Textbook of Pain

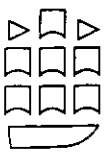
EDITED BY

Patrick D. Wall
DM, FRCP
Director, Cerebral Functions Research Group;
Professor, Department of Anatomy,
University College London, London, UK

Ronald Melzack
PhD
Professor, Department of Psychology, McGill
University, Montreal, Quebec, Canada

CLINICAL CONSULTANTS

John J. Bonica MD, DSc, FFARCS
Harold Merskey DM, FRCP, FRCP(C),
FRCPsych
Peter W. Nathan MD, FRCP
John P. O'Brien MBBS, PhD, FRCS (Edin),
FACS
William H. Sweet MD, DSc, OHC
C.B. Wynn Parry MBE, DM, FRCP, FRCS



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mechanosensory corpuscles (Iggo & Andres 1982) or to other types of peripheral mechanoreceptors in the deeper tissues (muscle spindles, Golgi tendon organs, Pacinian corpuscles, etc). These sensory neurones might have some biological characteristics in common, as they are specifically destroyed by the desensitising agent capsaicin (8 methyl-N-vanillyl-6-nonemide) when this is applied neonatally (Lawson & Nickels 1980). Antigenicity against the following peptides can be found in these neurones: substance P (Hökfelt et al 1976, Cuello et al 1978), somatostatin (Hökfelt et al 1976), gastrin-CCK (Larsson & Rehfeld 1979) and VIP (Lundberg et al 1978).

These immunoreactivities in cell bodies of primary sensory neurones are better expressed following the application of colchicine (an agent which blocks axonal transport), although in some species these are easily demonstrated without any previous manipulation. How all these peptide-specific neurones relate to each other and to central and peripheral targets is not entirely known. There is experimental evidence for separate sub-populations of substance P and somatostatin-containing sensory neurones (Hökfelt et al 1976) but also for possible co-existence of substance P and CCK in some cell bodies (Dalsgaard et al 1982).

A single process originates from the cell body of the ganglionic sensory neuron and after a few micrometres divides into a centrally and a peripherally-directed fibre. In our immunofluorescence studies we have sometimes noticed an initial axonal segment containing substance P immunoreactive material, or a fibre which may be derived from it, curling around the cell body (Matthews & Cuello 1983). It may be expected that such an arrangement will be related to some sort of autoregulatory mechanism, and might underlie the phenomenon of centrifugal firing of these neurones following synchronous activation.

Although there is some controversy as to whether the diameters of the central and peripheral branches are different (see Lieberman 1976) there are indications that the immediate axonal diameter close to the point of bifurcation, as seen in Golgi preparations, is greater for peripheral processes, particularly in the case of unmyelinated fibres (Ha 1970). We have also seen this in relation to substance P immunofluorescent material in neurones of lumbar dorsal root ganglia (Matthews & Cuello 1983) (Fig. 27). Since in this case we are not necessarily staining right up to the cell surface as in the Golgi preparation, but rather detecting an intracellular antigen, this reaction could represent simply larger quantities of the peptide carried in the peripheral branches. Such a contention is strongly supported by observations on the *in vivo* transport (Brimijoin et al 1980) and the *in vitro* biosynthesis and transport of substance P (Harmar & Keen 1982). Harmar & Keen monitored the accumulation of substance P immunoreactive material *in vitro* in the ganglion-nerve preparation, observing that there was differential accumulation of the peptide in favour of peripheral branches, reaching up to approximately 8-fold that of central branches 9 hours after the initiation of the experiment. They also established that the accumulated material was newly-synthesised peptide transported from the cell bodies, as denecoline (an agent which, like colchicine, disrupts microtubules and axonal transport) prevented it while accumulation of substance P immunoreactive material still occurred in cell bodies. These authors have calculated the peptide turnover time in the ganglion to be in the region of 3.6 h.

