < 0.001$, $n = 10$, paired *t* test). Following release of the tourniquet, tail temperature recovered to values which did not differ significantly from those before the cuff was applied ($P > 0.05$, $n = 10$, paired *t* test). Figure 1 represents change in

NSAIDS ABOLISH REPERFUSION HYPERALGESIA

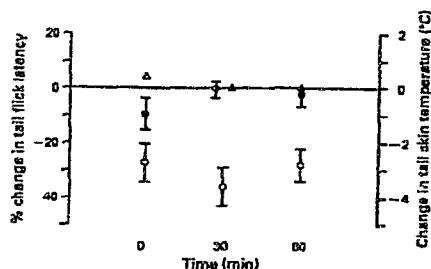


Figure 1 Percentage change in tail flick latency (mean, with s.e. shown by vertical bars, $n = 10$) (left y-axis) and change in tail temperature (mean $\pm$ s.e., $n = 10$) (right y-axis) from values evident before application of a tourniquet, measured during tail reperfusion following removal of the tourniquet. Temperature ($\circ$) did not change significantly ($P > 0.05$, paired *t* test). The tail flick latency (Δ) decreased significantly at all time intervals ($0.05 > P > 0.001$, paired *t* test with Bonferroni correction for repeated measures). Tail flick latency (Δ) predicted from the tail temperature, by use of the coefficient -0.25°C^{-1} (Milne & Gamble, 1989; Han & Rea, 1991).

tail flick latency and in tail temperature following release of the tourniquet. Also included in the figure are the changes in tail flick latency predicted from the changes in temperature, using the coefficient of -0.25°C^{-1} increase in temperature derived in previous work (Milne & Gamble, 1989; Han & Rea, 1991). We found no significant change in tail skin temperature, from that prevailing before application of the tourniquet, whereas there was a significant reduction in tail flick latency ($P < 0.01$, $n = 10$, paired *t* test with Bonferroni correction) at all three measurement times following release of the tourniquet. This hyperalgesia therefore was unrelated to changes in tail temperature.

The latency to coordinated escape behaviour following the induction of ischaemia in the absence of any of the drugs was 13.8 ± 0.7 min (mean $\pm$ s.e., $n = 50$). There was no significant difference in escape latency between different groups of rats ($P > 0.05$, $n = 10$, unpaired *t* test). None of the five different drugs had any significant effect on the escape latency to ischaemia at any dosage ($P > 0.05$, $n = 10$, unpaired *t* test, see Table 1). Also none of the drugs tested had any effect on the tail flick latency in the absence of ischaemia ($P > 0.05$, $n = 10$, unpaired *t* test).

Following reperfusion of the tail after a period of ischaemia, there was a significant hyperalgesia, as indicated by a reduction in tail flick latency, which was greatest immediately

Table 1 Effect of non-steroidal anti-inflammatory drugs (NSAIDs) on ischaemia and tail flick latency in the absence of ischaemia

Agents administered	Escape latency during ischaemia (min)	Tail flick latency (s)
Sterile water	14.8 $\pm$ 2.3	7.1 $\pm$ 0.4
Polyethylene glycol	13.1 $\pm$ 2.2	7.1 $\pm$ 0.3
Corn oil	15.6 $\pm$ 2.2	7.9 $\pm$ 0.3
Indomethacin 5 mg kg ⁻¹	15.4 $\pm$ 2.7	7.0 $\pm$ 0.3
Diclofenac sodium 50 mg kg ⁻¹	15.6 $\pm$ 2.1	7.6 $\pm$ 0.4
Ibuprofen 100 mg kg ⁻¹	12.0 $\pm$ 2.3	7.3 $\pm$ 0.2
Paracetamol 200 mg kg ⁻¹	15.1 $\pm$ 2.0	7.9 $\pm$ 0.3
Dipyron 200 mg kg ⁻¹	13.5 $\pm$ 1.9	7.1 $\pm$ 0.4

Escape latencies during ischaemia (mean $\pm$ s.e.) and tail flick latencies in the absence of ischaemia (mean $\pm$ s.e.), following intraperitoneal administration of vehicles and of the highest doses of each of the NSAIDs. The drugs did not affect escape latency or tail flick latency.

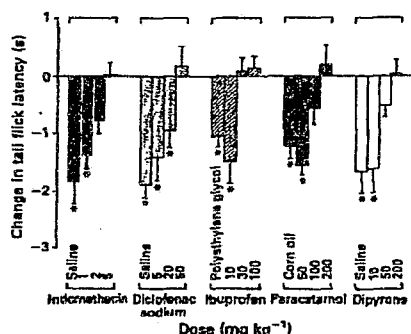
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Figure 2 Change in tail flick latency (mean with s.e. shown by vertical bars) measured immediately after relief of ischaemia, following treatment with vehicles and agents. The asterisks represent those groups in which treatment resulted in a significant decrease in tail flick latency from pre-treatment values ($P < 0.01$, $n = 10$, paired t test) that is groups in which there was a significant hyperalgesia. All agents abolished this hyperalgesia, at sufficient dose. All doses are mg kg^{-1} .

following removal of the tourniquet, and persisted for 60 min, after administration of the vehicles ($0.05 > P > 0.001$, $n = 10$, unpaired t test, with Bonferroni correction for repeated measures). Figure 2 represents the change in tail flick latency from that evident before application of the tourniquet, measured immediately after release of the tourniquet, for five different groups of rats, each treated with either the vehicle or one of the drugs. There were no significant differences between the values measured during treatment with the lowest dose of each of the drugs tested and during treatment with the corresponding vehicle ($P > 0.05$, $n = 10$, paired t test). However, the change in tail flick latency for both the vehicle and the lowest dose of each drug was significantly different from zero ($0.02 > P > 0.001$, $n = 10$, t test) indicating the presence of significant reperfusion hyperalgesia in each case. Administration of the highest dose of each of the drugs resulted in the tail flick latency during reperfusion being no different to that prevailing before ischaemia ($P > 0.05$, $n = 10$, paired t test). Therefore all the drugs abolished reperfusion hyperalgesia.

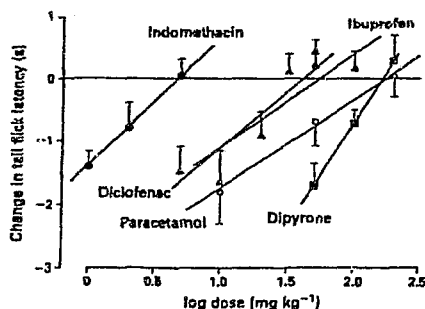


Figure 3 Change in tail flick latency (mean with s.e. shown by vertical bars) measured immediately after relief of ischaemia, plotted against log dose of the five agents. Reperfusion hyperalgesia was attenuated in a dose-dependent manner. Doses required to abolish hyperalgesia, calculated from regression analysis, were: indomethacin 5 mg kg^{-1} , diclofenac sodium 42 mg kg^{-1} , ibuprofen 34 mg kg^{-1} , dipyrone 168 mg kg^{-1} and paracetamol 170 mg kg^{-1} .

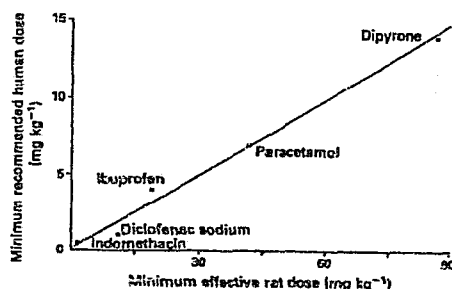


Figure 4 Minimum effective rat dose, calculated, from regression analysis, as the dose of agent which induces a change in tail flick latency following ischaemia equal to the change in tail flick latency induced by administration of the vehicle, plotted against minimum recommended human dose, taken from standard therapeutic references for physicians. Regression analysis gave $y = 0.056 + 0.164x$, $r = 0.993$, $P < 0.001$.

However, none of the drugs, at the doses we used, induced analgesia; tail flick latency never increased significantly.

Figure 3 shows change in tail flick latency, measured immediately after release of the tourniquet, plotted against the log dose of each drug. Reperfusion hyperalgesia was attenuated in a dose-dependent manner. Doses of each drug required to abolish hyperalgesia, calculated from the intercepts of the regression line with the x-axis, were: indomethacin 5 mg kg^{-1} , diclofenac sodium 42 mg kg^{-1} , ibuprofen 34 mg kg^{-1} , dipyrone 168 mg kg^{-1} and paracetamol 170 mg kg^{-1} .

Another measure of the relative potency of the drugs could be derived by calculating, from the regression lines, the doses at which the change in tail flick latency was equal to that produced by the vehicle. These doses could be considered the minimum effective doses, since any higher dose would attenuate the hyperalgesia. Figure 4 shows the correlation between these minimum effective doses and the minimum recommended human therapeutic doses for the same drugs. The minimum human therapeutic doses were taken from the manufacturer's information recorded in a compendium of such information for physicians (MIMS Desk Reference Vol. 22 1986/87), assuming a 75 kg patient. There was a very high degree of correlation between the experimental measure of potency and the human dose.

Discussion

Previous work indicates that tail flick latency is negatively correlated with tail skin temperature (Bergo *et al.*, 1988; Tjolsen *et al.*, 1989) and that some factors which alter tail flick latency, usually interpreted as affecting nociception, may really just alter tail skin temperature (Tjolsen *et al.*, 1988; Lund *et al.*, 1989). The induction of ischaemia in the rat's tail reduces blood flow and consequently tail skin temperature at the ambient temperature we used. However, following release of the tourniquet, tail temperature returned to pre-ischaemic values whilst tail flick latency remained significantly decreased. We have therefore established that the hyperalgesia observed on reperfusion of the previously ischaemic tail is not the result of a change in tail skin temperature.

Non-steroidal anti-inflammatory drugs attenuated the hyperalgesia evident on reperfusion of the rat tail following a period of ischaemia. We were able to rank the potency of a range of non-steroidal anti-inflammatory drugs by their ability to attenuate reperfusion hyperalgesia. Rank order of potency was: indomethacin > diclofenac sodium > ibuprofen > paracetamol > dipyrone. The behavioural response to the ischaemic stimulus itself was not altered

following administration of the drugs. This observation leads us to suggest that the mechanisms underlying nociception during ischaemia and reperfusion hyperalgesia are not the same, because doses of drugs which abolished hyperalgesia had no observable effect on nociception during ischaemia. Similarly, the same doses of the drugs did not appear to affect nociception during application of noxious heat to the tail. In experiments conducted in the absence of ischaemia, the tail flick latency was not altered by the administration of any of the non-steroidal anti-inflammatory drugs. It has been reported previously that diclofenac is not anti-nociceptive in the tail flick or hot-plate tests (Bjorkmann *et al.*, 1990). In our experiments, all five of the non-steroidal anti-inflammatory drugs exhibited an anti-algesic effect during reperfusion of the previously ischaemic tail, but did not exhibit any antinociceptive effects.

Ischaemia or hypoxia is believed to induce the release of prostaglandins and bradykinin which together stimulate afferent nerve endings (Szaszewska-Barczak *et al.*, 1976; Stebbins *et al.*, 1985; Pal *et al.*, 1989). Bradykinin is present in inflammatory exudates before prostaglandins (Juan, 1978; 1981; Terenius, 1981; Neugebauer *et al.*, 1989), and stimulates the release of prostaglandins, which, in turn, may sensitize the nociceptor to the algogenic action of other mediators released during tissue damage. In studies of cardiac ischaemic pain, the NSAIDs, aspirin and indomethacin, reduced bradykinin-induced excitation of afferent nerve endings (Vogt *et al.*, 1979; Sacchetti *et al.*, 1980). Arachidonic acid, a precursor of prostaglandins, accumulates in flow-deprived cardiac tissue and its concentration increases significantly in the first hour following reperfusion of the heart (Van der Vusse *et al.*, 1989). Prostaglandin concentrations measured in the brain after 15 min global cerebral ischaemia were at their highest levels in the 15–60 min reperfusion period, when compared with pre-ischaemic levels (Stevens & Yaksh, 1988). The hyperalgesia we observed following reperfusion of the tail was greatest immediately after release of the tourniquet and lasted for 60 min (Gelgor *et al.*, 1986b).

Arachidonic acid accumulation in ischaemic conditions can elevate the rate of prostaglandin synthesis only if the local oxygen concentration is sufficiently high (Lands, 1979). We believe that prostaglandin precursors accumulate during ischaemia, and are metabolised to prostaglandins, or other eicosanoids, when oxygen becomes available during reperfusion. NSAIDs, which inhibit the cyclo-oxygenase enzyme, consequently will have no effect during ischaemia itself, but will attenuate prostaglandin synthesis during reperfusion. We also conclude that prostaglandins do not play a role in the tail flick response.

Hyperalgesic assays currently used to assess the efficacy of non-steroidal anti-inflammatory drugs involve the administration of chemical irritants such as brewers' yeast, trypsin, formalin, kaolin, carrageenan, acetic acid or *Mycobacterium butyricum* in the adjuvant arthritis model (Randall & Selitto, 1957; Vinegar *et al.*, 1976; 1990; Menasse *et al.*, 1978; Ferreira *et al.*, 1978; Maier *et al.*, 1979; Van Kolschoten *et al.*, 1983; Okuyama & Aihara, 1984; Shibata *et al.*, 1989). In many cases

the hyperalgesia takes hours to develop and no simple intervention can terminate the noxious stimulus. The procedure of reperfusion of the rat tail following a period of ischaemia does not involve the administration of a chemical irritant to induce hyperalgesia, but rather relies on the endogenous release of humoral mediators. In the absence of a deliberately applied stimulus, like noxious heat, the animal is in no distress and the duration of the hyperalgesia is relatively short. Although the doses of NSAIDs required to abolish reperfusion hyperalgesia in rats are considerably higher than the therapeutic doses used in man, they were highly significant, and linearly correlated with minimum recommended human doses, so our procedure provides the basis for an assay which may be used to rank the potency of new non-steroidal anti-inflammatory drugs. Rank order of potency of the drugs we tested correlates with potency ranking in other studies which have employed the carrageenan paw oedema test, Randall-Selitto test, acetic acid writhing test and the adjuvant arthritis model (Vinegar *et al.*, 1976; Van Kolschoten *et al.*, 1983; Okuyama & Aihara, 1984; Tolman *et al.*, 1984; Vane & Botting, 1987; Weichman, 1989).

