nociceptive neurones, and is reversible (see Sessle 1996).

The "central sensitization" processes expressed in V nociceptive pathways may involve NMDA receptor mechanisms since the mustard oil-evoked increase in jaw muscle activity and the expansion of the neuronal mechanoreceptive field can be reduced by systemic or intracerebral application of the NMDA antagonist MK-801 in a dose-dependent manner (Yu et al. 1996). A role for NMDA mechanisms in central V nociceptive processing is supported by other V neuropharmacological and immunohistochemical data (see Sessle 1996) and by analogous findings in spinal dorsal horn that are outlined in the three articles. It is also of interest that the local application of MK-801 to TMJ tissues can block the mustard oil-evoked jaw muscle activity (Yu et al. 1996). These findings are consistent with data indicating that NMDA receptors may be located in peripheral tissues (for review, see Erdö 1991) and appear to be the first to document that NMDA antagonists may act peripherally to reduce a nociceptive reflex. Although the detailed mechanisms underlying such a peripheral action remain to be clarified, these findings do raise other potential approaches (e.g., peripherally applied NMDA antagonists) for inducing pre-emptive analgesia in addition to those outlined by CODERRE & KATZ (sect. 5) and DICKENSON (sect. 4.3).

Several other factors involved in the central sensitization process in the spinal nociceptive system have been outlined by these three target articles. One of these involves endogenous opioids. Central opioids have also been shown to be important modulators of V nociceptive processing (see Sessle 1996). Indeed, central opioids may be recruited to limit the duration and extent of central sensitization evoked by nociceptive barrages entering the CNS: once the jaw muscle and caudalis neuronal excitability increases induced by deep tissue injection (e.g., into TMJ) of mustard oil have dissipated (see above), the changes can be "rekindled" in a dose-dependent manner by administration of the opioid antagonist naloxone (Sessle 1996; Yu et al. 1994). The finding that naloxone administration in animals receiving the TMJ injection of vehicle (mineral oil) does not induce a recurrence of the increased muscle activity indicates that the increased activity is dependent on the previous occurrence of mustard oil-induced effects. Preliminary data (Tambeli et al., unpublished) that the specific mu opioid receptor antagonist CTOP replicates these effects of naloxone suggest that a central mu-receptor opioid mechanism is triggered by the mustard oil-evoked afferent input and limits the increase in muscle activity and associated central sensitization. Our findings appear to be consistent with the concept of opioid recruitment mentioned by DICKENSON (sect. 7.1) and by WIESENFELD-HALLIN et al. (sect. 4) but may also have relevance to the opioid insensitivity noted in these two articles since it is conceivable that central opioid dysfunction could result from prolonged opioid release induced by a sustained afferent barrage. Peripherally acting opioids such as those noted by DICKENSON (sect. 4.3) do not appear to be involved in these particular effects since the peripherally acting opioid antagonist methylnaloxone does not block the mustard oil-evoked changes (Yu et al. 1994).

Other neurochemical modulators have also been discussed in these three target articles; WIESENFELD-HALLIN et al. (sect. 3) especially emphasize the potential role of GABA mechanisms in central inhibitory mechanisms, dysfunction of which may lead to pain phenomena. In the V system, it is interesting in this light that the excrutiatingly painful condition of V neuralgia has allodynia-like features in that it can be triggered by a light tactile perioral stimulus and controlled by the $GABA_B$ agonist baclofen which also affects central inhibition in V brainstem neurones (Fromm 1991). ute to allodynia if there is dysfunction of central inhibitory mechanisms.

Role of capsaicin-sensitive afferent nerves in initiation and maintenance of pathological pain

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Abstract: This commentary provides experimental data in support of the critical role of capsaicin-sensitive primary afferent fibers in the initiation and maintenance of pathological pain. The demonstration of capsaicin-induced, centrally-evoked cutaneous hyperalgesia, and of neuroplastic changes elicited by the degeneration of C-fiber primary afferent terminals following peripheral nerve damage, indicates a significant contribution of capsaicin-sensitive sensory ganglion neurons in the development of pathological pain conditions. [CODERRE & KATZ]

In their target article, CODERRE & KATZ put forward the hypothesis that both peripheral and central neural mechanisms contribute significantly to pathological pain. The present commentary provides experimental data in support of a critical role of capsaicin-sensitive primary afferent fibers in the initiation and maintenance of pathological pain. This particular class of neurons is a morphologically and neurochemically well-defined population of primary sensory neurons with a unique dual functional trait. They are involved in the transmission of nociceptive impulses evoked by noxious mechanical, thermal, and chemical stimuli, they mediate somatic and visceral reflexes ("afferent function"), and, through the release of sensory neuropeptides from their peripheral endings, they participate in local regulatory functions of the innervated tissues ("efferent" or "local regulatory" function; Holzer 1991; Jancsó 1968; Jancsó et al. 1977; Lembeck 1983; Maggi & Meli 1988; Szolcsányi 1984). The available experimental evidence indicates that this particular class of afferent neurons may be involved in both the initiation and maintenance of painful conditions.