Ligation experiments have also provided abundant evidence for peripheral transport of substance P and other peptides in mixed nerves as detected both by radioimmunoassay and immunocytochemistry (Hökfelt et al 1977, Gamse et al 1979, Gilbert et al 1980, Brimijoin et al 1980). Although all these studies have been carried out in mixed nerves, there is every indication that the transported peptide is present mostly, if not exlusively, in sensory fibres (see also Fig. 27a). In rabbit and in cat vagus nerve the maximum accumulation of SP-immunoreactive material at 24 h was found just proximal to the point of ligation (Gamse et al 1979). In that study most of the immunoreactive material extracted from the nerves was seen to be identical to authentic substance P. The light microscopic analysis revealed that the SP immunoreactive material was present in dilated axons of seemingly small calibre, while fewer could be regarded as myelinated ones (Fig. 28). This transport is inhibited by colchicine (Gamse et al 1979) and more recently it has also been demonstrated that transport is inhibited by the local application of capsaicin (Gamse et al 1982). This inhibitory effect of capsaicin for the peripheral and central transport of neuroactive substances seems to be restricted to sensory elements, as the transport of catecholamine is not affected (Gamse et al 1982). The velocity of transport of the peptide varies according to the method of calculation and the species; in the cat it was expected to fall between 60 and 170 mm/d, a value which could compare well with other neurotransmitter substances.

Peptide immunoreactive fibres in the skin and other peripheral tissues

Hökfelt et al (1977b) first demonstrated the existence of substance-P-containing fibres in a large variety of peripheral structures. In the skin they noticed immunoreactive fibres singly or in bundles in the connective tissue, sometimes in close contact with the epidermis or even penetrating it. We have confirmed this type of location in human skin. Substance P immunoreactive fibres have also been located in salivary glands (Hökfelt et al 1977b). These fibres could probably elicit salivation as the peptide is known to possess a strong sialogogic action (von Euler & Gaddum 1931). Other locations for these fibres are sweat glands (fibres usually in pairs), glands of the nasal mucosa (Anggard et al 1979) and singly-dispersed fibres around small vessels. Less often isolated varicose fibres can be detected in deeper structures such as the facial muscles. In the trigeminal territory all these peripherally located fibres diminish in number following electrolytic lesions of the Gasserian ganglia (a procedure which seldom produces total loss of sensory fibres) and there is complete depletion following the sensory denervation of this territory (Cuello et al 1978; see Fig. 29). These investigations demonstrated experimentally that the substance P material seen in peripheral somatic nerve fibres was derived from sensory neurones and not from autonomic or other neural elements, and established the symmet-

from that of the postganglionic sympathetic neurones, as detected by immunoreactivity for dopamine ß-hydroxylase or by formaldehyde-induced catecholamine fluorescence, they were sensitive to capsaicin, they survived the removal of lower thoracic to lumbo-sacral spinal cord which spared dorsal root ganglia but they were lost when the lumbar splanchnic nerves were sectioned (Matthews & Cuello 1983).

The anatomy of peripheral substance-P-containing fibres corresponds well with the distribution of free endings classically regarded as the peripheral receptors for nociceptive information (Sweet 1959). The central ends of these neurones also terminate in the substantia gelatinosa of the spinal cord and the spinal trigeminal nucleus, an area of prime importance for the processing of nociceptive information (see Chapter 1.5). It is therefore conceivable that these fibres are carrying nociceptive information. Whether or not substance P is a 'pain transmitter' at their central ends is a matter of controversy (see Chapter 1.5). The peripheral fibres are presumably engaged in responding to nociceptive signals. Substance P *per se* does not seem to play a fundamental role in this particular function (Fitzgerald & Lynn 1979). It is not as yet clear if the peptide is indeed released from these sensory endings following physiological or pathological circumstances. Experimentally, radioimmunoassayable substance P can be recovered from the dental pulp after nerve stimulation (Olgart et al 1977). This group also demonstrated a 45% depletion of substance P content in the cat dental pulp following prolonged stimulation of the alveolar nerve (Gazelius et al 1981).

The peptide, as discussed in the introduction, is in some way involved in antidromic vasodilatation. Lembeck & Holzer (1979) reported that the application of substance P resulted in plasma extravasation which paralleled that observed following the antidromic stimulation of the saphenous nerve in the rat. We have observed that stimulation at 10 Hz for 3–5 min of the purely sensory mental nerve (a branch of the trigeminal nerve) results in a clear-cut vasodilatation in the mandibular region of the rat and that this response was preserved (even enhanced) following sympathectomy (Couture & Cuello 1983) (see Fig. 31).