Indomethacin is a potent inhibitor of prostaglandin synthesis in man and in animal anti-inflammatory assays (Ferreira & Vane, 1974; Robinson *et al.*, 1978), and, of the drugs we tested in our procedure, indomethacin proved to be the most potent. Also, the dosage range of efficacy of indomethacin in our study ($1.5\text{--}5\text{ mg kg}^{-1}$) correlates well with ED_{50} dosages reported in the carrageenan paw oedema assay, which were in the range of $1.3\text{--}4.5\text{ mg kg}^{-1}$ (Flower *et al.*, 1972; Vinegar *et al.*, 1976; Van Kolschoten *et al.*, 1983; Vane & Botting, 1987). Diclofenac sodium, in some tests, has been found to be equipotent to indomethacin (Menasse *et al.*, 1978; Maier *et al.*, 1979; Skoutakis *et al.*, 1988; Small, 1989), but was less potent in our hands. Different non-steroidal anti-inflammatory drugs inhibit prostaglandin synthesis to varying degrees in different tissues, and hence have different potential therapeutic effects (Flower & Vane, 1972). Paracetamol and dipyrrone are more potent in inhibiting prostaglandin synthesis in brain tissue and do not possess anti-inflammatory properties (Flower *et al.*, 1972; Ferreira *et al.*, 1978; Vane, 1983; Carlson *et al.*, 1986). High doses, 168 and 170 mg kg^{-1} respectively, were required to abolish reperfusion hyperalgesia.

In conclusion, we have shown that five NSAIDs attenuate reperfusion hyperalgesia at doses at which they do not influence the responses to a noxious ischaemic or noxious thermal stimulus. Our observations are consistent with a role for prostaglandins in nociception during hyperalgesia, but not during noxious ischaemia or thermal stimulation. We can interpret our results in terms of a peripheral action of prostaglandins, but they do not exclude a central action (Ferreira *et al.*, 1978; Jurna & Brune, 1990). Finally, we believe we have indicated a way to test potency of NSAIDs which has far fewer ethical problems than other existing procedures.

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CHAPTER 3

**INTRACEREBROVENTRICULAR MICRO-INJECTIONS OF
NON-STEROIDAL ANTI-INFLAMMATORY DRUGS
ABOLISH REPERFUSION HYPERALGESIA IN THE RAT'S
TAIL, PAIN, 50 (1992) 323-329.**

Basic Section

Intracerebroventricular micro-injections of non-steroidal anti-inflammatory drugs abolish reperfusion hyperalgesia in the rat's tail

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Summary Prostaglandins are mediators of reperfusion hyperalgesia; their site of action may be in the periphery, in the central nervous system, or both. We have investigated whether prostaglandins play a role in the central nervous system during reperfusion hyperalgesia, by intracerebroventricular (i.c.v.) micro-injection of non-steroidal anti-inflammatory drugs (NSAID), to inhibit local prostanoid synthesis. We induced tail ischaemia in conscious rats by applying an inflatable cuff at the base of the tail. The cuff was released at the first signs of co-ordinated escape behaviour. Responses to a noxious thermal stimulus were assessed, by measuring tail flick latency following immersion of the tail in water at 49°C, prior to and immediately after release of the tourniquet. Tail flick latency decreased significantly following ischaemia, that is there was post-ischaemic reperfusion hyperalgesia. Intracerebroventricular micro-injection of NSAID prior to applying the tourniquet had no effect on the co-ordinated escape behaviour during ischaemia or on the tail flick latency after application of a sham tourniquet (uninflated cuff). However all the drugs abolished the hyperalgesia evident during reperfusion. Doses required to abolish hyperalgesia were 0.001 mg/kg indomethacin, 0.08 mg/kg dipyron, 0.09 mg/kg ibuprofen, 0.2 mg/kg diclofenac sodium and 0.2 mg/kg paracetamol. These doses are 2–3 orders of magnitude less than those necessary to abolish reperfusion hyperalgesia when the same drugs are administered systemically. Our results indicate that the development of reperfusion hyperalgesia of the rat's tail depends on the synthesis of prostanoids within the central nervous system.

Key words: Reperfusion hyperalgesia; Central nervous system; Prostaglandins; Non-steroidal anti-inflammatory drugs

Introduction

We have shown that 5 different non-steroidal anti-inflammatory drugs (NSAID), administered systemically, abolish reperfusion hyperalgesia of the rat's tail (Gelgor et al. 1990). Our observations are consistent with the hypothesis that prostaglandins are released in previously ischaemic tissue (Stasweska-Barczak et al. 1976; Stebbins et al. 1985; Longhurst and Dittman 1987; Pal et al. 1989) and sensitize nociceptors locally

(Ferreira 1972; Willis and Cornelsen 1973), so inducing hyperalgesia; NSAIDs which block prostaglandin synthesis by inhibiting cyclo-oxygenase (Ferreira and Vane 1974; Montcada et al. 1975) would prevent this sensitization. However, our observations may also be consistent with another hypothesis, namely that hyperalgesia arises from the action of prostaglandins and NSAID within the central nervous system (CNS).

NSAIDs do have central effects on neuronal transmission (Chen and Chapman 1980; Hunskaar and Hole 1987; Willer et al. 1989; Braga 1990; Jurna and Brune 1990). Peripheral electrical stimulation induces the release of prostaglandins within the frog spinal cord (Ramwell et al. 1966b) and cat cerebral cortex (Ramwell and Shaw 1966a). Prostaglandins within the CNS may facilitate transmission in nociceptive pathways, an ac-

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tion which would be blocked by NSAID (Yaksh 1982; Taiwo and Levine 1986; Taiwo and Levine 1988; Attal et al. 1988; deBeaurepaire et al. 1990). More specifically, centrally acting prostaglandins have been implicated in the hyperalgesia provoked by carrageenin in the rat paw (Ferreira et al. 1978) and that evident in arthritic rats (Okuyama and Aihara 1984). Hence, reperfusion hyperalgesia might also arise, partly or totally, through an action of prostaglandins within the CNS. Systemically administered NSAID could block this action provided that they cross the blood-brain barrier.

To resolve whether the abolition of reperfusion hyperalgesia could depend on a central action of NSAID, we have now administered NSAID directly into the CNS before inducing experimental ischaemia and then reperfusing the rat's tail. We used very small doses of the drugs to eliminate any peripheral action arising from reverse diffusion across the blood-brain barrier. Some of the results have been reported briefly to the Physiology Society of Southern Africa (Gelgor et al. 1990b); centrally administered NSAID indeed did abolish reperfusion hyperalgesia.

Methods

Animals

Male Sprague-Dawley rats weighing 250–300 g were used. The animals were anaesthetized with 0.025 mg/kg fentanyl and droperidol (Innovar Vet, Janssen) administered intramuscularly. Sterile cannulae were implanted stereotactically into the lateral ventricle 7.7 mm anterior to the interaural line and 1.5 mm from the midline (Paxinos and Watson 1986) according to the method of Gray and Gorzalka (1979). The cannulae were fixed to the skull with dental cement and stainless steel screws and protruded 3.8 mm below the surface of the skull. We used 30-ga needles as obturators. The animals were allowed 3 days to recover from surgery prior to any experimentation.

Following surgery the rats were housed individually at an ambient temperature of 21–23°C on a 12-h light/12-h dark cycle and were allowed free access to standard rat chow and tap water.

Ischaemia

Ischaemia was induced by applying an inflatable cuff to the base of the restrained rat's tail, as described previously (Gelgor et al. 1986a,b). The cuff was connected to a sphygmomanometer which was inflated to a pressure of 200 mm Hg, well above the systolic pressure of the rat. The moment the rat exhibited an escape response, the cuff was deflated. The time between application of the tourniquet and the escape response, that is the escape latency, is a measure of the noxious effect of ischaemia. If the rat had not responded within 30 min, the cuff was deflated to avoid excessive tissue damage.

Application of a similar tourniquet to the arms of the authors and other human subjects caused an intense, aching, poorly localized pain. Following removal of the tourniquet there was hyperaemia, temporary paraesthesia, and then resolution of all pain within a few minutes. There were no sequelae.

Tail flick test

Nociception before ischaemia and during reperfusion was compared using the modified tail flick test which we have employed

previously (Gelgor et al. 1986a,b). The rat's tail was submerged in a water bath controlled at 49°C, and tail flick latency was measured on a stop watch, as the time from submergence to the first co-ordinated motor response indicated by flicking of the tail. A mean of 3 measurements 1 min apart was recorded as the latency. To avoid thermally induced tissue damage, animals which failed to respond within 20 sec had their tails removed from the water.

Experimental procedure

The rats were placed in clear perspex restrainers which allowed free movement of the tail and slightly restricted movement of the rest of the body. The rats were placed in these restrainers for 2–3 h/day on 2 consecutive days prior to any experimentation to allow them to habituate. On experimental days the animals were placed in restrainers for 15 min prior to any testing. At least 48 h was allowed between successive measurements on individual animals. Experiments were carried out between 09.00 and 13.00 h at an ambient temperature of 24°C.

Tail flick latencies were measured prior to any treatment, immediately after cuff deflation, then at 0.5-h intervals for 1 h. Control experiments were performed by placing an uninflated (sham) cuff on the tail for 12 min; this time is equal to the mean escape latency measured in previous experiments in our laboratory (Gelgor et al. 1986a,b).

Because tail flick latency varies with tail temperature, in a pilot experiment, we measured tail skin temperature, using copper-constantan thermocouples taped to the dorsal surface of the tail 30 mm from the base, before, during and after ischaemia. Temperatures were recorded on a data logger (MCS 120, MC Systems). Temperatures were recorded for 30 min prior to any testing and at 10-min intervals throughout the experiment. Specific temperatures were recorded during ischaemia and immediately before tail flick latencies were measured.

All agents as well as their vehicles were administered in a volume of 10 µl into the lateral ventricle using a 30-ga needle attached to a Hamilton syringe, 5 min before application of the tourniquet (test groups) or sham tourniquet (control groups). The vehicle was normal saline except for ibuprofen and paracetamol where it was a 20% ethanol in water solution. The drugs and dosages used were: indomethacin (Merck, Sharp and Dohme Research Laboratories) 0.002, 0.001, 0.002 mg/kg; diclofenac sodium (Ciba Geigy) 0.05, 0.25, 0.5 mg/kg; ibuprofen (Lennon) 0.03, 0.14, 0.3 mg/kg; paracetamol = 4-acetamidophenol (Lennon) 0.05, 0.23, 0.50 mg/kg; dipyrrone (Hoechst AG) 0.04, 0.19, 0.37 mg/kg. The Student's *t* test with Bonferroni correction for multiple comparisons and a 1-way analysis of variance were used for data analysis.

The experimental procedures were approved by the Animal Ethics Committee of the University of the Witwatersrand (Certificate no. 89/23/5) and complied with the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmerman 1980).

Results

Tail temperature decreased significantly during ischaemia ($P < 0.001$, $n = 10$, paired *t* test). Following release of the tourniquet, tail temperature recovered to values which did not differ significantly from those evident before the cuff was applied ($P > 0.05$, $n = 10$, paired *t* test). However, there was a significant reduction in tail flick latency ($P < 0.01$, $n = 10$, paired-*t* test with Bonferroni correction for repeated measures) at all measurement times following release of the tourniquet.

TABLE 1

ESCAPE LATENCIES DURING ISCHAEMIA (mean $\pm$ S.E.) AND TAIL FLICK LATENCIES IN THE ABSENCE OF ISCHAEMIA (mean $\pm$ S.E.), FOLLOWING I.C.V. ADMINISTRATION OF VEHICLES AND OF THE HIGHEST DOSES OF EACH OF THE NSAID

The drugs did not affect escape latency nor tail flick latency.

Agents administered		Escape latency during ischaemia (min)	Tail flick latency (sec)
Sterile water		14.2 $\pm$ 2.2	7.8 $\pm$ 0.7
Ethanol	20%	13.8 $\pm$ 2.8	7.8 $\pm$ 0.3
Indomethacin	0.002 mg/kg	12.7 $\pm$ 2.1	8.0 $\pm$ 0.6
Diclofenac sodium	0.5 mg/kg	11.0 $\pm$ 2.3	7.4 $\pm$ 0.5
Ibuprofen	0.3 mg/kg	11.6 $\pm$ 2.1	7.6 $\pm$ 0.4
Paracetamol	0.47 mg/kg	13.6 $\pm$ 2.7	8.2 $\pm$ 0.5
Dipyrone	0.37 mg/kg	13.3 $\pm$ 2.6	8.0 $\pm$ 0.6

The latency to co-ordinated escape behaviour following the induction of ischaemia in the absence of any drugs was 13.0 $\pm$ 0.3 min (mean $\pm$ S.E., $n = 50$). There was no significant difference in escape latency between different groups of rats ($P > 0.05$, $n = 10$, unpaired t test). None of the 5 different drugs significantly altered the escape latency at any dosage ($P = 0.88$ in 1-way analysis of variance, see Table 1). Also none of the drugs tested had any significant effect on the tail flick latency in the absence of ischaemia ($P = 0.23$ in 1-way analysis of variance, see Table 1).

Following reperfusion of the tail after a period of ischaemia, there was a significant decrease in tail flick latency of 1.25–1.84 sec or about 25–30%, indicative of hyperalgesia. The greatest decrease in tail flick latency was observed immediately after removal of the tourniquet, but a significant decrease persisted for 60 min after administration of the vehicles (0.01 $> P > 0.001$, $n = 10$, unpaired t test with Bonferroni correction for repeated measures).

Fig. 1 represents the change in tail flick latency, measured at half-hour intervals following release of the tourniquet, for rats treated with either the vehicle or different doses of indomethacin. Administration of both the vehicle and the lowest dose of indomethacin resulted in a significant decrease in tail flick latency from pre-ischaemic values ($P < 0.01$, $n = 10$, paired t test with Bonferroni correction for repeated measures). Hyperalgesia was still evident 60 min after reperfusion of the previously ischaemic tail. Administration of indomethacin at the 2 highest doses tested abolished the hyperalgesia, and tail flick latencies were not significantly different from pre-ischaemic values ($P > 0.05$, $n = 10$, paired t test with Bonferroni correction for repeated measures) throughout the measurement period.