It has long been known that application of capsaicin to the human skin produces marked mechanical and thermal hyperalgesia (Szolcsányi 1977; Tóth-Kása et al. 1986). Similarly, intradermal injection of capsaicin has been shown to produce cutaneous hyperalgesia; the findings indicated that central sensitization may be responsible for mechanical allodynia after capsaicin (Simone et al. 1991). Animal studies clearly demonstrate that central mechanisms are critically implicated in the initiation of mechanical hyperalgesia. There is experimental evidence that mechanical hyperalgesia can also be elicited merely by stimulation of the central terminations of capsaicin-sensitive primary afferents. Injection of minute amounts of capsaicin into the subarachnoid space brings about a characteristic sequence of vascular and behavioral responses in the rat (Gamse et al. 1984; Jancsó 1981). Intracisternal injection of capsaicin in rats anesthetized with ether elicited an immediate, short-lived cutaneous vasodilatation (i.e., chemically evoked dorsal root vasodilatation) and, after the anesthesia wore off, protective wiping movements. Following this acute excitatory phase, a characteristic mechanical hyperalgesia developed: light touching of the skin or even the hairs evoked vigorous protective reflex movements. It was interesting to note that during this period, which lasts up to 30 min, the areas inner-

vated by afferent nerves related to the affected medullary and spinal dorsal horn areas proved completely insensitive to noxious chemical irritants, including capsaicin and zingerone (Jancsó 1981: unpublished observations).

These findings strongly suggest that capsaicin-sensitive afferents are essential in the initiation but not in the maintenance of mechanical hyperalgesia. In addition, the fact that the mechanical hyperalgesia produced by intracisternal capsaicin is associated with a deprivation of nociceptive afferent input to the dorsal horn neurons, is in line with the suggestion by CODERRE & KATZ that once hyperalgesia is established, it does not need to be maintained by inputs from the periphery (sect. 2.3, para. 2). Further, neurohistological findings indicated an early degeneration of spinal and medullary primary afferent terminals (Jancsó 1981) similar to that seen in peripheral nerve endings upon exposure to capsaicin at concentrations which causes the release of neuropeptide(s) from them (Király et al. 1991).

It may accordingly be proposed that central sensitization is produced by the release of sensory neuron-derived mediator(s) from peptidergic capsaicin-sensitive afferents. This is supported by electrophysiological findings that the sensitization of spinal dorsal horn cells is critically dependent on substance P released from capsaicin-sensitive primary afferent terminals (Dray et al. 1994). Although further studies are needed to clarify this point, the finding that intracisternal injection of capsaicin is associated with an immediate marked cutaneous vasodilatation may indicate that substance P and/or calcitonin gene-related peptide is likely to be involved (Chahl 1988).

The possible morphological changes which may ensue after peripheral nerve damage in the central terminations of nociceptive primary afferent fibers were not addressed by CODERRE & KATZ. However, such changes have been shown to occur and capsaicin-sensitive afferents may also be significantly implicated in the development of pain induced by peripheral nerve damage.

Recent findings indicate that peripheral nerve injuries result in a progressive, delayed transganglionic degeneration of C-fiber capsaicin-sensitive primary afferent fibers (Jancsó 1992; Jancsó & Ambrus 1994; Jancsó & Lawson 1990). This may involve the initial release of sensory neuropeptides and thereby contribute to the development of sensory disturbances which follow peripheral nerve damage. It has been suggested that substantial C-fiber afferent deafferentation creates favorable circumstances for structural neuroplastic changes to occur, resulting in a reorganization of spinal dorsal horn neuronal connections (Jancsó 1992). Recent findings lend support to this assumption. It has been shown that after both perineural treatment with capsaicin or peripheral nerve section, which result in massive transganglionic degeneration of C-fiber primary afferent terminals (Jancsó 1992; Jancsó & Ambrus 1994; Jancsó & Lawson 1990), extensive sprouting of presumed thick primary afferents occurs within the substantia gelatinosa, which is normally devoid of myelinated afferent terminals (Mannion et al. 1996; Woolf et al. 1995). These profound structural neuroplastic changes are most probably initiated by degenerative changes in capsaicin-sensitive afferents and, by altering the connectivity of dorsal horn neurons, may contribute significantly to the development of pathological pain after nerve injuries (Jancsó 1992; Woolf et al. 1995).