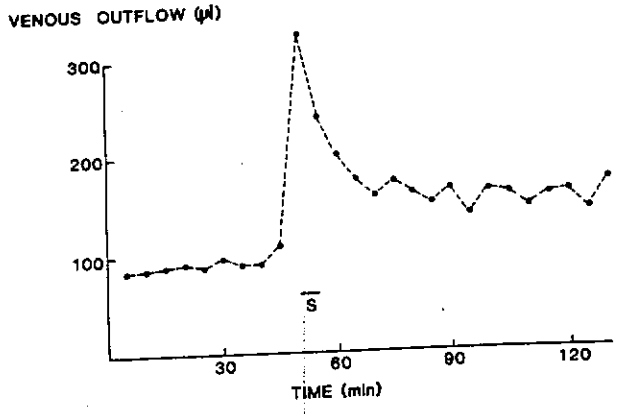

Fig. 31 Jugular venous outflow in the rat. Fractions collected at 5-minute intervals. S represents time and duration of antidromic stimulation of the mental nerve. (Couture & Cuello, 1983)

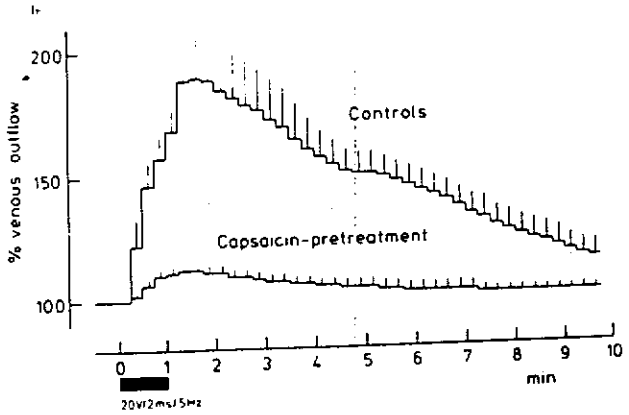

Fig. 32 Hind paw vasodilatation following antidromic stimulation of cut saphenous nerve 4 months after pretreatment with capsaicin or solvent (controls) on the second day of life. Means ± s.e.(mean); n = 5. (From Lembeck & Holzer 1979.)

The evidence for the participation of sensory nerves in this vasodilatation resulting from nerve stimulation is reinforced by the observation that it is not seen when the animals are neonatally treated with capsaicin (Lembeck & Holzer 1979) (see Fig. 32), a procedure which provokes the specific loss of small-diameter sensory neurones (Jancso et al 1977, Lawson & Nickels 1980) and also of substance P, CCK, VIP and somatostatin immunoreactivity in the substantia gelatinosa of the spinal cord and spinal trigeminal nucleus (Cuello et al 1981, Jancso et al 1981, Priestley et al 1982). Of the various peptides associated with sensory neurones, substance P seems to be the most effective vasodilator (Lembeck & Gamse 1982). A synthetic substance P analogue with antagonist characteristics on bioassay and electrophysiological models has recently been described by Rosell et al (1981). Use of this antagonist inhibited the vasodilatation of the dental pulp provoked by antidromic stimulation of the alveolar nerve (Rosell et al 1981). Both the neurally-activated and the substance-P-induced vasodilatation seem to be largely mediated by a histaminergic mechanism, as histamine blockers (cimetidine, methysergide) and depletors (48/80) substantially reduce the effects (Lembeck & Holzer 1979). Lembeck & Donnerer (1981) have observed that chronic denervation and capsaicin treatment both block the post-occlusive cutaneous vasodilatation ('reactive hyperaemia') by more than 60%. As for the antidromically-induced vasodilatation, the phenomenon is blocked by the histamine depletor 48/80 and it has been attributed to substance P release from peripheral sensory fibres (Lembeck & Donnerer 1981) acting probably via release of histamine from mast cells.