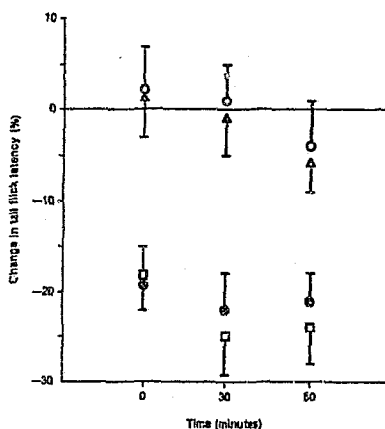


Fig. 1. Percentage change in tail flick latency (mean $\pm$ S.E.) measured at different time intervals after release of the tourniquet. Both saline ($\bullet$) and 0.0002 mg/kg indomethacin ($\square$) resulted in a significant decrease in tail flick latency at all time intervals tested (0.01 $< P < 0.05$, $n = 10$, paired t test with Bonferroni correction for repeated measures). Indomethacin 0.001 ($\circ$) and 0.002 (Δ) mg/kg abolished hyperalgesia, and tail flick latency did not differ significantly from pre-ischaemic values ($P > 0.05$, $n = 10$, paired t test).

Fig. 2 represents the change in tail flick latency from pre-ischaemic values, measured immediately following deflation of the tourniquet, for the 5 different groups of rats treated with either the appropriate vehicle or 1 of the drugs. As was the case with indomethacin,

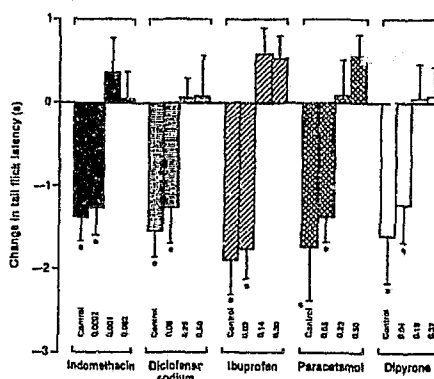


Fig. 2. Change in tail flick latency (mean $\pm$ S.E.) measured immediately after release of the tourniquet, following treatment with vehicles and agents. The asterisks represent those groups in which treatment resulted in a significant decrease in tail flick latency from pretreatment values ($P < 0.001$, $n = 10$, paired t test), that is groups in which there was significant hyperalgesia. All agents abolished this hyperalgesia at sufficient dose.

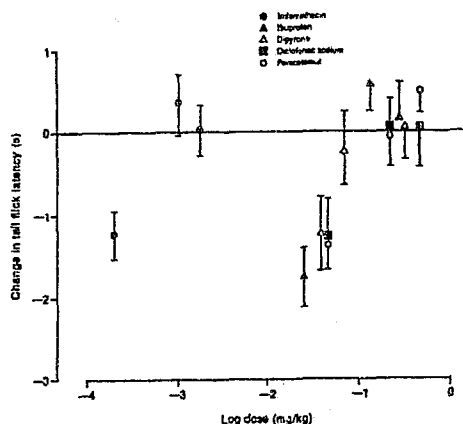


Fig. 3. Change in tail flick latency (mean $\pm$ S.E.) measured immediately after relief of ischaemia, plotted against log dose of the 5 agents. Doses required to abolish hyperalgesia, calculated from the points of intersection with the x axis were: 0.001 mg/kg indomethacin, 0.08 mg/kg dipyrrone, 0.09 mg/kg ibuprofen, 0.2 mg/kg diclofenac sodium and 0.2 mg/kg paracetamol.

following administration of either the vehicle or the lowest dose of each drug there was a significant decrease in tail flick latency from pre-ischaemic levels ($0.02 > P > 0.0001$, $n = 10$, t test), and there was no significant difference in the changes induced by the vehicle and the lowest dose of each drug ($P > 0.05$, $n = 10$, paired t test). Administration of the NSAIDs at the 2 higher doses tested for each of the drugs completely abolished hyperalgesia; tail flick latencies were not significantly different from those observed before induction of ischaemia ($P > 0.05$, $n = 10$, paired t test) nor did they differ significantly between the 2 doses ($P > 0.05$, $n = 10$, paired t test). Increasing the dose of NSAID above that sufficient to abolish reperfusion hyperalgesia brought about no further change in tail flick latency, confirming that the NSAIDs had no intrinsic action on the tail flick response, even during reperfusion.

Fig. 3 represents the change in tail flick latency, measured immediately after release of the tourniquet plotted against the log dose of each drug. Indomethacin proved to be the most potent of the drugs tested, with the other 4 drugs having a lower, but similar, potency. Doses of each drug required to abolish hyperalgesia were calculated from the intercept with the x axis where $y = 0$. The doses in order of potency were: 0.001 mg/kg indomethacin, 0.08 mg/kg dipyrrone, 0.09 mg/kg ibuprofen, 0.2 mg/kg diclofenac sodium and 0.2 mg/kg paracetamol.

Discussion

Previous reports indicate that tail flick latency is weakly negatively correlated with tail skin temperature (Berge et al. 1988; Milne and Gamble 1989; Tjolsen et al. 1989; Han and Ren 1991) and that some factors which alter tail flick latency, usually interpreted as affecting nociception, may really just alter tail skin temperature (Tjolsen et al. 1988; Lund et al. 1989). Induction of ischaemia in the rat's tail reduces blood flow and consequently tail skin temperature at the ambient temperature we used. However, following release of the tourniquet, tail temperature returned to pre-ischaemic values whilst tail flick latency remained significantly decreased. We have therefore established that the hyperalgesia observed on reperfusion of the previously ischaemic tail is not the result of a change in tail temperature.

When 5 different NSAIDs produce the same physiological response, it is highly likely that the response originates from an action that the drugs share, namely inhibition of prostaglandin synthesis, rather than idiosyncratic actions each might have separately. We have shown that 5 NSAIDs abolish reperfusion hyperalgesia arising in the rat's tail without affecting nociception to a noxious thermal stimulus or to a noxious ischaemic stimulus. We therefore conclude that prostaglandins are synthesized during reperfusion of previously ischaemic tissue, and contribute to the hyperalgesia, but do not play a vital role in the behavioural response to noxious heat or ischaemia in the absence of hyperalgesia.

We have shown previously (Gelgor et al. 1990a) that the NSAID abolishes reperfusion hyperalgesia when administered systemically, an observation potentially consistent with either a peripheral or a CNS site of prostaglandin synthesis. We have now shown, by micro-injection of very much lower doses of the same drugs directly into the cerebrospinal fluid, that inhibition of central prostaglandin synthesis is sufficient to abolish reperfusion hyperalgesia. This observation does not exclude the possibility of both peripheral and central prostaglandin synthesis contributing to the hyperalgesia, but it does confirm that the development of reperfusion hyperalgesia, like other hyperalgesias (Hardy et al. 1950; Coderre and Melzack 1987), depends on neural interactions within the CNS.

In a previous study (Gelgor et al. 1990a), the doses of NSAID required to abolish reperfusion hyperalgesia were in order of potency: 5 mg/kg indomethacin, 42 mg/kg diclofenac sodium, 54 mg/kg ibuprofen, 168 mg/kg dipyrrone and 170 mg/kg paracetamol. The rank order of potency for indomethacin, ibuprofen and paracetamol follows similar trends after i.c.v. and i.p. administration, but dipyrrone and diclofenac sodium appeared to act differently centrally and systemically.

We have shown that potency following peripheral administration correlates well with anti-inflammatory potency in man (Gelgor et al. 1990a). The discrepancies on i.c.v. administration may arise from differing activity on cyclo-oxygenase iso-enzymes in different tissues (Flower and Vane 1972; Laburn et al. 1980), or differing accessibility of the drugs to the CNS sites at which prostaglandins may be synthesized, which include hypothalamus (Dubas and Parker 1971), periaqueductal grey matter (Carlsson et al. 1986), thalamus (Carlsson et al. 1988; Jurna and Brune 1990) and spinal cord (Yaksh 1982; Taiwo and Levine 1988). Indomethacin remained the most potent of the NSAIDs in inhibiting reperfusion hyperalgesia; it is a very powerful inhibitor of prostaglandin synthesis in anti-inflammatory assays (Ferreira and Vane 1974; Robinson et al. 1978). Paracetamol and dipyrrone are considered to be weak anti-inflammatories, but relatively strong inhibitors of prostaglandin synthesis in brain tissue (Flower and Vane 1972; Ferreira et al. 1978; Vane 1983; Carlsson et al. 1986). Dipyrrone indeed ranked high in ability to attenuate reperfusion hyperalgesia when administered i.c.v. but not peripherally. Contrary to expectations, however, paracetamol was a weak inhibitor of hyperalgesia regardless of the route of administration. It has also been reported to be a weak inhibitor of adjuvant-induced hyperalgesia (Okuyama and Aihara 1984) and carrageenin-induced hyperalgesia (Ferreira et al. 1978) when injected i.c.v. in rats; its relative inactivity may result from its apparent inability to inhibit prostaglandin synthesis in rat CNS tissue (Abdel-Halem et al. 1978), in contrast to the brain tissue of other species (Flower and Vane 1972).

The observation that NSAIDs block reperfusion hyperalgesia, whether administered systemically (Gelgor et al. 1990a) or now centrally, means that the proposition that the NSAIDs are antinociceptive only in inflammatory states (Ferreira et al. 1978) is too restrictive; they will be antinociceptive in any state in which there is increased prostaglandin synthesis, which we consider to include the state of reperfusion after transient ischaemia. Our results support the view that NSAIDs, whether administered systemically or centrally, are not antinociceptive against uncomplicated noxious stimulation of the skin. Indomethacin and paracetamol (as well as aspirin and phenacetin), injected i.c.v. or i.p., do not affect paw withdrawal in the Randall-Sellito test (Ferreira et al. 1978), and i.c.v. or systemic indomethacin and paracetamol attenuate vocalization during noxious electrical stimulation only at very high doses (Okuyama and Aihara 1984). Diclofenac was not antinociceptive against noxious thermal stimuli in the rat when injected systemically, i.c.v., or into the spinal cord (Bjorkmann et al. 1990). There is one report (Taiwo and Levine 1988) that both indomethacin and aspirin, while being inactive if injected

systemically, block reactions to noxious thermal and mechanical stimuli in rats when injected directly into the spinal cord, and another report that ketoprofen blocks reactions to noxious heat when injected into brain parenchymal tissue at sites not known to be involved in nociception (deBeaurepaire et al. 1990).

If one accepts the hypothesis that NSAID efficacy implies the involvement of prostaglandins in the nociception, then 2 other forms of noxious stimulation contend for inclusion with inflammatory hyperalgesia and reperfusion hyperalgesia. They are mechanical distension of the viscera, where the resultant writhing is blocked by i.c.v. and s.c. diclofenac (Bjorkmann et al. 1990) and i.p. indomethacin (Deleo et al. 1989), and direct electrical stimulation of afferent nerve trunks, the neuronal responses to which are blocked by i.v. dipyrrone, lysine acetylsalicylate and paracetamol (Carlsson et al. 1988).

That prostaglandin synthesis within the CNS is involved in the development of hyperalgesia now seems established for both inflammatory and reperfusion hyperalgesia, and central activity of NSAIDs is sufficient to account for their attenuation of hyperalgesia. Our results, and those of others (Okuyama and Aihara 1984), demonstrate that the CNS doses of NSAIDs required to abolish hyperalgesia are so small that penetration through the blood-brain barrier may be enough to account for the action of NSAIDs administered systemically. However, aspects of hyperalgesia can be mimicked by the local peripheral injection of prostaglandins (Willis and Cornelsen 1973; Taiwo and Levine 1990). We assume, provisionally, that in reperfusion hyperalgesia, as in other hyperalgesia (Ferreira et al. 1978), prostaglandin synthesis occurs and NSAIDs act both centrally and peripherally.

Acknowledgements

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CHAPTER 4

**MODALITY-SPECIFIC HYPERSENSITIVITY OF DORSAL
HORN CONVERGENT DORSAL HORN NEURONES DURING
REPERFUSION OF THEIR RECEPTIVE FIELDS ON THE
RAT'S TAIL, PAIN, 55 (1993) 305-312.**

FAIN 02390

Modality-specific hypersensitivity of dorsal horn convergent neurones during reperfusion of their receptive fields on the rat's tail

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Summary In rats anaesthetised with enflurane, we examined the responses of convergent neurones in the dorsal horn of the spinal cord to noxious thermal and mechanical stimulation and to innocuous brushing, during reperfusion of their receptive fields on the tail, following transient ischaemia. Neurones were included if they responded, before induction of ischaemia, to both pinching and brushing of receptive fields restricted to the tail. Ischaemia was induced by occluding the blood supply to the tail for 30 min using a tourniquet.

Compared to their own responses before ischaemia, during reperfusion almost all the neurones (17 of 20) exhibited significantly increased activity to noxious pinching and innocuous brushing of their receptive fields, following 30 and 60 min of reperfusion. Receptive field size increased markedly in 16 of 20 of the neurones tested. Only 13 of 35 of the neurones responded to noxious thermal stimulation of the tail before induction of ischaemia, and of these only two exhibited enhanced sensitivity to thermal stimulation during reperfusion.

Our results indicate that there is a population of convergent neurones that demonstrates hypersensitivity to mechanical stimulation of the rat's tail, but not to noxious thermal stimulation, during reperfusion of their receptive fields following transient ischaemia.

Key words: Hyperexcitability; Ischaemia; Reperfusion; Noxious stimulation; Convergent neurones; (Rat)

Introduction

There are at least two classes of dorsal horn neurone that receive input from peripheral nociceptors, high-threshold or nociceptive-specific neurones, and convergent or wide-dynamic-range neurones, which are activated by noxious and innocuous stimuli but more powerfully by noxious stimuli (Dubner and Bennett 1983). The properties of convergent neurones are considered more appropriate for encoding noxious thermal stimuli, and changes in intensity of mechanical stimuli, than those of nociceptive-specific neurones (Chung et al. 1986; Cervero et al. 1988; Maimner et al. 1988; Dubner et al. 1989).

In a previous study conducted in our laboratory, convergent dorsal horn neurones with receptive fields in the rat's hind leg exhibited increased excitability to noxious mechanical stimulation during ischaemia, but not to innocuous stimulation of the same receptive field (Sher and Mitchell 1990). We have now examined the responses of convergent dorsal horn neurones with receptive fields in the rat's tail to noxious and innocuous mechanical stimulation, as well as noxious thermal stimulation, during reperfusion of the tail following relief of ischaemia. During reperfusion, the neurones became more sensitive to mechanical stimuli, but not to noxious thermal stimuli.

Materials and methods

Animal preparation

Male Sprague-Dawley rats weighing 180–225 g were used in all experiments. We induced anaesthesia with sodium pentobarbitone

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(50 mg/kg, i.p.) and administered atropine sulphate (0.25 mg/kg) to reduce tracheal secretions. A tracheal cannula was inserted and anaesthesia maintained, using spontaneous respiration, with enflurane (Ethrane, Abbott Laboratories) in nitrous oxide/oxygen (67:33%), at concentrations of 2–3% for surgery and 0.6–1% for maintenance (Sher and Mitchell 1990). We monitored reflexes and pupil diameter throughout the experiment to ensure adequate anaesthesia. Core body temperature was maintained at 37°C by a heating pad controlled from a rectal thermistor.