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Sex differences in pain: And now for something completely different

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Abstract: The belief that women report more somatic complaints than men is not new. Many centuries B.C., the Egyptians and the Greeks already made an association between female pains and *hysteria*, which is Greek for "wandering womb." Despite the commonly held belief that women are more sensitive to pain than men, the issue of sex differences in pain has received little attention from the scientific community in general. It is the merit of BERKLEY to draw our attention to this large gap in our scientific knowledge.

> Alas, our frailty is the cause, not we. For such as we are made of, such we be.
>
> *William Shakespeare, Twelfth Night*

As is convincingly shown in BERKLEY's target article, the human literature on sex differences in pain is equivocal, possibly as a consequence of the myriad factors that are operative. Indeed, for nearly every study reporting a sex difference, one can find another reporting none. Consider the following two striking examples. In two controlled studies (Bush et al. 1993 and Feine et al. 1991; both cited by Berkley) on sex differences in response to experimental heat pain, the authors came to opposing conclusions. Although the experimental procedures used in the two studies were very similar (and both groups have extensive experience with psychophysical testing procedures), Feine et al. found that women are more sensitive to experimental pain than men, whereas Bush et al. found no sex differences. A second example concerns the effectiveness of spinal cord stimulation (SCS) in the relief of chronic nonmalignant pain. Whereas North (1991) found a significantly better therapeutic result of SCS in female patients, Kupers et al. (1994) found that men responded better than women.

This large variability in results should warn us against reacting with too much enthusiasm whenever a new sex difference is put forward. For example, in a recent study, Gear et al. (1996) found that kappa-opioids produce significantly greater analgesia in women than in men. In view of the above-mentioned inconsistency in the results on sex-related differences in pain derived from different laboratories, this finding should be interpreted with caution and be replicated in other independent studies.

Although in BERKLEY's review not much attention was paid to animal studies on sex differences, here we find the same inconsistency as in the human literature. For instance, some studies failed to show gender-related baseline differences in hot plate and tail-flick tests and after intraperitonial injection of acetic acid; but other studies showed gender-related differences in the formalin test. Another example: whereas some studies reported that female rats are more susceptible to the development of neuropathic pain after nerve constriction, others showed that after dorsal root section, male rats were more susceptible to the deafferentation pain syndrome.

Despite the enormous variability of gender differences in pain in both clinical and experimental pain studies, the only constants seem to be:

1. *Clinical pain syndromes:* there are significantly more studies showing that women report a higher incidence of endogenous pain compared to men than there are studies showing the opposite.

2. *Experimentally induced pain:* studies either show that women rate experimentally applied stimuli as more painful or no

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Evidence for an inhibition by endogenous galanin of neurogenic cutaneous vasodilatation in the pigeon

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Abstract

The effect of high affinity galanin antagonist M35 on neurogenic cutaneous vasodilatation has been studied in the pigeon using a Laser Doppler Imager. Cutaneous application of mustard oil or antidromic electrical stimulation of a cutaneous nerve produced a small increase in skin blood flow. Close arterial injection of M35 prior to chemical or electrical stimulation resulted in a marked augmentation of the vasodilatory response. This effect was abolished by chronic denervation. The results suggest a nerve-mediated inhibitory effect of endogenous galanin on neurogenic cutaneous vasodilatation in the pigeon skin and provide the first experimental evidence for an inhibitory local regulatory function of cutaneous sensory nerves at least in the avian skin. © 1998 Published by Elsevier Science Ireland Ltd.

Keywords: Antidromic vasodilatation; Galanin; Skin; Sensory nerves; Pigeon; Mustard oil