Substance P immunoreactive fibres in the eye

Substance P immunoreactive fibres have been found in the anterior stroma of the cornea and, as observed in the skin, some also in the basal layers of the anterior epithelium (Tervo et al 1981). In the same study substance P immunoreactive fibres were also detected in the iris, especially in the sphincter muscle, a localisation which is in line with the observation of Bill et al (1979) that trigeminal nerve

shown in Figure 9. Responses to histamine are reliably found, but only a minority of units respond to substance P (Fitzgerald & Lynn 1979). PMN units also respond well to bradykinin, serotonin (5HT) and capsaicin (Beck & Handwerker 1974, Szolcsanyi 1980, Kenins 1982); some also respond to itch powder (Tuckett 1980). Responses to bradykinin (and also to heat) are enhanced by prostaglandin PGE_1 (Handwerker 1976). More information on the pharmacology of cutaneous receptors will be found in Lynn (1983).

Heat sensitisation is not enhanced by stopping the local blood flow and so probably does not depend on the release of a stable, diffusible substance such as 5HT (Lynn 1979). It is also not significantly affected by aspirin (a prostaglandin synthesis inhibitor) (Perl et al 1976) or by antihistamines (Fitzgerald & Lynn, unpublished). At present the most probable hypothesis concerning the mechanism of heat sensitisation is that it is due to the simultaneous effects of several mediators, and that if only one of these is blocked sensitisation is not much affected. In favour of this view is the finding of King et al (1976) that a cocktail of blocking agents (including an anti-histamine, an anti-5HT agent, a PG synthesis inhibitor and a peptidase to break down kinins) did substantially reduce sensitisation in the isolated, perfused rabbit ear.

In view of their sensitivity to histamine and to itch powder, PMN units are likely to be involved in generating itch as well as pain. Recent micro-stimulation studies (Torebjork & Ochoa 1981) show that itch responses may be due to a specific sub-population of PMN units. If this is confirmed, then the nociceptor designation will clearly have to be changed!

No specific morphology has been proposed for PMNs. However, such endings are very common in the skin. For example, calculations for the rat hind limb similar to those described above for HTMs give a value of 3 PMN units/mm^2 for the leg and 11/mm^2 for the foot. Given such a high density, it would appear certain that if the endings had a characteristic morphology at the light microscopic level they would have been identified easily. It seems more likely that, as is usually suggested, PMN endings are relatively unspecialised and have the appearance of free nerve endings.

Other types of cutaneous nociceptor unit

As well as the two major classes described above, several other types of insensitive, presumably nociceptive, units from skin have been described. Some C units respond to strong pressure but are not heat responsive (Bessou & Perl 1969). These units thus show similar responses to A-delta HTM units, but they do not have the characteristic multipoint fields of the HTMs. This group of C units have not been well characterised. Some may respond to extreme cooling of the skin, a stimulus that is not usually routinely used by investigators in this field (Burgess & Perl 1973).

Several studies have used the term heat nociceptor or thermal nociceptor to describe units responding well to noxious heat but poorly to pressure (e.g. Beck et al 1974, Iggo 1977). However, most of these units are sensitive to strong pressure and therefore would fit into the PMN class. Since the responses to mechanical stimulation are likely to be of more relevance to the animal's normal environment than those to heat, the polymodal designation appears more appropriate. Some heat nociceptors have been described that failed to respond to strong pressure. However, it seems possible that these were simply at the most insensitive end of the mechanical sensitivity range of PMNs. A comparison of the mechanical and thermal thresholds of 78 nociceptive C fibres in the rat revealed no correlation between thermal and mechanical thresholds and no distinct sub-group with low heat and high mechanical thresholds (Lynn & Carpenter 1982).

Units with A-delta axons that respond to heat, pressure and irritant chemicals have been found in experiments using percutaneous recordings from human skin nerves (Adriaensen et al 1980), and similar units have also been reported in the monkey (Georgopoulos 1976). These relatively fast-conducting, heat-sensitive units are presumably responsible for the quick reaction times in man following noxious heating of the skin (Price et al 1977). This group of units are clearly similar in most respects (except axonal type) to C fibre PMN units. C fibres form a smaller proportion of the fibres in skin nerves in primates than in smaller mammals (Ranson et al 1935). Cold-sensitive thermoreceptors have C-axons in non-primates but A-delta axons in primates. The findings of A-delta units of the PMN type may represent a similar trend for nociceptors.

