NEUROGENIC MODULATION OF CUTANEOUS VASCULAR REACTIONS BY GALANIN

Ph. D. Thesis

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INTRODUCTION

MORPHOLOGICAL CHARACTERISTICS OF THE CUTANEOUS MICROVASCULATURE

The skin of mammals and avian species consists of two layers of distinctive morphology, functional properties and embryological origin. The major part of the skin is the dermis or corium, mainly build up by connective tissue, which is covered by stratified layers of keratinocytes, the epidermis. Below the dermis lies the superficial fascia or subcutaneous layer, which is bound to the underlying deep fascia. The epidermis, especially its outermost squamous layer forms the primary barrier, which protects the body from the harmful mechanical, chemical or microbiological insults. The mechanical strength of the skin is due to the high proportion of collagen fibres intermingled with fibroblasts and fibrocytes in the dermis. Mast cells and tissue macrophages participating in the inflammatory and defence mechanisms of the skin are also present. The vascular and many neural elements of the skin are also embedded in the connective tissue of the dermis. A rich network originating from unmyelinated and thinly myelinated axons serves the epidermis as "free nerve endings".

The blood supply of the skin derives mainly from small arteries penetrating the underlying skeletal muscles. They form a weak arterial network the so-called rete cutaneum or deep plexus close to the border of the dermis and the subcutaneous tissue. Close to the dermo-epidermal interface there is a more prominent vascular plexus called rete subpapillare or superficial plexus which gives rise to the dense capillary network lying directly under the epidermis. Skin appendages (hair or feather follicles and the sweat glands) have a rich vascular supply, too. Venules and veins collecting blood from skin capillaries usually form three distinct vascular plexa. Close to the subpapillary arteriosus plexus lies the superficial venous plexus, an intermediate venous plexus is present in the middle part of the reticular layer of the dermis, and a deep venous plexus lies at the junction of the dermis and the superficial fascia. Arteriovenosus anastomoses common in the acral skin are situated in the deeper layers of the skin (Grant, 1930; Mescon, 1956). The venous blood is finally drained into the deep collecting veins either directly or after passing through the superficial veins.

FUNCTIONAL PRINCIPLES OF THE CUTANEOUS CIRCULATION

The total blood flow through the skin may vary over a wide range from about 300 ml/min up to 8 l/min (Rowell, 1974). Cutaneous circulation is strongly affected by autonomic and thermoregulatory effector mechanisms of the body, whereas local metabolic changes are of minor importance. The heat exchanger function of the cutaneous blood vessels is essential in maintaining a stable body temperature in homoiterm animals like mammals and birds. Therefore, the neuro-humoral control of cutaneous circulation is strictly coupled with thermoregulatory mechanisms (Rowell, 1974; Johnson, 1989). The skin may also serve as a potential blood reservoir since large volumes of blood can be stored primarily in the cutaneous veins. Consequently, regulation of the storage capacity of the cutaneous vascular bed can significantly influence the distribution of the circulating blood volume (Rowell, 1974; Johnson, 1989; Crossley, 1966). In contrast to these systemic circulatory responses, neuro-humoral mechanisms operating in the skin produce local changes in vascular functions. Noxious stimuli, threatening the integrity of the skin and the underlying tissues induce localised vascular reactions which support the protective functions of the skin. A striking example for such a mechanism is the local regulatory function of cutaneous sensory nerves. They participate not only in the detection of noxious stimuli but they may also initiate circulatory/inflammatory reactions which help to eliminate or alleviate the consequences of tissue injury.

NEURAL REGULATION OF THE CUTANEOUS CIRCULATION

Sympathetic innervation of the blood vessels

The cutaneous arteries, arterioles and veins of mammals and birds are densely innervated by sympathetic postganglionic nerve fibres (Norberg, 1964). These fibres arise from the paravertebral ganglia and contain noradrenaline as a principal transmitter. The varicose unmyelinated noradrenergic fibres are located predominantly in the adventitia of the vessels but fibres can be also found in the media (Bjorklund et al. 1986; Roth, and Kummer, 1994). They can be demonstrated either by fluorescence histochemistry using formaldehyde or glyoxylic acid (Falck, 1962) or by the detection of tyrosine hydroxylase, the rate limiting enzyme in noradrenaline synthesis (Pickel et al. 1976). Adenosine 5'-phosphat was the first candidate for

a co-transmitter in the sympathetic system (Burnstock, 1976). Immunohistochemical studies revealed the presence of somatostatin (Schultzberg et al. 1979), enkephalin (Schultzberg et al. 1979) and neuropeptide Y (NPY; Lundberg et al. 1982) in perivascular sympathetic fibres. Noradrenaline exerts vasoconstriction, mediated by the α_1 and/or by α_2 receptors located on vascular smooth muscle cells (Ekenvall et al. 1988). NPY co-localised with noradrenaline may effectively modulate the noradrenaline-induced vasoconstriction (Morris, 1994; Hökfelt et al. 1989).

Electrophysiological studies demonstrated that even at rest, the sympathetic fibres exhibit a low frequency firing (1-3 Hz). This resting activity ensures a constant release of noradrenaline which maintains the cutaneous resistance vessels in a permanent constricted state, referred to as sympathetic tone. Changes in sympathetic tone therefore can produce either vasoconstriction or vasodilatation; an increase in sympathetic activity produces vasoconstriction whereas a decrease results in vasodilatation. In heat stress the marked cutaneous vasodilatation is mainly due to the withdrawal of the sympathetic tone, although in non-apical skin an additional sympathetic nerve mediated active vasodilator mechanism was also proposed (Rowell, 1974; Roddie, 1983). Thermoregulatory hypothalamic centres also control the reflex reduction in sympathetic activity. They detect the elevation of the core temperature and might be also influenced by signals arriving from cutaneous warm receptors (Peter and Riedel, 1982).

General activation of the sympatho-adrenal system increases both the systemic vascular resistance and the total skin vascular resistance (Crossley, 1966). The increased sympathetic discharge exerts a dual effect on cutaneous blood vessels. The arterial vasoconstriction reduces cutaneous blood flow decreasing the partition of the skin from the cardiac output. In parallel with this, the increasing sympathetic tone lowers the compliance of the capacitance vessels, which in turn reduces the blood volume stored in the venous plexus, ensuring a mobilisation of these depots into the systemic circulation (Rowell, 1974).

Under experimental conditions it is possible to reduce or even abolish the function of sympathetic efferents using the selective neurotoxins guanethidine and 6-hydroxydopamine (Thoenen and Tranzer, 1968; Bloom et al. 1969). Administration of single high doses or repeated injections of these compounds results in a rapid defunctionalization of postganglionic sympathetic nerve fibres which is followed by degeneration and elimination of these fibres from the target tissues.

Peptidergic innervation of the blood vessels

Cutaneous blood vessels receive not only nerve fibres originating from the sympathetic ganglia but also fibres of sensory origin (Furness et al. 1982; Gibbins et al. 1985; Cuello et al. 1978; Ishida-Yamamoto et al. 1989; Weihe and Hartschuh, 1988). These are the peripheral branches of the primary sensory neurones situated in the corresponding dorsal root ganglia (Molander et al. 1987). These unmyelinated small diameter sensory fibres form perivascular or paravascular nerve bundles which might penetrate the adventitia and enter the underlying smooth muscle layer (Gibbins et al. 1987). A significant population of cutaneous sensory fibres exhibits neuropeptide-like immunoreactivity (Weihe, and Hartschuh, 1988; Lawson, 1996). These neuropeptides involve primarily tachykinins i.e. substance P (SP; Cuello et al. 1978; Furness et al. 1982) and neurokinin A (NKA), as well as calcitonin gene-related peptide (CGRP) (Gibbins et al. 1985; Kruger et al. 1989), vasoactive intestinal polypeptide (VIP) (Wallengren et al. 1987), galanin (Ju et al. 1987) and somatostatin (Johansson and Vaalasti, 1987). The sensory fibres supplying the cutaneous blood vessels have a dual function. These fibres are able to detect and convey sensory signals derived either from the blood stream or from the close environment of the blood vessels. They are especially sensitive to certain algogenic substances; close arterial or intravenous injection of a wide spectrum of chemical irritants can elicit pain sensation or pain reaction. Neuropeptides present in these fibres can act on vascular smooth muscle cells or on the endothelium and they are involved in the mechanism of the sensory nerve-mediated vascular reactions. In contrast to perivascular sympathetic nerve fibres, sensory nerves do not show any resting activity, therefore, is likely that they do not exert a permanent action on the cutaneous vascular bed. Activation of sensory nerves, however, leads to a release of sensory neuropeptides which produce profound, though localised circulatory responses.

THE SENSORY NERVE-MEDIATED VASCULAR REACTIONS IN THE SKIN

The sensory nerve-mediated vasodilatation

The early observations of Stricker and Bayliss that stimulation of the peripheral ends of transected spinal dorsal roots elicited vasodilatation provided the first evidence for a vasoregulatory action of sensory nerves (Stricker 1876, Bayliss 1901). According to the Bell-Magendi law, this vasodilatation was considered to be sensory in origin, caused by the antidromic activation of afferent nerve fibres. Investigating the inflammatory effect of chemical irritants, Bruce found that vasodilatation induced by mustard oil is decreased or abolished following the administration of local anaesthetics or after chronic sensory denervation (Bruce 1913). To explain this efferent function of cutaneous sensory nerves he proposed an axon reflex mechanism. Bruce hypothesised that collaterals of sensory axons possess an efferent vasodilatatory function which can be activated either by stimuli originating from the periphery or by antidromic impulses propagating from the central part of the axon.