In the mammalian skin stimulation of chemosensitive afferent fibres induces local vascular changes involving vasodilatation and plasma extravasation [1,2,12,13]. These responses are mediated by the release of neuropeptides, primarily substance P (SP) and calcitonin gene-related peptide (CGRP) from stimulated C-fibre nerve endings [9]. Previous studies have shown that in avian species neither antidromic electrical stimulation of afferent nerves nor direct stimulation of sensory nerve endings produce similar vascular responses [15,16]. Dorsal root ganglion (DRG) neurons of pigeons and chickens contain CGRP and SP in a quantity similar to rats and other mammals [3,7,8], and these neuropeptides have also been demonstrated in small nerve fibres in the skin of the chicken [17]. Furthermore, intravasal application of tachykinins and CGRP increases skin blood flow in the chicken [17]. One possible explanation for the lack of vascular responses in the avian skin after stimulation of afferent nerves may be an inappropriate release of peptides from stimulated fibres. Several peptides, including galanin, have been shown to inhibit the release of tachykinins and CGRP from mammalian sensory fibres [6,20]. Recent findings demonstrating that in the pigeon approximately 20% of DRG neurons contain galanin-like immunoreactivity [8] support a possible modulatory role for galanin. Noteworthy, in mammals galanin is present only in less than 5% of DRG neurons [4,10.18]. Therefore, we assumed that in the pigeon skin endogenous galanin might inhibit the release of tachykinins and/or CGRP from sensory nerves and modulate the vascular effects of these peptides. To test this hypothesis, in the present experiments we studied the effect of a high affinity galanin antagonist M35 [19] on cutaneous vascular responses elicited by mustard oil, or by antidromic electrical stimulation of a cutaneous nerve.

The experiments were performed on 30 domestic pigeons, anaesthetized with an isoflurane-oxygen gas mixture and kept at a constant body temperature of 40°C. The external iliac artery and vein were cannulated for measurement of the mean arterial blood pressure and for continuous electrolyte infusion, respectively. The ulnar artery was cannulated for close arterial infusion of the galanin antagonist Galanin (1–13)-bradykinin (2–9) amide (M35. Neosystem) dissolved in saline. Cutaneous blood flux (SBF) was mea-

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sured on the defeathered wing skin by a Laser Doppler Imager (LDI; Lisca Development). Blood pressure values and LDI signals were recorded, stored, and analyzed by a computer program. In the first series of experiments M35 (0.5, 1 and 5 nM) or its vehicle (0.9% saline) were injected 20 min after surgery in a volume of 500 μl into the ulnar artery within 10 min. Sixteen consecutive LDI images of a standardized 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 six cases the radial nerve serving the investigated skin area was transected on both sides 4–6 days before the experiments (chronic denervated group). In a second series of experiments ($n = 7$) antidromic nerve stimulation was used to induce cutaneous vascular responses in animals pretreated with guanethidine (20 mg/kg s.c.) 1 day before the experiment to prevent sympathetic vasoconstriction. The nervus cutaneous antebrachii lateralis was transected and the distal end placed on a pair of steel electrodes. Following the intraarterial (i.a.) infusion of 1 or 5 nM M35 or its vehicle, sequential LDI images were taken from a skin area of 50 mm^2 with a frequency of 8 per min for 8 min. After a control period of 1 min, nerve stimulation was started with rectangular pulses of 10 Hz, 20 V and 0.5 ms for a period of 20 s.

Values of SBF were determined by calculation of the average flux values of single images. To demonstrate the time course of skin perfusion, the relative changes of 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).

Slow retrograde i.a. infusion (500 μl/10 min) of either vehicle or low doses of M35 (0.5 and 1 nM) did not cause any change in SBF or blood pressure. In two animals a higher dose of M35 (5 nM) produced a slight decrease in blood pressure without any effect on SBF. Epicutaneous application of mustard oil after an i.a. infusion of 0.9% NaCl resulted in a slight increase in SBF (Fig. 1 and Table 1). After i.a. infusion of 1 or 5 nM M35, mustard oil elicited a marked dose-dependent increase in SBF (Fig. 1). Statistical evaluation of the data with one way ANOVA indicated a significant difference between the three groups ($P = 0.01$), while the post-hoc analysis (Student-Newman–Keuls) revealed significant differences between control and M35 pretreated groups. Mustard oil induced a similar small increase of SBF in normal and chronically denervated skin. In contrast, the effect of M35 was significantly attenuated in the denervated skin: the mustard oil induced vasodilatation was significantly smaller than the flux elevation measured in the intact skin after administration of M35 (Table 1). The two-way ANOVA confirmed the interaction between the effects of M35 pretreatment and denervation.

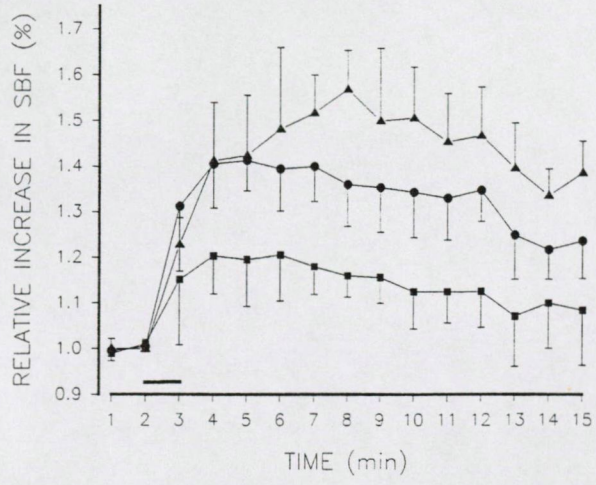

Fig. 1. Effect of epicutaneous application of mustard oil on skin blood flow (SBF) in control (■) and M35 pretreated animals after i.a. infusion of saline and 1 nM (●) or 5nM (▲) M35, respectively. The bar indicates the period of mustard oil application. For clarity, SD values are shown only in one direction.

