FUNCTIONAL ANATOMY OF CUTANEOUS CAPSAICIN-SENSITIVE AFFERENT NERVES

Ph.D. Thesis

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Papers related to the subject of this thesis

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II. Dux M., Jancsó G., Sann H.: Inhibition of cutaneous neurogenic inflammation by lidocaine. Inflammation Research 45: 10-13, 1996.

III. Sann H., Dux M., Schemann M., Jancsó G.: Neurogenic inflammation in the gastrointestinal tract of the rat. Neuroscience Letters 219: 147-150, 1996.

IV. Jancsó G., Juhász A., Dux M., Sántha P., Domoki F.: Axotomy prevents capsaicin-induced sensory ganglion cell degeneration. Primary Sensory Neuron (in press)

V. Jancsó G., Dux M., Sántha P.: Role of capsaicin-sensitive afferent nerves in initiation and maintenance of pathological pain. Behavioral and Brain Sciences (in press)

CONTENTS

INTRODUCTION

MORPHOLOGY OF MAMMALIAN CUTANEOUS RECEPTORS

The mammalian skin is a major sensory organ, which contains different receptors of sensory nerve fibres reporting on the mechanical and thermal state of the body surface, including the presence of noxious or damaging stimuli. Cell bodies of these specialized sensory end organs lie in the spinal and cranial sensory ganglia. Central branches of these primary afferent neurons enter the central nervous system where sensory modalities are conveyed by anatomically separate pathways of the somatosensory system.

Mechanoreceptors of the skin may be either rapidly adapting endings, responding to deformational changes evoked by mechanical stimulation, or slowly adapting endings reacting to steady deformations. Examples of the former are the palisade endings near hair follicles, subcutaneous large lamellated Pacinian endings and the tactile Meissner's corpuscles adjacent to the epidermis. Slowly adapting receptors include Merkel and Ruffini endings (Schmidt, 1978; Williams et al.1989).

Nerve endings associated with hair follicles are Aβ myelinated fibres (according to the classification of Erlanger & Gasser). In palisade endings fibres approach the follicle from different directions in the deep dermal layer. They divide and run parallel to the hair in the outer follicular layer. Some of them give off branches which encircle the hair and terminate as free endings among collagen bundles.

The large lamellated corpuscles of Vater-Pacini (Pacinian corpuscles) are oval and relatively big. Each has a capsule and a central core containing the axon terminal. These endings are present in both the glabrous and the hairy skin of mammals.

Tactile corpusles of Meissner are found in the dermal papillae of the glabrous skin. They are cylindrical in shape with their long axes perpendicular to the skin surface. Each corpusce is supplied by a thick myelinated Aβ nerve fibre. These rapidly adapting mechanoreceptors (Pacini and Meissner corpuscles) of the skin are active in vibration and touch sensations.

Slowly adapting mechanoreceptors of the hairy skin (Ruffini endings) and those of the glabrous skin (Merkel's receptors) are the highly branched endings of Aβ afferents. Slowly adapting receptors of the skin are active mainly in pressure sensation (Schmidt, 1978).

Thermoreceptors of both hairy and glabrous skin are responsive to moderate cold or heat stimuli. They are located immediately under the epidermis at separate points. Warmth

receptors are presumed to be free nerve endings and warmth signals are transmitted mainly over type C unmyelinated nerve fibres. Cold sensations are transmitted by branches of thin Aδ myelinated nerve fibres.

Nociceptor fibres have similar free endings of Aδ and C fibres, responding to various damaging stimuli, signalled centrally as pain, discomfort or irritation of the skin. Some of them respond only to noxious thermal or mechanical stimuli, others can be stimulated by more than one type of noxious stimuli. The latter type of nociceptors are called polymodal nociceptors. Stimulation of Aδ nociceptor fibres is responsible for the sharp, pricking component of pain sensation, while the activation of C-fibres induces a slow, burning type of pain. Nociceptor endings may often be activated by the release of inflammatory mediators from surrounding tissues, producing prolonged pain or itch (Tóth-Kása et al.1986; Kruger, 1988; Lynn, 1992).

The morphology of sensory endings may vary with their location and with species. However, broadly similar arrays of sensory endings occur in different areas of the skin, ranging from the complex encapsulated endings of large myelinated nerve fibres, to free endings of fine myelinated or unmyelinated fibres. The type and the pattern of activation of cutaneous sensory nerve endings are responsible for the particular quality and intensity of the sensation (Schmidt, 1978; Williams et al.1989; Kandel et al.1991).

MORPHOLOGY AND CHEMICAL PHENOTYPES OF PRIMARY SENSORY NEURONS

Primary sensory neurons of cranial and dorsal root ganglia (DRG) can be characterized not only by their receptive properties. Division of sensory ganglion cells into morphological subtypes has been the subject of studies for nearly 100 years. Most reports have favored two main subgroups (Andres, 1961; Lieberman, 1976; Duce, and Keen, 1977; Rambourg et al.1983), called types A and B (Andres, 1961) or, more recently, mainly larger "light" neurons and "small dark" neurons. The morphological classification into two main categories was based on ontogenetic, morphological and morphometric analyses (Lawson et al.1974; Lawson, 1979). Statistical analysis of cell size measurements has shown that in mouse and rat DRG the A and B neurons each have a normal distribution of cell size, and these two distributions overlap. The light neuron distribution extends over the entire size range of ganglion, whereas the small dark neurons are limited to the lower end of the distribution (Lawson et al.1974).

The cytoplasm of the light neurons is characterized by uneven staining due to clumps of

Nissl substance (aggregation of ribosomes and rough endoplasmic reticulum) interspersed with lightly staining regions of the cytoplasm that contain microtubules and large amounts of neurofilament (NF) (Yamadori, 1970; Duce and Keen, 1977). The small neurons have more evenly and darkly staining cytoplasm because of a more even, denser distribution of organelles, few neurofilaments and a greater number of Golgi bodies.

Neurofilament (NF) immunoreactivity can be used to distinguish between the two cell types since small dark cells are NF-poor and large light cells are NF-rich (Sharp et al.1982; Lawson et al.1984). RT97, a monoclonal antibody against the phosphorylated form of the 200 kD NF subunit, was shown to label the light but not the small dark neuron population (Lawson and Waddell, 1991).

Immunocytochemical and histochemical techniques have been used extensively to study the presence of peptides, enzymes and carbohydrate epitopes in DRG neurons, while in situ hibridization has permitted the localization of specific mRNAs. The findings suggested that DRG neurons, in particular "dark" cells, may be divided into further subpopulations. These will be dealt with in some detail in the following section (Robertson et al.1991; Lawson, 1992; Holford et al.1994).

MAJOR POPULATIONS OF PEPTIDE-CONTAINING SENSORY GANGLION CELLS

The preprotachykinin A gene expressed in primary sensory neurons can give rise to three types of mRNAs, all of which produce posttranslational products containing substance P (SP), whereas only two give rise to neurokinin A and only one to neuropeptide K (Weihe, 1989).

Substance P-like immunoreactivity (SP-LI) is present in about 20% of rat lumbar DRG neurons. SP-LI neurons are mainly small but a few intermediate-sized neurons also show SP-LI (Hökfelt et al.1976; Tuchscherer, and Seybold, 1985; Mccarthy, and Lawson, 1989). The great majority of SP-immunoreactive cells also exhibit calcitonin-gene-related peptide (CGRP)-LI. This implies a close functional linkage of these peptides (Lee et al.1985; Sundler et al.1985; Carr and Nagy, 1993; Kashiba et al.1996).

In rat lumbar DRGs the conduction velocity of SP-LI neurons has been measured: about 50% of C-fibre cells, 20% of Aδ -cells, and no fast conducting A-fibre cells showed SP-LI (Lawson et al.1993).

Calcitonin gene-related peptide is derived from the calcitonin gene as a result of alternative processing of mRNA. It occurs in 30-50% of rat DRG neurons. They are mainly small, with some medium and large cells. CGRP-LI has been shown to represent at least two

forms: αCGRP and βCGRP. The predominant form is αCGRP, which is in small, medium and large neurons, whereas βCGRP is in small and medium-sized neurons (Mulderry et al.1988; Noguchi et al.1990). In rat lumbar DRGs about 30% of CGRP-LI neurons are RT97-positive (Mccarthy, and Lawson, 1990) and these include the medium-sized to large CGRP-LI neurons. The percentage of CGRP-LI neurons with SP-LI in rats is about 50% (small neurons). CGRP-LI may coexist also with somatostatin-, vasoactive intestinal polypeptide- and bombesin-LI. DRG neurons with CGRP-LI have a wide range of conduction velocities, consistent with their size and RT97 labelling (Lawson, and Waddell, 1991). In rat lumbar DRGs 40% of C-fibre cells, 33% of Aδ-fibre cells and 17% of fast A-fibre cells exhibit CGRP-LI (Mccarthy, and Lawson, 1990; Lawson et al.1996).

Somatostatin (SOM)-LI is seen in about 5-15% of rat DRG neurons, the percentage being highest in the lumbar region. The size of DRG neurons with SOM-LI in rats is mostly small (Ju et al.1987), although the mean cell size was larger than that of SP-LI neurons (Price, 1985; Kawatani et al.1986; Garry et al.1989). The lack of RT97 positivity indicates that SOM-LI is in small neurons which probably have C-fibres (Lawson, and Waddell, 1991) . There is little or no coexistence of SOM-LI with SP-LI in rats, but all SOM-LI cells have CGRP-LI (Lawson et al.1993).

Vasoactive intestinal polypeptide (VIP)-LI neurons are rare in intact animals, but peripheral axotomy enhances the expression of VIP-LI in injured dorsal root ganglion neurons in rats (Shehab and Atkinson, 1986; Doughty et al.1991). In rats VIP-LI coexists with CGRP-LI.

Galanin (GAL)-LI has been demonstrated in lumbosacral DRGs in rats. (Ch'ng et al.1985) The immunoreactive neurons were small and comprised 15% of all neurons. Peripheral axotomy causes a dramatic increase in the percentage of DRG neurons with GAL-LI in rats (Hökfelt et al.1987; Villar et al.1989; Kashiba et al.1994). In this species some GAL-immunoreactive neurons have SP-LI, but few (0-2%) showed SOM-LI (Lawson, 1992).

Bombesin/gastrin releasing peptide-LI has been found in rat DRG neurons in coexistence with SP-LI and CGRP-LI but not with SOM-LI (Lawson, 1992).

GAL, VIP and Neuropeptide Y (NPY) are referred to as "injury peptides" of the DRG cell (Jancsó, 1992). They are very rare in intact rat DRGs. Peripheral axotomy, however, results in a significant increase of GAL, VIP and NPY-LI and the expression of corresponding mRNAs in sensory ganglion cells. After peripheral nerve injury GAL-LI was found in many small, medium and some large neurons. VIP is upregulated in many small to medium sized neurons, while NPY-LI could be detected mostly in large and many medium sized neurons (Jancsó, 1992).

ENZYMATIC MARKERS OF DRG NEURONS

Different enzymes have been demonstrated in sensory neurons with both histochemical and immunocytochemical methods.

Acetylcholinesterase (AChE) activity can be demonstrated in DRGs of a variety of species. Its activity seems to be stronger in the small than in the large cells (Kalina, and Wolman, 1970; Lawson, 1992).

Acid phosphatases are found in many small DRG neurons. One class of these enzymes is fluoride-resistant acid phosphatase (FRAP), which is an extralysosomal enzyme found in many small neurons in rat DRG (Knyihár-Csillik, and Csillik, 1981; Lawson, 1992). Little or no overlap was found between FRAP and either SP-LI or SOM-LI in rat DRG neurons (Nagy and Hunt, 1982). Approximately 50% of FRAP neurons showed CGRP-LI (Lawson, 1992).

Thiamine monophosphatase (TMP) activity is seen exclusively in small rat DRG neurons, is also fluoride-resistant and has a distribution similar to FRAP. Hence it has been suggested that FRAP and TMP are identical enzymes (Knyihár-Csillik et al.1986; Bucsics et al.1988; Tomiyasu, and Inomata, 1991;).

Adenosine deaminase has been demonstrated in a small proportion (7-13%) of exclusively small neurons in rat DRG (Nagy et al.1984). All these neurons also showed SOM-LI, but none showed SP-LI, nor did any have positive histochemical reactions for FRAP (Nagy, and Daddona, 1985; Lawson, 1992).

Tyrosine hydroxylase, activity is present in only 1% of adult rat DRG cells (Price, and Mudge, 1983). These cells belong to the small dark cells (Lawson, 1992).

Carbonic anhydrase is present in 20-38% of rat DRG neurons which are large and medium-sized. All these neurons are RT97-positive cells, and therefore probably all have myelinated fibres (Lawson, and Waddell, 1991; Prabhakar and Lawson, 1995).

Protein kinase C -LI was found in 45% of rat lumbar DRG neurons, mainly in the larger neurons, and in fibres that were mainly myelinated (Roivainen et al.1990; Lawson, 1992).

DRG CELL POPULATIONS CONTAINING SPECIFIC PROTEINS

Growth-associated protein (GAP 43/B-50/neuromodulin) is a membrane proteine involved in axonal growth and regeneration. mRNA for GAP 43 is high in about 50% of intact lumbar DRG neurons in rats. There is a virtually complete correspondence of neuronal populations expressing high levels of GAP 43 mRNA and high-affinity nerve growth factor

(NGF) receptor, and low GAP 43 mRNA and SOM-LI or TMP activity respectively (Verge et al.1990). After axotomy nearly all DRG neurons expressed mRNA for GAP 43 (Li et al.1995).

Calcium-binding proteins, calbindin D-28k and parvalbumin have been demonstrated immunocytochemically in 22% and 14% respectively, of rat DRG neurons, including small, medium and large neurons. Among small cells calbindin-immunoreactive neurons outnumber parvalbumin positive neurons (Carr et al.1990; Ichikawa et al.1994). These two calcium-binding proteins coexist extensively with each other and with carbonic anhydrase in rat DRG neurons (Carr et al.1990). They have low coexistence with CGRP-LI (Carr et al.1989; Ichikawa et al.1994). Recently another calcium-binding protein, calretinin has also been localized in DRG neurons (Ichikawa et al.1994).

DRG CELL POPULATIONS EXPRESSING CARBOHYDRATE EPITOPES

Lectins specific for carbohydrate epitopes of glycoconjugates label subpopulations of DRG and other sensory neurons. It has been suggested that these glycoconjugates may be involved in development, axon guidance or cell-to-cell recognition and contact (Streit et al.1985; Alvarez et al.1989; Scott et al.1990; Plenderleith et al.1992).

A large population (about 75%) of small DRG neurons and some intermediate-sized neurons in rats are labelled by the Peanut lectin, Griffonia simplicifolia I-B_4 lectin, Ricinus communis lectin and Glycine max (Soybean) lectin (SBA) which recognize terminal D-galactose residues and label laminae I and II in the spinal dorsal horn where central terminals of primary sensory neurons are distributed (Streit et al.1985). A large proportion of SBA-labelled neurons showed SP-LI and all SP-LI neurons were labelled with SBA (Streit et al.1986; Nagao et al.1992). Tomato lectin which has a specificity for poly-N-acetyllactosamine residues labels approximately 60% of DRG neurons. These tomato lectin-positive neurons belong exclusively to the population of small sensory neurons (Ambrus et al.1994).

Larger neurons are rarely stained by galactose binding lectins, but they are stained by the lectins Griffonia simplicifolia II lectin, and Lens culinaris lectin, which indicate the presence of glycoconjugates with N-glycosidically linked oligosaccharides and glycogen (Streit et al.1985).

SPECIFIC RECEPTORS EXPRESSED BY DRG NEURONS

A protein tyrosine kinase of ≈ 140 kD molecular weight, encoded by the proto-oncogen trkA, has been identified as the high affinity nerve growth factor (NGF) receptor (gp 140-trkA) (Molliver et al.1995). TrkA immunoreactivity was observed in small and medium sized ganglion cells. In lumbar L4 and L5 ganglia trkA-immunoreactive cells constitute 40% of DRG ganglion cells and range in size from 15 to 45 μm in diameter. Double labelling using markers for various dorsal root ganglion subpopulations revealed that virtually all (92%) trkA-immunoreactive cells express CGRP. 18 % of trkA-immunoreactive cells belong to the large light cell-population, identified by their strong immunostaining with the neurofilament antibody RT97. Non-peptide-containing small cells, which constitute approximately 30% of DRG cells are not trkA-immunoreactive and therefore most probably are functionally independent of nerve growth factor. (Averill et al.1995; Kashiba et al.1996).

The ganglioside GM 1 is a membrane receptor with high affinity for cholera toxin. Immunohistochemical demonstration of the binding of the B subunit of cholera toxin (CB) revealed that most cells of the light, RT97-positive population in rat DRG neurons are CB-positive. Eighty-five percent of CB-positive neurons also show carbonic anhydrase activity, but only a few of them have SP-LI, CGRP-LI or FRAP. This is so far the only marker for the majority of light neurons other than antineurofilament antibodies (Perry et al.1991; Robertson et al.1991; Robertson et al.1992).

Beside the markers characterizing specific populations of DRG cells, there are neuronal markers which are characteristic not only for smaller populations of DRG cells but all the primary sensory neurons express them. These general neuronal markers include the cytoplasmic protein, protein gene product 9.5 (PGP 9.5, Navarro et al.1995; Reynolds and Fitzgerald, 1995), neuron specific enolase (NSE, Vega et al.1990), and synaptophysin (Navone et al.1986).

THE SYSTEM OF CAPSAICIN-SENSITIVE PRIMARY AFFERENT NEURONS

Pseudo-unipolar neurons of spinal and cranial sensory ganglia have been classified according to a variety of morphological or functional criteria. Electrophysiological and histological studies have revealed a close correlation between axonal conduction velocity, fibre type and cell body size by showing that axons with low and high conduction velocities relate to small dark (type B) and light (type A) sensory cells, respectively (Harper and

Lawson, 1985; Mense, 1990).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) the pungent agent of red pepper is generally recognized as a substance affecting a well-defined population of small diameter primary afferent neurons (Jancsó et al.1977; Jancsó et al.1980a; Scadding, 1980; Nagy et al.1983; Jancsó et al.1985b; Lawson, 1987b). Early pharmacological studies by Miklós Jancsó indicated that a particular population of primary sensory neurons may be selectively stimulated and subsequently functionally inactivated by capsaicin (Jancsó, 1955; Jancsó and Jancsó-Gábor, 1959; Jancsó, 1968; Jancsó et al.1968). Local application of capsaicin onto the skin surface or oral mucosa induces a painful burning "hot" sensation which is due to excitation of specific afferent nerve endings (Jancsó, 1968).