Local vascular responses elicited by noxious stimulation of the skin

The stimulation of the human skin with noxious heat, mechanical or chemical stimuli produces a characteristic sequence of circulatory changes which were described first by Lewis (1924). The triple response comprises local vasodilatation, oedema formation (wheal) and a spreading vasodilatation called flare. The flare component was abolished after chronic but not after acute denervation of the skin. This observation strongly suggested the involvement of an axon reflex mechanism in the origin and propagation of the flare response. It was also suggested that the fiber population responsible for sensory antidromic vasodilatation and the fibres producing the axon reflex flare should be identical. The flare can be evoked by noxious heat (>43 °C), mechanical stimulation (punctate stimulus app. 30-60 g/mm^2) (Meyer et al. 1991; Treede et al. 1995; Lynn and Cotsell, 1992), or application of algogenic chemicals such as histamine, bradykinin, serotonin, etc. The flare response was first described on the human skin, but a similar cutaneous response can be observed on the pig skin (Pierau et al. 1991). However, no flare reaction of this type could be observed in small rodents, including rats.

Neurogenic cutaneous inflammatory responses

Chemical or antidromic electrical stimulation of cutaneous nerves of rats results in an increased microvascular permeability and plasma protein leakage in the skin (Jancsó, 1960; Lembeck and Holzer, 1979; Chahl, 1979, Jancsó et al. 1980). This can be demonstrated either by using colloidal substances or by dyes combined with plasma albumin (Jancsó, 1947; Jancsó 1955; Jancsó 1980). Colloidal silver particles injected into the systemic circulation pass the endothelium of permeable blood vessels, and accumulate at the abluminal surface of the endothelium entrapped by the basal lamina. This phenomenon referred to as vascular labelling (Jancsó, 1947; Jancsó 1955; Majno 1961) allows the direct visualisation and identification of leaky blood vessels. The labelled blood vessels proved to be (predominantly) small postcapillary venules and veins (Majno, 1961; Jancsó, 1984; Kenins et al. 1984). Application of algogenic substances like capsaicin, mustard oil, xylene etc. onto the skin of rats directly activates cutaneous chemosensitive nerve endings and produces plasma protein extravasation similar to that evoked by antidromic nerve stimulation (Jancsó, 1960; Jancsó et al. 1977). This reaction is neurogenic in origin since it is abolished by chronic denervation of the skin. The permeability change of cutaneous blood vessels is accompanied by an increase in regional blood flow due to arterial vasodilatation. Since hyperaemia and increased vascular permeability are leading signs of acute inflammation, this phenomenon is referred to as neurogenic inflammation (Jancsó, 1960) to emphasise its neuronal origin. Neurogenic inflammation can be observed in small laboratory rodents but it is absent in many mammals and birds (Pierau et al. 1987).

After the initiation of neurogenic inflammation, in parallel with the hemodynamical and vascular permeability changes, the activation of mast cells (Foreman and Jordan, 1983; Kowalski and Kaliner, 1988) tissue macrophages (Lotz et al. 1988; Hosoi et al. 1993), neutrophyl granulocytes (Helme et al. 1987; Ohlen et al. 1989; Perianin et al. 1989), platelets (Ohlen et al. 1989; Dimitriadou et al. 1992; Gecse et al. 1996) and vascular endothelial cells (Smith et al. 1993) takes place. As a consequence of these interactions, the inflammatory process which was elicited purely by the activation of cutaneous nerves can be amplified and prolonged by the active contribution of other inflammatory/immunocompetent cells (Jancsó et al. 1980; Jancsó, 1984; Payan, 1989; Weihe and Hartschuh, 1988).

THE ROLE OF CAPSAICIN-SENSITIVE PEPTIDERGIC AFFERENT NERVES IN CUTANEOUS VASCULAR RESPONSES

A large population of primary sensory neurones which innervate different organs, including the skin are sensitive to the excitatory effect of capsaicin and related compounds referred to as vanilloids. Capsaicin, the pungent component of the Capsicum species selectively activates these neurones via the opening of a recently cloned non-selective cation channel, the so-called vanilloid type 1 (VR1) receptor (Caterina et al. 1997). The discovery of the selective neurotoxic action of capsaicin largely promoted the study of the morphological traits and organisation of this particular class of primary sensory neurones. Administration of capsaicin to newborn animals resulted in a selective degeneration of about 50% of small, type B sensory ganglion cells giving rise to unmyelinated and, to a lesser extent, thinly myelinated axons (Jancsó et al. 1977; Jancsó and Király, 1981; Jancsó et al. 1984). It has been proposed that capsaicin sensitive primary sensory neurones form a separate afferent system since these neurons share common morphological, functional and pathobiological characteristics which distinguish them from other somatic or visceral afferents (Jancsó, 1990; Prechtl and Powley 1990). It has been demonstrated that a significant proportion of the capsaicin sensitive sensory neurones contain the neuropeptides SP (Nagy et al. 1980; Gamse et al. 1980), NKA, CGRP (Franco-Cereceda et al. 1987), VIP, cholecystokinin (CCK), somatostatin and galanin (Papka and Traurig, 1989; Kar et al. 1990; Skofitsch and Jacobowitz, 1985). Already the early studies by Nicholas Jancsó revealed the dual function of capsaicin-sensitive neurones showing their involvement in pain signalling and inflammatory responses (Jancsó, 1960; Jancsó, 1984).

Application of capsaicin onto the skin, conjunctiva, or other mucous membranes induces an intense burning pain sensation in humans (Jancsó, 1960; Jancsó 1967) and elicits pain behaviour and protective reflexes in animals. In addition, capsaicin produces local changes in tissue microcirculation eliciting local vasodilatation and flare in humans, and vasodilatation accompanied with local oedema formation in rats (Jancsó, 1960). However repeated applications of capsaicin decrease and finally eliminate these vascular reactions (Jancsó, 1955; Jancsó and Jancsó-Gábor, 1959; Jancsó, 1968). Similarly, chemogenic pain and nociceptive reflexes evoked by chemical irritants are markedly reduced in humans and in animals, respectively (Jancsó and Jancsó-Gábor, 1959; Jancsó, 1968). These phenomena, collectively known as capsaicin desensitisation were first described by Nicholas Jancsó (Jancsó and Jancsó-Gábor, 1959; Jancsó, 1968; Jancsó, 1994; Holzer, 1991). He concluded that the pain

producing and the vascular effects of capsaicin should be functionally coupled, and the sensory neurones activated by capsaicin may be identical to the neural elements participating in the sensory nerve-mediated vascular reactions.

THE EFFERENT FUNCTIONS OF CUTANEOUS SENSORY NEURONS

Experiments utilising capsaicin desensitisation and/or the selective neurotoxic effect of neonatal capsaicin treatment provided deeper insights into the efferent functions of primary sensory neurones (Jancsó, 1967; Jancsó et al. 1977; Maggi, and Meli, 1988; Holzer, 1992). The concept of the sensory efferent function involves not only the contribution of sensory nerves to the local control of tissue circulation but also their trophic, proliferative and pro-inflammatory influence on the adjacent non-neural cells. Capsaicin-induced chronic sensory denervation of the skin results in skin damages and loss of fur (Maggi et al. 1987; Thomas et al. 1994). The contribution of sensory nerves to wound healing (Kishimoto, 1984) and transplanted skin flap survival (Kjartansson and Dalsgaard, 1987) was also proposed. There is strong experimental evidence indicating the involvement of sensory fibres in the inflammatory reactions induced by various mediators such as histamine, bradykinin, serotonin etc. (Jancsó et al. 1980; Jancsó, 1984; Jancsó et al. 1985; Tóth-Kása et al. 1984; Payan, 1989), pointing to a pro-inflammatory function of cutaneous sensory nerves. The sensory nerves can also interact with mast cells (Foreman and Jordan, 1983; Newson et al. 1983), Langerhans cells (Hosoi et al. 1993) and mononuclear leukocytes. The mediators of the sensory efferent functions are the neuropeptides synthesised in the perikarya of primary sensory neurones. These peptides are transported via the fast axonal transport (Morton and Chahl, 1980; Gamse et al. 1982) toward the peripheral and central terminals of the sensory ganglion cells where they are stored and may be released upon stimulation (White and Helme, 1985; Amann et al. 1990; Yonehara et al. 1991; Jancsó et al. 1997).