Table 1

Relative increases in skin blood flow induced by mustard oil application in intact or chronically denervated skin

	Control	1 nM M35	5 nM M35
Intact skin	15.27 ± 5.34	29.74 ± 9.68*	40.4 ± 13.07*
Denervated skin	16.12 ± 7.97	19.09 ± 7.59	20.68 ± 7.81

Data are the mean ± SD of increases in skin blood flow (per cent) following mustard oil application on intact or chronically denervated skin in control and M35 pretreated animals from five to seven experiments. *Significantly different from control, $P < 0.05$.

The galanin antagonist also affected the cutaneous vasoreaction to antidromic nerve stimulation. In animals without guanethidine pretreatment, antidromic nerve stimulation resulted in a marked decrease in SBF, most probably due to the activation of sympathetic efferents. Pretreatment with guanethidine completely prevented this effect, unmasking a slight increase in SBF lasting for about 70 s. This small vasoreaction was not affected by i.a. infusion of 0.9% saline, but was changed markedly after M35 pretreatment (Fig. 2).

After infusion of 1 nM M35, antidromic nerve stimulation caused an immediate short increase in SBF which was followed by a long lasting second elevation after a latency of about 90 s. This biphasic flux response turned into an immediate monophasic increase of SBF with a long plateau after pretreatment with 5 nM M35 (Fig. 2 and Table 2).

The present experiments furnish evidence for the existence of neurogenic cutaneous vasodilator responses in the pigeon. Application of mustard oil onto the skin elicits a weak vasodilatory response which was not affected by denervation, indicating that this reaction is not of neurogenic origin. A similar non-neurogenic phlogogenic effect of mustard oil has been shown in the rabbit [14] and pig skin [11]. The vasodilatory effect of mustard oil was greatly enhanced by close arterial administration of the specific

galanin antagonist M35. The effect of the galanin antagonist was almost completely abolished by chronic denervation of the skin. These results strongly indicate that the augmentation of the vasodilatory action of mustard oil by pretreatment with the galanin antagonist is dependent on an intact cutaneous innervation. In addition, we obtained direct evidence for a neurogenic vasodilator mechanism in the skin of the pigeon by showing that the slight increase in SBF resulting from antidromic stimulation of a cutaneous nerve was significantly enhanced by prior administration of the galanin antagonist. These observations indicate that neurogenic vasodilatory responses elicited either chemically or by antidromic electrical stimulation of a cutaneous nerve in the skin of pigeons are under the inhibitory influence of endogenous galanin. The results also suggest that sensory nerves are intimately involved in this mechanism. It is tempting to suggest that in the intact (innervated) skin galanin may exert an inhibitory influence on the release of vasodilator sensory neuropeptides. Further studies are needed to clarify the possible source of galanin and the factors which influence its release. Since the primary aim of the present study was to reveal a presumed local regulatory or sensory efferent function of sensory nerves in an avian species, no attempt has been made to identify the possible mediator(s) of the observed vasodilatory effect. It can be suggested, however, that tachykinins and/or CGRP may be involved in this response. These peptides have been localized in avian dorsal root ganglion neurons [3,7,8] and cutaneous nerve fibres [17]. In addition, SP and CGRP showed potent vasodilator effects in the chicken skin [17]. Further experiments utilizing specific peptide antagonists and/or immunoblockade may clarify this point.