Summary: cutaneous nociceptors

In the hairy skin of non-primates, two distinct classes of nociceptive afferent unit occur, A-HTMs and C-PMNs. HTMs have large, overlapping, multi-point receptive fields with PMNs have fields comprising a single small zone or point. In primates significant numbers of A delta units with polymodal response profiles occur. A-HTMs appear specialised for detecting dangerous mechanical stresses and for triggering rapid protective responses. C-PMNs also respond to strong mechanical stimuli, but in addition show a sensitivity to chemicals released in damaged or inflamed skin. Thus, as well as reinforcing the immediate responses of HTM units to mechanical stress, they can also signal the presence of damaged or inflamed areas and perhaps promote their protection and rest. The sensitivity of all classes of nociceptor increases following mild injury and this increase is probably a major factor in producing hyperalgesia in injured skin.

PROPERTIES OF NOCICEPTORS IN TISSUES OTHER THAN SKIN THAT ARE INNERVATED BY SOMATIC NERVES

Skeletal muscle

Skeletal muscle is innervated by afferent fibres with a wide diameter range. The largest myelinated fibres (Group I), most of the medium-sized myelinated fibres (Group II) and a few of the smallest ones (Group III, A delta) arise from sensitive stretch receptors (muscle spindles and Golgi tendon organs) (Stacey 1969, Matthews 1972). It is unlikely that these highly specialised mechanoreceptor units signal

central terminals where its presence can be detected histologically in the light and electron microscope (e.g. Proshansky & Egger 1977, Light & Perl 1979, Gobel et al 1981). HRP can also be crushed into peripheral nerves and transported transganglionically to central nerve terminals (Mesulam & Brushart 1979, Grant et al 1979, Koerber & Brown 1980, Morgan et al 1981).

Undamaged nerve terminals will take up HRP from surrounding tissue. This method is improved if HRP is conjugated to wheat-germ agglutinin (HRP–WGA) which is actively transported along the nerve to the central nerve terminals (Carson et al 1982). Uptake by peripheral terminals means that afferent projections from particular organs can be studied (Kalia & Mesulam 1980b, Panneton & Burton 1981). HRP can also be injected into individual axons by intracellular penetration. Used in this way, in combination with electrophysiology, it is an extremely powerful technique (see p 000).

HRP, is not the only transported label that can be observed. Fluorescent lectins have been used similarly. Injection of FITC–WGA for example into the snout of a mouse produces fluorescence in the facial nucleus and the trigeminal ganglion (Nennesmo & Kristensson 1981).

Histochemical methods

These methods involve using the chemistry of primary sensory neurons to map their central terminations. These methods, so far have only been used for C fibres but the recent introduction of antibodies to such chemicals as glutamate (Storm-Mathisen et al 1982) which is thought to be concentrated in large myelinatated afferents, means that such techniques may soon also be used for A fibres.

a) Fluoride resistant acid phosphatase (FRAP)

FRAP has been shown to be present in the small 'B-type' dorsal root ganglion cells of rodents and transported centrally to their terminals in the CNS (see Fig. 16B). Its distribution can be therefore localised histochemically. FRAP, disappears from the spinal cord following dorsal root section (Knyihar et al 1974, Coimbra et al 1974) but more curiously it also disappears following peripheral nerve section (Schoenen et al 1968, Knyihar & Csillik 1976). This property has been used to map the distribution of afferent terminals of different peripheral nerves (Rustioni et al 1971, Devor & Claman 1980).

b) Peptides

Substance P (SP), somatostatin (SOM) and cholecystokinin-like octapeptide (CCK) are all manufactured in the DRG and transported to central terminals (Hökfelt et al 1975). They too disappear from these terminals following peripheral nerve section and root section. (Barber et al 1979, Jessell et al 1979, Barbut et at 1981). Their distribution can be observed using immunocytochemistry (Hökfelt et al 1975). These peptides disappear from C fibre primary afferent terminals following destruction of small DRG cells with capsaicin (Nagy et al, 1981) and this has been used to map terminal fields (Nagy et al, 1981; Jancso et al, 1981).