In the skin, nociceptive C-fibres of the polymodal class are the primary targets of the excitatory effect of capsaicin. Most other types of C-fibre afferents, e.g., sensitive C-mechanoreceptors or cold-sensitive thermoreceptors are not excited (Szolcsányi, 1985; Szolcsányi, 1987). However, excitation of warm-sensitive thermoreceptors has been reported (Foster, and Ramage, 1981; Konietzny, and Hensel, 1983). The relatively uncommon Aδ mechano-heat nociceptors in the hairy skin are also excited by capsaicin.

Similar excitatory actions occur in the airways and in many visceral organs (Sann et al.1996). After repeated applications this acute stimulatory effect of capsaicin is lost. This phenomenon has been generally referred to as capsaicin-desensitization (Jancsó, 1955; Jancsó and Jancsó-Gábor, 1959; Jancsó, 1968; Jancsó et al.1968). Capsaicin applied repeatedly to the human skin or tongue, after the initial violent stimulation, renders sensory nerve endings insensitive to pain-producing chemical irritants (Jancsó-Gábor, and Szolcsányi, 1969) and in several mammalian species capsaicin-desensitization abolished protective reflexes in response to painful stimuli evoked by chemical irritants (Jancsó, and Jancsó-Gábor, 1959). In addition, capsaicin-desensitization also markedly reduced the inflammatory reactions induced by different skin irritants acting via the neurogenic route (Jancsó et al.1967; Jancsó et al.1968; Jancsó-Gábor, and Szolcsányi, 1969; Jancsó-Gábor, and Szolcsányi, 1972). Functional impairments brought about by capsaicin were of long duration.

MORPHOLOGICAL IDENTIFICATION OF CAPSAICIN-SENSITIVE PRIMARY AFFERENT NEURONS

The discovery of the selective neurotoxic action of capsaicin in newborn rats, which causes permanent functional disturbances, made possible the direct morphological identification of

primary sensory neurons sensitive to capsaicin (Jancsó et al.1977). A single systemic injection of 50 mg/kg capsaicin results in the loss of pain sensation induced by chemical irritants. This functional impairment results from the degeneration of dorsal root and cranial sensory ganglion cells of the rat. Light microscopic examination indicated increased nuclear and cytoplasmic basophilia of the affected neurons. The perikarya showed severe vacuolisation. Disruption of the nuclear membrane and disorganization of the structure of cytoplasmic organelles are indicative of irreversible neuronal damage. These degenerative changes affected small "B" type sensory ganglion cells which make up about half of the total neuronal population of the rat spinal sensory ganglia (Jancsó et al.1977; Jancsó and Király, 1980; Jancsó et al.1981; Jancsó et al.1985b; Jancsó et al.1985c).

Chemosensitive i.e. capsaicin-sensitive primary sensory neurons (CPSNs) correspond to primary sensory ganglion cells which contain neuropeptides such as substance-P, vasoactive intestinal polipeptide, somatostatin and cholecystochinin. Systemic capsaicin given to neonatal rats causes the loss of up to 90% of small dark (RT97-negative) neurons and up to 30% of light neurons (Lawson, 1981; Lawson, 1987a; Lawson, 1987b). This loss included 60-85% of SP-LI neurons, 46-71% of CGRP-LI neurons (Lawson, 1987b) and 65% of FRAP-containing neurons (Carr et al.1990). Other neuronal subgroups known to be depleted by neonatal capsaicin-treatment involved neurons which can be labelled with lectins Griffonia simplicifolia, Soybean agglutinin and Tomato lectin (Streit et al.1986; Ambrus et al.1994). Besides the loss of DRG cells, 70% of the unmyelinated nerve fibres and approximately 10% of the Aδ fibres present in the peripheral sensory nerves are also lost (Jancsó et al.1980a; Nagy, 1982). This finding is in line with the observation that systemic neonatal capsaicin-treatment affects not only small dark DRG cells (with C-fibres), but also some of the large light cells (with Aδ fibres) (Hiura, and Sakamoto, 1987; Lawson, 1987a; Lawson, 1987b).

The finding that capsaicin-sensitive neurons involve peptidergic primary sensory neurons provided further evidence for the selective neurotoxic effect of capsaicin since intrinsic peptidergic neurons of the spinal cord were not affected by capsaicin-treatment (Hökfelt et al.1981; Jancsó et al.1981; Hökfelt et al.1982; Hökfelt et al.1983).

Distribution of chemosensitive primary sensory afferents in the central nervous system was also studied. It was shown that these afferents terminate in Rexed's laminae I and II of the spinal dorsal horn. In the brainstem capsaicin-sensitive fibres were found to be distributed in the spinal trigeminal nuclei and the solitary system. In the caudal part of the trigeminal nucleus caudalis degeneration was noted both in the subnucleus marginalis and subnucleus gelatinosus, while in the rostral part of the nucleus degeneration was almost exclusively confined to the subnucleus gelatinosus. In addition, a small number of degenerating

terminals were found in the nucleus oralis. Heavy terminal degeneration was observed in the nucleus of the solitary tract and the adjoining nucleus comissuralis. Degenerating structures were also found in a small region in the dorsolateral part of the area postrema.
Degenerating afferent fibres terminating in the sensory nuclei of cranial nerves and the spinal cord represent central projections of chemosensitive primary sensory neurons, supplying the skin, mucous membranes, and different visceral areas by the Vth, IXth, and Xth cranial and spinal nerves respectively (Jancsó et al.1978; Jancsó and Király, 1980; Nagy et al.1981; Jancsó et al.1985c; Jancsó and Lawson, 1987; Jancsó and Lawson, 1990a; Hiura and Ishizuka, 1994; Hiura and Ishizuka, 1995).

CAPSAICIN-SENSITIVE PRIMARY AFFERENT NEURONS AND NEUROGENIC INFLAMMATION

Capsaicin-sensitive primary afferent neurons which give rise almost exclusively to unmyelinated C-fibres possess a dual function. On the one hand, they transmit nociceptive impulses towards the central nervous system (CNS) and, on the other hand, by the release of different neuropeptides from their stimulated peripheral nerve endings they are responsible for local effector responses of the innervated tissues. These "efferent" or local regulatory functions of capsaicin-sensitive primary sensory neurons include axon reflex vasodilatation, smooth muscle contraction, increased plasma protein extravasation, mast cell degranulation and recruitment of inflammatory cells (Jancsó et al.1967; Szolcsányi, 1984; Jancsó et al.1987a; Holzer, 1988; Maggi and Meli, 1988; Holzer, 1991).
Neurogenic inflammation refers to the phenomenon of vasodilatation and edema formation that results from the stimulation of capsaicin-sensitive primary sensory neurons (Jancsó, 1968; Jancsó et al.1968; Jancsó et al.1977; Jancsó et al.1980a; Jancsó et al.1985b; Chahl, 1988). Nociceptive capsaicin-sensitive type B neurons that contain SP, CGRP and some other neuropeptides are implicated in the development of the flare reaction (arteriolar vasodilatation) and wheal formation (increased venular permeability) in the human skin after thermal (Lundberg et al.1985; Helme et al.1986), mechanical (Salt, and Hill, 1983) and chemical (Jancsó et al.1980a; Szolcsányi, 1984) injury (Pedersen-Bjergaard et al.1991; Ekblom et al.1993; Lynn et al.1996). Neuropeptides released from sensory nerves may act directly on blood vessels (Kowalski, and Kaliner, 1988) to produce flare and wheal reactions (Lembeck et al.1982) and/or indirectly on mast cells, which, in turn, release inflammatory mediators (Lembeck and Holzer, 1979; Gamse et al.1980; Pedersen-Bjergaard et al.1991; Ekblom et al.1993; Lynn et al.1996). Application of local

anaesthetics, including the highly specific Na^+ channel-blocking agent tetrodotoxin, leads to a reversible blockade of the conduction of nerve impulses. Local anaesthetics inhibit one component of the efferent function of capsaicin-sensitive primary afferent neurons: antidromic activation of axon collaterals i.e. axon reflex flare. Since tetrodotoxin has no effect on the generator potential of sensory receptors (Matzner, and Devor, 1993) or on chemically evoked excitation-secretion coupling (e.g. by mustard-oil) in the nerve endings (Kuraishi et al.1991), it seems that the release of different mediators (e.g. SP, CGRP) from stimulated peripheral terminals of capsaicin-sensitive primary afferent neurons is a tetrodotoxin-resistant process. Although the release of sensory neuropeptides from the stimulated terminals is not affected, application of the local anaesthetic lidocaine strongly inhibits cutaneous plasma extravasation induced by the neurogenic route (e.g. mustard oil), or by histamine stimulation (Dux et al.1996). These observations suggest that the site of the inhibitory action of lidocaine is beyond the sensory nerve terminal, presumably at the endothelium of small blood vessels.

Current theories on neurogenic inflammation suggest an involvement of mast cells which appear to be in close contact with nerves containing neuropeptides (Foreman, and Jordan, 1983; Newson et al.1983; Skofitsch et al.1983). It is suggested that discrete sensory neuron-mast cell units may exist (Caulfield et al.1990; Mio et al.1991). Activation of these neurons resulting in the release of neuropeptides is believed to induce mast cell degranulation; discharge of vasoactive substances contributes to the development of neurogenic inflammation. However, the early phase of neurogenic inflammation in rat skin may proceed without mast-cell degranulation (Kowalski, and Kaliner, 1988; Inoue et al.1996). It is generally accepted that CGRP and SP liberated from stimulated sensory nerve endings cause arteriolar vasodilatation and enhanced vascular permeability of small postcapillary venules with diameters of 7-20 μm, respectively (Brain et al.1985; Brain et al.1992; Cambridge and Brain, 1992).

Since activation of unmyelinated cutaneous polymodal nociceptor afferents leads to an increase in vascular permeability in the immediate vicinity of stimulated nerve endings, demonstration of skin areas with increased vascular permeability provides information on the topographical distribution and functional condition of capsaicin-sensitive nerve endings. A dye such as Evans blue which readily binds to plasma proteins, provides a convenient way of showing up areas of plasma extravasation. This approach, combined with antidromic nerve-stimulation, has been used to demonstrate the cutaneous distribution of polymodal nociceptor afferents travelling in the stimulated nerve. Extravasated Evans blue dye can be extracted from the tissue and the dye content of the samples can be quantitatively determined. (Jancsó et al., 1967; Jancsó-Gábor et al.1967; Jancsó and Király, 1983b).

Visualization of the neurogenic inflammatory response has become a valuable approach to study the peripheral innervation territories and the regenerative properties of cutaneous C-fibres since (1) the area demarcated by the extravasation of Evans blue labelled albumin corresponds exactly the innervation territory of the nerve, as assessed by electrophysiological techniques (Pertovaara, 1988); (2) the phenomenon of plasma extravasation evoked by antidromic stimulation of peripheral nerves is a distinctive feature of capsaicin-sensitive C-fibre polymodal afferents (Jancsó, 1960; Jancsó et al.1967; Jancsó, 1968; Jancsó et al.1968; Jancsó et al.1977; Kenins, 1981; Jänig and Lisney, 1989); (3) indirect evidence indicates that the amount of extravasated dye, which can be measured quantitatively, is proportional to the number of afferent fibres innervating the tissue (Nagy, 1982; Jancsó, 1984; Brenan et al.1988) and (4) the data are easily reproducible.
Although the Evans blue technique has proved to be of considerable value in studies of the regenerative properties of cutaneous C-fibres (Jancsó and Király, 1983a; Jancsó and Király, 1983b; Jancsó and Király, 1984; Brenan, 1986; Brenan et al.1988; Kinnman et al.1992), it is less suited for histological studies and for exploring the exact localization or the overlapping of innervation territories.
Vascular labelling is a phenomenon which can be observed at sites of increased vascular permeability after the administration of a suitable colloidal substance into the bloodstream. The colloid particles accumulate in the wall (in the basement membrane) of leaky blood vessels, which can therefore be clearly distinguished in histological preparations (Jancsó, 1947; Jancsó, 1955; Jancsó, 1959; Jancsó, 1960; Majno et al.1961; Joris et al.1982). Vascular labelling is a salient histological feature of the neurogenic inflammatory response and is entirely dependent on the integrity and functional condition of peptidergic sensory nerves (Jancsó et al.1967; Gamse et al.1980; Jancsó et al.1980a; Jancsó, 1982; Jancsó, 1984).

DESENSITIZATION OF CAPSAICIN-SENSITIVE PRIMARY AFFERENT NEURONS

Capsaicin-induced excitation may be followed by desensitization of primary sensory neurons. Since the sensory neuron becomes unresponsive not only to capsaicin itself but also to noxious stimuli, this condition can be denoted as defunctionalization (Holzer, 1991). These seems to be the result of loss of the capsaicin binding sites (Szallasi and Blumberg, 1992; Goso et al.1993), the block of nerve conduction (Jancsó and Such, 1983; Welk et al.1983; Baranowski et al.1986; Such and Jancsó, 1986; Waddell and Lawson, 1989), the block of axonal transport (Jancsó et al.1980b; Gamse et al.1982; Jancsó et al.1987b)

leading to nerve growth factor deprivation and contributing to the depletion of releasable neuropeptide pool in nerve endings (Miller et al.1982; Jancsó, 1992; Winter et al.1993; Jancsó and Ambrus, 1994; Hu-Tsai et al.1996; Jancsó et al., 1997).

Capsaicin-desensitization is a complex phenomenon which encompasses different stages and, most likely, different mechanisms of action (Buck and Burks, 1986; Jancsó et al.1987a; Holzer, 1991). Following systemic administration to adult or newborn animals capsaicin can produce ultrastructural (e.g. mitochondrial swelling) (Joó et al.1969; Jancsó et al.1984; Chiba et al.1986) or even toxic (cell death) effects on primary afferent neurons (Joó et al.1969; Jancsó et al.1977; Nagy et al.1980; Jancsó and Király, 1981; Nagy et al.1981; Nagy, 1982; Jancsó et al.1984; Dinh and Ritter, 1985; Chiba et al.1986; Jancsó et al.1987a; Ritter and Dinh, 1988; Jancsó and Lawson, 1990b; Jancsó, 1992; Ritter and Dinh, 1992; Hiura and Ishizuka, 1994; Hiura and Ishizuka, 1995). The extent of this damage may vary depending on factors such as the dose, the way of application, the species and the developmental age of the animal. Obviously, systemic administration of capsaicin affects the whole system of capsaicin-sensitive nerves supplying to different organs of the body. After capsaicin-treatment of both neonatal and adult animals the number of small "B" type DRG cells is strongly reduced, the number of unmyelinated fibres in peripheral nerves is reduced and some of the thin myelinated Aδ fibres are also lost. It seems that young animals are more sensitive to the neurotoxic effect of capsaicin since the proportion of affected DRG cells (and axons in peripheral nerves) are higher after neonatal capsaicin-treatment than after capsaicin-treatment of adult animals (Jancsó et al.1978; Nagy et al.1981; Chung et al.1985b; Jancsó et al.1985a; Jancsó et al.1985c; Arvidsson and Ygge, 1986; Jancsó and Lawson, 1990a).

Capsaicin is thought to open ligand-gated cation channels that seem to be permeable to both monovalent and divalent cations with a limited selectivity for Ca^{2+} (Wood et al.1988; Bevan and Geppetti, 1994; Arbuckle and Docherty, 1995; Feigin et al.1995; Oh et al.1996). Activation of these channels results in an inward Ca^{2+} and Na^{+} current while K^{+} ions leave the cells (Wood et al.1988; Dray, 1992). Inward current leads to membrane depolarization and to direct stimulation of transmitter release by Ca^{2+} (Holzer, 1991). Persistent stimulation leads to high intracellular Ca^{2+} concentrations which may activate Ca^{2+} dependent proteases and impair mitochondrial functions. These effects combined with the osmotic shock which follows intracellular NaCl accumulation lead to irreversible damage and finally to cell death (Bevan et al.1987).

Topical application of capsaicin to peripheral nerves is an adequate and reliable technique to produce the selective chemical lesion of chemosensitive afferent nerves supplying a particular organ or some part of it (Jancsó et al.1980b; Jancsó and Such, 1983; Chung et

al.1985a; Baranowski et al.1986; Jancsó and Lawson, 1987; Jancsó et al.1987b; Lynn and Shakhanbeh, 1988; Wood et al.1988; Jancsó and Lawson, 1990a; Pini et al.1990; Pini and Lynn, 1991; Bevan and Geppetti, 1994; Arbuckle and Docherty, 1995; Feigin et al.1995; Oh et al.1996). This method has the advantage that its effects are confined to primary sensory neurons whose peripheral branches run in the treated nerve (Jancsó et al.1980b; Jancsó et al.1987c). Functional impairment following application of capsaicin onto a peripheral nerve includes a complete abolition of the neurogenic inflammatory response and marked thermal and chemical analgesia in the skin area supplied by the treated nerve (Jancsó et al.1980b; Gamse et al.1982; Jancsó et al.1985a). Functional studies have indicated that the effects of capsaicin applied to the peripheral nerve can be separated into three subsequent phases (Jancsó, and Such, 1983). The first phase is characterized by selective activation of both Aδ and C afferent fibres. The second phase consists of a non-specific and reversible blockade of impulse conduction of both Aδ and C fibres (Jancsó, and Such, 1983; Such, and Jancsó, 1986). The third phase is characterized by a highly selective impairment of the function of unmyelinated afferents; postganglionic efferent fibres are not affected (Gamse et al.1982; Jancsó and Such, 1983; Jancsó et al.1987b).

Changes induced by the local application of capsaicin are dose-dependent and apparently permanent (Fitzgerald, and Woolf, 1982; Chung et al.1985a). Electrophysiological studies have extended these observations and indicated that the perineural application of capsaicin may be regarded as a specific chemodenervation technique which produces a permanent and selective loss and/or blockade of C-fibre polymodal nociceptor afferents (Baranowski et al.1986; Such, and Jancsó, 1986; Lynn et al.1987; Marsh et al.1987). Furthermore, the marked alterations observed in the functional properties of spinal and medullary dorsal horn neurons provide evidence for significant central changes following perineural capsaicin-treatment (Wall and Fitzgerald, 1981; Fitzgerald, 1982; Fitzgerald and Woolf, 1982; McMahon et al.1984).