We performed a laminectomy of the 2nd to 5th lumbar vertebrae and removed the dura mater from the cord. Vertebrae rostral and caudal to the exposed cord were clamped rigidly, to minimise movement at the recording site.

Recording technique and characterisation of cells

Single-unit extracellular recordings were made from dorsal horn neurones 50–1100 µm (mean: 371; standard error: 63) the surface of the cord, the depths being determined by the position taken of a stepping microdrive, a method which has been shown to have an accuracy of 65 µm in the rat lumbar cord (Mitchell and Hellon 1977). The majority of the neurones (59%) lay in the superficial dorsal horn (<280 µm from the surface), with the remaining neurones being located in deeper laminae. Analysis of neurones with respect to depth showed no difference in response between neurones recorded in the superficial and deeper laminae, so the results have been presented as mean responses of all convergent neurones recorded. Only neurones which responded to both noxious pinching and innocuous brushing of the tail were pursued.

We searched for responsive neurones by brushing the tail. We therefore avoided damaging the tail by searching with a noxious stimulus. If brushing elicited a response we then manually pinched the tail. Neurones responding to brush or light mechanical stimulation only were excluded. Once a potentially responsive neurone had been identified, its receptive field was mapped out using a brush and its spontaneous firing rate was measured. We excluded neurones with receptive fields at the tip of the tail, because this portion of the tail became excessively damaged when stimulated repeatedly; we rarely encountered neurones with receptive fields in the tip of the tail.

Once a stable spontaneous firing rate had been established, we applied a noxious pinch, using serrated forceps, of 10 sec duration and 1.7 N force as described previously (Sher and Mitchell 1990), to the centre of the receptive field. Innocuous stimulation was achieved by brushing the tail with a camel hair paint brush along the entire length of the receptive field, with repeated strokes for a 10 sec period and about 0.2 N force, as measured on a balance. We also heated the receptive field using a Peltier thermo-electric thermode connected to a temperature controller (Sensortek TS4, Physitemp Instruments, NJ) which enabled us to ramp up the temperature of the receptive field in 2°C steps between 40 and 50°C, a range based on our previous behavioural studies of hyperalgesia (Gelgor et al. 1986, 1988). A separate thermocouple on the surface of the tail measured the actual tail temperature. The temperature of the tail was maintained at 35°C between stimuli to ensure that the neuronal responses observed were not the result of changes in tail temperature, although we have shown previously in behavioural experiments that reperfusion hyperalgesia is independent of changes in tail skin temperature (Gelgor et al. 1992). We then induced tail ischaemia by applying a tourniquet in the form of an inflatable cuff at the base of the tail, and inflating it to a pressure of 200 mm Hg, so occluding the arterial blood supply to the tail. The cuff was deflated after 30 min, and the receptive field was mapped out again and stimulated immediately, as described above, and then at 30 min and 60 min following relief of ischaemia. Control experiments followed the same experimental procedure but without inflation of the cuff. Because of potential consequences of repeated noxious stimulation, we recorded

only 1 neurone from each animal. The animals were subsequently killed by anaesthetic overdose, without recovery of consciousness.

All recordings were made using glass-coated tungsten electrodes. Amplified potentials were fed through a spike processor (Digitimer D130, Digitimer, UK) into a laboratory interface (CED 140, Cambridge Electronic Designs, Cambridge) attached to a microcomputer for storage and analysis. To ensure that the same unit was being recorded from for the full duration of an experiment, the amplitude and shape of each impulse were monitored, as well as the consistency of response to stimulation of the centre of the receptive field.

Statistical analyses were made using the paired Student's *t* test with the Bonferroni correction for multiple comparisons.

All procedures were approved by the Animal Ethics Committee of the University of the Witwatersrand (Clearance certificate number 89/181/12).

Results

We recorded 35 neurones which responded to both noxious pinch and innocuous brushing of the tail within the same receptive field. Of these only 13 responded to graded noxious thermal stimulation of the receptive field. In 24 animals, we applied the tourniquet, but we failed to recover 4 neurones following removal of the tourniquet. In the other 11 animals we applied a sham tourniquet.

The mean spontaneous firing rate of 20 neurones, before application of the tourniquet was 1.2 ± 0.3 spikes/sec; there was no difference between those which responded to thermal stimuli and those which did not. The majority of the neurones (32 of 35) had a spontaneous firing rate less than 2 spikes/sec with the remaining 3 neurones exhibiting a spontaneous firing rate of 5–10 spikes/sec.

During ischaemia, the firing rate increased significantly ($P < 0.001$, paired *t* test with Bonferroni correction for repeated measures, $n = 20$) (Fig. 1). The latency of response of the neurones following application of the tourniquet was 11.3 ± 1.5 min ($n = 20$). This value correlates well with the response latency of thalamic neurones to the same manoeuvre (Gelgor et al. 1988), as well as with the escape latency observed in conscious animals following application of a tourniquet to the tail (Gelgor et al. 1988; Sher et al. 1992). The mean spontaneous firing rate during reperfusion of the tail was lower than that prevailing during ischaemia, but still significantly different from that prevailing before application of the tourniquet. Fig. 1 also shows the mean spontaneous firing rate of 11 neurones before, during, and following application of an uninflated (sham) tourniquet; there was no significant change in firing rate associated with the sham tourniquet.

Fig. 2 shows the response of one of the neurones we tested to pinch and brush, before application of the tourniquet and following its release (top panel), as well as the response to thermal stimulation of the same receptive field (bottom panel). Before application of

the tourniquet, the responses of the neurone to pinch and brush, at the intensities we employed, were similar which was the case in 20% (7 of 35) of the rats. Immediately following release of the tourniquet the response to both pinch and brush was markedly decreased. This decrease in response immediately following ischaemia was evident in 45% (9 of 20) of the neurones; in 10 of 20 neurones there was no change in sensitivity at this time. However, at 30 and 60 min after release of the tourniquet, the response to both pinching and brushing of the receptive field was clearly greater than that evident before application of the tourniquet. The firing rate of this neurone did not change significantly with increases in temperature, either before application of the tourniquet or during reperfusion of the tail.

The selective hypersensitivity to mechanical stimuli, exhibited by the neurone of Fig. 2, was evident in most of the neurones we encountered. Fig. 3 illustrates the

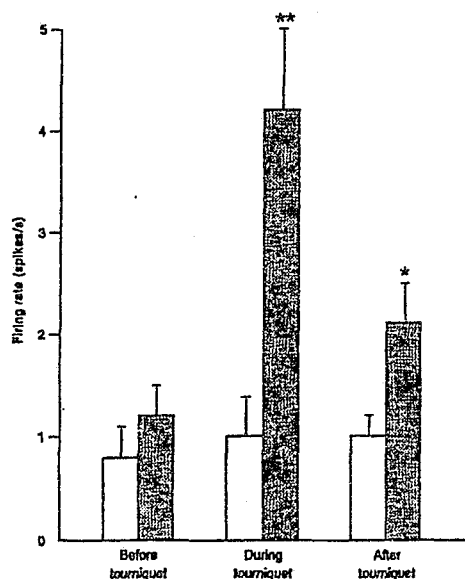


Fig. 1. Firing rate of convergent neurones with tail receptive fields before, during, and after application of a tourniquet (filled bars, mean $\pm$ S.E.M., $n = 20$) or sham tourniquet (open bars, $n = 11$) to the tail. During application of the tourniquet the firing rate increased significantly above the firing rate prevailing before application of a tourniquet ($P < 0.001$, paired t test). The spontaneous firing rate following release of the tourniquet was lower and significantly different to that prevailing before ($P < 0.05$) and during application of a tourniquet ($P < 0.02$, paired t test, with Bonferroni correction for repeated measures). Application of a sham tourniquet had no effect on spontaneous firing rate ($P > 0.05$, $n = 11$, paired t test).

characteristics of a neurone which developed hypersensitivity to thermal as well as mechanical stimulation of the receptive field. This neurone responded more to pinching than to brushing of the receptive field, a pattern evident in 28 of 35 (80%) of neurones we examined. The neurone of Fig. 3 was the only neurone that exhibited enhanced sensitivity to mechanical and thermal stimuli immediately after release of the tourniquet; it was even more sensitive after 30 and 60 min of reperfusion. Whilst 17 of 20 (85%) of neurones we examined had a greater response to noxious and innocuous stimuli of the receptive field after 30 and 60 min of reperfusion than before application of the tourniquet, only one other neurone exhibited an increase in response to thermal stimulation of the receptive field during reperfusion.

Fig. 4 consolidates the responses of the population of neurones, including those which also responded to thermal stimuli, to noxious pinch and innocuous brushing, before application of a tourniquet or sham tourniquet, and following their removal. Immediately following release of the tourniquet, the mean responses of the neurones to both pinch and brush did not differ significantly from the responses before the tourniquet was applied. However, after 30 and 60 min of reperfusion, there was a significant increase in response to both pinch and brush. There was no significant change in response, at any time, associated with the sham tourniquet.

Only a subpopulation of 13 neurones exhibited an increase in firing rate with increasing tail temperature, before we applied a real or sham tourniquet. Most of the thermally responsive units (62%) were located in the superficial dorsal horn. The spinal neurones we examined fired at about 2 spikes/sec at receptive field temperatures between 42 and 44°C. Based on analysis of individual neurones, the threshold temperature above which firing increased was $44.6 \pm 1.0^\circ\text{C}$ (mean $\pm$ S.E.M.). The firing rate reached a maximum of about 8 spikes/sec at a temperature of $48.0 \pm 0.8^\circ\text{C}$ (mean $\pm$ S.E.M.), a rate very similar to that recorded in thermally sensitive spinothalamic tract cells (Palecek et al. 1992), but considerably lower than that of the neurones in the ventrobasal complex of the rat thalamus initially selected for their responsiveness to noxious thermal stimulation of the tail (Gelgor et al. 1988).

Following release of the tourniquet, 2 of the 13 neurones exhibited an increase in firing rate with increasing temperature which was greater than their response prior to ischaemia. Eight other neurones did not change their firing rates at any temperature tested, nor did they change threshold temperature, during the reperfusion of their receptive fields ($P > 0.05$, $n = 8$, paired t test with Bonferroni correction for repeated measures). Also, none of the neurones which were unresponsive to noxious thermal stimuli before appli-

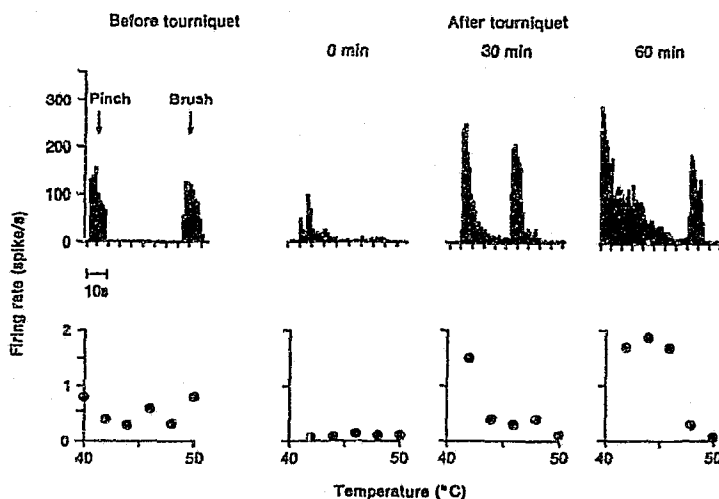


Fig. 2. Response of a convergent neurone in the dorsal horn to pinch, brush and graded thermal stimulation of the tail before application of the tourniquet, immediately after release of the tourniquet, and 30 and 60 min later. The top panel represents the rate histogram of the neurone over 1 sec epochs. The bottom panel shows the mean firing rate of the neurone at each temperature tested. Immediately following release of the tourniquet, the response to pinch and brush decreased, but then increased with time, with hypersensitivity evident after 30 and 60 min of reperfusion of the tail. This neurone did not respond significantly to thermal stimulation of its receptive field; note that the firing rate never exceeded 2 spikes/sec. The mean spontaneous firing rate of this neurone was 0.8 spikes/sec before application of the tourniquet and 1.5 spikes/sec during reperfusion.

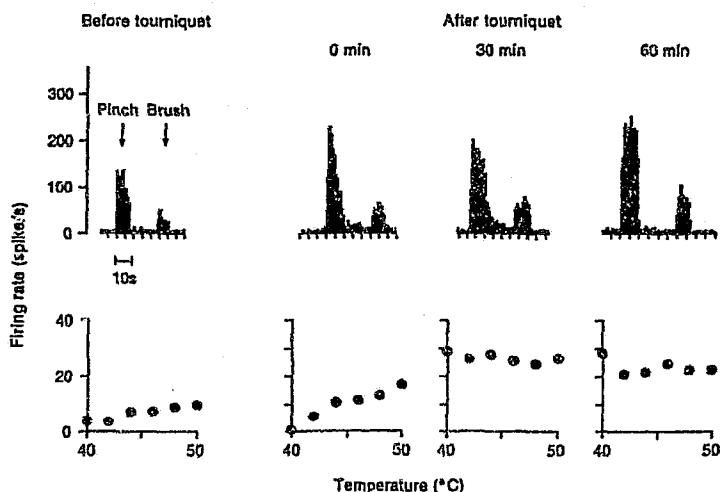


Fig. 3. Characteristics of a neurone which exhibited a response to graded thermal stimulation as well as a response to pinch and brush of the receptive field before application of the tourniquet. This was the only neurone which exhibited hypersensitivity to noxious stimulation immediately following release of the tourniquet. Details as in Fig. 2; note the change in scale of firing rates in response to thermal stimulation (maximum of 2 Hz in Fig. 2, but 40 Hz here).

cation of the tourniquet became responsive during reperfusion of the tail.