Electrophysiological methods

a) Intracellular recording from afferents and injection of HRP

Single afferent fibres can be impaled and intracellular recordings made with microelectrodes filled with HRP. The great advantage of this technique is that the physiological properties of the afferent can be established (modality, receptive field etc) and then the fibre filled with HRP by passing current through the electrode. The HRP is transported through to the central terminals and the anatomical distribution of a single afferent can be determined (see Brown 1981a, b for details).

b) Stimulation of primary afferent terminals

A stimulating electrode is placed in various regions of the CNS and extracellular recordings made from single afferents in the root, DRG or peripheral nerve. The sites can then be mapped where antidromic stimulation effectively activated the primary afferent at the lowest threshold (Wall et al 1955, Wall 1958, Fitzgerald & Woolf 1981). These are presumed to be the sites of their central terminals.

c) N waves

The arrival of an afferent volley in the spinal cord produces one or more negative waves (N waves). The area of peak negativity in the cord corresponds to the region where most interneurons are activated by the volley. Current flows from the extracellular space into sinks produced at the somata and dendrites by EPSPs and action potentials. These areas of maximum activity are presumed to be the areas of densest termination of the incoming afferent fibres (Howland et al 1955, Beall et al 1977).

d) Recording from cells in the CNS

It is difficult to record from primary afferent terminals in the CNS, although it can be done with spike triggered averaging using a single afferent spike as the trigger (Sypert et al 1980). It is much easier, technically, to record from cell bodies in the CNS. The responses of second order cells to an afferent input can give information about the afferent fibre terminations. Therefore, if a cell receives monosynaptic connections (fixed latency and tightly coupled), then it is likely that the afferent terminates in the region of the cell soma or proximal dendritic field.

Problems with the methods

All the methods described above are associated with technical problems, some more than others.

With Golgi staining there is no control over which afferent or afferents are stained, and myelinated fibres are harder to impregnate than unmyelinated ones (Beal 1979). Also, the dorsal root origin of the fibres must be verified

selves are particularly apparent in the unmyelinated fibres. We therefore wondered what would be the effect of lesions limited almost entirely to unmyelinated fibres. We have done this using capsaicin. If given as a single dose to neonatal rats or mice this compound permanently destroys some 95% of the C fibres. For a more subtle and controlled effect we have applied capsaicin for 15 minutes to a single nerve in adult rats (Ainsworth et al 1981, Wall & Fitzgerald 1981). In this case, the myelinated fibres are intact and so are the unmyelinated afferents. They are capable of conducting nerve impulses but the central excitatory effect of the C impulses is reduced. There are marked chemical changes in the chemistry of the central terminals of the C fibres which mimic the effects of nerve section. There is a marked expansion of the receptive field of the cell supplied by the treated nerve in spite of the fact that the A fibres remain intact and able to drive the cell (Wall et al 1982). We have repeated these experiments on the mouse infraorbital nerve in order to be able to take advantage of the most precise somatotopic map known (Wall et al 1982). In normal mouse somatosensory cortex there are cylinders of cells, the barrels, clearly visible with light microscopy. Developmental and physiological studies show that each barrel is related to a single whisker. If mice are treated with capsaicin the barrels are present anatomically but now the receptive fields of cells have expanded to incorporate many whiskers.

These results taken together suggest a new role for the unmyelinated fibres and the following hypothesis. C fibres could be continually transporting chemicals from the periphery which constitute messages about the nature and state of the tissue in which the fibres terminate. If the tissue is damaged or if the nerve is cut across a new chemical message will be transmitted since the fibre ends will now lie in a novel chemical environment. We know that the chronic

IMMEDIATE

Highly variable relation of injury to pain.

SECONDARY

The mode of search for relief. Agitation.

TERTIARY

The mode of recovery. Inaction.

Fig. 5 The sequence of phases seen in man after injury. The drawing is modified from the famous diagram of sensory mechanisms which appeared in René Descartes's *L'Homme* (C. Angot, Paris, 1664)