VASCULAR EFFECTS OF SENSORY NEUROPEPTIDES IN THE SKIN

The effects of tachykinins

SP was the first peptide whose involvement in the mediation of the sensory-efferent functions was verified (Lembeck and Holzer, 1979). This peptide is a member of the tachykinin peptide family together with NKA and B, sharing structural and functional similarities (Fong, 1996). The tachykinins act on G-protein coupled transmembrane receptors, known as neurokinin (NK) receptors (Guard, 1991; Regoli et al. 1994; Maggi, 1995), which are present on the target cell surface (Deguchi et al. 1989). Receptor binding studies revealed three subclasses of NK receptors with different affinity toward the various tachykinins (Regoli et al. 1987; Guard, 1991). SP binds with the highest affinity to the NK1, NKA to the NK2 and NKB to the NK3 receptor. In the rat skin, SP induces a strong increase in microvascular permeability as indicated by the increased plasma protein leakage from postcapillary venules (Lembeck and Holzer, 1979; Chahl, 1979; Kenins et al. 1984). This effect can be inhibited by using selective NK1 receptor antagonists (Chahl, 1984; Devor et al. 1989; Xu et al. 1991). NK1 receptor antagonists also inhibit extravasation of plasma proteins elicited by antidromic electric stimulation of the cutaneous nerve (Xu et al. 1991). The permeability increasing effect of SP is probably due to the facilitation of interendothelial gap formation (McDonald, 1994) or an increased transcellular vesicular transport of albumin (Dimitriadou et al. 1992). SP can also activate cutaneous mast cells via an NK receptor independent mechanism causing the release of histamine (Bueb et al. 1990; Holzer, 1992). The involvement of other tachykinins and tachykinin receptors in cutaneous oedema formation was also demonstrated in other mammalian species (Hua et al. 1984; Devillier et al. 1986; Fuller et al. 1987) including humans (Devillier et al. 1986; Fuller et al. 1987). The data on the direct vasodilatatory effect of SP are somewhat controversial (Eklund, 1977; Brain and Williams, 1988; Brain and Williams, 1989) but it was demonstrated in rat and chick (Sann 1996) skin.

The effects of calcitonin gene-related peptide

Large numbers of cutaneous sensory fibres exhibit CGRP immunoreactivity, partly co-localised with SP (Gibbins et al. 1985; Franco-Cereceda et al. 1987). CGRP is encoded by the calcitonin gene and results from an alternative splicing process (Amara et al. 1982). It proved

to be a very potent vasodilator in the human skin and in the skin of many other animals (Brain et al. 1985b; Brain et al. 1986). Administration of CGRP (8-37), a potent competitive CGRP antagonist (Chiba et al. 1989; Dennis et al. 1990), inhibits cutaneous vasodilatation evoked either by antidromic electric stimulation of the rat saphenous nerve or by topical administration of capsaicin (Escott and Brain, 1993). It appears that the flare response is also mediated by CGRP in the human and porcine skin (Schmelz et al. 1997; Sann and Pierau, 1998). The vasodilatatory effect of CGRP is mediated by a direct action on smooth muscle cells of the precapillary arterioles via a nitric oxide independent mechanism (Ralevic et al. 1992). CGRP alone is not able to induce oedema formation, but it can significantly augment the permeability increasing effect of tachyikinins, probably as a consequence of increased local blood flow (Brain and Williams, 1985a; Brain and Williams, 1989; Gamse and Saria, 1985).

INHIBITORY MODULATION OF THE SENSORY NERVE-MEDIATED VASCULAR REACTIONS

The modulation of sensory nerve mediated vascular reactions might take place at three distinct levels: at the level of peptide release, at the level of peptide action, and finally, at the level of peptide inactivation. Numerous endogenous and exogenous substances can influence the release process of neuropeptides from sensory nerve terminals. Opioids have been shown to have a marked inhibitory effect on peptide release from stimulated terminals (Brodin et al. 1983; Lembeck and Donnerer, 1985), presumably due to a binding to their receptors located on the prejunctional membrane (Laduron, 1984; Stein et al. 1990). It has been demonstrated that opiate agonists inhibit neurogenic plasma extravasation and oedema formation induced by activation of cutaneous sensory fibres (Morton and Chahl, 1980b; Barthó and Szolcsányi, 1981; Smith and Buchan, 1984; Lembeck and Donnerer, 1985). The natural source of opioid peptides under in vivo conditions might be the cutaneous leukocytes, which can produce endogenous opioid peptides under inflammatory conditions (Stein et al. 1990).

Cathecholamines may also reduce neurogenic inflammatory processes by acting on the α_2 adrenergic auto-receptors present both on the central and peripheral terminals of sensory neurones (Lindgren et al. 1987). This mechanism might permit a regulatory interaction between the autonomic and the somatosensory systems (Donnerer et al. 1991).

Galanin, a neuropeptide present in numerous locations throughout the nervous system (Ch'ng et al. 1985, Bartfai et al. 1993), including a small population of mammalian dorsal root

ganglion cells (Skofitsch et al. 1985; Ju et al. 1987; Hökfelt et al. 1987), has been shown to inhibit neurogenic plasma extravasation. Administration of exogenous galanin significantly reduced the neurogenic inflammatory reactions in the skin (Xu et al. 1991) and joints (Green et al. 1992) of the rat. In the skin, it was demonstrated that the observed inhibition of the sensory efferent function is partly postjunctional in nature but in the other models a prejunctional action was suggested (Giuliani et al. 1989; Green et al. 1992). Similarly, a prejunctional inhibitory action of somatostatin on SP release was also reported (Gazelius, 1981). Endogenous peptides have not been implicated in an inhibitory modulation of cutaneous vascular responses. However, recent studies on pigeon dorsal root ganglia demonstrated that a large population of sensory ganglion cells contains galanin (Hildesheim et al. 1996). This observation has led to the suggestion that, at least in this species, endogenous galanin may play a role in the modulation of sensory nerve-mediated vascular responses.

SPECIES DIFFERENCES IN CUTANEOUS SENSORY-NERVE MEDIATED VASCULAR RESPONSES

In mammalian species sensory efferent functions seems to be a general feature of a subset of primary sensory neurones which are also sensitive to capsaicin (Jancsó et al. 1977; Maggi and Meli 1988; Holzer, 1992). However, the nociceptor system of avian species differs fundamentally in this respect from that of mammals. Early investigations with capsaicin showed that birds are completely insensitive to the algogenic and inflammatory effects of this compound (Pierau et al. 1986; Szolcsányi et al. 1986; Sann et al. 1987). It was also demonstrated that administration of capsaicin failed to induce sensory ganglion cell degeneration in avian species (Pierau et al. 1987; Harti et al. 1989; Rossler et al. 1993). In birds the antidromic stimulation of cutaneous nerves produces only a slight transient increase in cutaneous blood flow (Pierau et al. 1988; Pintér, 1990), and plasma protein leakage was either absent (Pierau et al. 1987) or restricted to the wattle innervated by the trigeminal nerve (Gentle and Hunter, 1993). Epicutaneous application of chemical algogens like capsaicin, mustard oil or xylene onto the skin of chicken failed to induce neurogenic inflammation (Pierau et al. 1987). The absence of the sensory nerve-mediated cutaneous vascular response in birds cannot be attributed to the absence of vasoactive sensory peptides, since similarly to mammals the existence of SP and CGRP was demonstrated in avian sensory ganglion cells (Pierau et al. 1987; Castrignano et al. 1990) and cutaneous nerves (Harti et al. 1989; Sann et al. 1996).

Close arterial or intravenous administration of these peptides induced marked vasodilatation in the chick skin indicating the responsiveness of the cutaneous vascular bed to these peptides (Sann et al. 1996).

AIM OF THE STUDY

The principal objective of this study was to explore the possibility of a neurogenic inhibitory modulation of cutaneous vascular responses in the pigeon. Sensory nerve-mediated neurogenic vasodilatation and plasma extravasation (neurogenic inflammation) comprise the characteristic vascular responses of the mammalian and, in particular of the rat skin (Jancsó, 1967; Jancsó et al. 1977; Maggi and Meli, 1988; Holzer, 1992). However similar vascular reactions cannot be elicited in avian species (Pierau et al. 1987; Pierau et al. 1988). Previous histological and pharmacological studies demonstrated galanin-like immunoreactivity in a large proportion of pigeon sensory ganglion cells (Hildesheim et al. 1996) and an inhibitory effect of exogenous galanin on neurogenic inflammatory responses in rats, respectively (Xu et al. 1991; Green et al. 1992). These findings provided the basis of our hypothesis that, at least in the pigeon, endogenous galanin may play a role in the inhibitory modulation of inflammatory reactions. The studies presented in this Thesis were initiated in an attempt to provide experimental evidence for this assumption. To explore and characterise presumed galanin-mediated mechanisms, a high affinity specific galanin receptor antagonist, M35 (galanin-(1-13)-bradykinin-(2-9)-amide; Ogren, 1992; Bártfai, 1992) has been employed. Experiments were performed to study the vascular reactions elicited by chemical irritants and by antidromic stimulation of cutaneous nerves. These responses and the effect of the galanin antagonist thereupon were studied both in intact and denervated skin to explore the role of galanin and cutaneous nerves in inflammatory reactions. Finally, functional histological studies were performed to explore the mechanism(s) of the increase in vascular permeability by the identification of the types and density of leaky cutaneous blood vessels after the administration of histamine in the intact and denervated skin, or after prior pretreatment with the galanin antagonist.

MATERIALS AND METHODS

Experimental animals

Experiments were performed on adult domestic pigeons (*Columba livia*) of either sex. The animals were kept in open-air cages and they were given standardised avian food and water ad libitum. The experiments were conducted in accord with the German and Hungarian Animal Protection Acts.

Anaesthesia and surgery

Animals were anaesthetised with isofluran gas (Forene®) administered by an open system apparatus. Although the pharmacological effects of isofluran are similar to halothane, its pharmacokinetic characteristics permit a faster elimination and, in turn, a more accurate dosage. Isofluran was vaporised in oxygen by using a vaporiser (Ohmeda) and the gas mixture was conducted via a plastic tube to a mask covering the beak and the face. The expired gas and anaesthetic were eliminated through a suction bell covering the animal's head and connected via a plastic tube to a high performance vacuum system. The flow of the inspired oxygen-isofluran gas mixture (1-12 l/h) was adjusted with a Drager flowmeter. For induction of anaesthesia, isofluran was administered at a concentration of 4-5% which was reduced after the onset of the narcosis (2-5 min) to 1.5-2%. The gas flow and the concentration of the anaesthetic were adjusted with respect to the monitored physiological parameters (arterial blood pressure, heart rate, respiration) and the behavioural signs.