The present findings point to a new type of modulation of cutaneous inflammatory responses which may operate at the level of the sensory nerve ending by the release of inhibitory peptides. The results indicate that galanin may represent

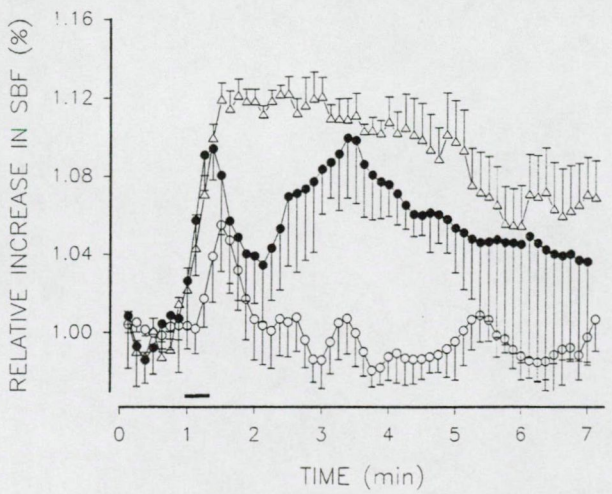

Fig. 2. Effect of antidromic nerve stimulation on the skin blood flow (SBF) in control animals (i.a. infusion of 0.9% NaCl, ○) and after infusion of 1 nM (●) or 5 nM M35 (△). The bar indicates the period of stimulation.

Table 2

Maximal increases in skin blood flow induced by antidromic nerve stimulation in control and M35 pretreated animals

Time (min post-stimulation)	Control	1 nM M35	5 nM M35
0–1	5.15 ± 5.61	9.91 ± 1.22	12.6 ± 1.78*
1–5	1.16 ± 3.44	10.99 ± 2.77*	13.79 ± 1.59*
6–7	−0.59 ± 3.11	3.63 ± 5.15	6.88 ± 4.33*

Data are the mean ± SD of maximal increases in skin blood flow (per cent) measured for three different consecutive periods after the start of antidromic nerve stimulation (0 min) from five to seven experiments. *Significantly different from control, $P < 0.05$.

such an endogenous peptide modulator. This assumption is in line with previous findings showing an inhibitory effect of exogenous galanin on neurogenic inflammatory reactions of the skin and joints [6,20]. It has been suggested that galanin is a potent endogenous inhibitor of neurogenic inflammation [6] and that the inhibitory effect of galanin may be due to an inhibition of the release of vasoactive peptides from sensory nerve terminals [5,6]. Although inhibition of the release of neural vasoactive mediators by galanin seems to be the most likely explanation for the effect observed in the present study, an interaction of galanin with vascular/endothelial mechanisms cannot be excluded.

In conclusion, the present study revealed that in the pigeon antidromic electrical stimulation of a cutaneous nerve or direct application of a chemical irritant onto the skin evoke neurogenic vasodilatory responses which may be masked by an inhibitory action of endogenous galanin-like substance(s). The observations provide the first evidence that sensory nerves may be implicated in an inhibitory local regulatory function in the avian skin.

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Inhibitory modulation of cutaneous vascular responses by endogenous galanin in the pigeon

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Abstract

The possible role of endogenous galanin in modulation of cutaneous vascular responses was studied in pigeons. Chemically induced plasma extravasation and regional skin blood flow changes were measured simultaneously with a capillary perfusion technique and a laser Doppler imager, respectively. Perfusion with both histamine and bradykinin increased plasma protein extravasation which was dose-dependently and significantly augmented by co-administration of M35, a specific galanin antagonist. This effect of M35 was abolished after chronic cutaneous denervation. In intact but not denervated skin, M35 increased the vasodilatatory effect of histamine, too. It is suggested that galanin-containing nerves may play an inhibitory efferent role in the modulation of cutaneous inflammatory responses.

Keywords: Inflammation; Skin; Galanin; Nerves; Blood flow; Plasma extravasation

In mammalian skin stimulation of chemosensitive afferent fibres induces local vascular changes involving vasodilatation and plasma extravasation [3,10,12,15]. These responses are mediated by the release of neuropeptides, primarily tachykinins and calcitonin gene related-peptide, from stimulated nerve terminals [3]. Previous studies have shown that in avian species antidromic electrical stimulation of cutaneous nerves or direct chemical stimulation of sensory nerve endings produced only moderate vascular responses [4,16]. Immunohistochemical studies revealed that in birds, similar to mammals, dorsal root ganglion neurones of pigeons contain substance-P and CGRP [7,16]. However, it has been demonstrated also that, in the pigeon, the proportion of galanin-immunoreactive cells is considerably higher compared to mammals [8]. Exogenous galanin has been shown to inhibit neurogenic inflammatory processes in rats [6,20] and available experimental evidence indicates that endogenous galanin of neural origin inhibits neurogenic cutaneous vasodilatation in the pigeon [17]. Therefore, we assumed that the failure of chemical irritants to produce neurogenic vascular responses in the avian skin may be related to an inhibitory action of endogenous galanin. To test this hypothesis, in the present experiments we studied the effect of a specific galanin antagonist on chemically-induced cutaneous vascular responses in the pigeon.