In addition to investigating the effect of reperfusion on firing rate, we investigated receptive field size during reperfusion. Fig. 5 demonstrates individual examples of receptive field topography before application of the tourniquet and after its release. Receptive fields initially were mostly bilateral, encompassed both the dorsal and ventral surface of the tail, measured 32 ± 10 mm (mean $\pm$ S.E.M., $n = 35$), along the tail's major axis and were distributed throughout the tail. Receptive field size increased significantly ($P < 0.02$, paired t test) with length increasing to 84 ± 21 mm (mean $\pm$ S.E.M., $n = 20$), immediately following tourniquet release in those neurones the receptive field of which were subject to ischaemia. During reperfusion the receptive field sometimes extended to the entire tail,

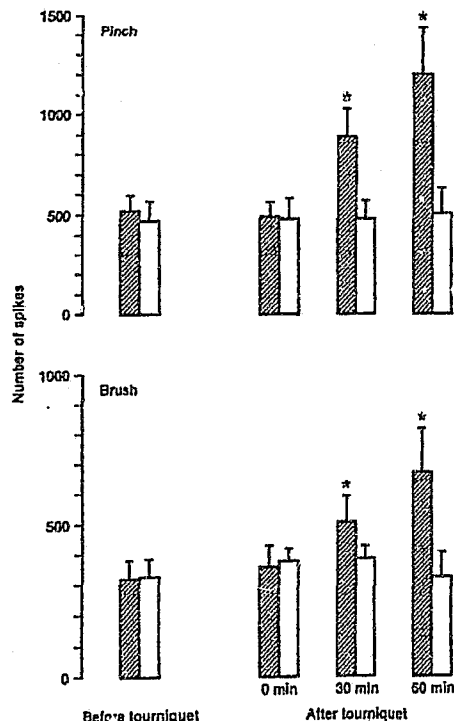


Fig. 4. Response of the population of convergent neurones to noxious pinching (top panel) and innocuous brushing (bottom panel) of the receptive field, before application and following release of the tail tourniquet (filled bars, mean $\pm$ S.E.M., $n = 20$) or sham tourniquet (open bars, $n = 11$). The asterisks signify responses significantly different from the responses before application of the tourniquet ($P < 0.02$, paired t test with Bonferroni correction for repeated measures).

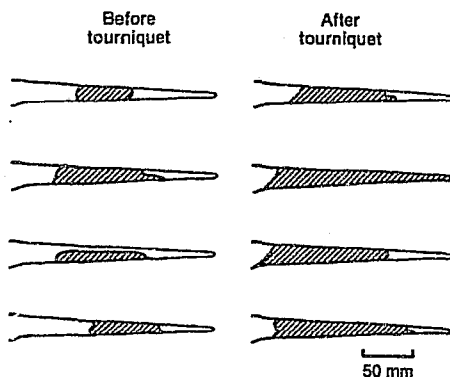


Fig. 5. Individual examples showing receptive field size before ischaemia and immediately following release of the tourniquet. Receptive field size increased in 16 of 20 (80%) of the neurones tested.

although a few receptive fields did not change in size. The receptive fields never extended beyond the tail before or after application of the tourniquet. No change in receptive field size was observed following application of the sham tourniquet.

Discussion

The population of neurones we encountered exhibited hypersensitivity to both noxious and innocuous mechanical stimulation, during reperfusion of the tail. Of the neurones, which were selected because they responded to noxious and innocuous mechanical stimulation of their receptive fields, and only two of these exhibited an increased responsiveness to noxious thermal stimulation during reperfusion of the tail. The population of dorsal horn neurones therefore exhibited modality-specific hypersensitivity; even those which were sensitive to thermal stimuli did not exhibit enhanced thermal sensitivity when they exhibited enhanced mechanical sensitivity.

The increased responsiveness to mechanical stimuli usually was not evident immediately after removal of the tourniquet, but the response to both pinch and brush had doubled after 60 min reperfusion. Concomitant with the increased responsiveness there was a marked increase in receptive field size of most of the neurones, which indeed was evident immediately following release of the tourniquet. The hypersensitivity and increased receptive field size were not the consequence of repeated noxious stimulation of the tail, because they were absent when the same stimuli were applied before and after application of a sham tourniquet.

Occluding the blood supply to the tail, in the absence of other stimuli to the tail, produced a 3-fold increase in spontaneous activity. Following removal of the tourniquet, the spontaneous firing rate decreased, but did not return to the pre-ischaemic value within the 60 min of our recordings. Ischaemia appears to excite specific afferent nociceptive pathways (Chabel et al. 1990; Schaible et al. 1990; LaMotte et al. 1991), and activity in these pathways may contribute to the increased spontaneous activity evident during and after ischaemia. The increase in spontaneous firing rate during tail ischaemia, and the receptive field enlargement immediately after release of the tourniquet, presumably resulted from C-fibre input to the dorsal horn neurones, because they occurred when conduction in A fibres would have been blocked by the tourniquet (Kojo and Pertovaara 1986; Cline et al. 1989). During tourniquet-induced ischaemia of the rat hindlimb, activity developed in fibres with conduction velocities in the C-fibre range that were not active prior to tourniquet inflation (Chabel et al. 1990), and such fibres may exist in pathways from the tail too. Increases in receptive field size like those we observed have been reported following repeated noxious stimulation of, or injury to, the periphery (Cervero et al. 1984; McMahon and Wall 1984; Cook et al. 1987; Woolf and King 1990). The mechanism underlying receptive field enlargement is not simply modification of afferent input, but also depends on events which take place in the dorsal horn of the spinal cord (Coderre and Melzack 1987; Cook et al. 1987; Cervero and Laird 1988; Hoheisel and Mense 1989; Hylden et al. 1989; Woolf and King 1990).

The activation of novel afferent pathways during and after ischaemia may also contribute to the hypersensitivity to mechanical stimuli which the convergent neurones exhibited during reperfusion. The activation is likely to arise from chemosensors stimulated by tissue metabolites released peripherally during and after ischaemia (Lindahl 1974; Sicuteri et al. 1974; Lynn 1977). However, once activated, neurones in the pathways develop sensitivity to mechanical stimuli which is not evident in the absence of ischaemia (Schaible et al. 1990; LaMotte et al. 1991). Such neurones, if they exist in afferent pathways from the tail, may excite the dorsal horn neurones.

In the context of our results, we need to consider how convergent dorsal horn neurones might exhibit enhanced responsiveness to mechanical stimuli but not to thermal stimuli during reperfusion. One possibility is that the hypersensitivity is specific to a particular afferent fibre type. Shir and Seltzer (1990) have proposed that, in causalgiform pain disorder in rats, mechanical hyperalgesia is mediated via A-fibre afferents and thermal hyperalgesia by C-fibre afferents. Although A fibres may contribute to thermal sensitivity in afferent

nociceptive pathways at nociceptor temperatures greater than 49°C (Beitel and Dubner 1976), over the temperature range we used C fibres are most likely responsible for the transmission of thermal nociceptive information (Handwerker et al. 1975; Beitel and Dubner 1976; LaMotte 1984). These thermosensitive C fibres may not converge on to dorsal horn neurones, even if chemosensitive C fibres indeed do so.

Another possible explanation for the modality-specific hypersensitivity is that the hypersensitivity is confined, not to specific afferent fibre types, but to a specific mediator, released either in primary afferent fibres or in the spinal cord, which facilitates the transmission of a specific stimulus modality (Follenfant et al. 1989; Fleetwood-Walker et al. 1990; Kellstein et al. 1990; Noguchi et al. 1991). Excitatory amino acids appear likely candidates; they are not involved in the thermally excited tail flick reflex (Yasphal et al. 1991; Sher et al. 1992) but specifically mediate the response to mechanical stimulation, and mechanical allodynia (Zieglansberger and Herz 1971; Salt and Hill 1983; Dougherty and Willis 1991a,b). Previous studies in our laboratory have shown that excitatory amino acids in the spinal cord play a role in nociception during ischaemia and in reperfusion hyperalgesia (Sher and Mitchell 1990; Sher et al. 1992). Recruitment of low-threshold mechanoreceptors which use excitatory amino acids as transmitters (Hill and Salt 1980) could underlie the hyperalgesia evident to noxious and innocuous mechanical stimuli during reperfusion of the tail. Moreover, the hypersensitivity in which excitatory amino acids are involved is not confined to A-fibre afferent input; C-fibre activity is also enhanced by excitatory amino acids (Dickenson and Sullivan 1987).

Other investigators have proposed that it is the convergent dorsal horn neurones which account for the hyperalgesia to noxious heat and encode information arising from thermal nociceptors (Dubner et al. 1989; Kenshalo et al. 1989; Maixner et al. 1989; Simone et al. 1991). Our results do not support these proposals. The population of neurones we examined exhibited hypersensitivity only to mechanical stimuli, during reperfusion following ischaemia to the rat tail.

Acknowledgements

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CHAPTER 5

**PROSTANOID SYNTHESIS IN THE SPINAL CORD
ENHANCES EXCITABILITY OF DORSAL HORN
CONVERGENT NEURONES DURING REPERFUSION OF
ISCHAEMIC RECEPTIVE FIELDS ON THE RAT'S TAIL,
PAIN, 60 (1995) 181-187.**

PAIN 2633

Prostanoid synthesis in the spinal cord enhances excitability of dorsal horn convergent neurones during reperfusion of ischaemic receptive fields on the rat's tail

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Summary In 40 rats anaesthetized with enflurane, we identified convergent dorsal horn neurones responding to both noxious (pinch) and innocuous (brush) mechanical stimulation of their receptive fields on the tail. We recorded extracellular activity before and during ischaemia of the receptive fields, as well as during subsequent reperfusion. Two NSAIDs, indomethacin and diclofenac sodium, or saline were applied locally to the spinal cord before the induction of ischaemia. During ischaemia, spontaneous activity of the neurones increased significantly, and the responses to both pinch and brush were reduced significantly; indomethacin and diclofenac sodium had no effect on either spontaneous activity or sensitivity to mechanical stimuli. The neurones became hypersensitive to both pinch and brush during reperfusion of their receptive field, and receptive field size increased. Application of indomethacin and diclofenac sodium to the spinal cord abolished both the hypersensitivity and the increase in receptive field size. Our results indicate that spinal cord prostanoid synthesis facilitates the enhanced excitability of dorsal horn convergent neurones to both noxious and innocuous mechanical stimuli during reperfusion of their receptive fields, but does not affect the neurones' responses to receptive field ischaemia, nor their responses to mechanical stimuli in the absence of a conditioning stimulus.

Key words: Prostaglandin; Reperfusion hyperalgesia; Convergent neurone; Spinal cord; Ischaemia; (Rat)

Introduction

Hyperalgesia following peripheral tissue damage may result from increased sensitivity of nociceptors at the site of injury (Beitel and Dubner 1976; Bessou and Perl 1976), or from increased excitability of central nervous system (CNS) neurones (Woolf 1983; Wall and Woolf 1984), or both. The antinociceptive action of non-steroidal anti-inflammatory drugs (NSAIDs) following tissue injury or inflammation has been widely attributed to their ability to inhibit the synthesis of prostanoids peripherally, thus preventing prostanoid-mediated sensitization of nociceptors (Rang et al. 1991).

However, prostaglandins also facilitate nociceptive transmission by an action in the CNS (Braga 1990; Jurna and Brune 1990). Peripheral electrical stimulation induces the release of prostaglandins within the frog spinal cord (Ramwell et al. 1966) and intrathecal administration of low doses of prostaglandins causes a reduction in nociceptive thresholds (Ferreira et al. 1978; Ferreira 1983; Taiwo and Levine 1988). Blocking spinal prostanoid synthesis by intrathecal administration of NSAIDs attenuates the hyperalgesia which follows peripheral injection of an irritant, at doses which are too low to exert a systemic effect (Yaksh 1982; Malmberg and Yaksh 1992b).

We have now investigated how spinal prostanoid synthesis affects the electrical activity of neurones in nociceptive pathways in the spinal cord. We examined the responses of convergent dorsal horn neurones to noxious and innocuous mechanical stimuli, during ischaemia and subsequent reperfusion of their receptive

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fields on the rat's tail. We administered two NSAIDs, diclofenac sodium and indomethacin, directly on to the exposed spinal cord, to block local prostanoid synthesis.

Material and methods

Animal preparation

Male Sprague-Dawley rats weighing 180–250 g were used. We induced anaesthesia with sodium pentobarbitone (50 mg/kg, i.p.) and administered atropine sulphate (0.25 mg/kg) to reduce tracheal secretions. A tracheal cannula was inserted and anaesthesia maintained, using spontaneous respiration, with enflurane (Eithane, Abbott Laboratories) in nitrous oxide/oxygen (67:33%), at concentrations of 2–3% for surgery and 0.6–1% for maintenance (Sher and Mitchell 1990). We monitored reflexes and pupil diameter throughout the experiment to ensure adequate anaesthesia. Core body temperature was maintained at 37°C by a heating pad controlled from a rectal thermometer.

We performed a laminectomy of the second to the fifth lumbar vertebrae and removed the dura mater from the cord. Vertebrae rostral and caudal to the exposed cord were clamped rigidly, to minimise movement at the recording site. Muscle flaps were raised to form a small pool around the exposed cord for drug administration.

Recording technique and characterisation of cells

Single-unit extracellular recordings were made from dorsal horn neurones 200–1400 µm (mean: 688; SE: 60) below the surface of the cord, the depths being determined by the position of a stepping microdrive, a method which has been shown to have an accuracy of 65 µm in the rat lumbar cord (Mitchell and Hellon 1977). The majority of the neurones (90%) were located in deeper laminae with the remaining 10% located in the superficial dorsal horn (< 290 µm from the surface). Analysis of neurones with respect to depth showed no difference in response between neurones recorded in the superficial and deeper laminae, so the results have been presented as mean responses of all convergent neurones recorded. Two of the neurones were more than 1000 µm below the surface, and so were likely to be outside the anatomical dorsal horn; their characteristics were not distinguishable from those of the other neurones.

We searched for responsive neurones by brushing the tail. If brushing elicited a response we then manually pinched the tail. Only neurones which responded to both noxious pinching and innocuous brushing of a receptive field on the tail were pursued. Once a qualifying neurone had been identified, its receptive field was mapped out using a brush. We abandoned neurones with receptive fields at the tip of the tail, because this portion of the tail became excessively damaged when stimulated repeatedly.

We measured spontaneous firing rate and then applied a noxious pinch, using serrated forceps, of 10 sec duration and 1.7 N force as described previously (Sher and Mitchell 1990), to the centre of the receptive field. To apply standard innocuous stimulation, we brushed the receptive field with a camel hair paint brush for 10 sec with repeated strokes, at about 0.2 N force. Three sets of stimuli were applied 10 min apart. We then induced tail ischaemia by applying a pneumatic tourniquet at the base of the tail and inflating it to a pressure of 200 mm Hg, thereby occluding the arterial blood supply to the tail. The receptive field was stimulated at 10 and 20 min after the induction of ischaemia. The tourniquet was removed after 30 min, and the receptive field was mapped out again and stimulated immediately, as described above, and at 10 min intervals for a further 60 min. Control experiments followed the same experimental procedure but without inflation of the tourniquet. Because of potential

consequences of repeated noxious stimulation (Sher and Mitchell 1990), we recorded only 1 neurone from each animal.