One day before the experiments the animals were anaesthetised and the wings were fixed. The feathers covering the dorsal surface of the wing (brachial and antebrachial region) were removed. Special care was taken to avoid any injury to the skin. The animals were allowed to recover from anaesthesia and then were returned to the animal house. The following day the pigeons were anaesthetised again and prepared for the experiment. Fine polyethylene cannulas were inserted into the right external iliac artery and vein for the measurement of mean arterial blood pressure with a pressure transducer (Hellige) and infusion of physiological saline (0.3-1 ml/h) using a perfusion pump, respectively. Retrograde intraarterial injections (500 µl/10 min) into the radial artery supplying the investigated skin area were given through a cannula inserted into the ulnar artery. In pilot experiments injection of Evans blue dye through this cannula

resulted in a blue coloration of the dorsal skin of the wing indicating that drugs administered through this cannula reached the skin area under investigation. The body and skin temperature of the animals were kept at 38.5 ± 1.4 and 36.2 ± 1.7 °C, respectively.

Measurement of skin blood flow

Skin blood flow (SBF) was measured with a Laser Doppler Imager (LDI, Lisca Development, Sweden or LDI, Moor Instruments, U.K.). After close arterial injection of saline or different doses of M35 sixteen consecutive LDI images of a standardised skin area of 225 mm^2 were recorded at 1 min intervals. After determination of the basal SBF value, a piece of filter paper (10 mm in diameter) moistened with 5% mustard oil was placed onto the skin. In 6 cases the radial nerve serving the investigated skin area was transected on both sides 4-6 days before the experiments (chronic denervation). In another series of experiments (n=7) antidromic nerve stimulation was used to induce cutaneous vascular responses in animals pre-treated with guanethidine (20 mg/kg s.c.) one day before the experiment to prevent sympathetic vasoconstriction. The cutaneous antebrachii lateralis nerve was transected and the distal end placed on a pair of steel electrodes. Following the close arterial infusion of saline or M35 (1 and 5 nmol), sequential LDI images were taken from a skin area of 50 mm^2 with a frequency of 8/min for 8 min. After a control period of 1 min, nerve stimulation was started with rectangular pulses of 10 Hz, 20 V, 0.5 ms for a period of 20 seconds.

Values of SBF were determined by calculation of the average flux values of single images. To demonstrate the time course of changes in skin perfusion, the relative changes in SBF were plotted as a function of time. The total blood flow elevation elicited by mustard oil application was determined by calculation of the area under the flux curve for the total time of measurement (15 min).

Measurement of plasma protein extravasation

In experiments aimed at the study of histamine- or bradykinin-induced plasma protein extravasation, plasmapheresis capillaries (diameter: 0.4 mm, cut off size: 3000 kD, Asahi, Japan) were inserted intracutaneously with the aid of a guiding cannula and connected by a fine tube (Tygon®, Novodirekt, Germany) to a perfusion pump. The capillaries were perfused with Ringer's solution at a flow rate of 3.25 µl/min. After an equilibration period of 60 min,

histamine (Sigma, 500 μM) or bradykinin (Neosystem, 200 μM) dissolved in Ringer's solution were perfused through the capillaries for 40 min. The galanin antagonist M35 (0.2, 2 and 10 μM) or galanin (Neosystem, 10 μM) were co-perfused with bradykinin or histamine to examine their effect on vascular reactions evoked by these vasoactive agents. Thereafter, Ringer's solution was perfused for a further period of 60 minutes. Fractions of the effluent were collected every 20 minutes for the determination of their protein content according to Bradford (Bradford, 1976). Aliquots (20 μl) of each sample were mixed with 200 μl reagent containing Coomassie Brillant Blue (Merck) and the absorbance was determined at 595 nm using an MRX microplate reader (Dynatech, Germany). The average absorbance of 3 parallel measurements was used to estimate the protein content of the effluent. To compare the effects of different compounds, the total protein content of samples collected for 60 minutes after irritant challenge was also calculated.

Changes in SBF were also monitored using a LDI. Consecutive images were recorded from a skin area of 0.6 cm^2 with a frequency of 1/min and evaluated as described above.

Visualisation of leaky blood vessels using the vascular labelling technique

To demonstrate the vascular changes associated with increased vascular permeability, the vascular labelling technique (Jancsó 1947; Majno et al. 1961; Jancsó et al. 1980) was used for the histological visualisation of the distribution and type(s) of leaky blood vessels in the intact and chronically denervated skin. In 5 animals different doses of histamine (1 or 10 μg in 100 μl Tyrode's solution) were injected intracutaneously 10 min after injections of 100 μl of Tyrode's solution (control) or M35 (10 μM) at the same sites. Immediately after the injection of histamine, infusion of a 1% solution of colloidal silver (Merck; 1 ml/100 g b.w.) was started at a rate of 0.5 ml/min. Twenty min later the animals were sacrificed by an overdose of the anaesthetic and skin samples were removed, fixed in methanol for overnight, cleared in xylene and mounted in Canada balsam for light microscopic examination. The density of leaky blood vessels was determined by measuring the lengths of silver labelled vessels (mm/mm^2) with the aid of a camera lucida and a digitising tablet using a computer program (Sigma-Scan®, Jandell Scientific). The diameters of silver-labelled blood vessels were measured using an ocularmicrometer.

Denervation of the wing skin

The dorsal skin of the wing was denervated by sectioning of the ulnar and radial nerves 4-6 days before the experiment. Animals were anaesthetised with isofluran and the feathers were removed from the dorsal and brachial aspects of the wing. The ulnar and radial nerves were exposed in the axillary and brachial regions, ligated and transected distally. The skin wounds were closed with fine atraumatic surgical thread. After recovery from anaesthesia, the animals were returned to the animal house.

Statistical evaluation of experimental data

The statistical analysis of the experimental data was performed using SigmaStat® (Jandel Scientific) and SPSS® (SPSS Corp.) statistical software. Mean values of paired groups were compared with independent t-test at $p<0.05$ level of significance. For comparison of means of multiple groups one- or two way ANOVA models ($p<0.05$) were used. For additional post-hoc analysis the Student-Newman-Keuls test was applied ($p<0.05$).

RESULTS

CUTANEOUS VASODILATATORY RESPONSES ELICITED BY MUSTARD OIL

One day after the removal of the feathers covering the dorsal skin of the wing no signs of irritation, cutaneous lesions or inflammation could be observed. Epicutaneous application of 5% mustard oil induced a slight increase in SBF reaching its maximum 2 min after the application of the irritant (19.82 ± 3.24%). The calculated total increase in skin perfusion measured for 14 min amounted to 15.72 ± 2.39%. This vasodilatatory response appeared to be restricted to the site of application of the irritant.

CUTANEOUS VASODILATATORY RESPONSES ELICITED BY ANTIDROMIC NERVE STIMULATION

Slow retrograde infusion of 500 µl saline into the ulnar artery did not influence the SBF measured on the defeathered skin region. In animals without guanethidine pre-treatment antidromic nerve stimulation elicited an immediate marked decrease in SBF due to the activation of sympathetic efferent vasomotor fibres. Pre-treatment with guanethidine completely abolished this effect and unmasked a weak vasodilatatory response. Antidromic stimulation for 20 s resulted in an average increase of SBF by 5.15 ± 2.51% lasting for about 70 s. Systemic blood pressure was unaffected by antidromic nerve stimulation.

CUTANEOUS VASODILATATORY RESPONSES ELICITED BY HISTAMINE AND BRADYKININ

To study the effect of vasoactive agents on SBF, histamine and bradykinin were perfused through intracutaneously inserted microcapillaries. Perfusion with Ringer's solution for 60 min failed to affect SBF in the close proximity of the perfused skin area; SBF values were similar to those measured in adjacent skin regions. However, perfusion of histamine or bradykinin resulted in an increase of SBF. The concentrations of these vasoactive substances producing reliable and repeatable increases in SBF were determined in pilot experiments. Perfusion of

histamine at concentrations of 100 and 500 μM produced moderate increases in SBF by 19.17 ± 3.17 and 28.43 ± 4.19%, respectively. The elevation in SBF remained close to this level during the entire period of the measurement. Infusions of bradykinin at concentrations of 20 and 200 μM resulted in a maximal increase in SBF by 15.07 ± 1.3 and 22.04 ± 2.9%, respectively. Elevation in SBF reached its maximum after 2-4 minutes followed by a decline toward the basal SBF value.

THE EFFECT OF CHRONIC DENERVATION ON CUTANEOUS VASODILATATORY RESPONSES

Chronic denervation of the dorsal skin of the wing failed to affect significantly the increase in SBF elicited by mustard oil. In the denervated skin epicutaneous application of mustard oil produced a maximal increase in SBF by 16.12 ± 3.56% which is not significantly different from that measured in the intact (innervated) skin (19.82 ± 3.24%).

The vasodilatatory effect of intracutaneously perfused histamine was significantly reduced by chronic cutaneous denervation. Application of histamine (500 μM) elicited a maximal increase in SBF only by 17.81 ± 5.33% in the denervated skin. The effect of bradykinin (200 μM), however, was unaffected by denervation; it induced a maximal increase by 22.62 ± 3,23%, which is similar to that measured in the intact skin.