The experiments were performed on 46 domestic pigeons (*Columba livia*) anaesthetised with an isoflurane-oxygen gas mixture. The dorsal surface of the wing was defeathered one day prior to the experiments. Mean arterial blood pressure and body temperature were continuously monitored. Plasmapheresis capillaries (diameter: 0.4 mm, cut off size: 3000 kDa, Asahi, Japan) were inserted intracutaneously with the aid of a guiding cannula and connected through a fine tubing (Tygon, Novodirekt, Germany) to a perfusion pump. The capillaries were perfused with Ringer's solution at a flow rate of 3.25 μl/min. Fractions of the effluent were collected every 20 min for the determination of their protein content with Bradford's method [2] using an MRX microtiter-plate reader (Dynatech, Germany). After an equilibration period of 60 min, histamine (Sigma) or bradykinin (Neosystem) dissolved in Ringer's solution were perfused through the capillaries for 40 min. M35, a specific galanin antagonist (galanin-1-16-bradykinin-2-9-amide [1], Neosystem) was co-perfused with bradykinin or histamine to examine their effect on plasma protein extravasation and vasodilatation evoked by these vasoactive agents. Cutaneous blood flow was measured with a laser Doppler imager (LDI, Moor Instruments). Consecutive perfusion images

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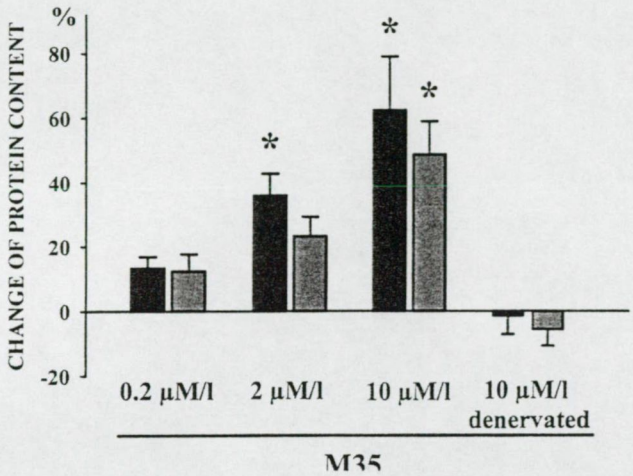

Fig. 1. Effect of M35, a specific galanin antagonist on plasma protein extravasation induced by histamine (500 μM/l, black columns) and bradykinin (200 μM/l, gray columns) in the innervated and denervated skin of the pigeon. Values are expressed as percentage increases (mean ± SEM) in total protein content of the perfusate.

were captured of a skin area of 0.6 cm^2 with a frequency of 1/min. Photographic and perfusion images were processed as described previously [17]. To study the role of cutaneous nerves in the mechanism of cutaneous vascular responses elicited by these agents, the skin was denervated by sectioning the ulnar and radial nerves 5–7 days before the experiment.

Perfusion of the skin with Ringer's solution resulted in a gradual decrease of protein concentration of the perfusate. Pilot experiments showed that histamine and bradykinin produced dose-dependent increases in the plasma protein content of the perfusate (data not shown). In further experiments concentrations of 200 and 500 μM/l of bradykinin and histamine, respectively, were chosen which produced similar and reproducible increases in cutaneous vascular permeability. The maximal increases of the protein content of the perfusate induced by histamine and bradykinin amounted to 282±22 and 233±18 μg/ml, respectively. The calculated total protein content of the fractions collected for 60 min after the start of the perfusion were significantly higher after the administration of either histamine (48.65±3.36 mg) or bradykinin (43.73±3.36 mg), as compared with the control, i.e. infusion of Ringer's solution (20.91±1.29 mg). Co-administration of increasing doses of the galanin antagonist, M35 caused dose-dependent increases in histamine-induced plasma protein extravasation (Fig. 1). Similarly, co-perfusion of M35 with bradykinin dose-dependently enhanced the protein content of the perfusate. Calculation of the total protein content of the perfusates collected for a 60-min period after co-administration of M35 showed significant dose- and drug-dependent increases for both histamine and bradykinin (two-way ANOVA $P < 0,001$; post-hoc analysis with Fisher LSD method $P < 0.05$). In contrast, in the chronically denervated skin co-administration of M35 failed to affect the responses to either histamine or bradykinin (Fig. 1).

Both histamine and bradykinin produced significant increases in cutaneous blood flow as measured by LDI, although bradykinin produced a less marked effect. Co-administration of M35 resulted in moderate increases in the vasodilatatory response to both agents which reached significance in the case of histamine but not bradykinin (Fig. 2). Denervation markedly reduced the vasodilatatory effect of histamine but failed to significantly affect the response to bradykinin. The pro-vasodilatatory effect of M35 on histamine-induced vasodilatation was completely abolished in the chronically denervated skin (Fig. 2).