These functional alterations are accompanied by marked changes in the chemistry of primary sensory neurons. Histochemical and biochemical investigations have revealed a marked depletion of peptides and other sensory neurone-specific macromolecules from the central terminals and the perikarya of small sensory ganglion cells (Ainsworth et al.1981; Gamse et al.1982; Gibson et al.1982; Jancsó and Lawson, 1988; Anand et al.1990; Jancsó, 1992). Although these findings have indicated profound changes in the chemistry of the primary sensory neurons, they have yielded little information on the nature of these changes. In particular, it is unclear whether these changes are associated with an irreversible structural impairment of primary afferent terminals or merely reflect a depletion of these peptides and proteins from the neurons. Animal experiments have provided

evidence for a selective inhibition of the transmission of nociceptive impulses following the application of capsaicin onto peripheral nerves. Therefore, peripheral treatment with capsaicin may represent a possible therapeutic approach in the management of certain neurogenic pain syndromes (Jancsó et al.1980b; Jancsó and Lynn, 1987; Lynn, 1990; Maggi, 1991).

AIM OF THE STUDY

The morphological, neurochemical and functional traits of capsaicin-sensitive neurons are well established. However, there is little information on the morphology and neurochemistry of capsaicin-sensitive cutaneous nerve endings and their relation to other structures involved in neurogenic inflammatory responses. Experimental data on the precise extent and on the possible functional significance of overlapping innervation territories of peptidergic peripheral nerves supplying adjacent skin areas are scarce. Furthermore, the morphological changes of peripheral nerve endings associated with, and probably forming the basis of the antinociceptive and antiinflammatory effects of perineural capsaicin-treatment have not been examined yet. Therefore the aims of the present study were
(1) to explore the morphology, chemical phenotypes and patterns of afferent nerve fibres innervating the rat hairy skin;
(2) to determine the topographical relationship of axonal and vascular structures involved in neurogenic plasma extravasation and,
(3) to assess the changes in the morphology and chemistry of cutaneous sensory nerve endings after perineural capsaicin-treatment.

MATERIALS AND METHODS

ANIMALS

Experiments were performed on male Wistar rats weighing 200-300 g. In all series of experiments animals were anaesthetized either with an intraperitoneal injection of chloral hydrate (350 mg/kg b.w.) or with ether.

PRODUCTION OF THE NEUROGENIC INFLAMMATORY RESPONSE

To induce a neurogenic inflammatory response of the skin two different methods were used for different purposes. Neurogenic inflammatory response was induced either by antidromic electrical stimulation of peripheral nerves, or by direct stimulation of sensory nerve endings by painting the skin with the chemical irritant, mustard oil (Jancsó, 1960; Jancsó et al.1967; Jancsó, 1968; Jancsó et al.1977).

1. Production of the neurogenic inflammatory response by antidromic nerve stimulation: Body temperature of the anaesthetized rat was recorded with a thermoprobe inserted 5 cm into the rectum and was kept at 37.5 $\pm$ 0.5^{o}C. Either the saphenous or the peroneal nerve was exposed and cut centrally before being placed onto bipolar platinum wire electrodes for nerve stimulation. The nerves were stimulated with rectangular pulses for 6 min (15 V, 2 Hz, 2ms) to induce a neurogenic inflammatory response. To visualize the developing inflammatory response Evans blue dye (50 mg/kg) was injected intravenously into a lateral tail vein 1 min prior to nerve stimulation.

2. Production of the neurogenic inflammatory response by direct stimulation of sensory nerve endings: In this series of experiments, cutaneous inflammatory reaction was evoked by painting the dorsal surface of the hindpaw with 50 mg/ml mustard oil (allyl-isothiocyanate) in liquid paraffin. Evans blue dye (50 mg/kg) was injected intravenously into a lateral tail vein prior to the application of the inflammatory stimulus.

HISTOLOGICAL DEMONSTRATION OF THE NEUROGENIC INFLAMMATORY RESPONSE

The phenomenon of vascular labelling was utilized to demonstrate the neurogenic inflammatory response in the rat skin (Jancsó, 1955; Majno et al.1961; Joris et al.1982; Dux and Jancsó, 1994). In this series of experiments either antidromic electrical nerve stimulation with rectangular pulses (15 V, 2 Hz, 2ms) for 6 min, or application of mustard oil preceded by an intravenous injection of either a 1% colloidal silver solution (1 ml/ 100 g b.w.) or an aqueous suspension of 3% Monastral blue B (0.3 ml/100 g b.w.). Thirty minutes after the stimulation of chemosensitive nerves, the animals were bled and the dorsal skin of the hindpaw was carefully removed, fixed in methanol, cleared in xylene and mounted in Canada balsam for light microscopic examination.

MATCHING THE AREAS OF VASCULAR LABELLING AND EVANS BLUE EXTRAVASATION

To match the areas of vascular labelling and Evans blue extravasation, the injection of 50 mg/kg Evans blue dye was followed immediately by the injection of a 1% colloidal silver solution. A small area of the dorsal skin of the hindpaw (size: approx. 0.5 x 0.5 cm) was painted with 50 mg/ml mustard oil. The painted area was surrounded by unstimulated skin areas. Three minutes after the stimulation of chemosensitive nerve fibres, the animals were bled and the dorsal skin of the hindpaw was carefully removed, fixed with Zamboni's fixative for 4 hours at 4 ^{o}C. The tissue samples were washed in phosphate buffer (0.1 M, pH 7.4) and stored in 0.1 % Na-azid containing 0.1 M phosphate buffer. Pieces of fixed skin samples containing the whole mustard oil-treated skin area with the surrounding untreated areas were dissected and immersed in sucrose (30%) overnight. Longitudinal sections of the skin samples were cut at 20 μm on a cryostat and mounted onto chrome alum-coated slides.

The fluorescent microscopic evaluation was based on matching the localisation of silver labelled blood vessels and Evans blue extravasation in the skin sample. Both substances could be seen and compared simultaneously because of the autofluorescence of the Evans blue dye.

DEMONSTRATION OF OVERLAPPING CUTANEOUS INNERVATION AREAS

In this series of experiments, a cannula was inserted into the penile vein of the anaesthetized rat for intravenous injections. Body temperature was kept at 37.5 $\pm$ 0.5 ^{o}C with a heating pad during the whole experiment. The saphenous, peroneal and/or sural nerves on one side were exposed and cut centrally before antidromic nerve stimulation (parameters: see above). Either a 1% colloidal silver solution or a 3% Monastral blue B suspension has been injected intravenously before nerve stimulation begun. Successive nerve stimulations were separated by a period of 60 min. This was necessary for the labels to be removed from the circulation by the reticuloendothelial system before the next stimulation was begun. Indeed, pilot experiments showed that vascular labelling did not occur if a colloid was injected 60 min prior to nerve stimulation. Since the sural and saphenous nerves do not serve neighbouring skin regions, alternate injections of colloidal silver and the Monastral blue B suspension permitted the demonstration of the innervation territories of all the three nerves supplying the dorsal skin of the hindpaw. Thirty minutes after the last nerve stimulation, the animals

were bled and the dorsal skin of the hindpaw was carefully removed. Labelled vessels in the entire skin area whose proximal border was determined by a line connecting the internal and external ankles were examined. Tissue samples were fixed in methanol, cleared in xylene and mounted in Canada balsam for light microscopic examination.
Areas of interest were drawn and measured separately with a camera lucida. Quantitative evaluation of the experimental data were performed by a computerized system. Sizes of skin areas containing blood vessels labelled with only one label, either with colloidal silver or with Monastral blue B, and areas which were labelled with both substances were measured separately. Percentage distribution of these different innervation territories were calculated as the percentage of the total surface area of the rat dorsal hindpaw skin (Dux and Jancsó, 1994).

PERINEURAL CAPSAICIN-TREATMENT

Experimental animals were anaesthetized with ether. The sciatic nerves were exposed high in the thigh on both sides. A small piece of gelfoam moistened with 0.1 ml of a 1% solution of capsaicin (dissolved in 3% ethanol, 4% Tween 80 in physiological saline) was applied to the right sciatic nerve. The left sciatic nerve was treated with the vehicle for capsaicin. The skin supplied by the vehicle-treated nerve served as control. The wounds were closed and the animals were returned to the animal house (Jancsó et al.1980b).

IMMUNOCYTOCHEMISTRY

3, 14 or 42 days after local capsaicin-treatment of the sciatic nerves, the animals were given an intravenous injection of a 1% solution of colloidal silver under ether anaesthesia and the neurogenic inflammatory response was induced by painting the hindpaw skin with a 5% solution of mustard oil. After a survival time of 60 min, the animals were bled and the dorsal skin of the hindpaw was carefully removed. Skin samples were pinned flat at the bottom of a Petri dish that had a silicon elastomer bottom (Sylgard 184, Down Corning, USA) and fixed with Zamboni's fixative for 4 hours at $4^{o}C$. The tissue samples were washed in phosphate buffer (0.1 M, pH 7.4) and stored in 0.1 M phosphate buffer containing 0.1% Na-azid.
In our immunohistochemical studies the indirect immunofluorescence technique was used. Pieces of fixed skin samples containing both the control skin areas (supplied by the

untreated saphenous nerve) and areas supplied by the capsaicin-treated sciatic nerve were cut and immersed in sucrose (30% in 0.1 M phosphate buffer) overnight, sectioned at 20 μm with a cryostat and mounted onto chrome alum-coated slides.

Slides containing the sections were preincubated for 1 hour in phosphate-buffered saline (PBS 0.1 M) containing 0.5 % Triton X-100 and 4% goat serum. The preparations were exposed for 12-16 hours at room temperature to the primary antisera diluted in 0.1 M PBS containing 0.5 % Triton X-100 and 4% goat serum. Primary antisera against the following peptides and neuronal markers were used: protein gene-product 9.5 (PGP 9.5), growth associated protein (GAP 43), substance P (SP), calcitonin gene-related peptide (CGRP), somatostatin (SOM), and neurofilaments (RT97). Specification of the antibodies and antisera and the dilutions used in this study are given in Table 1. The tissues were then washed three times for 10 min in 0.1 % Na-azid containing 0.1 M phosphate buffer before they were incubated for 2 hours at room temperature with the secondary antibodies. The secondary antibodies (goat anti-rabbit and goat anti-mouse) were labelled with the fluorophore dichlorotriazinyl aminofluorescein (DTAF) or carboxymethylindocyanine (Cy3, both from Jackson Labs, purchased from Dianova, Germany). The DTAF- and Cy3-labelled antibodies were used at a dilution of 1:200 and 1:500. For double labelling, primary antibodies raised in different species were combined with species-specific secondary antibodies and contrasting fluorophores (DTAF and Cy3). After a further wash in PBS, the preparations were coverslipped with Citifluor. The preparations were examined with an Olympus fluorescence photomicroscope. Cy3 was visualized by using the filter cube G (exciting filter BP 545 + EO 530, dichroic mirror DM 570, barrier filter O 590; no DTAF fluorescence is visible with this filter/mirror combination). DTAF was visualized with the filter cube IB (excitation filter BP 495, dichroic mirror DM 505, barrier filter G 515 + G 520 IF; no Cy3 fluorescence is visible with this filter/mirror combination).

ANTIGEN	HOST SPECIES	DILUTION	SOURCE
PGP 9.5	rabbit	1:1000	Ultraclone, UK
GAP 43	mouse	1:200	Boehringer Mannheim Biochemica
SP	rabbit	1:1000	Harti et al.1989.
CGRP	rabbit	1:1000	Paesel and Lory, Germany
SOM	rabbit	1:1000	Peninsula
RT97	mouse	1:1000	John Wood, Sandoz Institute, London

Table 1.: List of primary antibodies and antisera used for immunohistochemistry

QUANTITATIVE DETERMINATION OF IMMUNOHISTOCHEMICALLY IDENTIFIED POPULATIONS OF CUTANEOUS NERVES

Skin areas of intact innervation exhibited a characteristic pattern of vascular labelling after painting the skin with mustard oil following an intravenous injection of a colloidal silver solution. In contrast, skin areas served by the capsaicin-treated nerve did not contain labelled blood vessels. This permitted the direct visualization of innervated and chemically denervated skin regions in the same section. In order to quantify the effects of capsaicin-treatment on immunoreactive nerve fibres, fibre counts in two different layers of the skin were performed. The density of the innervation of the epidermis and the subepidermal plexus was determined.

Thin varicose, presumably single axons were counted separately from the bundles (containing more than one immunoreactive nerve fibres). Since skin samples were streched to their original size during fixation, the fibre counts could be expressed as immunopositive nerve fibres/mm^2 of skin surface area.

Four sections of each skin samples were evaluated. Data (single fibres, nerve bundles and all fibres) of each animal were further used for the statistical comparison.

STATISTICS

Statistical comparisons were performed using Student's t-test. All values are expressed as mean $\pm$ S.E.M. A level of $p < 0.05$ was accepted as indicating a significant difference.

CHEMICALS

Capsaicin (Fluka)
Evans blue (Sigma)
Mustard oil (Merck)
Colloidal silver (Merck)
Monastral blue B (Sigma)
Triton X-100 (Sigma)
Citifluor (Citifluor UKC, Chem. Lab. Canterbury)

Type and source of the primary and secondary antibodies used are described above. All other materials used were of the highest grade available.

RESULTS

THE MORPHOLOGY OF AFFERENT NERVE FIBRES INNERVATING THE RAT HAIRY SKIN

General morphology of nerve fibres innervating the rat hairy skin

Antisera raised against different neuronal constituents (like PGP 9.5 and NSE) are available to identify the general innervation pattern of the skin (Rice et al.1993; Johnson et al.1994; Fundin et al.1995; Navarro et al.1995; Sisask et al.1995). By making use of PGP 9.5-immunohistochemistry we could identify large numbers of PGP 9.5-immunoreactive (PGP 9.5-IR) cutaneous nerve fibres. In the epidermis only single thin varicose or non-varicose axonal structures have been visualized ($956 \pm 52/mm^2$). They originated from prominent nerve bundles situated just underneath the epidermis. They took separate routes in the epidermis, most of them ran straight up to the superficial layers, some ran parallel to the skin surface. In the subepidermal layer - where the silver labelled vessels are localized - a rich network of PGP 9.5-IR fibres could be observed which was made up mainly of bundles of nerve fibres (Fig. 1.A). PGP 9.5-IR nerve fibres were localized around almost all the hair follicles. Separate types of perifollicular nerve fibres were observed. Some fibres ran parallel to the hair and formed pallisade like terminal structures while others surrounded the hair follicle in a ring-like fashion. Sometimes both types could be seen in the same hair follicle. PGP 9.5-IR was detectable even in the deeper regions of the dermis around small blood vessels and in the nerve bundles running in the deep dermal regions.
With the antibody RT97 raised against the 200 kD neurofilament protein (Lawson et al.1984) as a marker of capsaicin-insensitive afferents (Sann et al.1995), the myelinated sensory nerve fibres could be identified. No RT97-IR fibres could be seen in the epidermis. Approximately half of the RT97-IR nerve fibres present in the subepidermal layer were single fibres whereas the rest formed bundles. In the hairy skin of the rat RT97 immunoreactivity was localized mainly around hair follicles in both pallisade and circular fibres.

Chemical phenotypes of afferent nerve fibres innervating the rat hairy skin: quantitative aspects

Antibodies raised against the sensory neuropeptides SP, CGRP and SOM and against growth associated protein were used to identify chemically different populations of

cutaneous sensory axons of hairy skin of the rat hindpaw.

Substance P

Only few SP-immunoreactive nerve fibres were present in the epidermis of the rat hairy skin. This population of sensory nerve fibres was made up almost exclusively of single nerve fibres. In the subepidermal layer SP-IR fibres were more numerous (Fig. 1.E). In preparations obtained from animals in which a cutaneous neurogenic inflammatory response was elicited by mustard oil after a previous i.v. injection of a colloidal silver solution, the relationship of silver-labelled leaky blood vessels and nerve fibres could be clearly observed. Many SP-IR axons were closely associated with leaky blood vessels.

Calcitonin gene-related peptide

The CGRP-IR nerve fibres were more numerous in both the epidermis and subepidermal layer of the skin than the SP-IR nerve fibres. In the epidermis mainly single CGRP-immunoreactive fibres could be detected; many were associated with labelled blood vessels in the subepidermal layer. In the deeper regions of the skin, the majority of the CGRP-immunoreactive structures formed thick nerve bundles (Fig. 1.C).

Somatostatin

SOM-immunoreactivity could not be detected in the epidermis of the hairy skin. Some immunoreactive single fibres and just a few nerve bundles were present underneath the epidermis. In deeper layers of the skin, the proportion of SOM-immunoreactive nerve bundles was higher.

GAP 43

A large population of cutaneous axons showed GAP 43-IR. Under normal conditions the hairy skin of the rat contained a high number ($566 \pm 119/mm^2$) of single, often branching intraepidermal GAP 43-immunoreactive nerve fibres. The subepidermal plexus also contained a significant number of GAP 43-immunoreactive nerve fibres which were mainly arranged in nerve bundles. Almost all the hair follicles bore a rich GAP 43-positive innervation. GAP 43-immunoreactive nerve fibres could be detected also around the small blood vessels lying in the deep dermis.

The quantitative data on the densities of SP-, CGRP-, SOM- and GAP 43-immunoreactive nerve fibres of the epidermis and of the subepidermal layer of the rat hairy skin are presented in Table 2. The numbers of PGP 9.5- and RT97-IR axons representing the total axonal population and the population of myelinated fibres, respectively, are shown.

EPIDERMIS

	Single	Bundle	**All**
PGP 9.5	956±52	0	**956±52**
SP	35±7	0	**35±7**
CGRP	51±11	0	**51±11**
GAP 43	566±119	0	**566±119**
RT97	0	0	**0**
SOM	0	0	**0**

SUBEPIDERMIS

	Single	Bundle	**All**
PGP 9.5	10±1	314±19	**324±20**
SP	96±12	64±21	**160±33**
CGRP	132±31	123±4	**255±35**
GAP 43	36±7	354±13	**390±7**
RT97	46±3	17±4	**63±7**
SOM	26±10	3±2	**29±13**

Table 2.: Number of immunoreactive nerve fibres in the hairy skin of the rat. Single fibres, nerve bundles and all nerves (single + bundle) are calculated for different layers of the rat hairy skin. Values are mean/mm^2 ± S.E.M. from four to six animals with four counts per animal.