THE EFFECT OF M35, A SPECIFIC GALANIN ANTAGONIST ON CUTANEOUS VASODILATATORY RESPONSES

Retrograde intraarterial infusion (500 μl/10 min) of low doses (0.5 or 1.0 nmol) of M35, a specific galanin antagonist, affected neither cutaneous blood flow as assessed by LDI, nor mean arterial blood pressure. In two animals infusion of a higher dose of M35 (5 nmol) produced a slight decrease in mean arterial blood pressure without any significant change in skin perfusion. Intracutaneous perfusion of M35 even at the highest dose of 5 nmol failed to influence SBF or mean arterial blood pressure.

Epicutaneous application of mustard oil after an intraarterial infusion of M35 (1 or 5 nmol) resulted in a marked, dose-dependent increase in SBF (Fig. 1). The total SBF increase calculated as the area under the curve amounted to 29.74 ± 4.33 and 40.40 ± 5.85 % after the administration of 1 and 5 nmol M35, respectively (Fig. 3).

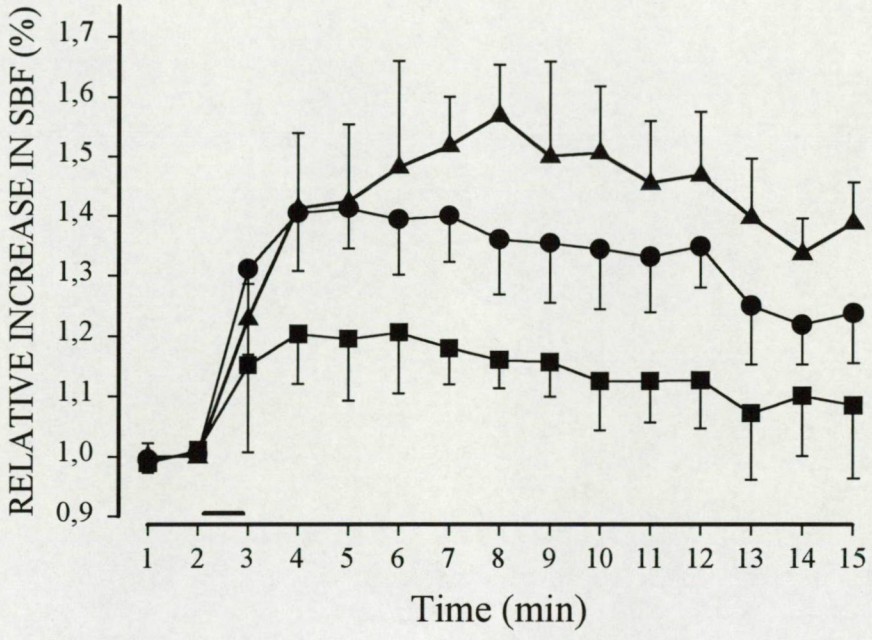

Fig. 1. Effect of epicutaneous application of mustard oil on skin blood flow (SBF) in control (■) and M35 pre-treated animals after i.a. infusion of saline and 1 nmol (●) or 5 nmol (▲) M35. The horizontal bar indicates the period of mustard oil application. For clarity, S.E.M. values are shown only in one direction.

The vasodilatatory response induced by antidromic electrical stimulation of the cutaneous antebrachii lateralis nerve was substantially increased following a close arterial infusion of M35. After infusion of 1 nmol M35 antidromic nerve stimulation elicited an immediate short increase in SBF (maximum increase: 9.91 ± 1.22%) which was followed by a long-lasting second elevation (10.99 ± 1.24%) after a latency of about 90 s (Fig. 2.). After pre-treatment with 5 nmol M35 this biphasic response turned into an immediate monophasic increase in SBF with a sustained plateau (maximum increase: 13.79 ± 0.71%).

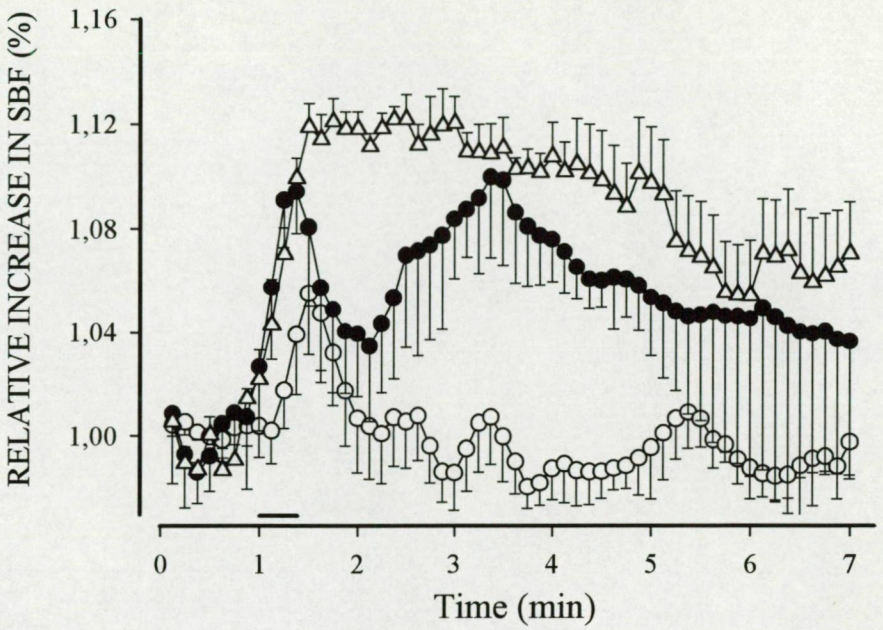

Fig. 2. Effect of antidromic nerve stimulation on the skin blood flow (SBF) in control animals (i.a. infusion of 0.9% NaCl, ○) and after infusion of 1 nmol (●) or 5 nmol M35 (△). The bar indicates the period of stimulation.

The vasodilatatory effect of intracutaneous histamine perfusion (500 μM) was significantly increased by co-perfusion of M35 (10 μM) resulting in a substantial elevation in SBF by 40.92 ± 6.25% (Fig. 4). The vasodilatation induced by intracutaneous perfusion of bradykinin (200 μM) was increased slightly but not significantly (26.41 ± 4.82%).

ABOLITION OF THE PRO-VASODILATATORY EFFECT OF M35 BY CHRONIC CUTANEOUS DENERVATION

Chronic denervation of the skin substantially attenuated the effect of M35 pre-treatment on the mustard oil-induced cutaneous vasodilatation in the intact skin. Unlike in the innervated skin, infusions of neither 1 nmol nor 5 nmol M35 enhanced the vasodilatatory response to mustard oil. Application of this irritant resulted in elevation in SBF by 19.09 ± 3.39% and 20.68 ± 3.49% which is not significantly different from values obtained in the intact or denervated skin without M35 pre-treatment (Fig. 3).

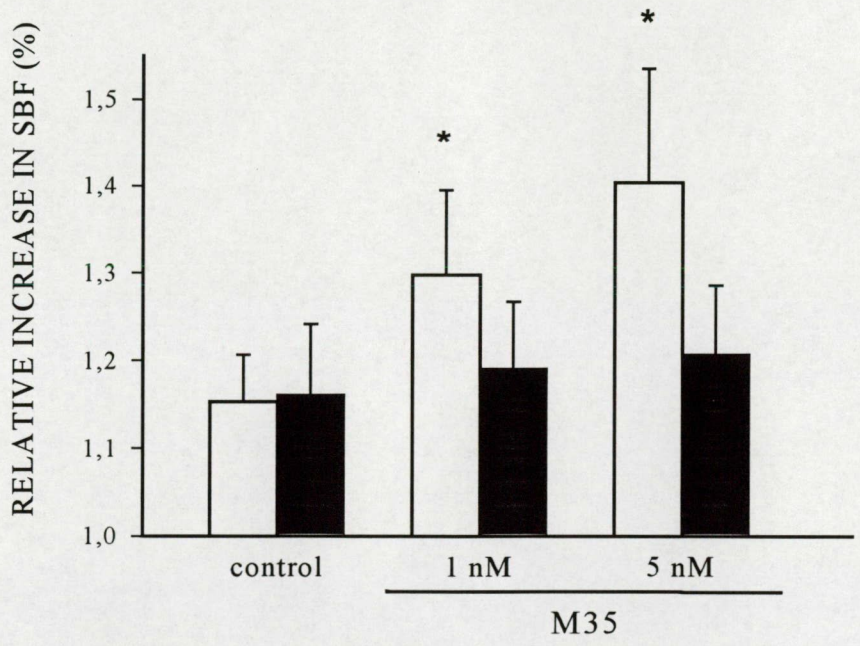

Fig. 3. Effect of M35 pre-treatment on the mustard oil induced cutaneous vasodilatation in intact (open columns) and chronically denervated skin (black columns). Columns represent the means + S.E.M. of relative perfusion increases measured during the 14 min poststimulatory period.

Similarly, chronic denervation completely abolished this potentiating effect of M35 on histamine-induced vasodilatation; elevation in SBF amounted to 19.32 ± 3.01% which is not different from values obtained in the denervated skin, but was smaller than in the innervated skin without M35 co-perfusion (Fig. 4). In the denervated skin bradykinin-induced vasodilatation was not affected by M35 co-perfusion (22.62 ± 3.23 %).