Extracellular spikes were recorded using glass electrodes with a tip diameter of approximately 2 µm. The electrodes were filled with a 3 M KCl solution and had a resistance of 2–5 MΩ. Amplified potentials were fed through a spike processor (Digitimer D130, Digitimer, UK) into a laboratory interface (CED 1401, Cambridge Electronic Designs, Cambridge) attached to a microcomputer for storage and analysis.

Drug administration

The drugs were applied directly on to the exposed spinal cord in 25 µl boluses, mimicking intrathecal administration, 5 min before application of the tourniquet or sham tourniquet. Control experiments for the drug administration were done by applying 25 µl of physiological saline to the cord in 10 animals and in 9 animals we did not apply anything on the cord. There were no significant differences in the properties of neurones of saline-treated and untreated animals, and the results of all control animals subsequently were combined.

Indomethacin (Merck, Sharp and Dohme Research Laboratories) was administered at a dose of 1 µg/kg and 0.2 µg/kg and diclofenac sodium (Ciba Geigy) at a dose of 0.25 mg/kg. The vehicle used for both agents was physiological saline. These doses derive from a previous study of behavioural responses of conscious rats (Gelgor et al. 1992b). To ascertain whether spinal prostanoids affected neuronal responses to mechanical stimuli in the absence of ischaemia, we administered indomethacin at a dose of 1 µg/kg in a group of animals following application of the sham tourniquet.

Statistics

Statistical comparisons were made using a 1-way analysis of variance (ANOVA) and Student's *t* test with the Bonferroni correction for multiple comparisons.

Ethics

All procedures were approved by the Animal Ethics Committee of the University of the Witwatersrand (Clearance certificate number 90/106/2).

Results

Fig. 1 demonstrates the typical response of a convergent neurone to pinch and brush, recorded in an animal given intrathecal saline, before receptive field ischaemia, during ischaemia and at 0 and 30 min after the termination of ischaemia. During ischaemia the response to both pinch and brush was diminished whilst spontaneous firing rate increased. A marked increase in the response to both pinch and brush developed during reperfusion of the receptive field. Fig. 2 shows the mean responses to pinch and brush stimuli of all animals given intrathecal saline, or no intrathecal injection, during ischaemia and reperfusion of the receptive fields. During ischaemia, there was a significant decrease in response to both noxious ($P < 0.001$, $n = 19$, paired *t* test,) and innocuous mechanical stimulation of the receptive field ($P < 0.001$, $n = 19$, paired *t* test). Immediately following release of the tourniquet, the responses of the convergent neurones to both pinch

and brush did not differ significantly from those prevailing before the tourniquet was applied ($P > 0.05$, $n = 19$, paired t test). However, following 10 min of reperfusion, the responses to both pinch and brush increased significantly ($P < 0.01$, $n = 19$, paired t test with Bonferroni correction for repeated measures), an effect sustained for the 60 min recording period. Such excitability changes may result from changes in tail skin temperature (Hole and Tjolsen 1993) but, as we have demonstrated previously (Gelgor and Mitchell 1993), changes in tail temperature do not account for the hypersensitivity of convergent neurones during reperfusion. Application of a sham tourniquet did not affect the response to pinch or brush ($P > 0.05$, $n = 8$, paired t test).

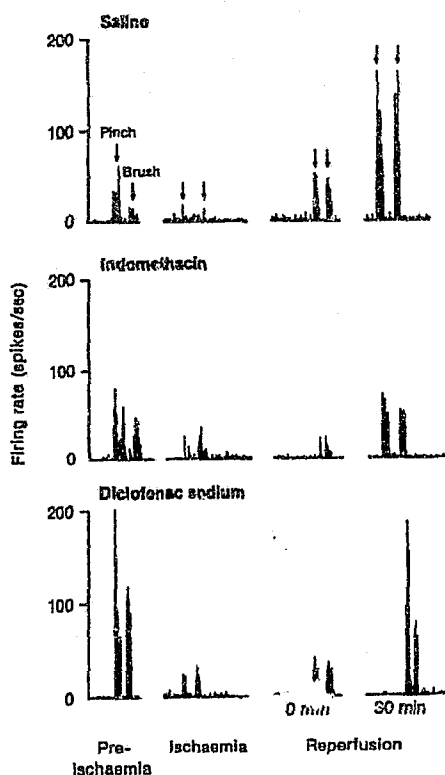


Fig. 1. Examples of the firing patterns of convergent dorsal horn neurones with receptive fields on the tail, before and during tail ischaemia, as well as during reperfusion of the tail, in animals given intrathecal saline, indomethacin (1 μ g/kg), or a diclofenac sodium (0.25 mg/kg). Arrows indicate stimulation of the receptive field.

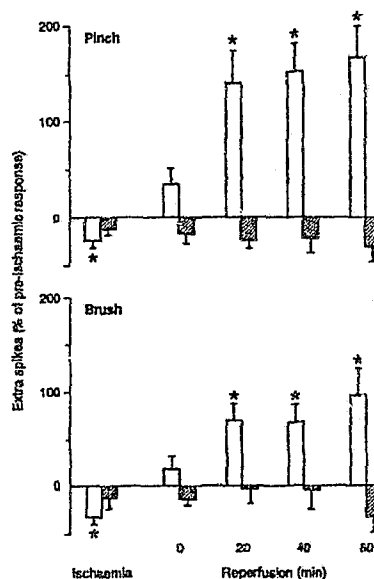


Fig. 2. Responses of convergent neurones to noxious pinching (top panel) and innocuous brushing (bottom panel) of the receptive field during tail ischaemia and reperfusion (open bars, mean $\pm$ SE, $n = 19$), or following application of the sham tourniquet (hatched bars, mean $\pm$ SE, $n = 8$), in animals given either intrathecal saline or no intrathecal injection. The responses were expressed as the percentage difference between the total number of spikes excited by the stimulus and the number recorded when the same stimulus was applied to the same animal immediately before application of the tourniquet. Asterisks signify values significantly different from zero ($P < 0.05$, t test with Bonferroni correction for multiple comparisons). The neurones showed reduced sensitivity to mechanical stimulation during ischaemia and increased sensitivity during reperfusion of the receptive field.

Inhibition of prostanoïd synthesis profoundly influenced the responses to mechanical stimuli. Fig. 1 illustrates, for individual neurones, how the firing pattern changed following treatment with intrathecal diclofenac sodium or indomethacin. Neither of the agents affected the increase in spontaneous firing rate evident during ischaemia, nor the responses to pinch and brush of the receptive fields during ischaemia. However, during reperfusion of the tail, the hypersensitivity to pinch and brush was attenuated by pretreatment with indomethacin or diclofenac sodium. Fig. 3 shows the mean responses. During receptive field ischaemia itself, the prostanoïd synthesis inhibitors had no significant effect on neuronal activity. However, both inhibitors, administered to the spinal cord, abolished the hypersensitivity to both pinch and brush which other-

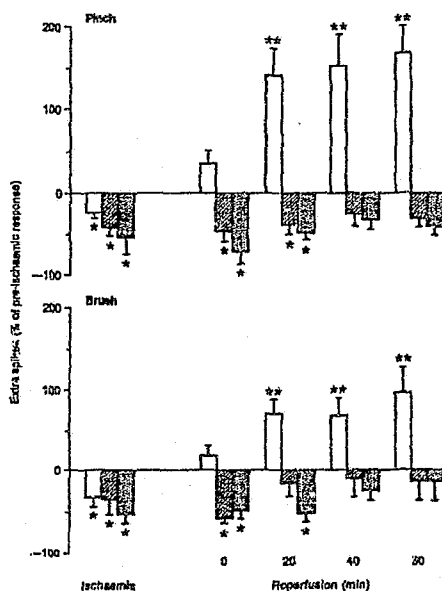


Fig. 3. Mean responses of convergent neurones to noxious pinching (top panel) and innocuous brushing (bottom panel) in animals given intrathecal diclofenac sodium 0.25 mg/kg (hatched bars), indomethacin 1 µg/kg (dotted bars), or no NSAID (open bars). The analysis was performed in the same way as that of Fig. 2. The asterisks represent values significantly different from zero (* $P < 0.05$, ** $P < 0.001$, t test with Bonferroni correction). The NSAIDs abolished the hypersensitivity evident during reperfusion but did not affect responses during ischaemia of the receptive fields.

wise developed during reperfusion of the receptive fields. In addition, both inhibitors retarded the transition from the firing pattern evident during ischaemia to that evident during reperfusion, and the neurones continued to exhibit reduced sensitivity to pinch and brush for the first 20 min of reperfusion. Indomethacin had no effect on the responses to pinch and brush following application of a sham tourniquet ($P > 0.05$, $n = 10$, paired t test, data not shown).

Prostanoids were involved not only in the neuronal response to mechanical stimuli during reperfusion, but also in the neurones' spontaneous activity. Fig. 4 shows the change in spontaneous firing rate following intrathecal administration of saline, diclofenac sodium, or indomethacin. During ischaemia the spontaneous firing rate increased significantly ($P < 0.001$, $n = 10$, paired t test) in all three treatment groups, and the increase did not differ significantly between the groups ($P > 0.05$, unpaired t test with Bonferroni correction). The latency to the onset of enhanced spontaneous

activity following application of the tourniquet was 11.4 ± 2.3 min ($n = 19$) in animals given intrathecal saline or no intrathecal agent. This value is the same as that found for convergent neurones with receptive fields in the foot, following occlusion of the femoral artery (Sher and Mitchell 1990). Intrathecal administration of indomethacin and diclofenac sodium did not affect the latency. In contrast to their lack of effect during ischaemia, intrathecal indomethacin and diclofenac sodium abolished the increased spontaneous firing rate otherwise evident during reperfusion of the tail.

During reperfusion, the prostanoid synthesis inhibitors affected not only the firing rate of convergent neurones, but also their receptive field sizes. Fig. 5 shows examples of receptive field size, in an animal from each of the three treatment groups. In the 19 animals receiving intrathecal saline or no intrathecal agent, receptive field size increased following relief of

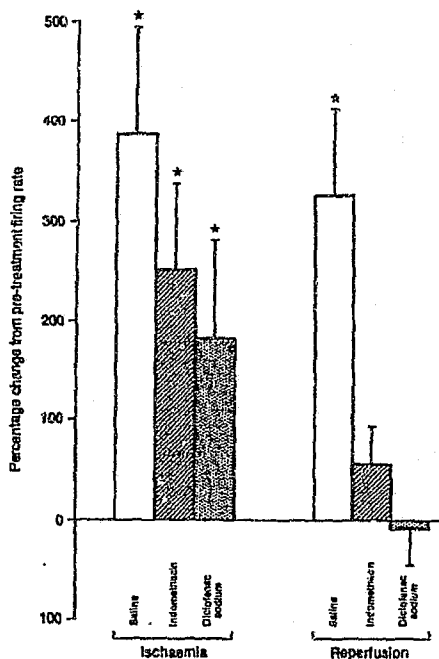


Fig. 4. Percentage change in spontaneous firing rate of convergent neurones, from pre-treatment firing rate, during ischaemia and reperfusion of their receptive fields. Indomethacin 1 µg/kg and diclofenac sodium 0.25 mg/kg blocked the increase in spontaneous firing rate evident in saline-treated animals during reperfusion ($P < 0.01$, unpaired t test), but had no significant effect on the increase during ischaemia itself. Asterisks represent values significantly different ($P < 0.001$) from zero.

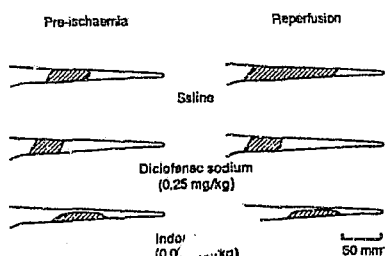


Fig. 5. Examples of receptive field size before the induction of ischaemia and immediately after release of the tourniquet, for animals within each treatment group. Intrathecal administration of diclofenac sodium and indomethacin prevented the increase in receptive field size which otherwise occurred during reperfusion of the tail.

ischaemia, with receptive field length doubling (63 ± 13 to 118 ± 16 , mean $\pm$ SE, $P < 0.02$, paired t test). Pre-treatment with indomethacin (9 of 10 animals) and diclofenac sodium (all animals tested) abolished the increase in receptive field size; receptive field length was not significantly different to that evident before application of a tourniquet. We observed no changes in receptive field size following application of a sham tourniquet.

Discussion

Convergent neurones become hypersensitive to mechanical stimulation when their receptive fields on the rat's tail are reperfused following transient ischaemia, and spinal administration of indomethacin and diclofenac sodium significantly attenuated the hypersensitivity to both noxious and innocuous mechanical stimulation during the first hour of reperfusion at least. Intrathecal application of the NSAIDs also attenuated the enlargement in receptive field size as well as the increase in spontaneous firing rate evident during reperfusion of the rat tail. The NSAIDs did not affect the activity of neurones during the transient receptive field ischaemia, nor did they affect responses to stimuli applied without prior ischaemia. We therefore conclude that prostanooids are synthesized in the spinal cord, and enhance the excitability of convergent neurones during reperfusion of receptive fields in the rat tail. However, prostanooids do not play a role in mediating the responses of the neurones during ischaemia of the receptive fields. Prostanooids also are not involved in the responses to mechanical stimuli in the absence of the conditioning stimulus of transient ischaemia. The responses of the convergent neurones to local application of NSAIDs are reminiscent of observations

we made in a previous study, in which we showed, using systemic administration of NSAIDs, that prostanooids had a special role in the behavioural responses of rats to noxious stimuli during, and only during, reperfusion of receptive fields (Gelgor et al. 1992a).

The convergent neurones increased their activity both during ischaemia and during reperfusion of their receptive fields, presumably because of altered afferent activity from the receptive fields. The altered afferent activity during reperfusion appeared to induce prostanooid synthesis in the spinal cord, while that occurring during ischaemia did not. It may be that central prostaglandin release during reperfusion of the tail depends on activation of nociceptors, and consequently afferent pathways, which are different to those excited during ischaemia of the tail. Metabolites released in the tail tissue during reperfusion, which may be similar to those present in inflammatory conditions, could excite previously silent nociceptors (Handwerker et al. 1991) or chemonociceptors such as those proposed by LaMotte et al. (1988). Such metabolites, and particularly prostanooids themselves, may not be released in ischaemic tissue (Gelgor et al. 1992a,b).