THE TOPOGRAPHICAL RELATIONSHIP OF AXONAL AND VASCULAR STRUCTURES INVOLVED IN NEUROGENIC PLASMA EXTRAVASATION

Light microscopic demonstration of leaky blood vessels

Utilization of the vascular labelling technique permitted the direct visualization of leaky blood vessels in histological preparations. Under the light microscope silver-labelled leaky blood vessels formed an elaborate network in the subepidermal layer of the hairy skin of the

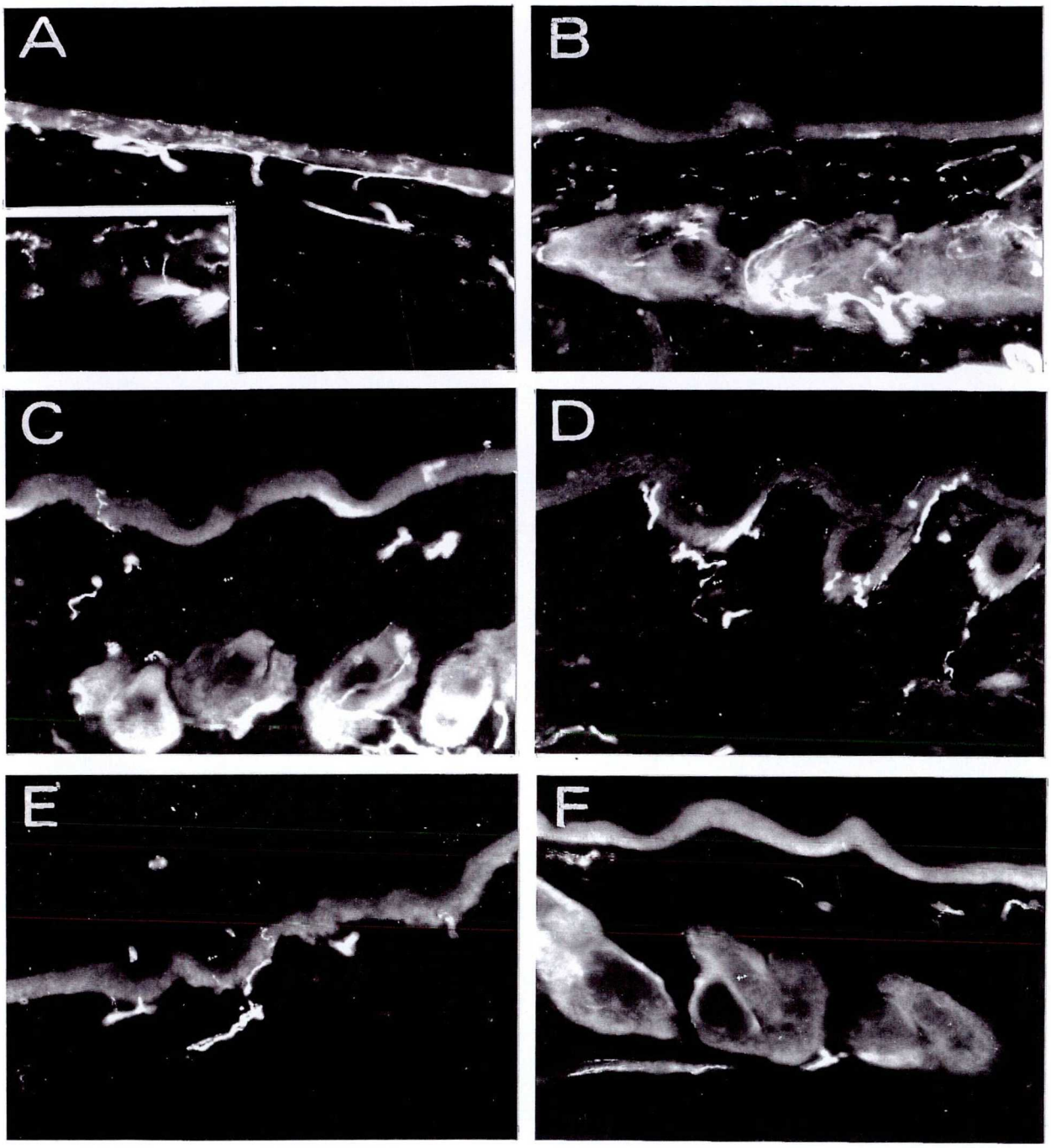

Fig. 1.: Fluorescence photomicrographs of control skin areas (A, C, E) and areas innervated by the capsaicin pre-treated (3 days previously) sciatic nerve (B, D, F) showing fibres immunoreactive for PGP 9.5 (A, B), CGRP (C, D) and SP (E, F). Magnification in A-F is x200, and x400 in the insert in A.

rat hindpaw. These vessels corresponded in size to postcapillary venules (7-20 μm in diameter).

In transverse cryostat sections of the hindpaw skin prepared for immunocytochemistry, silver-labelled blood vessels could also be clearly identified. Shorter or longer segments of such vessels were observed in the subepidermal layer adjacent to the epidermis.

By making use of the vascular labelling technique, the exact topographical localization of a chemically denervated skin area was also possible. Perineural application of capsaicin onto the sciatic nerve resulted in a complete abolition of vascular labelling in the lateral part of the rat dorsal hindpaw skin already 3 days after treatment (Fig. 2.A). The border of innervated and denervated skin areas, as judged from the topographical distribution of labelled blood vessels, was always very distinct. There was little difference in this respect among the 3 groups of rats with different survival times after perineural capsaicin-treatment. This indicates that reinnervation of the denervated skin area by means of collateral nerve sprouting was minimal or absent.

The topographical relationship of peptidergic nerve fibres and leaky blood vessels

The combination of the vascular labelling technique with the immunohistochemical demonstration of sensory nerve fibres permitted the direct investigation of the topographical relationship of leaky blood vessels and different types of sensory axons. Neuro-vascular association was studied in samples obtained from animals in which a neurogenic inflammatory response was elicited after an i.v. injection of a colloidal silver solution in the dorsal hindpaw skin. In sections prepared at a plane parallel to the skin surface a network of silver-labelled blood vessels were revealed. Silver-stained vessels measured 9-18 μm in diameter and they were situated in a narrow zone (100 μm) immediately underneath the epidermis. These findings suggest that these vessels may correspond to postcapillary venules known to be involved in the mechanism of mediator-type increase in vascular permeability. Immunohistochemistry revealed a close association of silver stained blood vessels and SP- and CGRP-IR axonal structures. In favorable sections it was possible to observe nerve fibres which followed labelled vessels for considerable distances (up to 40 μm). Considering the possible functional importance of this anatomical arrangement between sensory fibres and blood vessels in the initiation of a neurogenic inflammatory response, we refer to these anatomical formations as axo-venular units of the skin.

Matching areas of vascular labelling and Evans blue extravasation

These experiments were initiated in an attempt to ascertain that innervation territories of peripheral nerves determined with the Evans blue extravasation and the vascular labelling techniques, respectively, are comparable. To this end a direct comparison of the innervation territories as determined with these techniques were made in the same histological section. Comparison of cutaneous areas exhibiting silver stained blood vessels and Evans blue fluorescence using bright field illumination and fluorescence microscopy, respectively, revealed that innervation areas referred to the skin surface are virtually identical determined with the two methods (n=4).

Overlapping innervation territories of peripheral nerves as determined with the dual vascular labelling technique

Following intravenous injections of appropriate tracers and successive antidromic stimulation of hindlimb nerves, labelled segments of small blood vessels of different colours could be observed in transparent preparations of the dorsal skin of the hindpaw under the light microscope. Labelled vessels exhibited a distinct brown or blue colouration due to accumulation of colloidal silver or Monastral blue B in their walls. The label was evenly distributed within the wall of these blood vessels. As a result of the experimental paradigm employed in the present experiments, cutaneous territories served by different nerves were expected to be delineated by labelled blood vessels of different colours. Indeed, most vessels appeared either brown or blue, indicating the presence of only one of the injected substances in their walls. In accord with previous studies using the Evans blue technique, the medial and lateral parts of the dorsal paw skin exhibited vascular labelling after stimulation of the saphenous and peroneal nerves, respectively. In the lateralmost portion of the dorsal paw skin, labelling was seen after stimulation of the sural nerve. However, numerous blood vessels were labelled with both substances; the presence of the two labels could be clearly seen under high power. The areas containing these double-labelled blood vessels were regarded as overlapping innervation territories.

Four different areas could be distinguished with respect to the characteristics of the labelled blood vessels: (1) non-overlapping area, i.e., an area displaying clear labelling with only one of the substances injected, representing the main innervation territory of the stimulated nerve; (2) area of direct overlap, i.e. an area showing double-labelled vessels at the border of the innervation territories of two adjacent nerves; (3) area of remote overlap ("insular overlap"), i.e. a separated, well-circumsribed area showing double-labelled vessels distant

from the area of direct overlap within the area of skin innervated by one of the nerves; (4) "alien insular area", i.e. a usually small but well-demarcated area lying distant from the border zone within the innervation area of a nerve and innervated by another nerve supplying the neighbouring skin area.

The total innervation area of the dorsal hindpaw skin was 549 $\pm$ 62 mm^2. Quantitative estimation disclosed a significant but variable degree of overlap, ranging up to 10-20% of the innervation territories of individual hindlimb nerves. The results revealed an overlap of about 4.05% between the saphenous and peroneal and about 0.81% overlap between the peroneal and sural innervation territories. The significance of this apparently limited overlap lies in the fact that these represent approximately 10 and 20% of the total innervation territories of the saphenous and sural nerves, respectively.

Examination of the topographical distribution of overlapping cutaneous innervation areas demonstrated some characteristic features. The largest overlap between the innervation territories of the saphenous and peroneal nerves was recorded in an area overlying the second and third metatarsals and in a more proximal region close to the ankle joint.

EFFECT OF PERINEURAL CAPSAICIN-TREATMENT ON POPULATIONS OF NERVE FIBRES IN THE RAT HAIRY SKIN

Effect of perineural capsaicin-treatment on neurogenic inflammation

Investigation of histological specimens of the rat hindpaw which included areas related to the intact and capsaicin-treated nerves revealed that skin areas of intact innervation exhibited the characteristic pattern of vascular labelling after painting the skin with mustard oil following an intravenous injection of a colloidal silver solution. In contrast, skin areas served by the capsaicin-treated nerve were devoid of labelled blood vessels (Fig. 2.A). This approach has the advantage over the Evans blue extravasation technique that innervated and chemically denervated cutaneous areas may be clearly differentiated in immunostained histological sections.

Effect of perineural capsaicin-treatment on nerve fibre populations of the rat hindpaw skin

In sections stained for the sensory neuropeptide SP (thought to be primarily responsible for inducing neurogenic plasma extravasation in the rat skin), we observed a significant, almost complete loss of immunoreactive nerve fibres in the skin supplied by the capsaicin-treated

nerve. Persistent loss of SP-immunoreactivity was noted in both the epidermis and the subepidermal layer (Fig. 1.F).

In sections stained for another major sensory neuropeptide, CGRP, a significant loss of immunoreactive nerve fibres was noted in the epidermis 3, 14 and 42 days after perineural capsaicin-treatment. The number of CGRP-IR nerve fibres of the subepidermal plexus was also influenced by the treatment (Figs. 1. D, and 2. B). There was a significant loss of immunoreactive nerve bundles in all the three groups of animals.

Perineural capsaicin-treatment had no significant effect on the densities of cutaneous SOM- and RT97-immunoreactive fibres (Fig. 2. C).

PGP 9.5 is regarded as a general neuronal marker which is present in all kinds of peripheral nerve fibres irrespective of their functional and neurochemical characteristics. Staining of skin samples with an antiserum raised against PGP 9.5 revealed a significant loss of epidermal PGP 9.5 immunoreactive single nerve fibres in all the three groups of experimental animals which were sacrificed 3, 14 and 42 days after perineural treatment with capsaicin. In the subepidermal layer of the skin, the number of PGP 9.5-IR nerve bundles was significantly less in all the three experimental groups (Fig. 1. B). Similarly, the number of GAP 43-immunoreactive epidermal nerve fibres was strongly reduced.

The results of the quantitative evaluation of our immunohistochemical data are summarized in Table 3.

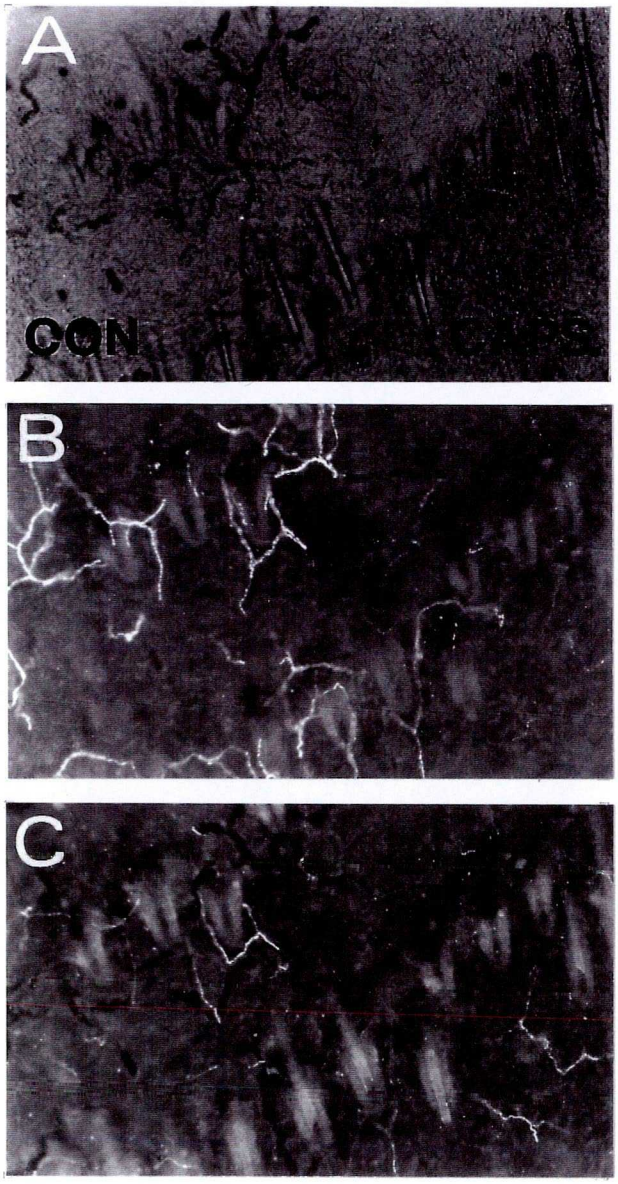

Fig. 2.: Photomicrographs taken from a section cut parallel to the surface of the dorsal skin of the rat hind paw showing adjacent innervation territories of the intact (saphenous, CON) and the capsaicin treated (sciatic, CAPS) nerves. In the bright field photomicrograph (A) the presence of colloidal silver-labelled blood vessels indicates the skin area with intact sensory innervation. B and C show fluorescence photomicrographs of the same field after staining with antibodies against CGRP (B) and RT97 (C). Note that CGRP- but not RT97-IR fibres are depleted from the skin area served by the capsaicin treated nerve (x 60).

	EPIDERMIS			SUBEPIDERMIS		
	Single	Bundle	**All**	Single	Bundle	**All**
PGP 9.5						
Control	956±52	0	**956±52**	10±1	314±19	**324±20**
Caps 3d	98±34***	0	**98±34*** **	62±17	174±28**	**236±43**
Caps 14d	23±9***	0	**23±9*** **	42±12	147±16***	**189±22****
Caps 42d	62±14***	0	**62±14*** **	88±19	151±26***	**239±27***
SP						
Control	35±7	0	**35±7**	96±12	64±21	**160±33**
Caps 3d	1±0.7***	0	**1±0.7*** **	9±2***	0***	**9±2*** **
Caps 14d	0.6±0.5***	0	**0.6±0.5*** **	11±5**	4±3**	**14±5****
Caps 42d	5±2**	0	**5±2****	7±2***	5±2*	**12±4****
CGRP						
Control	51±11	0	**51±11**	132±31	123±4	**255±35**
Caps 3d	17±6*	0	**17±6***	108±15	56±8**	**164±16***
Caps 14d	25±1*	0	**25±1***	143±15	48±16*	**192±13**
Caps 42d	3±1*	0	**3±1***	103±9	30±7**	**133±11***
GAP 43						
Control	566±119	0	**566±119**	36±7	354±13	**390±7**
Caps 3d	26±6**	0	**26±6****	79±10	251±33	**331±40**
Caps 14d	5±2**	0	**5±2****	114±13	218±32*	**333±40**
Caps 42d	5±2**	0	**5±2****	99±15	226±35*	**325±43**
RT97						
Control	0	0	**0**	46±3	17±4	**63±7**
Caps 3d	0	0	**0**	39±9	8±3	**47±12**
Caps 14d	0	0	**0**	49±6	14±0.7	**64±6**
Caps 42d	0	0	**0**	47±6	27±10	**74±15**
SOM						
Control	0	0	**0**	26±10	3±2	**29±13**
Caps 3d	0	0	**0**	35±7	11±4	**46±11**
Caps 14d	0	0	**0**	22±4	9±3	**32±6**
Caps 42d	0	0	**0**	16±3	4±1	**20±5**

Table 3.: Number of immunoreactive nerve fibres in the rat hairy skin after perineural capsaicin treatment. Values are mean±S.E.M. from four to nine animals with four counts per animal. Significant differences (t-test) between control and capsaicin pretreatment are indicated (* $P<0.05$, ** $P<0.01$, *** $P<0.001$)

DISCUSSION

NERVE FIBRE POPULATIONS INNERVATING THE RAT HAIRY SKIN

Reaction of an organism to changes in its environment requires the presence of suitable receptors controlling the parameters of body functions important for survival. These changes are recorded by cells which have differentiated to respond selectively and with great precision. Sensory receptors are such cells; they take many forms but in mammals, with minor exceptions, they have a common pattern. Neuronal receptors are parts of primary sensory neurons, with a soma in a cranial or spinal sensory ganglion and a peripheral axon whose end is the sensory terminal. All cutaneous sensors are of this type, but the sensory terminals may be encapsulated or linked to special mesodermal or ectodermal elements forming a part of the sensory apparatus. These non-neural cells create the proper environment for the excitation of the nerve ending or modify its excitation in some way. The morphological types of cutaneous receptors are well established. Although they show great variety in their microscopic structure, there is no clear-cut correlation between their structure and functional character. Indeed, cutaneous sensory end organs of entirely different structure may serve the same function (e.g. mechanoreceptors) and conversely, receptors of similar morphology may serve broadly different functions (e.g. free nerve endings). Hence, beyond the morphological distinction, nerve endings should be classified by using additional cues. Separation of distinct subgroups of sensory neurons based on their putative transmitter content and/or pharmacological characteristics may help to identify functionally different classes of sensory neurons grouped together on the basis of morphological techniques. Recent correlative electrophysiological and immunohistochemical findings support this notion. Further, sensitivity of mammalian primary afferent neurons to capsaicin is generally regarded as a characteristic trait of a population of mammalian primary sensory neurons (Williams et al.1989). In rats, perineural treatment with capsaicin - similar to that performed in this study - has been shown to result in a selective loss of C-fibre afferents which correspond to polymodal nociceptors and comprise up to 80% of cutaneous C-fibre afferents in rats (Jancsó and Lawson, 1987; Jancsó and Lawson, 1990a; Pini et al.1990; Pini and Lynn, 1991). Therefore, comparative studies on the cutaneous innervation patterns of skin samples obtained from intact animals and from animals in which the nerve serving the skin area was treated with capsaicin, respectively, may reveal the morphological and neurochemical characteristics of this particular group of cutaneous afferents.