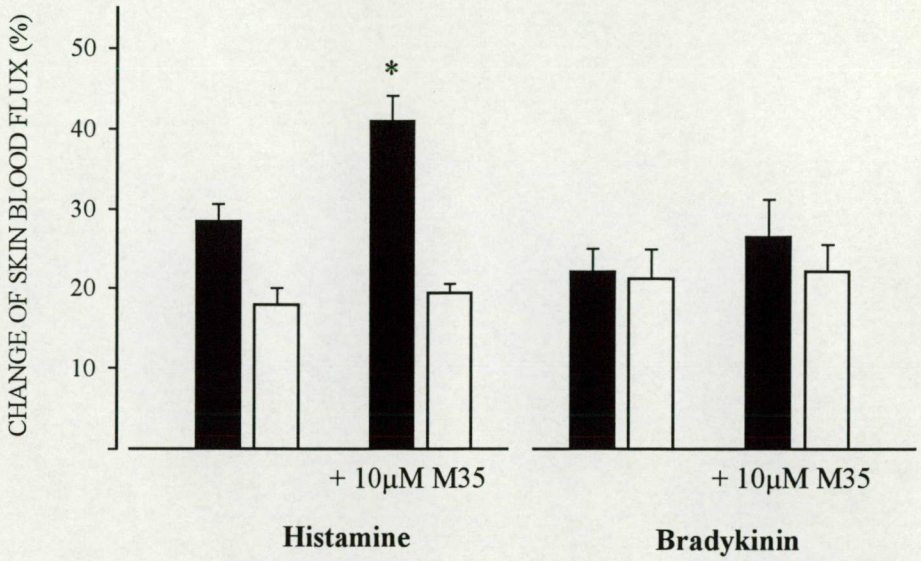

Fig. 4. Effect of M35 (10 μM), a specific galanin antagonist on cutaneous vasodilatation elicited by histamine (500 μM) and bradykinin (200 μM) in the innervated (black columns) and denervated (open columns) skin of the pigeon. Columns represent the means + S.E.M. of relative perfusion increases measured during the 14 min poststimulatory period.

CUTANEOUS PLASMA EXTRAVASATION ELICITED BY HISTAMINE AND BRADYKININ

In control animals, after perfusion with Ringer's solution for 60 min, the average protein concentration of the fraction collected for 20 min yielded 138.95 ± 9.35 μg/ml (which corresponds to the start of irritant perfusion in other specimens) which fell to 87.21 ± 6.97 μg/ml after perfusion for 120 min (last fraction collected). The perfusion of histamine (500 μM) and bradykinin (200 μM) for 40 minutes increased the protein concentration of the fractions collected (maximal protein concentration: 282 ± 22 μg/ml and 233 ± 18 μg/ml) which indicated a marked increase in vascular permeability of cutaneous vessels. To evaluate the permeability increasing effects of the different substances used in our experiments we calculated the protein content of the fluid samples collected for 60 min (3 fractions) after the start of the stimulation. These values amounted to 48.64 ± 3.35 and 43.73 ± 3.36 μg for histamine and bradykinin, respectively. These values were significantly different from the

control, i.e. from values obtained after perfusion with Ringer's solution alone (20.91 ± 1.29 μg).

THE EFFECT OF M35 ON THE PERMEABILITY INCREASING EFFECT OF HISTAMINE AND BRADYKININ

Perfusion of M35 alone failed to cause any increase in the protein concentration of the perfusates, resulting in values similar to the control, i.e. perfusion with Ringer's solution. Co-perfusion of M35 at increasing concentrations of 0.2, 2.0 and 10.0 μM with histamine (500 μM) resulted in a dose-dependent augmentation of the permeability increasing effect of histamine by a maximum of 62.27 ± 16.6% (Fig. 5). Similarly, co-perfusion of M35 with bradykinin (200 μM) dose-dependently increased plasma protein extravasation by a maximum of 48.49 ± 10.24% (Fig. 5).

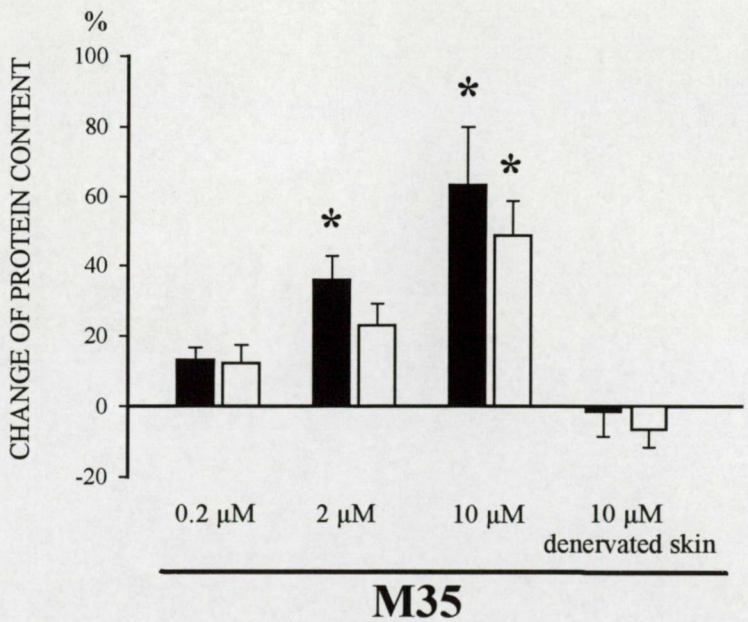

Fig. 5. Effect of M35, a specific galanin antagonist on plasma protein extravasation induced by histamine (500 μM, black columns) and bradykinin (200 μM, open columns) in the innervated and denervated skin of the pigeon. Values are expressed as percentage increases (mean ± S.E.M.) in total protein content of the perfusate.

THE EFFECT OF CHRONIC DENERVATION ON PLASMA EXTRAVASATION EVOKED BY HISTAMINE AND BRADYKININ

In the chronically denervated skin, perfusion with histamine (500 μM) produced significantly larger plasma protein extravasation (total protein content: 74.61 ± 7.45 μg) than in the intact skin (Fig. 6). However, bradykinin-induced plasma protein extravasation was slightly but not significantly decreased (39.63 ± 2.16 μg) after chronic denervation.

INFLUENCE OF CHRONIC DENERVATION ON THE EFFECT OF M35 ON PLASMA PROTEIN EXTRAVASATION INDUCED BY HISTAMINE AND BRADYKININ

Co-administration of M35 at the highest concentration used in these experiments (10 μM) failed to affect histamine-induced extravasation in the denervated skin (73.48 ± 5.76 μg) (Fig. 5, 6). Similarly, co-perfusion of M35 did not influence the permeability increasing effect of bradykinin (36.97 ± 1.98 μg) in the chronically denervated skin (Fig. 5).

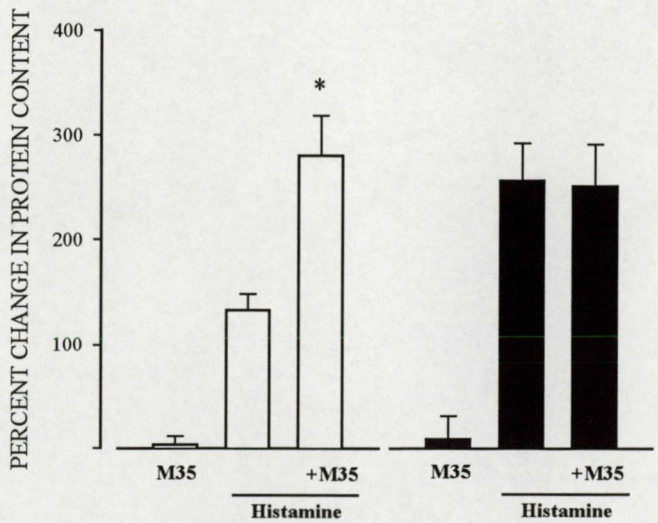

Fig. 6. Effect of M35, on histamine-induced (500 μM) cutaneous plasma protein extravasation in intact (open columns) and chronically denervated (black columns) skin. Values represent the total protein content of the perfusate (mean ± S.E.M.).
*: Significantly different from corresponding control value, $p < 0.05$.

HISTOLOGICAL EXAMINATION OF LEAKY BLOOD VESSELS CONTRIBUTING TO PLASMA PROTEIN LEAKAGE

In the innervated skin, the sites of histamine injections showed marked vascular labelling: small blood vessels displayed conspicuous silver depositions in their walls which is a characteristic histological feature of leaky venules. An especially dense network of labelled vessels could be observed adjacent to the feather follicles. Quantitative analysis revealed a significantly increased number of leaky blood vessels at sites of injections of the galanin antagonist M35 and histamine as well as at sites of injections of histamine in the denervated skin (Table 1). Injection of the galanin antagonist on its own failed to cause vascular labelling. Measurement of silver-labelled blood vessels revealed a significant increase in their diameters following either the co-administration of histamine and M35 or after chronic cutaneous denervation (Table 1).

Table 1. Quantitative histological evaluation of the effect of M35 and of chronic denervation on the histamine-induced increase in cutaneous vascular permeability

	Lengths of labelled blood vessels (mm/mm^2)	Diameters of labelled blood vessels (μm)
Histamine	8.31 ± 2.54	22.1 ± 0.9
Histamine + M35	16.80 ± 2.69*	28.5 ± 2.4*
Histamine + denervation	14.25 ± 2.80*	28.7 ± 1.8*

Data represent the means ± S.E.M. of lengths per unit surface area and diameters of silver-labelled blood vessels of the skin. Histamine and M35 were administered at concentrations of 500 μM and 10 μM, respectively.
* Significantly different from histamine, $p < 0.05$, $n = 6$.