Chapman and Dickenson (1992) recently also reached the conclusion that spinal prostanooid synthesis influences the activity of convergent neurones only after a noxious conditioning stimulus had been applied to their receptive fields. In their case, the conditioning stimulus was the inflammatory irritant formalin administered into the receptive field on the hindpaw of the rat, a procedure which elicits a characteristic biphasic excitation of dorsal convergent neurones (Dickenson and Sullivan 1987). Intrathecal indomethacin reduced the responses of convergent dorsal horn neurones to activation of afferent fibres following formalin conditioning of the receptive fields, but had no effect on their responses to the same activation in the absence of the formalin.

Conditioning procedures which activate C-fibre afferents produce profound changes in the excitability and receptive field properties of convergent neurones (Cook et al. 1987; Dougherty and Willis 1991; Woolf and Thompson 1991), and Chapman and Dickenson (1992) deduced that it was indeed C-fibre afferents that were affected by formalin. We believe that activation of C-fibre afferents also takes place during tail ischaemia and reperfusion. Our deduction is based on previous descriptions of the neuronal response to ischaemia. Applying a tourniquet to a rat hindlimb causes C-fibre afferents, silent before application of the tourniquet, to fire (Chabel et al. 1990). Ischaemia also causes C-fibre activity in the cornea *in vitro* (MacIver and Tanelian 1992). Moreover, while exciting C-fibre afferents, ischaemia blocks conduction in A fibres (Fink and Cairns 1982; Kojo and Pertovaara 1988; Cline et

al. 1989), so disqualifying A fibres as the source of spinal excitation.

Though we believe that both ischaemia and reperfusion activate C fibres, only the activation resulting from reperfusion appears to induce spinal prostanoid release. Similarly, Chapman and Dickenson (1992) found that C-fibre wind-up did not induce prostanoid release in the cord, whereas C-fibre activation following formalin administration indeed did so.

Our results, and those of Chapman and Dickenson (1992), implicate prostanoid synthesis in the CNS in a special way in nociception. Others have previously used intrathecal administration of NSAIDs to demonstrate that prostanoids must play an important role in the transmission of nociceptive information in the spinal cord, without identifying that role (Taiwo and Levine 1988; Jurna et al. 1992; Malmberg and Yaksh 1992a,b). The prostanoids involved seem to be prostaglandins; intrathecal administration of the prostaglandins PGE₂, PGD₂ and PGF₂ in conscious rats induces behavioural hyperalgesia which may be attenuated by intrathecal NSAIDs (Yaksh 1982; Taiwo and Levine 1986; Uda et al. 1990). The precise role of CNS prostanoid synthesis in nociception is still not established. However, our results are in agreement with those of others (Taiwo and Levine 1988; Chapman and Dickenson 1992; Malmberg and Yaksh 1992a,b) who suggest that prostanoids are involved with the transmission of nociceptive information in the dorsal horn of the spinal cord following application of a conditioning stimulus which initiates a C-fibre afferent barrage.

Acknowledgements

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CHAPTER 6

CONCLUSIONS

6.1 Role of prostaglandins in reperfusion hyperalgesia

I have shown that both central and systemic administration of NSAIDs, before the induction of ischaemia attenuates behavioural evidence of hyperalgesia which occurs during reperfusion of the rat's tail. Although NSAIDs are a chemically diverse group of drugs with multiple modes of action [McCormack and Brune 1991], it is generally accepted that a property shared by NSAIDs is their ability to inhibit the enzyme cyclo-oxygenase thereby inhibiting the synthesis of prostaglandins. Since I administered five different NSAIDs which all attenuated reperfusion hyperalgesia, I have concluded that it is from an action they all have in common and that prostanoids play a role in the development of reperfusion hyperalgesia.

None of the NSAIDs I administered, either systemically or centrally at doses which attenuated reperfusion hyperalgesia were antinociceptive during ischaemia, indicating that the mechanisms underlying nociception during ischaemia and reperfusion hyperalgesia differ. Similarly, the same doses of NSAIDs did not affect the responses to an acute noxious thermal stimulus conducted in the absence of prior ischaemia. The lack of antinociceptive effect of NSAIDs on tail flick latency are consistent with the observation that diclofenac is not antinociceptive in the tail flick or hot plate tests [Bjorkmann et al 1990].

I have shown that prostanoids play a role in facilitated nociception which is not dependant on the development of inflammation. Histological examination of cross sections of the rat tail taken from the area under the tourniquet and from the receptive field after 30 min of ischaemia, showed no microscopic changes from samples of tails where only a sham tourniquet was applied [I. Dal Mas unpublished observations]. In particular, there was no oedema, leukocyte infiltration or any other visible sign of inflammation.

The antinociceptive effects of NSAIDs have mostly been demonstrated in experimental models which depend on the presence of inflammation and concomitant hyperalgesia [Coderre et al 1990; Malmberg and Yaksh 1992 b]. Systemic administration of NSAIDs in my study shows that prostanoids are involved in the development of non-inflammatory hyperalgesia, but that involvement could be central or peripheral. The central administration of NSAIDs shows that involvement of prostanoids is largely (or entirely) central. My observation that NSAIDs attenuate reperfusion hyperalgesia following central administration, adds to the growing body of evidence that prostanoids are released in the central nervous system in circumstances where there is amplification of nociceptive responses [Capetola et al. 1983; Ferreira et al. 1978; Malmberg and Yaksh 1992 a;b].

Following intracerebroventricular administration the rank order of potency of the individual NSAIDs differed in attenuating reperfusion hyperalgesia when

compared with systemic administration (Ch. 6, Fig.1). The doses required to attenuate reperfusion hyperalgesia, following central administration, were uniformly several orders of magnitude less than those required following systemic administration. Penetration of NSAIDs through the blood brain barrier following systemic administration, therefore may be sufficient to inhibit prostaglandin synthesis in the central nervous system. Malmberg and Yaksh [1992b] also observed that the intrathecal doses of a range of NSAIDs were considerably less than the systemic doses required to suppress the second phase of the formalin response. It is possible, therefore, that central prostaglandin release may account for the amplification of pain in many forms of hyperalgesia.

My data does not necessarily indicate that prostaglandins are released only centrally during reperfusion hyperalgesia. Local administration of prostaglandins induces behavioural evidence of hyperalgesia [Ferreira et al 1990; Taiwo and Levine 1988] and sensitizes peripheral nociceptive fibres to thermal and mechanical stimuli [Handwerker 1976; Martin et al 1988; Raja et al 1990]. A marked, transient increase in prostaglandins has been found in reperfused vascular tissue [Ward et al 1994]. It would, therefore, be of value to measure prostaglandins in the tail at various time intervals following release of the tourniquet, particularly PGI_2 and PGE_2 as they are considered to play a more prominent role in mediating hyperalgesia than the other metabolites of the cyclo-oxygenase pathway of arachidonic acid [Taiwo and Levine 1990b].

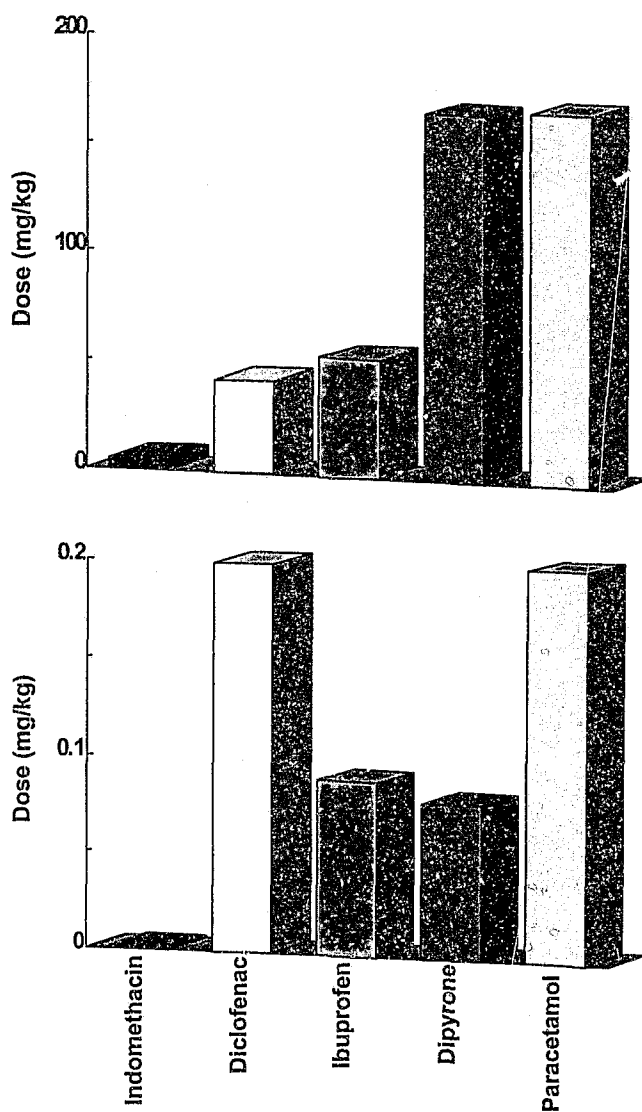


Fig. 1 The top panel represents the doses of NSAIDs required to abolish reperfusion hyperalgesia, after systemic administration of the drugs (Chapter 2) and the bottom panel represents the doses required to abolish reperfusion hyperalgesia following i.c.v. administration (Chapter 3). Rank order of potency differs with the different routes of administration of the NSAIDs.

6.2 Neuronal substrates of reperfusion hypersensitivity

The population of convergent neurones I examined exhibited a significant increase in responsiveness to noxious and innocuous mechanical stimulation, an increase in spontaneous firing rate, as well as receptive field enlargement, during reperfusion of receptive fields on the rat tail. The increased excitability of the convergent dorsal horn neurones was not evident in the absence of ischaemia, control experiments followed the same experimental procedure but without inflation of the tourniquet on the rat tail. Therefore the enhanced excitability of these neurones did not result simply from the passage of time and must have occurred directly as a result of the ischaemia induced conditioning stimulus.

Noxious stimulation at sufficient intensity to activate C afferent fibres triggers an increase in the excitability of spinal cord neurones, changing the responsiveness of these neurones to subsequent afferent inputs [Woolf 1983], and this central sensitization contributes to the hypersensitivity following injury [Woolf and Chong 1993]. I believe that ischaemia and reperfusion triggers a C-fibre barrage since the changes in sensitivity of convergent neurones I observed following tourniquet release are consistent with other studies following a C-afferent conditioning stimulus [Cook et al 1987; Dickenson and Sullivan 1987 a; Ménétrey and Besson 1982; Ménétrey et al 1989; Schaible et al 1987; Simone et al 1991 a; Woolf and Thompson 1991].

There are distinct differences between the characteristics of the convergent dorsal horn neurones and the behavioural responses to noxious thermal stimulation during reperfusion of the tail. Most of the convergent neurones I examined did not respond to noxious thermal stimuli before or after ischaemia. Those that did respond exhibited no subsequent hypersensitivity to thermal stimuli during reperfusion of the tail. Behaviourally, the rats showed hyperalgesia to thermal stimuli which was maximum immediately following release of the tourniquet. Studies using the chronic sciatic nerve constriction model have also reported no increase in responsiveness of dorsal horn neurones to thermal stimuli despite the fact that the rats displayed thermal hyperalgesia [Laird and Bennett 1993; Palechek et al 1992]. There are some methodological problems associated with comparing the responses of a single neurone with the integrated behavioural response observed in the conscious animal.

However, the behavioural responses of rats to thermal stimulation during reperfusion of the tail paralleled the electrophysiological properties of nociceptive specific neurones, in the ventrobasal complex of the rat thalamus [Gelgor et al 1988]. These neurones exhibited hypersensitivity to a noxious thermal stimulus, greatest immediately following release of the tourniquet and gradually recovering over an hour. There must therefore, be a population of distinct and different spinal cord neurones projecting to thalamic regions [for review, Craig 1995], which I did not discover. Nor was I able to learn anything about the response and receptive field properties of these neurones. It is likely that NSAIDs block hypersensitivity

in that pathway too. The block could be peripheral or central. To my knowledge there are no studies describing the effects of NSAIDs on thermally sensitive dorsal horn neurones in other hyperalgesic models.

The convergent dorsal neurones exhibited an enhanced responsiveness to both noxious and innocuous mechanical stimulation of their receptive fields on the tail. The increased response to mechanical stimulation was not evident immediately following tourniquet removal, but responses to pinch and brush had doubled following 60 min of reperfusion. The spontaneous firing rate also remained elevated throughout the recording period. I did not record from these neurones for longer than one hour, based on the time course I observed in behavioural studies where the rats exhibit hyperalgesia to a noxious thermal stimulus, which is maximal following tourniquet release and resolves within an hour [Gelgor et al 1986 a;b]. The time course and the progressive increase in neuronal activity to noxious and innocuous mechanical stimuli in convergent dorsal horn neurones during reperfusion resembles the phenomenon of progressive tactile sensitivity in inflamed tissue described by Ma and Woolf [1996]. Further neurophysiological and behavioural studies are required to elucidate the time course of both neuronal hyperexcitability and hypersensitivity to mechanical stimuli during reperfusion of the tail. The increased responsiveness of the convergent neurones to mechanical stimuli at an hour, when thermal hyperalgesia has resolved, implies that mechanical hyperalgesia might outlast thermal hyperalgesia.

It would be of value to investigate the responses of conscious rats to noxious and innocuous mechanical stimuli during reperfusion of the tail. It is, however, difficult to apply noxious mechanical stimuli repeatedly without risking local tissue damage and trauma-induced sensitization [Handwerker and Kobal 1993], and this may confound the assessment of reperfusion hyperalgesia. Pilot experiments are currently under way to establish a working behavioural model which uses mechanical stimuli to assess reperfusion hyperalgesia. Studies where a chemical irritant has been used to induce hyperalgesia, have observed that the responses of convergent dorsal horn neurones closely parallel the behavioural responses in conscious animals [Calvino et al 1987; Dickenson and Sullivan 1987; Dougherty et al 1992].

I selected neurones on the basis of their response to mechanical stimulation. My search procedure was not modified in this study as I was of the opinion that even those neurones that were thermally unresponsive before ischaemia might become active during reperfusion as a result of nociceptor sensitization, leading to a corresponding increase in activity in dorsal horn neurones [Handwerker et al 1991]. In future experiments, therefore, 1) a thermal search stimulus should be used, and 2) the responses of nociceptive specific neurones in response to thermal and mechanical stimulation both before and after the induction of ischaemia should be examined.