In this study cutaneous afferent nerves were classified according to their sensitivity to capsaicin and their chemical phenotype. In addition, by making use of a combined morpho-functional approach, an attempt has been made to identify a particular population of sensory fibres which may be responsible for the initiation of the neurogenic inflammatory response.
Using antibodies to PGP 9.5, a general neuronal marker, we have demonstrated separate populations of afferent nerve fibres innervating the epidermis and the subepidermal layer of the rat hairy skin. A great number of PGP 9.5-immunoreactive nerve bundles and single fibres were localized in the subepidermal layer. Almost all of these fibres branch and penetrate into the epidermis. The great majority of these nerve fibres were not detectable by antibodies to neuropeptides. This alludes to the possibility that the great majority of afferent fibres innervating the epidermis as classical free nerve endings do not contain the major sensory neuropeptides investigated in the present study. This is somewhat surprising, since approximately 10-15 and 19-60% of DRG neurons which project to the skin contain SP and CGRP, respectively. These findings raise the possibility that neuropeptides synthesized in the perikaryon may not reach the most distant terminal portions of unmyelinated afferent axons or non-peptidergic C-fibres branch more extensively than peptidergic ones.
GAP 43-IR axonal structures comprised a significant proportion of PGP 9.5 positive nerve fibres; they appeared to be exclusively localized to cutaneous neuronal structures. Comparative study with PGP 9.5 indicated that approximately half of the intraepithelial nerve fibres and apparently all the subepidermal nerves expressed GAP 43-IR in the rat skin under normal conditions. GAP 43 expression has been strongly implicated in axonal development (Skene, and Willard, 1981a; Freeman et al.1986) and axonal regeneration (Skene, and Willard, 1981b; Tetzlaff et al.1989), but it is difficult to make such a correlation in adult rat skin. GAP 43 is known to be a major substrate for neuronal protein kinase C (Zwiers et al.1980) and its phosphorylation may be related to neurotransmitter release (Dekker et al.1989). It may influence transmembrane signal transduction since GAP 43 is also involved in the modulation of the phosphatidylinositol second-messenger pathway in synaptic membranes (Gispen, 1986). In adults, high basal levels of GAP 43 have been found in those neuronal systems which possess significant functional and morphological plasticity in the mature nervous system (Neve et al.1987; McGuire et al.1988). With regard to our results, it is tempting to speculate that the expression of GAP 43 in normal adult rat nerves might be related to a continuing capacity for neuronal regeneration and/or reorganization. It is interesting to note that these fibres also possess NGF receptor-immunoreactivity and NGF is known to stimulate the synthesis of GAP 43 (Van Hoof et al.1986; Karns et al.1987; Federoff et al.1988).
Our findings on the distribution of RT97-IR nerve fibres correspond to earlier observations

showing that RT97 immunoreactivity is characteristic for myelinated nerves. In accord with earlier findings we failed to demonstrate RT97-IR fibres in the epidermis. It seems that neurofilament-IR nerve fibres do not contribute to perivascular plexa, although RT97 immunoreactive fibres often run parallel with them.

In the rat hairy skin intraepidermal fibres are clearly stained with antisera against SP and CGRP. Intraepithelial nerve endings immunoreactive for SP or CGRP amounted to only small fractions of PGP 9.5 positive nerves. In comparison to PGP 9.5- or GAP 43-IR nerves, neither SP- nor CGRP-IR fibres had so numerous branches within the epidermis. In the subepidermal layer more CGRP-IR fibres could be observed than SP-IR fibres. This is in agreement with previous findings showing that although SP is often co-localized with CGRP, there are some thick fibres in peripheral tissues which do not contain SP, but express CGRP immunoreactivity. These findings corroborate previous observations indicating that CGRP is the major sensory neuropeptide in peripheral tissues (Kruger et al.1989; Silverman and Kruger, 1989). The results also disclose that only a relatively small proportion of cutaneous afferents contains SP. In addition to SP and CGRP, SOM-IR nerves could be demonstrated in the skin, too. These were present in the subepidermal layer whereas the epidermis was devoid of such fibres.

POSSIBLE STRUCTURAL CORRELATES OF THE NEUROGENIC INFLAMMATORY RESPONSE

Neurogenic inflammation is a fundamental manifestation of the efferent function of capsaicin-sensitive primary afferent neurons. This response can be elicited in the skin by noxious stimuli, in particular by irritant chemicals, tissue damage and by antidromic electrical stimulation of sensory nerves (Jancsó, 1959; Jancsó, 1960; Jancsó et al.1967; Jancsó, 1968; Jancsó et al.1968; Jancsó et al.1977; Jancsó et al.1980a; Jancsó, 1984; Tóth-Kása et al.1984; Jancsó et al.1985b; Chahl, 1988; Jancsó, 1994). Recent findings suggest that tachykinins and CGRP co-released from stimulated sensory nerve terminals may mediate the multiple effects associated with the neurogenic inflammatory response (Gamse and Saria, 1985; Gibbins et al.1985; Lundberg et al.1985; Franco-Cereceda et al.1987; Gamse et al.1987; Alvarez et al.1988; Brain and Williams, 1988; Brain and Williams, 1989; Louis et al.1989; O'Brien et al.1989; Siney and Brain, 1996). The increased permeability of small blood vessels is thought to be caused by the local release of SP from the sensory nerve endings. SP may act either directly on blood vessels or indirectly on the neighboring mast cells which, in turn, release vasoactive substances (e.g. histamine) that

mediate the vascular response. The experimental findings concerned with the mechanisms of neurogenic inflammation are not without contradictions. In addition, it appears that there are significant differences in the mechanism of the response among different tissues. In this study we have utilized a combined approach to visualize leaky blood vessels and peptidergic nerves by the vascular labelling technique and immunocytochemistry, respectively. This permitted the direct visualization of the topographic relationships of peptidergic nerves and leaky blood vessels in the rat skin. A close association of SP-IR nerves and colloidal silver labelled leaky blood vessels (their diameters correspond to the size of small postcapillary venules) was observed. Many SP-IR fibres were seen running parallel to labelled vessels. This could be especially well observed in favourable sections cut parallel to the skin surface.

The association of CGRP-immunoreactive nerve fibres with silver labelled blood vessels was less pronounced. Although more CGRP-IR fibres could be observed, not all of them were associated with leaky blood vessels. This is in agreement with earlier observations showing that CGRP is the most abundant peptide in sensory nerves. Practically all SP-IR fibres also show CGRP-IR, whereas numerous CGRP-IR fibres lack SP-IR. Although the principal neuropeptide involved in the permeability enhancing effect of antidromic nerve stimulation is SP, the significance of CGRP may be the potentiation of SP action by inhibiting a SP endopeptidase. (Le Greves et al.1985)

Our observation that most leaky blood vessels were in close association with peptidergic nerves suggests that these peptides, especially SP, may play a key role in the mechanism of neurogenic plasma extravasation (Skofitsch et al.1985; Barnes et al.1986; Church et al.1989). This suggestion is supported by recent findings showing the localization of NK1 receptors on postcapillary venules (Amann et al.1995; Inoue et al.1995).

VISUALIZATION OF CUTANEOUS INNERVATION TERRITORIES OF PERIPHERAL NERVES USING THE VASCULAR LABELLING TECHNIQUE

The double labelling technique offers a convenient and reliable method for the morphofunctional mapping of the innervation territories of capsaicin-sensitive nociceptive peptidergic afferent fibres. Most importantly, the results show that this new technique permits, for the first time, a direct, simultaneous and quantitative demonstration of individual and overlapping cutaneous innervation territories of rat hindlimb nerves. Existing techniques are incapable of the direct demonstration of overlapping cutaneous innervation territories. An indirect method which has been used to determine the extent of overlap

between adjacent innervation territories of peripheral nerves is rather innacurate; it involved measurements of the innervation areas of the two nerves on the ipsi- and contralateral sides, from which the possible overlap of the innervation territories on one side was calculated (Wiesenfeld-Hallin, 1988). However, this approach is inaccurate because the innervation territories of homologous peripheral nerves are not symmetrical and show considerable differences in the rat hindpaw.

Our findings indicate a variable, but significant degree of overlap between the innervation territories of rat hindlimb nerves. Interestingly, small overlapping innervation areas have been observed not only at the border of adjacent skin areas served by different peripheral nerves, but also distant to that border zone. This may reflect innervation areas of branching sensory axons running in separate peripheral nerves (Taylor, and Pierau, 1982).

These findings are of particular relevance with respect to investigations into the mechanisms of cutaneous nerve regeneration. The significance and extent of collateral sprouting in the reinnervation of a denervated skin area is somewhat controversial. Such sprouting varies with skin type and the methods used to asses nerve regeneration. Extensive collateral sprouting of intact nerves has been suggested by some anatomical (Kinnman et al.1992) and functional (Pertovaara, 1988) studies, whereas other studies utilizing the Evans blue extravasation (Jancsó, and Király, 1983b) and electrophysiological (Seltzer et al.1987) techniques disclosed only moderate collateral sprouting. An underestimation of the normally existing overlap of the innervation territories of cutaneous nerves might explain these conflicting results, at least in part. Because of the lack of an appropriate experimental method, this possibility could not be investigated satisfactorily previously. Hence, the double-labelling technique described here may help to resolve some of the conflicting findings. By showing a significant overlap between the innervation territories of peripheral nerves which supply the hindpaw skin, the present results support the notion that the existence of normally overlapping cutaneous innervation territories should be considered in studies where collateral nerve sprouting of intact nerves is examined (Wiesenfeld-Hallin, 1988). It is worthy of mention that, in our study, the greatest overlaps were found in areas where the most extensive collateral sprouting has been reported to occur, i.e. close to the ankle and just proximal to the third and fourth digits (Brenan et al.1988). We suggest, therefore, that after peripheral nerve injury the contribution of collateral nerve sprouting in the reinnervation of a denervated skin area may depend on the extent of the normally existing overlap between the innervation areas of the affected nerves.

CHEMICAL PHENOTYPES OF NERVE FIBRE POPULATIONS INNERVATING THE RAT HAIRY SKIN: SENSITIVITY TO CAPSAICIN

Capsaicin-sensitive primary afferent neurons form a significant division of the sensory nervous system. These neurons correspond to type B sensory ganglion cells giving rise almost exclusively to unmyelinated axons. Most of these neurons are peptidergic and are involved in the transmission of nociceptive impulses. In addition to this "classical" afferent function, they bear a unique "sensory efferent" or local regulatory function. Neurogenic plasma extravasation is a prominent manifestation of the activity of these neurons; hence visualization of this inflammatory response provides information on the functional state and the innervation territory of peripheral nerves. Using the technique of vascular labelling it is possible to distinguish between intact, innervated and denervated skin areas in histological sections. Perineural treatment with capsaicin results in a selective impairment of the function of this particular class of capsaicin-sensitive afferent nerves. Previous studies demonstrated profound changes in the morphology and chemistry of primary afferent neurons following perineural treatment with capsaicin. These involved depletion of peptides and degeneration of a substantial proportion of unmyelinated axons. However, changes which commence in the innervation of the skin area served by the affected nerve have not been studied. In the present study comparison of the innervation patterns of intact and chemodenervated skin areas permitted the determination of the different types of cutaneous nerve fibres affected by capsaicin.

Staining with antibodies to PGP 9.5 was used to study the general pattern of cutaneous innervation following perineural treatment with capsaicin. PGP 9.5-IR nerve fibres were abundant in the intact skin. They were observed in great numbers in the subepidermal layer either forming nerve bundles or occurring as single fibres. Many fibres course towards and penetrate the epidermis where they can be observed as branching single fibres reaching the most superficial layers of the epithelium. It has been revealed that the majority of intraepidermal nerve fibres can be demonstrated with this antibody but not with antibodies produced against different neuropeptides. Perineural treatment with capsaicin resulted in a rapid, striking reduction in the number of PGP 9.5-IR fibres in the epidermis. Changes in the number of PGP 9.5-IR fibres were less pronounced in the subepidermal layer, significantly decreased number of PGP 9.5-IR nerve bundles was combined with an increased frequency of single nerve fibres. These findings indicate that the overwhelming majority of intraepithelial nerve fibres are capsaicin-sensitive. The results may also indicate that perineural capsaicin-treatment resulted in a profound degeneration of at least the terminal portions of nerves innervating the epidermis. This loss of intraepithelial axons is

apparently permanent and possibly irreversible, since no recovery was seen during the time-span of the study (42 days). This is in accord with previous investigations showing a selective, substantial loss of unmyelinated axons from rat peripheral nerves six weeks after capsaicin-treatment. Loss of intraepidermal fibres may underlie the permanent functional disturbances brought about by perineural capsaicin. Staining with antibodies against GAP 43 yielded quantitative results essentially similar to those obtained for PGP 9.5. GAP 43 also seems to be a reliable and sensitive marker for capsaicin-sensitive epidermal nerves.

In the skin areas supplied by the capsaicin-treated nerve we observed a significant, almost complete loss of SP-immunoreactive nerve fibres in the epidermis. Only few SP-IR fibres were localized in the deeper dermal regions. These findings may explain the increase in chemical and thermal nociceptive thresholds and the abolition of the neurogenic inflammatory response. They also provide further support for the role of SP in the function of nociceptive afferent fibres. The presence of SP-containing nerve fibres in the deeper dermis may bear a significant role in the possible re-innervation of the superficial skin layers supplied by a damaged nerve.

Loss of CGRP-immunoreactive nerve fibres after perineural capsaicin-treatment was less pronounced although the number of both the epidermal single fibres and the nerve bundles in the subepidermal region were reduced. The moderate loss of these fibres is in line with previous findings showing a relative sparing of CGRP-IR neurons after capsaicin-treatment. This can be explained, at least in part, by the fact that CGRP is also contained in large light, capsaicin-insensitive DRG neurons.

SOM is thought to be present in capsaicin-sensitive small DRG neurons different from those containing SP. Our results support this notion by showing the occurrence of SP- but not SOM-IR nerves in the epidermis. It should be noted that changes, probably because of the relatively small number of SOM-positive cutaneous nerve fibres, were not significant.

RT97-IR nerve fibres have been reported to belong to capsaicin-insensitive primary afferent neurons. The quantitative results of the present study give further support to this view by showing that perineural capsaicin-treatment failed to affect the number of RT97-IR cutaneous nerves.

The quantitative immunohistochemical data on the effect of perineural capsaicin-treatment on different populations of cutaneous nerves revealed a substantial loss of mostly intraepidermal axons. SP- and CGRP-IR nerve fibres were significantly reduced in the subepidermal layer. This is in agreement with the notion that SP is the main mediator of the neurogenic inflammatory response. This is also consistent with the finding that leaky blood vessels demonstrated in this study with the vascular labelling technique are also situated in this layer. Loss of intraepidermal axons may well explain the elevation of chemical and heat

pain thresholds observed after perineural treatment with capsaicin. The quantitative data also point to the possibility that most polymodal nociceptor afferents which terminate in the epidermis do not contain the major sensory neuropeptides and are most probably non-peptidergic.

Taken together, these findings strongly suggest that the functional changes produced by perineural capsaicin may be accounted for by an irreversible degeneration of intraepidermal, mostly non-peptidergic axons and destruction of SP-IR subepidermal and epidermal fibres. The results also indicate that capsaicin may result in a distinct degeneration of the most distal, terminal portions of the afferent fibre. Further studies are needed to explore the possibility whether chemically damaged afferent axons are capable of notable functional regeneration.

SUMMARY

The morphology, distribution, chemical phenotypes and possible neuro-vascular relationships of axonal structures of the rat hairy skin were studied utilizing morpho-functional and immunohistochemical methods. The individual innervation territories of hindlimb nerves supplying the hairy skin of the rat hindpaw were identified by virtue of C-fibre afferent nerves to produce a neurogenic inflammatory response within their innervation areas upon orthodromic or antidromic stimulation. Leaky blood vessels were visualized histologically by means of the vascular labelling technique using colloidal silver as a marker. A new approach employing sequential administration of different markers was utilized to demonstrate directly overlapping innervation territories of hindlimb nerves. The vascular labelling technique also permitted investigations into the topographical relationship of axonal structures and the blood vessels which participate in the neurogenic inflammatory response. To identify capsaicin-sensitive sensory nerve fibres, the pattern of cutaneous innervation was also analyzed after topical capsaicin-treatment of the nerves supplying the skin area studied.

Localization of protein gene product 9.5- (PGP 9.5) immunoreactivity was used to reveal the general pattern of cutaneous innervation. In the dermis, nerve fibres were observed either as single axons or as bundles of nerve fibres. Specialized nerve endings, except perifollicular circular and pallisade nerve fibres, were not noted in the upper dermis. Occasionally, nerve fibres associated with arteries and veins were seen. In sections cut parallel to the skin surface silver-labelled small blood vessels were often accompanied by thin single axons. Many subepidermal fibres running parallel to the skin surface gave rise to

fibres penetrating the epidermis where only individual axons were seen. Nerve fibres immunoreactive to an antibody raised against growth associated protein 43 (GAP 43) were abundant both in the dermis and epidermis. Almost one third of all epidermal axons as revealed by PGP 9.5 immunohistochemistry showed GAP 43-IR.

Immunohistochemical localization of sensory neuropeptides revealed subpopulations of cutaneous sensory fibres. Calcitonin gene-related peptide (CGRP) proved to be the most abundant peptide in afferent nerves. CGRP-IR axons were localized both in the epidermis and in the dermis where they were often seen to be closely associated with silver-labelled blood vessels. Substance P- (SP) IR axons were less numerous but were frequently related to leaky vessels. In comparison to PGP 9.5-IR nerve fibres, the proportions of CGRP- and SP-IR axons reaching the epidermis were moderate. Somatostatin- (SOM) IR fibres were seen only in the dermis.