DISCUSSION

MEASUREMENT OF CUTANEOUS BLOOD FLOW BY LASER DOPPLER IMAGER

Laser Doppler flowmetry permits a non-invasive, highly sensitive, continuous measurement of cutaneous blood flow in man and experimental animals (Stern, 1975; Stern, 1975; Nilsson et al. 1980). Introduction of this technique provided new insights into the mechanisms participating in the regulation of cutaneous blood flow under physiological and pathophysiological conditions. However, conventional equipment using a single beam laser probe have several drawbacks. The size of the area which can be studied with these probes is very limited which makes the determination of the extent of a cutaneous vascular reaction impossible. Furthermore, local structural variations of the skin or the course of blood vessels may significantly affect the signal detected by the probe (Tenland et al. 1983). In addition, the technique is very sensitive to even small movements of the investigated skin area. These limitations are largely overcome by using a Laser Doppler Imager (Essex and Byrne, 1991). This enables the study of vascular changes within a sizeable skin area which permits the analysis of the temporo-spatial characteristics of these responses (Quinn et al. 1991). It also greatly enhances the reliability and reproducibility of the measurements. Moreover, the technique makes comparative studies possible by the simultaneous measurement of vascular responses induced by different stimuli in adjacent skin areas under practically identical experimental conditions (Lynn et al. 1996). Laser Doppler imaging is also less sensitive to artefacts induced by small displacements of the skin as compared to single point measurement of SBF.

COMPARATIVE ASPECTS OF CUTANEOUS VASCULAR RESPONSES IN MAMMALIAN AND AVIAN SPECIES

Sympathetic efferent nerves play a fundamental role in the regulation of the vascular tone of cutaneous blood vessels both in mammalian and avian species. This is supported by our findings showing that electrical stimulation of peripheral nerves produced a marked decrease in cutaneous blood flow in the pigeon. Pre-treatment with guanethidine, an adrenergic neurone blocking agent, resulted in a failure of nerve stimulation to produce vasoconstriction. In contrast, previous studies indicated that stimulation of sensory nerves produce clear-cut

responses in mammalian species but elicit only small changes in avian species (Pierau et al. 1987; Pierau et al. 1988; Pintér 1990, Gentle and Hunter, 1993). Vascular reactions elicited by antidromic electrical stimulation of afferent nerves or by direct excitation of sensory nerve endings with chemical irritants are brought about via the release of vasoactive peptides from capsaicin-sensitive afferent nerves (Jancsó et al. 1977; Chahl, 1988; Holzer, 1992; Brain and Williams, 1985b; Lembeck and Holzer, 1979). There appears to be a general agreement in the literature that vasodilatation evoked by antidromic electrical stimulation of dorsal roots (Habler et al. 1999) or peripheral nerves is mediated largely by calcitonin gene-related peptide (Brain et al. 1985a; Louis et al. 1989; Escott and Brain, 1993), whereas stimulation-induced release of SP is responsible for the production of plasma extravasation (Lembeck and Holzer, 1979; Kenins et al. 1984). Sensory nerve-mediated vasodilatation and plasma extravasation are cardinal symptoms of an acute inflammatory reaction and are collectively referred to as the neurogenic inflammatory response (Jancsó, 1967; Jancsó 1980; Jancsó et al. 1977; Chahl, 1988; Holzer, 1992). The apparent absence of neurogenic inflammation in avian species cannot be accounted for by a lack of sensory neuropeptides in birds. Immunohistochemical studies demonstrated the occurrence of both SP and CGRP in chicken and pigeon sensory ganglion cells (Pierau et al. 1987; Harti et al. 1989; Castrignano et al. 1990; Sann et al. 1996; Hildesheim et al. 1996). In addition, close arterial administration of SP and CGRP evoked marked cutaneous vasodilatatory responses in the chicken (Sann et al. 1996). Therefore, an insufficient release of these peptides was suggested as a possible cause of the failure of antidromic nerve stimulation to produce significant vascular responses in the avian skin (Pierau et al. 1988).

Our present findings corroborated these earlier observations by showing weak neurogenic vasodilatatory responses both to antidromic electrical stimulation of a peripheral nerve and direct epicutaneous application of a chemical irritant. In fact, application of mustard oil produced similar (weak) vasodilatation both in the intact and the chronically denervated skin. These findings suggest that neural mechanisms are not involved, and the vasodilatatory effect of mustard oil may possibly be attributed to a non-neurogenic direct action on cutaneous blood vessels (cf. Lynn and Shakhanbeh, 1988).

Experiments with intracutaneously administered vasoactive substances including histamine and bradykinin also indicated that neurogenic mechanism are of minor importance in their moderate vasodilatatory action; unlike in rats (Jancsó, 1980; Jancsó, 1984), chronic (sensory) denervation failed to reduce markedly the vasodilatatory action of these mediators.

This may indicate that contribution of (sensory) nerves to the vasodilatatory effect of histamine and bradykinin may be of minor importance in the intact pigeon skin.

DEMONSTRATION OF A TONIC INHIBITORY MODULATION OF CUTANEOUS VASCULAR RESPONSES BY GALANIN IN THE PIGEON

Recent immunohistochemical observations indicated that similarly to mammalian species, avian sensory ganglion cells contain the major sensory neuropeptides including SP, CGRP and galanin (Pierau et al. 1987; Castrignano et al. 1990; Hildesheim et al. 1996). However, quantitative analysis revealed that unlike rodent sensory ganglia, pigeon dorsal root ganglia contain a sizeable subpopulation of galanin-containing neurones, which amounted over 25% of the total neuronal population (Hildesheim et al. 1996). It is worth noting that exogenous galanin has been shown to exert a significant inhibitory effect on cutaneous and synovial neurogenic inflammatory responses in the rat (Xu et al. 1991; Green et al. 1992). These observations prompted us to hypothesise that in the pigeon endogenous galanin may interfere with the effects of (neurogenic) vasoactive mediators.

The findings presented in this thesis are in support of this assumption. Close arterial infusion of a specific galanin antagonist, M35 resulted in a substantial enhancement of the vasodilatatory effect of epicutaneously administered mustard oil. Mustard oil, at suitable concentrations, has long been known to be a specific stimulus for the production of neurogenic inflammatory responses (Jancsó, 1960; Jancsó et al. 1977; Lynn and Shakhanbeh, 1988; Jancsó et al. 1993). Mustard oil acts through the release of vasoactive peptides from the stimulated sensory nerve endings and, therefore, fails to elicit an inflammatory response in the chronically denervated skin (Jancsó, 1960; Jancsó et al. 1980). The present findings showing that chronic denervation completely abolished the effect of M35 in increasing the vasodilatatory effect of mustard oil administration strongly indicate the involvement of galanin-containing (afferent) nerves in this response. This conclusion is strongly supported by our further findings showing that administration of M35 markedly and significantly enhanced the vasodilatatory effect of antidromic electrical nerve stimulation. Similarly, the vasodilatatory actions of histamine and bradykinin were significantly increased by prior administration of M35. Chronic denervation abolished the effect of M35 on histamine- and bradykinin-induced vasodilatation.

These findings indicate that galanin may exert a tonic inhibitory modulation on vasodilatatory responses and this involves a significant neurogenic mechanism. We suggest that

in the intact skin galanin may inhibit the neural release of vasoactive sensory neuropeptides known to be stored in sensory nerves of the pigeon. In turn, administration of M35 prevents the effect of galanin on the neural release of pro-inflammatory sensory neuropeptides, thereby resulting in an enhanced vasodilatatory response. This suggestion is strongly supported by previous findings showing a significant inhibition of neurotransmitter and hormone release by galanin (Bártfai et al. 1993; Green et al. 1992; Xu et al. 1991). In addition, an amplification of vascular responses to inflammatory mediators by neuropeptides released from sensory nerves is well-documented (Jancsó et al. 1980; Jancsó, et al. 1985).

SP and CGRP are the most likely candidates for vasoactive peptides released from sensory nerves. Both peptides have been shown to produce marked vasodilatation upon intraarterial administration also in avian species (Sann et al. 1996). However, only direct determination of the peptide(s) using e.g. a sensitive radioimmunoassay may provide a definite answer as to the nature and exact mechanism of vasodilatation produced by antidromic electrical nerve stimulation or direct chemical stimulation of sensory nerve endings in the presence of a galanin antagonist in the pigeon (cf. White and Helme, 1985; Amann et al. 1990; Yonehara et al. 1991).

The possible role of galanin in the modulation of cutaneous inflammatory reactions is further supported by our observations on the vascular permeability increasing effects of histamine and bradykinin. We demonstrated an enhancement of the permeability-increasing effect of histamine in the chronically denervated skin. Histological examinations revealed that the increased protein leakage might result either from recruitment of small blood vessels of similar type and/or from the involvement of topographically different segments of the vascular tree in the inflammatory process. This denervation-induced change may be explained by the abolition of a neurogenic galanin-mediated inhibition of vascular responses. This assumption is supported by our findings, which demonstrated that exogenous galanin reduced the permeability increasing effect of histamine to control levels in the denervated skin.