As discussed in Chapter 4 the modality-specific hypersensitivity evident in the population of neurones that I examined may be due to peripheral input from a specific fibre type. Thermal hyperalgesia has been attributed to excitation of A-fibre afferents and mechanical hyperalgesia to C-fibre afferents [Shir and Seltzer 1990]. However, at the temperature range used in my studies it is likely that C-fibres were responsible for conveying thermal nociceptive information [Beitel and Dubner 1976; Handwerker et al 1975; LaMotte 1984]. These thermosensitive C-fibres may not have converged onto the population of WDR neurones I examined. In contrast to my findings, thermal hyperalgesia following capsaicin injection in humans correlates well with the responses of WDR neurones [Simone et al 1991c]. The mechanical hypersensitivity of WDR neurones observed during reperfusion could have resulted from the input from chemosensitive afferents [Handwerker et al 1991; Meyer et al 1991] or possibly from A β afferents [Ma and Woolf 1996].

There is also evidence that certain mediators released either in primary afferent fibres or in the spinal cord may modulate the transmission of a specific stimulus modality [Duggan et al 1988; Fleetwood-Walker et al 1990; Meller and Gebhart 1994]. Behavioural studies show, for example, that the intrathecally applied peptide galanin facilitated nociceptive responses to noxious mechanical stimulation but not to noxious thermal stimulation, whereas vasoactive intestinal peptide facilitated responses to noxious heat [Cridland and Henry 1988; Lundeberg et al 1993]. According to comprehensive studies by Meller and

Gebhart [1994] thermal hyperalgesia is dependant on activation of NMDA receptors and the subsequent involvement of PKC and nitric oxide whereas mechanical hyperalgesia is dependant on the activation of non-NMDA receptors, phospholipase A₂ and arachidonic acid leading to prostanoid synthesis. However, my studies show that thermal hyperalgesia during reperfusion of the rat tail is attenuated by NSAIDs, which is consistent with those observed by Malmberg and Yaksh [1992a]. They showed, that cyclooxygenase inhibitors attenuate the behavioural response produced by intraplantar formalin. Meller and Gebhart [1994], used zymosan or carrageenan to induce hyperalgesia and the intracellular mediators involved in different nociceptive models may not be the same, reperfusion hyperalgesia appears to resemble the formalin test. Furthermore, Malmberg and Yaksh [1992] also reported that thermal hyperalgesia produced by intrathecal administration of AMPA and NMDA was attenuated by cyclooxygenase inhibitors. Meller and Gebhart [1994] used lower concentrations of AMPA and NMDA than Malmberg and Yaksh and the duration of the thermal hyperalgesia in the two studies differed.

6.3 Differences between ischaemia and reperfusion hypersensitivity

Spinal administration of indomethacin and diclofenac abolished the enhanced excitability of convergent dorsal horn neurones during reperfusion. They did not, however, alter the responses of the neurones to noxious and innocuous mechanical stimulation or the spontaneous firing rate during tourniquet application. From my

studies it seems that prostaglandins do not play a role in mediating nociception during ischaemia.

While my results are consistent with the hypothesis that central release of prostaglandins is sufficient to account for the changes evident during reperfusion, my data are not sufficient to exclude a peripheral role. Thus, there could be central and peripheral prostaglandin release but if there is, the factors activating the release are probably not the same, the spinal cord itself was never ischaemic.

Ischaemia is characterized by a lack of oxygen, leading to various biochemical alterations at the cellular level in the ischaemic tissues [Welbourn et al 1991].

There is a marked increase in intracellular calcium during ischaemia which is further increased during subsequent reperfusion of the tissue [Ernster 1988].

Increased intracellular calcium results in phospholipase A₂ activation, leading to increases in intracellular arachidonic acid, a precursor of prostanoids. Once released arachidonic acid is metabolized by two types of enzyme, one of which is cyclo-oxygenase. Molecular oxygen is required for the conversion of arachidonic acid to prostanoids by cyclooxygenase [Lands 1979; Welbourn et al 1991].

Prostaglandin synthesis can occur during ischaemia providing the oxygen concentrations are sufficient. It is probable that there is a build up of arachidonic acid during ischaemia of the tail with no subsequent breakdown to prostaglandins due to insufficient oxygen. Following release of the tourniquet, and the availability of oxygen, arachidonic acid is now converted to prostaglandins which

together with the other metabolites released during ischaemia could sensitize different peripheral C-fibre nociceptors to those activated during ischaemia, possibly chemonociceptors.

There is evidence that spinal prostaglandin release results from the activation of both tachykinin [Uda et al 1990] and NMDA receptor sites [Minami et al 1994 a;b]. The C-afferent fibres sensitized during reperfusion most likely results in the activation of the NMDA receptor by glutamate [Dougherty and Willis 1991] and tachykinin receptor by substance P and neurokinin A [Nagy et al 1993].

Activation of both the tachykinin and NMDA receptors sites induces cellular changes in the spinal cord, resulting in enhanced excitability of spinal cord neurones [for reviews see Coderre et al 1993; Yaksh 1994]. One of the likely consequences of afferent C-fibre stimulation is depolarization of the neurones in the spinal cord and consequent opening of voltage operated Ca^{2+} channels, or an increase in the release of a neurotransmitter such as glutamate could lead to Ca^{2+} influx through the NMDA receptor operated channel leading to subsequent phospholipase A_2 activation [Dumuis et al 1993]. Phospholipase A_2 activation leads to an increase in intracellular arachidonic acid which in the presence of the enzyme cyclo-oxygenase is converted to prostaglandins.

The C-fibres activated during ischaemia and reperfusion must be qualitatively and quantitatively different. There must be C-fibres firing during ischaemia despite their receptive fields and fibre tracts being hypoxic. I have confirmed that such

fibres exist because the WDR neurones increased firing during ischaemia.

Further evidence of C-fibre activation during ischaemia is provided by the fact that application of a tourniquet to human subjects causes intense pain. During reperfusion, however, there is no ongoing pain only hypersensitivity probably initiated by the C-fibre barrage during the ischaemic phase. I have no evidence that C-fibres are activated during reperfusion. Concomitant with the cellular changes in the spinal cord during reperfusion, there is a recovery in both A β and A δ fibres shortly following release of the tourniquet, which may be responsible for the enhanced responsiveness to both noxious and innocuous stimuli since the dorsal horn neurones are now in a sensitized state following ischaemia.

6.4 Clinical significance of my study

There are clinical implications related to ischaemia, to the use of tourniquets, the timing of NSAID administration and the site of NSAID administration. None of the NSAIDs I used affected the escape latency to ischaemia, implying that NSAIDs, no matter how potent, cannot be used to treat clinical ischaemic pain such as angina and intermittent claudication. Previous studies in our laboratory have found only opiates to exhibit antinociception during ischaemia [Sher et al 1992].

Although NSAIDs are effective in some patients with primary dysmenorrhoea, a large percentage are not responsive probably due to the degree of hypoxia, since

prostaglandin synthesis only occurs in ischaemic conditions if there is sufficient oxygen available.

The C-fibre barrage triggers changes in excitability of neurones in the spinal cord outlasting the afferent input [Cook et al 1987; Wall and Woolf 1984; Woolf 1983]. My observations show that the afferent barrage is not blocked by general anaesthesia and that the subsequent hypersensitivity induced by ischaemia outlasts the duration of the input. The spatial, temporal and threshold changes of the receptive fields of spinal cord neurones closely parallel the post-injury hypersensitivity changes found in humans and animals [Treede et al 1992; Woolf and Chong 1993]. An important outcome of my study is that NSAIDs applied to the surface of the cord before ischaemia was able to block the receptive field spread during reperfusion of the rat tail.

The findings by Woolf [1983], that peripheral sensory input from damaged tissue during surgery increases the excitability of central nervous system neurones, has important implications for the management of post-operative pain. Clinical studies have been designed to assess whether treatment with opioids, NSAIDs or a local anaesthetic before surgery can pre-empt postoperative pain by preventing the establishment of central sensitization [for review see Woolf and Chong 1993].

NSAIDs are used extensively in the treatment of postoperative pain. They are commonly associated with adverse side effects on the kidney and gastrointestinal

tract [for review see Merry and Power 1995]. Based on my observation that spinally administered NSAIDs before the induction of ischaemia attenuates the hyperexcitability of spinal cord neurones, implies that spinal administration of low doses of NSAIDs may reduce the adverse effects prevalent following systemic administration. My study and those of others [Malmberg and Yaksh 1992b; Okuyama and Aihara 1984], show that the doses of NSAIDs required to attenuate hyperalgesia following central administration are so small that penetration through the blood brain barrier may be sufficient to account for their action following systemic administration. This phenomenon may explain why paracetamol is regarded clinically as a good antinociceptive, but a poor antiinflammatory, agent.

The tourniquet is often used for up to 3 hours in orthopaedic, micro-neurosurgical and other surgical procedures requiring a blood free environment [Concepcion et al 1988; Hidalgo and Jones 1990; Kerrigan and Stotland 1993]. Some of the orthopaedic procedures are carried out under spinal anaesthesia and the pain induced by the tourniquet is well documented [Concepcion et al 1988; Egbert and Deas 1962; Hagenouw et al 1986]. The mechanisms underlying tourniquet pain during an otherwise adequate spinal anaesthesia are unclear. Tourniquet pain has been related to the dose and type of anaesthetic used [Egbert and Dias 1962; Rocco et al 1984], the level of sensory anaesthesia [DeJong and Cullen 1963], and the baricity of the spinal anaesthetic solution [Bridenbaugh et al 1986]. Concepcion et al [1988] suggest that during spinal anaesthesia both A and C fibres are equally inhibited but as the concentration of local anaesthetic in the

cerebrospinal fluid declines C fibres may become unblocked before A fibres resulting in tourniquet pain but no change in sensory anaesthesia to pin prick. The pain usually subsides immediately following release of the tourniquet [Hageaou et al 1986] but can be so severe so as to necessitate supplemental general anaesthesia [Bridenbaugh et al 1988]. I have shown that tourniquet application to the rat tail for a brief period produces profound changes in excitability of spinal cord neurones under general anaesthesia. Thus the C-fibre barrage during ischaemia is not blocked by general anaesthesia. A-fibres would, most likely, be blocked as a result of the tourniquet and not necessarily related to the level of anaesthesia. Whilst there is no pain following tourniquet release, the consequent hypersensitivity outlasts the duration of the stimulus. My results show that even under general anaesthesia, the use of tourniquets during surgery would be expected to exacerbate post-surgical hypersensitivity.

My studies indicate that NSAIDs would not alleviate the pain arising from the tourniquet itself, as they do not stop the C-fibre barrage but prevent the consequent hyperalgesia. To eliminate the C-fibre barrage would require infiltration of the wound area with local anaesthetic. Administration of NSAIDs before tourniquet application would most likely attenuate post-surgical hypersensitivity. It is possible that following systemic administration of NSAIDs, they would act both peripherally and centrally to reduce central sensitization.

The value of some clinical studies emphasizing the benefit of administration of NSAIDs before surgery, compared with placebo, has been questioned [McQuay 1994]. Since many studies, have compared administration of NSAIDs with placebo before surgery but have not compared the same dose administered before with a dose subsequent to surgery. In my study only the effect of NSAID administration before the conditioning stimulus was evaluated. The lack of comparison, however, does not minimize my findings that NSAIDs administered before the ischaemia conditioning stimulus acts pre-emptively to eliminate reperfusion hypersensitivity. I believe that it would be of value, though in future studies, to change the timing of NSAID administration, to assess the effects on reperfusion hypersensitivity by administering NSAIDs during ischaemia and immediately following tourniquet removal. The duration of central sensitization varies depending on the nature and duration of the afferent input. I applied the tourniquet for 30 min in neurophysiological studies, it would be of interest to examine the responses of spinal cord neurones to mechanical stimuli after applying the tourniquet for different time periods. The tourniquet is often used for a few hours in orthopaedic surgical procedures.

It is important to evaluate pre-emptive treatment in different pain situations [McQuay 1994], since the underlying mechanisms may not be the same and thus pre-emptive treatment may not be effective in all circumstances. Pre-emptive analgesic effects have been demonstrated in animals in the formalin test [Coderre et al 1990; Malmberg and Yaksh 1992a], after peripheral nerve lesion [Puke and

Wiesenfeld-Hallin 1993] and now following ischaemia. None of these animal models, however, resembles the time frame of clinical postoperative conditions [McQuay 1994]. Clinical surgical procedures may involve inflammation, nerve damage and use of a tourniquet.

6.5 A new assay of hyperalgesia

The ischaemia/reperfusion technique, apart from improving the understanding of pain mechanisms, has introduced a new way for testing putative antinociceptive agents. Experimental ischaemic pain resembles clinical pain and reperfusion simulates clinical situations where there has been a C-fibre barrage.

The assay, that I with other colleagues have developed, complies more closely with the guidelines of the International Association for the Study of Pain 1983, in that the animal is able to indicate the need to terminate the stimulus and the stimulus can indeed be terminated immediately by the investigators. Furthermore, there are no sequelae so that the animal does not have to be killed.

Reperfusion hyperalgesia provides a simple and rapid way to assess the potency of NSAIDs as antinociceptives. The rank order of potency of the NSAIDs used to attenuate reperfusion hyperalgesia correlates with human potency rankings as discussed in Chapter 2, and is in agreement with potency rankings obtained in other animal studies [Okuyama and Aihara 1984; Tolman et al 1984; Vane and Botting 1987; Van Kolfshoten et al 1983; Vinegar et al 1976; Weichman 1989].

The combination of nociceptive tests that I used in this thesis, together with the evaluation of putative antinociceptive agents for antihyperalgesic properties, permits the evaluation of agents for analgesic properties dependant on their activity during ischaemia and on tail flick latency in the absence of the ischaemia induced conditioning stimulus [Sher et al 1992]. Thus, it is possible to test for analgesia and antihyperalgesia simultaneously, but also to separate them. Furthermore, the changes in the excitability of convergent dorsal horn neurones induced by ischaemia of the rat tail resemble those observed in many other models of hyperalgesia.

In particular, there are close similarities between the formalin procedure and ischaemia /reperfusion technique and therefore the results may be transferable. The formalin test also permits assessment of antinociceptive agents for antihyperalgesic and analgesic properties dependant on their action in the first or second phase of the response. Although the hyperalgesia in both the formalin and ischaemia assays are rather short-lived and not analogous to the clinical situation, these assays are nevertheless valuable tools for pharmacological pain research.

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