Perineural capsaicin-treatment resulted in a substantial but differential time-dependent reduction of axonal structures in the rat hairy skin. The most striking loss of nerve fibres was observed in the epidermis where the numbers of PGP 9.5-, GAP 43-, CGRP- and SP-IR axons dropped to 3-15 per cent of the control. GAP 43- and SP-IR axons proved to be the most vulnerable: there was an almost complete disappearance of these fibres from the epidermis. Silver labelled blood vessels were not observed in samples obtained from chemodenervated skin areas. These findings disclosed that the overwhelming majority of thin epidermal axons represent capsaicin-sensitive afferent nerves presumably of the polymodal nociceptor class. In addition, the findings indicate the existence of separate populations of capsaicin-sensitive afferent axons of different chemical phenotypes.

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REFERENCES

Ainsworth, A., P. Hall, P.D. Wall, G. Allt, M.L. MacKenzie, and Gibson (1981) Effects of capsaicin applied locally to adult peripheral nerve. II Anatomy and enzyme and peptide chemistry of peripheral nerve and spinal cord. Pain *11*:379-388.

Alvarez, F.J., C. Cervantes, I. Blasco, R. Villalba, R. Martin-Ezmurillo, J.M. Polak, and J. Rodrigo (1988) Presence of calcitonin gene-related peptide (CGRP) and substance P (SP) immunoreactivity in intraepidermal free nerve-endings of cat skin. Brain Res. *442*:391-395.

Alvarez, F.J., J. Rodrigo, T.M. Jessell, J. Dodd, and J.V. Priestly (1989) Morphology and distribution of primary afferent fibers expressing alpha-galactose extended oligosaccharides in the spinal cord and brain stem of the rat. Light microscopy. J.Neurocyt. *18*:611.

Amann, R., R. Schuligoi, P. Holzer, and J. Donnerer (1995) The non-peptide NK_1 receptor antagonist SR140333 produces long-lasting inhibition of neurogenic inflammation, but does not influence acute chemo- or thermonociception in rats. Naunyn Schmiedebergs Arch.Pharmacol. *352*:201-205.

Ambrus, A., G. Jancsó , and J. Fischer (1994) Tomato lectin: a new label for capsaicin-sensitive primary afferent neurons. In E. Driessche, J. Fischer, S. Beeckmans, and T.C. Bog-Hansen (eds): Lectins. Biology, Biochemistry, Clinical Biochemistry. Vol. 10. Hellerup, Denmark: Textop, pp. 3-6.

Anand, P., S.J. Gibson, F. Scaravilli, M.A. Blank, G.P. McGregor, O. Appenzeller, K. Dhital, J.M. Polak, and S.R. Bloom (1990) Studies of vasoactive intestinal polypeptide expression in injured peripheral neurons using capsaicin, sympathectomy and *mf* mutant rats. Neurosci.Lett. *118*:61-66.

Andres, K.H. (1961) Untersuchungen über den Feinbau von Spinalganglien. Z.Zellforsch. *55*:1-48.

Arbuckle, J.B. and R.J. Docherty (1995) Expression of tetrodotoxin-resistant sodium channels in capsaicin-sensitive dorsal root ganglion neurons of adult rats. Neurosci.Lett. *185*:70-73.

Arvidsson, J. and J. Ygge (1986) A quantitative study of the effects of neonatal capsaicin treatment and of subsequent peripheral nerve transsection in adult rat. Brain Res. *397*:130-136.

Averill, S., S.B. McMahon, D.O. Clary, L.F. Reichardt, and J.V. Priestley (1995) Immunocytochemical localization of trkA receptors in chemically identified subgroups of adult rat sensory neurons. Eur.J.Neurosci. *7*:1484-1494.

Baranowski, R., B. Lynn, and A. Pini (1986) The effects of locally applied capsaicin on conduction in cutaneous nerves in four mammalian species. Br.J.Pharmac. *89*:267-276.

Barnes, P.J., M.J. Brown, C.T. Dollery, R.W. Fuller, D.J. Heavey, and P.W. Ind (1986) Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. Br.J.Pharmacol. *88*:741-745.

Bevan, S. and P. Geppetti (1994) Protons: Small stimulants of capsaicin-sensitive sensory nerves. Trends Neurosci. *17*:509-512.

Bevan, S.J., I.F. James, H.P. Rang, J. Winter , and J.N. Wood (1987) The mechanism of action of capsaicin - a sensory neurotoxin. In P. Jenner (ed): Neurotoxins and their Pharmacological Implications: A Biological Council Symposium. New York: Raven Press, pp. 261-277.

Brain, S.D. and T.J. Williams (1988) Neuropharmacology of peptides in skin. Sem.Dermatol. *7*:278-283.

Brain, S.D. and T.J. Williams (1989) The interaction between the tachykinins and calcitonin gene-related peptide lead to the modulation of edema formation and blood-flow in rat skin. Br.J.Pharmacol. *97*:77-82.

Brain, S.D., H. Cambridge, S.R. Hughes, and P. Wilsoncroft (1992) Evidence that calcitonin gene-related peptide contributes to inflammation in the skin and joint. Ann.NY Acad.Sci. *657*:412-419.

Brain, S.D., T.J. Williams, J.R Trippins, H.R. Morris, and I. McIntyre (1985) Calcitonin-gene-related-peptide is a potent vasodilator. Nature *313*:54-56.

Brenan, A. (1986) Collateral reinnervation of skin by C-fibres following nerve injury in the rat. Brain Res. *385*:152-155.

Brenan, A., L. Jones, and N.R. Owain (1988) The demonstration of the cutaneous distribution of saphenous nerve C-fibres using a plasma extravasation technique in the normal rat and following nerve injury. J.Anat. *157*:57-66.

Buck, S.H. and T.F. Burks (1986) The neuropharmacology of capsaicin: review of some recent observations. Pharmalogical Reviews *38*:179-226.

Bucsics, A., D. Sutter, G. Jancsó, and F. Lembeck (1988) Quantitative assay of capsaicin-sensitive thiamine monophosphatase and β-glycerophosphatase activity in rodent spinal cord. J.Neurosci.Meth. *24*:155-162.

Cambridge, H. and S.D. Brain (1992) Calcitonin gene-related peptide increases blood flow and potentiates plasma protein extravasation in the rat knee joint. Br.J.Pharmacol. *106*:746-750.

Carr, P.A, T. Yamamoto, and J.I. Nagy (1990) Calcitonin gene-related peptide in primary afferent neurons of rat: Co-existence with fluoride-resistant acid phosphatase and depletion by neonatal capsaicin. Neuroscience *36*:751-760.

Carr, P.A. and J.I. Nagy (1993) Emerging relationships between cytochemical properties and sensory modality transmission in primary sensory neurons. Brain Res.Bull. *30*:209-219.

Carr, P.A., T. Yamamoto, G. Karmy, and K.G. Baimbridge (1989) Parvalbumin is highly colocalized with calbindin D28K and rarely with calcitonin gene-related peptide in dorsal-root ganglia neurons of the rat. Brain Res. *497*:163-170.

Carr, P.A., T. Yamamoto, G. Karmy, K.G. Baimbridge, and J.I. Nagy (1990) Analysis of parvalbumin and calbindin d28k-immuoreactive neurons in dorsal root ganglia of rat in relation to their cytochrome-oxidase and carbonic anhydrase content. Neurosci. *33*:363-371.

Caulfield, J.P., S. El-Lati, G. Thomas, and M.K. Church (1990) Dissociated human foreskin mast cells degranulate in response to anti-IgE and substance P. Lab.Invest. *63*:502-510.

Ch'ng, J.L.C., N.D. Christofides, P. Amand, S.J. Gibson, Y.S. Allen, H.C Su, and K. Tatemoto (1985) Distribution of galanin immunoreactivity in the central nervous system and the responses of galanin-containing neuronal pathways to injury. Neuroscience *16*:343-354.

Chahl, L.A. (1988) Antidromic vasodilatation and neurogenic inflammation. Pharm.Ther. *37*:275-300.

Chiba, T., S. Masuko, and H. Kawanso (1986) Correlation of mitochondrial swelling after capsaicin treatment and substance p and somatostatin immunoreactivity in small neurons of dorsal root ganglion in the rat. Neurosci.Letts. *64*:311-316.

Chung, J.M., K.H. Lee, Y. Hori, and W.D. Willis (1985a) Effects of capsaicin applied to a peripheral nerve on the responses of primate spinothalamic tract cells. Brain Res. *329*:27-38.

Chung, K., R.J. Schwen, and R.E. Coggeshall (1985b) Ureteral axon damage following subcutaneous administration of capsaicin in adult rats. Neurosci.Letts. *53*:221-226.

Church, M.K., M.A. Lowman, P.H. Rees, and R.C. Benyon (1989) Mast-cells, neuropeptides and inflammation. Agents and Actions *27*:9-16.

Dekker, L.V., P.N.E. DeGraan, D.H.G. Versteeg, A.B. Oestreicher, and W.H. Gispen (1989) Phosphorylation of B-50 (GAP43) is correlated with neurotransmitter release in rat hippocampal slices. J.Neurochem. *52*:24-30.

Dinh, T.T. and S. Ritter (1985) Capsaicin induces neuronal degeneration in the brain and spinal cord of adult rats. Soc.Neurosci.Abstr. *11*:349.

Doughty, S.E., M.E. Atkinson, and S.A.S. Shehab (1991) A quantitative study of neuropeptide immunoreactive cell bodies of primary afferent sensory neurons following rat sciatic nerve peripheral axotomy. Regul.Pept. *35*:59-72.

Dray, A. (1992) Mechanism of action of capsaicin-like molecules on sensory neurons. Life Sci. *51*:1759-1765.

Duce, I., and P. Keen (1977) An ultrastructural classification of the neuronal cell bodies of the rat dorsal root ganglion using zinc iodine-osmium impregnation. Cell Tissue Res. *185*:263-277.

Dux, M. and G. Jancsó (1994) A new technique for the direct demonstration of overlapping cutaneous innervation territories of peptidergic C-fibre afferents of rat hindlimb nerves. J.Neurosci.Meth. *55*:47-52.

Dux, M., G. Jancso, H. Sann, and F.K. Pierau (1996) Inhibition of the neurogenic inflammatory response by lidocaine in rat skin. Inflamm.Res. *45*:10-13.

Ekblom, A., T. Lundeberg, and C.-F. Wahlgren (1993) Influence of calcitonin gene-related peptide on histamine- and substance P-induced itch, flare and weal in humans. Skin Pharmacol. *6*:215-222.

Federoff, H.J., E. Grabczyk, and M.C. Fishman (1988) Dual regulation of GAP-43 gene expression by nerve growth factor and glucocorticoids. J. Biol. Chem. *263*:19290-19295.

Feigin, A.M., E.V. Aronov, B.P. Bryant, J.H. Teeter, and J.G. Brand (1995) Capsaicin and its analogs induce ion channels in planar lipid bilayers. Neuroreport *6*:2134-2136.

Fitzgerald, M. (1982) Alterations in the ipsi- and contralateral afferent inputs of dorsal horn cells produced by capsaicin treatment of one sciatic nerve in the rat. Brain Res. *248*:92-107.

Fitzgerald, M. and C.J. Woolf (1982) The time course and specificity of the changes in the behavioural and dorsal horn responses to noxious stimuli following peripheral nerve capsaicin tretament in the rat. Neuroscience *7*:2051-2056.

Foreman, J., and C. Jordan (1983) Histamine release and vascular changes induced by neuropeptides. Agents Actions *13*:105-116.

Foster, R.W., and A.G. Ramage (1981) The action of some chemical irritants on somatosensory receptors of the cat. Neuropharm. *20*:191-198.

Franco-Cereceda, A., H. Henke, J.M. Lundberg, J.B. Petermann, T. Hökfelt, and J.A. Fischer (1987) Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. Peptides *8*:399-410.

Freeman, J.A., S. Bock, M. Deaton, B. McGuire, J.J. Norden, and G.J. Snipes (1986) Axonal and glial proteins associated with development and response to injury in the rat and goldfish optic nerve. Exp.Brain.Res. *13*:34-47.

Fundin, B.T., J. Arvidsson, and F.L. Rice (1995) Innervation of nonmystacial vibrissae in the adult rat. J.Comp.Neurol. *357*:501-512.

Gamse, R. and A. Saria (1985) Potentiation of tachykinin induced plasma protein extravasation by calcitonin gene-related peptide. Eur.J.Pharm. *114*:61-66.

Gamse, R., M. Posch, A. Saria, and G. Jancsó (1987) Several mediators appear to interact in neurogenic inflammation. Acta Physiol.Hung. *69*:343-354.

Gamse, R., P. Holzer, and F. Lembeck (1980) Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br.J.Pharmacol. *68*:207-213.

Gamse, R., U. Petsche, F. Lembeck, and G. Jancsó (1982) Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Res. *239*:447-462.

Garry, M.G., K.E. Miller, and V.S. Seybold (1989) Lumbar dorsal root ganglia of the cat: A quantitative study of peptide immunoreactivity and cell size. J.Comp.Neurol. *284*:36-47.

Gibbins, I.L., J.B. Furness, M. Costa, I. MacIntyre, C.J. Hillyard, and S. Girgis (1985) Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea-pigs. Neurosci.Letts. *57*:125-130.

Gibson, S.J., G. McGregor, S.R. Bloom, J.M. Polak, and P.D. Wall (1982) Local application of capsaicin to one sciatic nerve of the adult rat induces a marked depletion in the peptide content of the lumbar dorsal horn. Neuroscience *7*:3153-3162.

Gispen, W.H. (1986) Phosphoprotein B-50 and phosphoinositides in brain synaptic plasma membranes: a possible feedback relationship. Biochem. Soc. Trans. UK *14*:163-165.

Goso, C., G. Piovacari, and A. Szallasi (1993) Resiniferatoxin-induced loss of vanilloid receptors is reversible in the urinary bladder but not in the spinal cord of the rat. Neurosci.Lett. *162*:197-200.

Harper, A.A. and S.N. Lawson (1985) Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurones. J.Physiol. *359*:31-46.

Harti, G., K.A. Sharkey, and Fr.-K. Pierau (1989) Effects of capsaicin in rat and pigeon on peripheral nerves containing substance P and calcitonin gene-related peptide. Cell.Tiss.Res. *256*:465-474.

Helme, R.D., G.M. Korschorke, and M. Zimmerman (1986) Immunoreactive substance P release from skin nerves in the rat by noxious thermal stimulation. Neurosci.Lett. *63*:295-299.

Hiura, A. and H. Ishizuka (1994) Early morphological changes of primary afferent neurons and their processes in newborn mice after treatment with capsaicin. Exp.Brain Res. *101*:203-215.

Hiura, A. and H. Ishizuka (1995) Central terminals of capsaicin-sensitive primary afferent make synaptic contacts with neuronal soma in the mouse substantia gelatinosa. Experientia *51*:551-555.

Hiura, A., and I. Sakamoto (1987) Quantitative estimation of the effects of capsaicin on the mouse primary sensory neurons. Neurosci.Lett. *76*:101-106.

Hökfelt, T., J.M. Lundberg, L. Terenius, G. Jancsó, and J. Kimmel (1981) Avian pancreatic polypeptide (APP) immunoreactive neurons in the spinal cord and spinal trigeminal nucleus. Peptides *2*:81-87.

Hökfelt, T., L. Skirboll, C.-J. Dalsgaard, O. Johansson, J.M. Lundberg, G. Norell , and G. Jancsó (1982) Peptide neurons in the spinal cord with special reference to descending systems. In B. Sjölund and A. Björklund (eds): Brain Stem Control of Spinal Mechanisms. Amsterdam: Elsevier, pp. 89-117.

Hökfelt, T., L. Skirboll, J.M. Lundberg, C.-J. Dalsgaard, O. Johansson, B. Pernow , and G. Jancsó (1983) Neuropeptides and pain pathways. In J.J. Bonica (ed): Advances in Pain Research and Therapy, Vol. 5. New York: Raven Press, pp. 227-246.

Hökfelt, T., R. Elde, O. Johansson, R. Luft, G. Nilsson, and A. Arimura (1976) Immunohistochemical evidence for separate populations of somatostatin-containing and substance P containing primary afferent neurons in the rat. Neuroscience *1*:131-136.

Hökfelt, T., Z. Wiesenfeld-Hallin, M.J. Villar, and C. Molander (1987) Increase of galanin-like immunoreactivity in rat dorsal root ganglion cells after peripheral axotomy. Neurosci.Lett. *83*:217-220.

Holford, L.C., P. Case, and S.N. Lawson (1994) Substance P, neurofilament, peripherin and SSEA4 immunocytochemistry of human dorsal root ganglion neurons obtained from post-mortem tissue: A quantitative morphometric analysis. J.Neurocytol. *23*:577-589.

Holzer, P. (1988) Local effector functions of capsaicin-sensitive sensory nerve-endings - involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience *24*:739-768.

Holzer, P. (1991) Capsaicin: Cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol.Rev. *43*:143-201.

Hu-Tsai, M., C. Woolf, and J. Winter (1996) Influence of inflammation or disconnection from peripheral target tissue on the capsaicin sensitivity of rat dorsal root ganglion sensory neurones. Neurosci.Lett. *203*:119-122.

Ichikawa, H., T. Deguchi, T. Nakago, D.M. Jacobowitz, and T. Sugimoto (1994) Parvalbumin, calretinin and carbonic anhydrase in the trigeminal and spinal primary neurons of the rat. Brain Res. *655*:241-245.

Inoue, H., N. Nagata, and Y. Koshihara (1995) Involvement of substance P as a mediator in capsaicin-induced mouse ear oedema. Inflamm.Res. *44*:470-474.

Inoue, H., N. Nagata, and Y. Koshihara (1996) Involvement of tachykinin receptors in oedema formation and plasma extravasation induced by substance P, neurokinin A, and neurokinin B in mouse ear. Inflamm.Res. *45*:316-323.

Jancsó, G. (1982) Neonatal capsaicin treatment impairs neurogenic inflammatory response, chemical and heat pain sensitivity in a dose dependent manner. Neuroscience *7, Suppl.*:S102.

Jancsó, G. (1984) Sensory nerves as modulators of inflammatory reactions. In L.A. Chahl, J. Szolcsányi, and F. Lembeck (eds): Antidromic Vasodilatation and Neurogenic Inflammation. Budapest: Akademiai Kiado, pp. 207-222.