Taken together, these findings may be interpreted in terms of a tonic galanin-mediated modulation of afferent nerve function. There are several mechanisms, which may be implicated in this modulatory/inhibitory action of endogenous galanin. The most likely explanation of the present findings involves an inhibition of the release of vasodilatatory peptide(s) from stimulated sensory nerves. Further studies are needed, however, to furnish direct evidence for a galanin-mediated modulation of sensory neuropeptide release upon nervous or chemical stimulation.

The present experiments also revealed that endogenous galanin may effectively modulate cutaneous inflammatory responses elicited by some mediators of the acute inflammatory reaction. The permeability enhancing effects of histamine and bradykinin were markedly and significantly increased after the administration of a specific galanin antagonist. This effect of M35 was largely abolished after chronic denervation suggesting an involvement of afferent nerves in this phenomenon. Sensory nerves play an important role in the mechanism of the permeability enhancing effect of inflammatory mediators (Jancsó et al. 1980; Jancsó, 1984; Payan, 1989). Specific sensory denervation by capsaicin resulted in diminished dye leakage responses of several vasoactive agents including histamine and bradykinin (Jancsó et al. 1980; Jancsó, 1984). It has been suggested that these vasoactive agents exert their effects, in part, by the stimulation of sensory nerve endings, resulting in a release of vasoactive peptides. These results in an augmentation of the primary response elicited by non-neurogenic vasoactive agents (Tóth-Kása et al. 1984; Jancsó, 1984; Payan, 1989). Our findings indicate that a similar amplification of cutaneous inflammatory responses is absent in the pigeon. However, the results support a new, hitherto unrecognised type of inhibitory peptidergic modulation of cutaneous inflammatory reactions. This inhibitory modulation involves a significant neurogenic component, since the effect of M35 was abolished after chronic denervation of the skin. A possible explanation of these findings is that administration of the galanin antagonist promoted the permeability increasing effect of histamine and bradykinin by blocking the inhibitory action of galanin on sensory neuropeptide release and/or by directly inhibiting their vascular effects.
Exogenous galanin has been shown to inhibit cutaneous and synovial inflammatory responses in the rat (Xu et al. 1991, Green et al 1992). It has been suggested that this effect may be attributed to an inhibition of the release of pro-inflammatory peptides from these nerves. However, a direct postjunctional, i.e. vascular effect could not be excluded (Xu et al. 1991).Our findins support a physiological, inhibitory role for endogenous galanin in the modulation of vascular reactions. The exact source of tissue galanin, however was not established in the present experiments. A neurogenic origin of galanin is supported by our present findings showing an abolition of the effect of M35 after chronic denervatin. In addition, galanin-containing perivascular nerves, in association with SP-containing nerve fibres have been demonstrated in the pigeon skin (Sántha et al. 1998). Further studies are needed to reveal the exact mechanism(s) of galanin-mediated inhibition of inflammatory reactions (Fig. 7). Our observations may have important implications as to the mechanism(s) of the nervous modulation of inflammatory reactions also in species other than the pigeon. Indeed, neurogenic

inflammatory responses are well-documented in small rodents but not in other mammalian species (cf. Szallasi and Blumberg, 1999). The findings showing that galanin is the most abundant peptide among rat sensory nerve fibres (Klein et al. 1990) strongly support this notion. Hence, it may be assumed that neurogenic inhibitory modulation of sensory nerve-mediated (vascular) effector responses may operate also in mammalian species. Further studies are needed to reveal the exact cellular origin(s) and the factors which may determine the release of pro-inflammatory (excitatory) and anti-inflammatory (inhibitory) sensory neuropeptides under physiological/pathophysiological conditions. In conclusion, the experiments

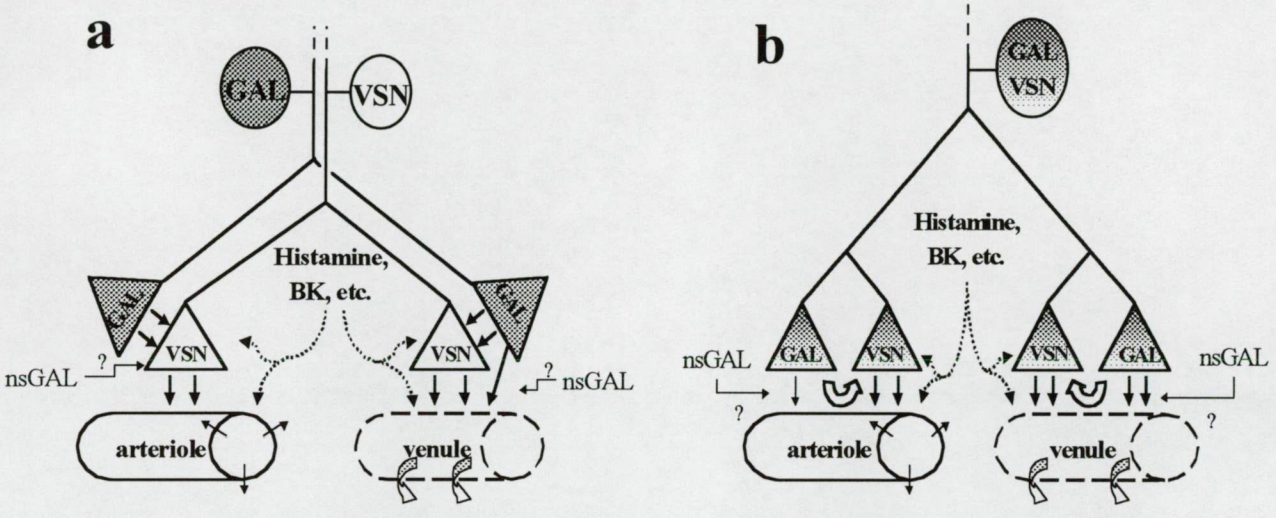

Fig. 7. Possible mechanisms of galanin-mediated inhibition of cutaneous vascular responses. GAL: galanin; nsGAL: galanin of non-sensory origin; VSN: vasoactive sensory neuropeptide(s); BK: bradykinin

presented in this thesis using M35, a highly specific galanin antagonist provided firm evidence for a new type of tonic modulatory/inhibitory role of endogenous galanin in cutaneous vascular reactions involving neurogenic vasodilatation and plasma extravasation. These findings indicated for the first time that the efferent function of sensory nerves may involve not only excitatory but inhibitory modulatory functions as well. It is suggested that similar sensory nerve-mediated inhibitory modulation of peptidergic local regulatory responses may exist in mammalian species as well.

SUMMARY

Vascular responses elicited by antidromic nerve stimulation and by direct cutaneous application of vasoactive agents were studied in the skin of the pigeon. In the intact, innervated skin antidromic nerve stimulation evoked only moderate increases in skin blood flow. After close intraarterial injection of a specific galanin antagonist, galanin (1-13) - bradykinin (2-9) - amide (M35) this small vasodilatatory response was greatly augmented. Similarly, the vasodilatatory responses elicited by superfusion of the skin with histamine or bradykinin were also significantly and dose-dependently increased following a previous administration of M35. Chronic cutaneous denervation abolished the pro-inflammatory effect of the galanin antagonist.

Histamine and bradykinin elicited dose-dependent increase in cutaneous vascular permeability as assessed by the quantitative Evans blue method and by the histological demonstration of leaky blood vessels employing the vascular labelling technique. Plasma extravasation elicited by histamine or bradykinin was significantly amplified by the co-administration of M35. Denervation per se resulted in an augmentation of the response to histamine which was significantly reduced by the administration of galanin.

The findings indicate that endogenous galanin may exert a tonic neurogenic inhibitory action on cutaneous vascular responses. The results provide evidence for a new, anti-inflammatory (inhibitory) local regulatory (sensory-efferent) function of cutaneous sensory nerves which may significantly contribute to the maintenance of the structural and functional integrity of the skin.

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APPENDIX

Publications related to the subject of this thesis:

I. Jancsó G., Dux M. and Sántha P., Role of capsaicin-sensitive afferent nerves in initiation and maintenance of pathological pain, Behavioral and Brain Sciences, 20 (1997) 454-455

II. Sántha P., Pierau Fr.-K. and Jancsó G., Evidence for an inhibition by endogenous galanin of neurogenic cutaneous vasodilatation in the pigeon, Neuroscience Letters, 243 (1998) 101-104

III. Sántha P., Pierau Fr.-K. and Jancsó G., Inhibitory modulation of cutaneous vascular responses by endogenous galanin in the pigeon, Neuroscience Letters, 273 (1999) 64-66

IV. Sántha P., Pierau Fr.-K. and Jancsó G.,Galanin mediated inhibitory nervous modulation of cutaneous vascular reactions, Acta Physiologica Hungarica, 86(3-4) (1999) 277-283

V. Jancsó G., Sántha P., Horváth V. and Pierau Fr.-K., Inhibitory neurogenic modulation of histamine induced cutaneous plasma extravasation in the pigeon, Regulatory Peptides (in press)

Other publications:

Jancsó G., Juhász A., Dux M., Sántha P. and Domoki F., Axotomy prevents capsaicin-induced sensory ganglion cell degeneration, Primary Sensory Neuron, 2 (1997) 159-165

Jancsó G., Domoki F., Sántha P., Varga J., Fischer J., Orosz K., Penke B., Becskei A., Dux M., Tóth L., β-Amyloid (1-42) peptide impairs blood-brain barrier function after intracarotid infusion in rats, Neuroscience Letters, 253 (1998) 139-141