Jancsó, G. (1992) Pathobiological reactions of C-fibre primary sensory neurones to peripheral nerve injury. Exp.Physiol. *77*:405-431.

Jancsó, G. (1994) Histamine, capsaicin and neurogenic inflammation. A historical note on the contribution of Miklós (Nicholas) Jancsó (1903-1966) to sensory pharmacology. In I. Berczi and J. Szelényi (eds): Advances in Psychoneuroimmunology. New York: Plenum Press, pp. 17-23.

Jancsó, G. and A. Ambrus (1994) Capsaicin-sensitivity of primary sensory neurones and its regulation. In J.M. Besson, G. Guilbaud, and H. Ollat (eds): Peripheral neurons in nociception: physio-pharmacological aspects. Paris: John Libbey Eurotext, pp. 71-87.

Jancsó, G. and B. Lynn (1987) Possible use of capsaicin in pain therapy. Clin.J.Pain *3*:123-126.

Jancsó, G. and E. Király (1980) Distribution of chemosensitive primary sensory afferents in the central nervous system of the rat. J.Comp.Neurol. *190*:781-792.

Jancsó, G. and E. Király (1981) Sensory neurotoxins: chemically induced selective destruction of primary sensory neurons. Brain Res. *210*:83-89.

Jancsó, G. and E. Király (1983a) Regenerative properties of peptide-containing cutaneous sensory nerves. Irish Journal of Medical science *152,suppl.1.*:35-36.

Jancsó, G. and E. Király (1983b) Cutaneous nerve regeneration in the rat: reinnervation of the denervated skin by regenerative but not by collateral sprouting. Neurosci.Lett. *36*:133-137.

Jancsó, G. and E. Király (1984) Regeneration of peptidergic sensory nerves in the rat skin. Acta Physiol.Hung. *63*:239-240.

Jancsó, G. and G. Such (1983) Effects of capsaicin applied perineurally to the vagus nerve on cardiovascular and respiratory functions in the cat. J.Physiol.(Lond.) *341*:359-370.

Jancsó, G. and S.N. Lawson (1987) Perineural capsaicin treatment of the sciatic nerve in adult rat causes transganglionic changes in the spinal cord dorsal horn. J.Physiol.(Lond.) *394*:109P.

Jancsó, G. and S.N. Lawson (1988) Ganglionic changes associated with transganglionic degeneration of capsaicin-sensitive primary sensory afferents: a quantitative morphometric and immunohistochemical study. Reg.Peptides *22*:97.

Jancsó, G. and S.N. Lawson (1990a) Transganglionic degeneration of capsaicin-sensitive C-fiber afferent terminals. Neurosci. *39*:501-511.

Jancsó, G. and S.N. Lawson (1990b) Transganglionic degeneration of capsaicin-sensitive C-fiber primary afferent terminals. Neuroscience *39*:501-511.

Jancsó, G., A. Juhász, M. Dux, P. Sántha, and F. Domoki (1997) Axotomy prevents capsaicin-induced sensory ganglion cell degeneration. Primary Sensory Neuron (in press)

Jancsó, G., E. Király, and A. Jancsó-Gábor (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature *270*:741-743.

Jancsó, G., E. Király, and A. Jancsó-Gábor (1980a) Chemosensitive pain fibers and inflammation. Int.J.Tiss.Reac. *2*:57-66.

Jancsó, G., E. Király, and A. Jancsó-Gábor (1980b) Direct evidence for an axonal site of action of capsaicin. Naunyn Schmiedeberg's Arch.Pharmacol. *313*:91-94.

Jancsó, G., E. Király, F. Joo, G. Such, and A. Nagy (1985c) Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in the adult rat. Neurosci.Lett. *59*:209-214.

Jancsó, G., E. Király, G. Such, F. Joó, and A. Nagy (1987a) Neurotoxic effect of capsaicin in mammals. Acta Physiol.Hung. *69*:295-314.

Jancsó, G., F.J. Obál, I. Tóth-Kása, M. Katona, and S. Husz (1985b) The modulation of cutaneous inflammatory reactions by peptide-containing sensory nerves. Int.J.Tiss.Reac. *7*:449-457.

Jancsó, G., G. Sávay, and E. Király (1978) Appearance of histochemically detectable ionic calcium in degenerating primary sensory neurons. Acta Histochem. *62*:165-169.

Jancsó, G., G. Such , and C. Rödel (1987c) A new approach to selective regional analgesia. In F. Sicuteri, L. Vecchiet, and M. Fanciullacci (eds): Trends in Cluster Headache. Amsterdam, New York, Oxford: Elsevier Science Publishers B. V., pp. 59-68.

Jancsó, G., G. Such , and C. Rödel (1987b) A new approach to selective regional analgesia. In F. Sicuteri, L. Vecchiet, and M. Fanciullacci (eds): Trends in Cluster Headache. Amsterdam, New York, Oxford: Elsevier Science Publishers B. V., pp. 59-68.

Jancsó, G., M. Ferencsik, G. Such, E. Király, A. Nagy , and M. Bujdoso (1985a) Morphological effects of capsaicin nad its analogues in newborn and adult mammals. In R. Hankanson and F. Sundler (eds): Tachykinin Antagonists. Amsterdam, New York, Oxford: Elsevier, pp. 35-44.

Jancsó, G., S. Karcsú, E. Király, A. Szebeni, L. Tóth, E. Bácsy, F. Joó, and . Párducz (1984) Neurotoxin induced nerve cell degeneration: possible involvement of calcium. Brain Res. *295*:211-216.

Jancsó, G., T. Hökfelt, J.M. Lundberg, E. Király, N. Halasz, G. Nilsson, L. Terenius, J. Rehfeld, H. Steinbusch, A. Verhofstad, R. Elde, S. Said, and M. Brown (1981) Immunohistochemical studies on the effect of capsaicin on spinal and medullary peptide and monoamine neurons using antisera to substance P, gastrin/CCK, somatostatin, VIP, enkephalin, neurotensin and 5-hydroxy-tryptamine. J.Neurocyt. *10*:963-980.

Jancsó, M. (1947) Histamine as a physiological activator of the reticulo-endothelial system. Nature *160*:227-228.

Jancsó, M. (1959) A kémiai fájdalomérzés tartós kikapcsolása farmakológiai úton és a neurogén gyulladás problémája. MTA Biol.Orv.Oszt.Közl. *10*:261-283.

Jancsó, N. (1955) Speicherung. Stoffanreichung im Retikuloendothel und in der Niere. Budapest: Akadémiai Kiadó.

Jancsó, N. (1960) Role of the nerve terminals in the mechanism of inflammatory reactions. Bull.Millard Fillmore Hosp.(Buffalo,N.Y.) *7*:53-77.

Jancsó, N. (1968) Desensitization with capsaicin as a tool for studying the function of pain receptors. In R.K.S. Lim (ed): Pharmacology of pain. (Proceedings of the 3 rd Int. Pharmacol. Meeting 1966). Oxford: Pergamon Press, pp. 33-55.

Jancsó, N. and A. Jancsó-Gábor (1959) Dauerausschaltung der chemischen Schmerzempfindlichkeit durch Capsaicin. Arch.Exp.Path.Pharmak. *236*:142.

Jancsó, N., A. Jancsó-Gábor, and J. Szolcsányi (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br.J.Pharmacol.Chemother. *31*:138-151.

Jancsó, N., A. Jancsó-Gábor, and J. Szolcsányi (1968) The role of the sensory nerve endings in neurogenic imflammation induced in human skin and in the eye and paw of the rat. Br.J.Pharmacol.Chemother. *33*:32-41.

Jancsó-Gábor, A., and J. Szolcsányi (1969) The mechanism of neurogenic inflammation. In A. Bertelli, and J.C. Houck (eds): Inflammation biochemistry and drug interaction. Amsterdam: Excerpta Medica Foundation, pp. 210-217.

Jancsó-Gábor, A., and J. Szolcsányi (1972) Neurogenic inflammatory responses. J.Dent.Res.Suppl. to No. 2 *51*:264-269.

Jancsó-Gábor, A., J. Szolcsányi, and N. Jancsó (1967) A simple method for measuring the amount of azovan blue exuded into the skin in response to an inflammatory stimulus. J.Pharm.Pharmacol. *19*:486-487.

Jänig, W. and S.J.W. Lisney (1989) Small diameter myelinated afferents produce vasodilatation but not plasma extravasation in rat skin. J.Physiol.(Lond.) *415*:477-486.

Johnson, P.C., J.L. Beggs, A.G. Olafsen, and C.J. Watkins (1994) Unmyelinated nerve fiber estimation by immunocytochemistry. Correlation with electron microscopy. J.Neuropathol.Exp.Neurol. *53*:176-183.

Joó, F., J. Szolcsányi, and A. Jancsó-Gábor (1969) Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long-lasting sensory disturbance induced by capsaicin. Life Sci. *8*:621-626.

Joris, I., U. DeGirolami, K. Wortham, and G. Majno (1982) Vascular labelling with Monastral Blue B. Stain Techn. *57*:177-183.

Ju, G., Th. Hökfelt, E. Brodin, J. Fahrenkrug, J.A. Fischer, P. Frey, R.P. Elde, and J.C. Brown (1987) Primary sensory neurons of the rat showing calcitonin gene related peptide immunoreactivity and their relation to substance P-, somatostatin-, galanin-,vasoactive intestinal polypeptide- and cholecystokinin - immonoreactive ganglion cells. Cell Tissue Res. *247*:417-431.

Kalina, M., and M. Wolman (1970) Correlative histochemical and morphological study on the maturation of sensory ganglion cells in the rat. Histochemie *22*:100-108.

Kandel, E.R., J.H. Schwartz , and T.M. Jessel (1991) Principles of neural science. New York: Elsevier.

Karns, L.R., J.A. Freeman, and M.C. Fishman (1987) Cloning of complementary DNA for GAP-43, a neuronal growth-related protein. Science *236*:597-600.

Kashiba, H., K. Noguchi, Y. Ueda, and E. Senba (1994) Neuropeptide Y and galanin are coexpressed in rat large type A sensory neurons after peripheral transection. Peptides *15*:411-416.

Kashiba, H., Y. Ueda, and E. Senba (1996) Coexpression of preprotachykinin-A, α-calcitonin gene-related peptide, somatostatin, and neurotrophin receptor family messenger RNAs in rat dorsal root ganglion neurons. Neuroscience *70*:179-189.

Kawatani, M., J. Nagel, and W.C. de Groat (1986) Identification of neuropeptides in pelvic and pudendal nerves afferent pathways to the sacral spinal cord of the cat. J.Comp.Neurol. *249*:117-132.

Kenins, P. (1981) Identification of the unmyelinated sensory nerves which evoke plasma extravasation in response to antidromic stimulation. Neurosci.Letts. *25*:137-141.

Kinnman, E., H. Aldskogius, O. Johansson, and Z. Wiesenfeld-Hallin (1992) Collateral reinnervation and expansive regenerative reinnervation by sensory axons into "foreign" denervated skin: An immunohistochemical study in the rat. Exp.Brain Res. *91*:61-72.

Knyihár-Csillik, E., A. Bezzegh, S. Boti, and B. Csillik (1986) Thiamine monophosphatase: A genuine marker for transganglionic regulation of primary sensory neurons. J.Histochem.Cytochem. *34*:363-371.

Knyihár-Csillik, E., and B. Csillik (1981) Histochemistry of the primary nociceptive neuron. Prog.Histochem.Cytochem. *14*:1-137.

Konietzny, F., and H. Hensel (1983) The effect of capsaicin on the response characteristics of human C-polymodal nociceptors. J.Therm.Biol. *8*:213-215.

Kowalski, M.L., and M.A. Kaliner (1988) Neurogenic inflammation, vascular permeability, and mast cells. J.Immunol. *140*:3905-3911.

Kruger, L. (1988) Morphological features of thin sensory afferent fibres: a new interpretation of nociceptor function. Progress in Brain Research *74*:253-257.

Kruger, L., J.D. Silverman, P.W. Mantyh, C. Sternini, and N.C. Brecha (1989) Peripheral patterns of calcitonin gene-related peptide general somatic sensory innervation - cutaneous and deep terminations. J.Comp.Neurol. *280*:291-302.

Kuraishi, Y., M. Minami, and M. Satoh (1991) Serotonin, but neither noradrenaline nor GABA, inhibits capsaicin-evoked release of immunoreactive somatostatin from slices of rat spinal cord. Neurosci.Res. *9*:238-245.

Lawson, S.N. (1979) The postnatal development of large light and small dark neurons in mouse dorsal root ganglia: A statistical analysis of cell numbers and size. J.Neurocyt. *8*:275-294.

Lawson, S.N. (1981) Dorsal root ganglion neurones and dorsal root: Effects of neonatal capsaicin. Spinal cord sensation

Lawson, S.N. (1987a) The morphological consequences of neonatal treatment with capsaicin on primary afferent neurones in adult rats. Acta Physiol.Hung. *69*:315-321.

Lawson, S.N. (1987b) Immunocytochemically defined populations of dorsal root ganglion neurons remaining in the rat after neonatal capsaicin. In L.M. Pubols and B. Sessle (eds): Effects of Injury on Trigeminal and Spinal Somatosensory Systems. New York: Alan R.Liss, pp. 125-132.

Lawson, S.N. (1992) Morphological and biochemical cell types of sensory neurones. In S.A. Scott (ed): Sensory neurones: diversity, development and plasticity. New York: Oxford University Press, pp. 27-59.

Lawson, S.N. and P.J. Waddell (1991) Soma neurofilament immunoreactivity is related to cell size and fibre conduction velocity in rat primary sensory neurons. J.Physiol.(Lond.) *435*:41-63.

Lawson, S.N., A.A. Harper, E.I. Harper, J.A. Garson, and B.H. Anderton (1984) A monoclonal antibody against neurofilament protein specifically labels a subpopulation of rat sensory neurons. J.Comp.Neurol. *228*:263-272.

Lawson, S.N., K.T. Caddy, and T.J. Biscoe (1974) Development of rat dorsal root ganglion neurones. Studies of cell birthdays and changes in mean cell diameter. Cell Tiss.Res. *153*:399-413.

Lawson, S.N., M.J. Perry, E. Prabhakar, and P.W. Mccarthy (1993) Primary sensory neurones: Neurofilament, neuropeptides, and conduction velocity. Brain Res.Bull. *30*:239-243.

Lawson, S.N., P.W. Mccarthy, and E. Prabhakar (1996) Electrophysiological properties of neurones with CGRP-like immunoreactivity in rat dorsal root ganglia. J.Comp.Neurol. *365*:355-366.

Le Greves, P., F. Nyberg, L. Terenius, and T.H. Hökfelt (1985) Calcitonin gene-related peptide is a potent inhibitor of substance P degradation. Eur.J.Pharm. *115*:309-311.

Lee, Y., Y. Kawai, S. Shiosaka, K. Takami, H. Kiyama, and C.J. Hillyard (1985) Coexistence of calcitonin gene-related peptide and substance P-like peptide in single cells of the trigeminal ganglion of rat: Immunohistochemical analysis. Brain Res. *330*:194-196.

Lembeck, F. and P. Holzer (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn Schmiedeberg's Arch.Pharmacol. *310*:175-183.

Lembeck, F., J. Donnerer, and L. Barthó (1982) Inhibition of neurogenic vasodilatation and plasma extravasation by substance P antagonists, somatostatin and (D-Met 2,Pro 5) enkephalinamide. Europ.J.Pharmacol. *85*:171-176.

Li, J.-Y., X.-E. Hou, and A. Dahlström (1995) GAP-43 and its relation to autonomic and sensory neurons in sciatic nerve and gastrocnemius muscle in the rat. J.Auton.Nerv.Syst. *50*:299-309.

Lieberman, A.R. (1976) Sensory ganglia. In D.M. Landon (ed): The peripheral nerve. London: Chapman and Hall, pp. 188-278.

Louis, S.M., A. Jamieson, N.J.W. Russel, and G.J. Dockray (1989) The role of substance P and calcitonin gene-related peptide in neurogenic plasma extravasation and vasodilatation in the rat. Neuroscience *32*:581-586.

Lundberg, J.M., A. Saria, E. Theodorsson-Norheim, E. Brodin, X.Y. Hua, C.-R. Martling, R. Gamse , and T. Hökfelt (1985) Multiple tachykinins in capsaicin-sensitive afferents: Occurence, release and biological effects with special reference to irritation of the airways. In R. Hakanson and F. Sundler (eds): Tachykinin Antagonists. Amsterdam: Elsevier, pp. 159-169.

Lundberg, J.M., A. Saria, S. Rosell, and K. Folkers (1985) Substance P antagonist inhibits heat-induced oedema in the rat skin. Acta Physiol.Scand. *120*:145-146.

Lynn, B. (1990) Capsaicin: actions on nociceptive C-fibres and therapeutic potential. Pain *41*:61-69.

Lynn, B. (1992) Capsaicin: Actions on C-fibre afferents that may be involved in itch. Skin Pharmacol. *5*:9-13.

Lynn, B. and J. Shakhanbeh (1988) Substance P content of the skin, neurogenic inflammation and numbers of C-fibres following capsaicin application to a cutaneous nerve in the rabbit. Neurosci. *24*:769-775.

Lynn, B., A. Pini , and R. Baranowski (1987) Injury of somatosensory afferents by capsaicin: selectivity and failure to regenerate. In L.M. Pubols, and B. Sessle (eds): Effects of injury on trigeminal and spinal somatosensory systems. New York: Alan R. Liss, pp. 115-124.

Lynn, B., S. Schütterle, and F.K. Pierau (1996) The vasodilator component of neurogenic inflammation is caused by a special subclass of heat-sensitive nociceptors in the skin of the pig. J.Physiol.(Lond.) *494*:587-593.

Maggi, C.A. (1991) Capsaicin and primary afferent neurons: From basic science to human therapy. J.Auton.Nerv.Syst. *33*:1-14.

Maggi, C.A. and A. Meli (1988) The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen.Pharmac. *19*:1-43.

Majno, G., G.E. Palade, and G.I. Schoefl (1961) Studies on Inflammation. II. The site of action of histamine and serotonin along the vascular tree: a topographic study. J.Biophys.Biochem.Cytol. *11*:607-625.

Marsh, S.J., C.E. Stansfeld, D.A. Brown, R. Davey, and D. McCarthy (1987) The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro. Neuroscience *23*:275-289.

Matzner, O., and M. Devor (1993) Method for distinguishing between drug action on impulse propagation versus impulse generation. J.Neurosci.Methods *49*:23-31.

Mccarthy, P.W., and S.N. Lawson (1989) Cell type and conduction velocity of rat primary sensory neurons with substance P-like immunoreactivity. Neuroscience *28*:745-753.

Mccarthy, P.W., and S.N. Lawson (1990) Cell type and conduction-velocity of rat primary sensory neurons with calcitonin gene-related peptide-like immunoreactivity. Neuroscience *34*:623-632.

McGuire, C.B., G.J. Snipes, and J.J. Norden (1988) Light-microscopic immunolocalization of the growth- and plasticity-associated protein GAP-43 in the developing rat brain. Dev. Brain Res. *41*:277-291.

McMahon, S.B., P.D. Wall, S.L. Granum, and K.E. Webster (1984) The effects of capsaicin applied to peripheral nerves on responses of a group of lamina I cells in adult rats. J.Comp.Neurol. *227*:393-400.

Mense, S. (1990) Structure-function relationships in identified afferent neurones. Anat.Embryol. *181*:19-30.

Miller, M.S., S.H. Buck, I.G. Lipes, H.I. Yamamura, and T.F. Burkes (1982) Regulation of substance P by nerve growth factor: disruption by capsaicin. Brain Res. *250*:193-196.

Mio, M., K. Izushi, and K. Tasaka (1991) Substance P-induced histamine release from rat peritoneal mast cells and its inhibition by antiallergic agents and calmodulin inhibitors. Immunopharmacology *22*:59-66.

Molliver, D.C., M.J. Radeke, S.C. Feinstein, and W.D. Snider (1995) Presence or absence of TrkA protein distinguishes subsets of small sensory neurons with unique cytochemical characteristics and dorsal horn projections. J.Comp.Neurol. *361*:404-416.

Mulderry, P.K., M.A. Ghatei, R.A. Spokes, P.M. Jones, A.M. Pierson, Q.A. Hamid, S. Kanse, S.G. Amara, J.M. Burrin, and S. Legon (1988) Differantial expression of alpha-CGRP and beta-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. Neuroscience *25*:195-205.

Nagao, M., H. Kamo, I. Akiguchi, and J. Kimura (1992) Soybean agglutinin binds commonly to a subpopulation of small-diameter neurons in dorsal root ganglion, vascular endothelium and microglia in human spinal cord. Neurosci.Lett. *142*:131-134.

Nagy, J.I. (1982) Capsaicin: A chemical probe for sensory neuron mechanism. In L.L. Iversen, S.D. Iversen, and S.H. Snyder (eds): Handbook of Psychopharmacology, Vol. 15. New York: Plenum Press, pp. 185-235.

Nagy, J.I. and S.P. Hunt (1982) Fluoride-resistant acid phosphatase-containing neurons in dorsal root ganglia are separate from those containing substance P and somatostatin. Neuroscience *7*:89-97.

Nagy, J.I., and P.E. Daddona (1985) Anatomical and cytochemical relationships of adenosine deaminase-containing primary afferent neurons in the rat. Neuroscience *15*:799-813.

Nagy, J.I., L.L. Iversen, M. Goedert, D. Chapman, and S.P. Hunt (1983) Dose dependent effects of capsaicin on primary sensory neurons in the neonatal rat. J.Neurosci. *3*:399-406.

Nagy, J.I., M. Buss, L.A. LaBella, and P.E. Daddona (1984) Immunohistochemical localization of adenosine deaminase in primary afferent neurons of the rat. Neurosci.Lett. *48*:133-138.

Nagy, J.I., S.P. Hunt, L.L. Iversen, and P.C. Emson (1981) Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin. Neuroscience *6*:1923-1934.

Nagy, J.I., S.R. Vincent, W.M.A. Staines, H.C. Fibriger, T.D. Reisine, and H.I. Yamamura (1980) Neurotoxic action of capsaicin on spinal substance P neurons. Brain Res. *186*:435-444.

Navarro, X., E. Verdú, G. Wendelschafer-Crabb, and W.R. Kennedy (1995) Innervation of cutaneous structures in the mouse hind paw: A confocal microscopy immunohistochemical study. J.Neurosci.Res. *41*:111-120.

Navone, F., R. Jahn, G. Di-Gioia, H. Stukenbrok, P. Greengard, and P. De-Camilli (1986) Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. J.Cell Biol. *103*:2511-2527.

Neve, R.L., N.I. Perrone-Bizzozero, S. Finklestein, H. Zwiers, E. Bird, D.M. Kurmit, and L.I. Benowitz (1987) The neuronal growth-associated protein GAP-43 (B-50, F1): neuronal specificity, developmental regulation and regional distribution of the human and rat mRNS. Molec.Brain Res. *2*:177-183.

Newson, B., A. Dahlstrom, L. Enerback, and H. Ahlman (1983) Suggestive evidence for a direct innervation of mucosal mast cells. An electron microscopic study. Neuroscience *10*:565-570.

Noguchi, K., E. Senba, Y. Morita, T. Sato, and M. Tohyama (1990) Co-expression of alpha-CGRP and beta-CGRP messenger RNAs in the rat dorsal root ganglion cells. Neurosci.Letts. *108*:1-5.

O'Brien, C., C.J. Woolf, M. Fitzgerald, R.M. Lindsay, and C. Molander (1989) Differences in the chemical expression at rat primary afferent neurons which innervate skin, muscles or joint. Neuroscience *32*:493-582.

Oh, U., S.W. Hwang, and D.H. Kim (1996) Capsaicin activates a nonselective cation channel in cultured neonatal rat dorsal root ganglion neurons. J.Neurosci. *16*:1659-1667.

Pedersen-Bjergaard, U., L.B. Nielsen, K. Jensen, L. Edvinsson, I. Jansen, and J. Olesen (1991) Calcitonin gene-related peptide, neurokinin A and substance P: Effects on nociception and neurogenic inflammation in human skin and temporal muscle. Peptides *12*:333-337.

Perry, M.J., S.N. Lawson, and J. Robertson (1991) Neurofilament immunoreactivity in populations of rat primary afferent neurons: A quantitative study of phosphorylated and non-phosphorylated subunits. J.Neurocytol. *20*:746-758.

Pertovaara, A. (1988) Collateral sprouting of nociceptive C-fibres after cut or capsaicin treatment of the sciatic nerve in adult rats. Neurosci Letts. *90*:248-253.

Pini, A. and B. Lynn (1991) C-fibre function during the 6 weeks following brief application of capsaicin to a cutaneous nerve in the rat. Eur.J.Neurosci. *3*:274-284.

Pini, A., R. Baranowski, and B. Lynn (1990) Long-term reduction in the number of C-fibre nociceptors following capsaicin treatment of a cutaneous nerve in adult rats. Eur.J.Neurosci. 2:89-97.

Plenderleith, M.B., L.L. Wright, and P.J. Snow (1992) Expression of lectin binding in the superficial dorsal horn of the rat spinal cord during pre- and postnatal development. Dev.Brain Res. *68*:103-109.

Prabhakar, E. and S.N. Lawson (1995) The electrophysiological properties of rat primary afferent neurones with carbonic anhydrase activity. J.Physiol.(Lond.) *482*:609-622.

Price, J. (1985) An immunohistochemical and quantitative examination of dorsal root ganglion neuronal subpopulations. J.Neurosci. *5*:2051-2059.

Price, J., and A.W. Mudge (1983) A subpopulation of rat dorsal root ganglion neurones is catecholaminergic. Nature *301*:241-243.

Rambourg, A., Y. Clermont, and A. Beaudet (1983) Ultrastructural features of six types of neurons in rat dorsal root ganglia. J.Neurocytol. *12*:47-66.

Reynolds, M.L. and M. Fitzgerald (1995) Long-term sensory hyperinnervation following neonatal skin wounds. J.Comp.Neurol. *358*:487-498.

Rice, F.L., E. Kinnman, H. Aldskogius, O. Johansson, and J. Arvidsson (1993) The innervation of the mystacial pad of the rat as revealed by PGP 9.5 immunofluorescence. J.Comp.Neurol. *337*:366-385.

Ritter, S. and T.T. Dinh (1988) Capsaicin-induced neuronal degeneration: Silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. J.Comp.Neurol. *271*:79-90.

Ritter, S. and T.T. Dinh (1992) Age-related changes in capsaicin-induced degeneration in rat brain. J.Comp.Neurol. *318*:103-116.

Robertson, B., B. Lindh, and H. Aldskogius (1992) WGA-HRP and choleragenoid-HRP as anterogradely transported tracers in vagal visceral afferents and binding of WGA and choleragenoid to nodose ganglion neurons in rodents. Brain Res. *590*:207-212.

Robertson, B., M.J. Perry, and S.N. Lawson (1991) Populations of rat spinal primary afferent neurons with choleragenoid binding compared with those labelled by markers for neurofilament and carbohydrate groups: A quantitative immunocytochemical study. J.Neurocytol. *20*:387-395.

Roivainen, R., J. Koistinaho, and A. Hervonen (1990) Localization of protein kinase C-beta-like immunoreactivity in the rat dorsal root ganglion. Neurosci.Res. *7*:381-384.

Salt, T.E., and R.G. Hill (1983) Neurotransmitter candidates of somatosensory primary afferent fibres. Neuroscience *10*:1083-1103.

Sann, H., M. Dux, M. Schemann, and G. Jancsó (1996) Neurogenic inflammation in the gastrointestinal tract of the rat. Neurosci.Lett. *219*: 147-150.

Sann, H., P.W. Mccarthy, G. Jancsó, and F.-K. Pierau (1995) RT97: a marker for capsaicin-insensitive sensory endings in the rat skin. Cell Tissue Res. *282*:155-161.

Scadding, J.W. (1980) The permanent anatomical effects of neonatal capsaicin on somatosensory nerves. J.Anat. *131*:473-484.

Schmidt, R.F. (1978) Fundamentals of neurophysiology. New York: Springer-Verlag.

Scott, S.A., N. Patel, and J.M. Levine (1990) Lectin binding identifies a subpopulation of neurons in chick dorsal root ganglia. J.Neurosci. *10*:336-345.

Seltzer, Z., R. Zeltser, A. Notzer, L. Cher, and V. Dor (1987) Lack of collateral sproutig of noci-thermoceptive C-fibres in the rat. Neuroscience *22*:719-719.

Sharp, G.A., G. Shaw, and K. Weber (1982) Immunoelectronmicroscopic localization of the three neurofilament triplet proteins along neurofilaments of cultured dorsal root ganglion neurons. Exp.Cell.Res. *137*:403-413.

Shehab, S.A.S. and M.E. Atkinson (1986) Vasoactive intestinal poypeptide increases in areas of the dorsal horn of the spinal cord from which other neuropeptides are depleted following peripheral axotomy. Exp.Brain R. *62*:422-430.

Silverman, J.D. and L. Kruger (1989) Calcitonin gene-related peptide-immunoreactive innervation of the rat head with emphasis on specialized sensory structures. J.Comp.Neurol. *280*:303-330.

Siney, L. and S.D. Brain (1996) Involvement of sensory neuropeptides in the development of plasma extravasation in rat dorsal skin following thermal injury. Br.J.Pharmacol. *117*:1065-1070.

Sisask, G., A. Bjurholm, M. Ahmed, and A. Kreicbergs (1995) Ontogeny of sensory nerves in the developing skeleton. Anat.Rec. *243*:234-240.

Skene, J.H.P., and M. Willard (1981a) Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous systems. J.Cell Biol. *89*:96-103.

Skene, J.H.P., and M. Willard (1981b) Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cells. J.Cell Biol. *89*:86-95.

Skofitsch, G., J. Donnerer, S. Petronijevic, A. Saria, and F. Lembeck (1983) Release of histamine by neuropeptides from the perfused rat hindquarter. Naunyn-Schmiedebergs Arch. Pharmakol. *332*:153-157.

Skofitsch, G., J.M. Savitt, and D.M. Jacobowitz (1985) Suggestive evidence for a functional unit between mast cells and substance P fibres in the rat diaphargm and mesentery. Histochem. *82*:5-8.

Streit, W.J., B.A. Schulte, J.D. Balentine, and S.S. Spicer (1986) Evidence for glycoconjugate in nociceptive primary sensory neurons and its origin from the golgi complex. Brain Res. *377*:1-17.

Streit, W.J., B.A. Schulte, J.D. Balentine, and S.S. Spicer (1985) Histochemical localization of galactose-containing glycoconjugates in sensory neurons and their processes in the central and peripheral nervous system of the rat. J.Histochem.Cytochem. *33*:1042-1052.

Such, G. and G. Jancsó (1986) Axonal effects of capsaicin: An electrophysiological study. Acta Physiol.Hung. *67*:53-63.

Sundler, F., E. Brodin, E. Ekblad, R. Hakanson , and R. Uddman (1985) Sensory nerve fibers: Distribution of substance P, neurokinn A and calcitonin gene-related peptide. In R. Hakanson and F. Sundler (eds): Tachykinin Antagonists. Amsterdam: Elsevier, pp. 3-14.

Szallasi, A. and P.M. Blumberg (1992) Vanilloid receptor loss in rat sensory ganglia associated with long term desensitization to resiniferatoxin. Neurosci.Lett. *140*:51-54.

Szolcsányi, J. (1984) Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. In L.A. Chahl, J. Szolcsányi, and F. Lembeck (eds): Neurogenic inflammation and antidromic vasodilatation. Budapest: Akademia Kiado, pp. 27-55.

Szolcsányi, J. (1985) Sensory receptors and the antinociceptive effects of capsaicin. In R. Hakanson and F. Sundler (eds): Tachykinin Antagonists. Amsterdam: Elsevier, pp. 45-54.

Szolcsányi, J. (1987) Capsaicin and nociception. Acta Physiol.Hung. *69*:323-332.

Taylor, D.C.M., and Fr.-K. Pierau (1982) Double fluorescence labelling supports electrophysiological evidence for dichotomizing peripheral sensory nerve fibres in rats. Neurosci.Letts. *33*:1-6.

Tetzlaff, W., H. Zwiers, K. Lederis, L. Cassar, and M.A. Bisby (1989) Axonal transport and localization of B-50/GAP-43-like immunoreactivity in regenerating sciatic and facial nerves of the rat. J.Neurosci. *9*:1303-1313.

Tomiyasu, K., and K. Inomata (1991) Enzyme-cytochemical study of small ganglion cells in experimental thiamine deficiency: Concerning the pain mechanism. Acta Neuropathol.(Berl.) *81*:396-400.

Tóth-Kása, I., G. Jancsó, A. Bognar, S. Husz, and F. Obal,Jr. (1986) Capsaicin prevents histamine-induced itching. Int.J.Clin.Pharmacol.Res. *6*:163-169.

Tóth-Kása, I., M. Katona, F.J. Obál, S. Husz , and G. Jancsó (1984) Pathological reactions of human skin: involvement of sensory nerves. In L.A. Chahl, J. Szolcsányi, and F. Lembeck (eds): Antidromic Vasodilatation and Neurogenic Inflammation. Budapest: Akademiai Kiado, pp. 317-328.

Tuchscherer, M.M., and V.S. Seybold (1985) Immunohistochemical studies of substance P, cholecystokinin-octapeptide and somatostatin in dorsal root ganglia of the rat. Neuroscience *14*:593-605.

Van Hoof, C.O.M., P.N.E. De Graan, J. Boonstra, A.B. Oestreichr, M.H. Schmidt-Michels, and W.H. Gispen (1986) Nerve growth factor enhances the level of the protein kinase C substrate B-50 in pheochromocytoma PC 12 cells. Biochem. Biophys. Res. Commun. *139*:644-651.

Vega, J.A., C. Rodriguez, M. Medina, and M.E. Del Valle (1990) Neuron-specific enolase (NSE)-like and neurofilament protein (NFP)-like immunoreactivities in the rat dorsal root ganglia and sciatic nerve. Cell.Mol.Biol. *36*:537-546.

Verge, V.M.K., W. Tetzlaff, P.M. Richardson, and M.A. Bisby (1990) Correlation between gap43 and nerve growth-factor receptors in rat sensory neurons. J.Neurosci. *10*:926-934.

Villar, M.J., R. Cortes, E. Theodorsson, Z. Wiesenfeld-Hallin, M. Schalling, J. Fahrenkrug, P.C. Emson, and T. Hokfelt (1989) Neuropeptide expression in rat dorsal-root ganglion cells and spinal-cord after peripheral-nerve injury with special reference to galanin. Neuroscience *33*:587-604.

Waddell, P.J. and S.N. Lawson (1989) The C-fibre conduction block caused by capsaicin on the rat vagus nerve in vitro. Pain *39*:237-242.

Wall, P.D. and M. Fitzgerald (1981) Effects of capsacin applied locally to adult peripheral nerve. I. Physiology of peripheral nerve and spinal cord. Pain *11*:363-377.

Weihe, E. (1989) Neuropeptides in primary afferent neurons. In W. Zenker, and W. Neuhuber (eds): The primary afferent neuron: A surgery of recent morpho-functional aspects. New York: Plenum, pp. 127-159.

Welk, E., U. Petsche, E. Fleischer, and H.O. Handwerker (1983) Altered excitability of afferent C-fibers of the rat distal to a nerve site exposed to capsaicin. Neurosci.Letts. *38*:245-250.

Wiesenfeld-Hallin, Z. (1988) Partially overlapping territories of nerves to hindlimb foot skin demonstrated by plasma extravasation to antidromic C-fiber stimulation in the rat. Neurosci.Lett. *84*:261-265.

Williams, P.L., R. Warwick, M. Dyson , and L.H. Bannister (1989) Gray's anatomy. London: Churchill Livingstone.

Winter, J., C.S.J. Walpole, S. Bevan, and I.F. James (1993) Characterization of resiniferatoxin binding sites on sensory neurons: Co-regulation of resiniferatoxin binding and capsaicin sensitivity in adult rat dorsal root ganglia. Neuroscience *57*:747-757.

Wood, J.N., J. Winter, I.F. James, H.P. Rang, J. Yeats, and S. Bevan (1988) Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J.Neurosci. *8*:3208-3220.

Yamadori, T. (1970) A light and electron microscopic study on the postnatal development of spinal ganglia in rats. Acta Anat.Nippon. *45*:191-204.

Zwiers, H., P. Schotman, and W.H. Gispen (1980) Purification and some characteristics of an ACTH-sensitive protein kinase and its substrate protein in rat brain membranes. J.Neurochem. *34*:1689-1699.