Mediators of the acute inflammatory reaction may produce their vascular effects not only by a direct action on vascular smooth muscle or endothelium, but also by an action on cutaneous nerves [3,9,11]. The release of sensory neuropeptides by bradykinin and histamine [3,5] significantly contributes to the permeability-enhancing effect of these agents [9,11]. Hence, the reduction of the vasodilatatory effect of histamine observed in the present experiments following denervation may be accounted for by a lack of release of vasodilatatory peptide(s) from cutaneous nerves. Denervation failed to affect the vasodilatatory effect of bradykinin which, therefore, may be attributed mostly to a direct action on vascular smooth muscle under the experimental conditions used.

The most striking observation of the present study was the marked increase of the vasodilatatory and, in particular, the vascular permeability enhancing effects of both histamine and bradykinin after the administration of M35, a specific galanin antagonist. This may suggest that release of vasoactive peptides from cutaneous nerves may be under a tonic galaninergic inhibition in this species. Several line of evidence support this suggestion. Exogenous galanin is known to inhibit the release of inflammatory neuropeptides

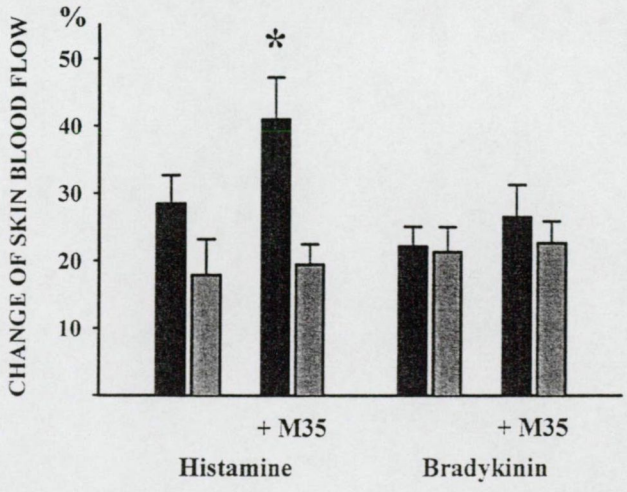

Fig. 2. Effect of M35, a specific galanin antagonist on cutaneous vasodilatation elicited by histamine (500 μM/l) and bradykinin (200 μM/l) in the innervated (black columns) and denervated (gray columns) skin of the pigeon.

from sensory nerves [6,20]. Furthermore, inhibition of the release of a number of classical and peptide transmitters and hormones by galanin is well documented [13]. Finally, galanin receptors were demonstrated on primary sensory neurons of several species [18,19]. In conclusion, the present findings strongly indicate that galanin-containing nerves may fulfil a novel, inhibitory sensory-efferent function of cutaneous nerves. The widespread presence of galanin in sensory fibres of the rat may indicate that similar mechanisms may operate in species other than the pigeon [14]. Further studies are needed to determine the factors which may influence and modify the release of peptides and/or other mediators from cutaneous nerves or non-neural elements under physiological conditions.

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Galanin mediated inhibitory nervous modulation of cutaneous vascular reactions

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Noxious stimulation induces local inflammatory responses in a variety of mammals but these reactions are only faint in avian species. The possibility that endogenous galanin inhibits neurogenic vascular responses in avians was tested in the wing skin of anaesthetized pigeons. Intraarterial infusion of nanomolar concentrations of the specific galanin antagonist M35 dose dependently enhanced the small mustard oil induced increase of skin blood flow measured by means of a Laser Doppler Imager. Similarly, the small transient vasodilatation following electrical stimulation of a cutaneous nerve was also enhanced by M35. The effect of M35 was not observed after chronic denervation. Coperfusion of M35 dose dependently augmented the histamine and bradykinin induced plasma extravasation revealed by skin microdialyses, but this effect was abolished in the chronically denervated skin. However, chronic denervation *per se* enhanced the plasma extravasation induced by histamine but not by bradykinin and this effect was diminished by coperfusion of galanin. The results suggest an inhibitory modulation of cutaneous neurogenic inflammatory reactions by endogenous galanin in the pigeon.

Keywords: galanin, inhibitory nervous modulation, cutaneous vascular reactions, avian species, galanin antagonist M 35, cutaneous nerve, histamine, bradykinin, pigeon

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A significant population of mammalian cutaneous afferent nerves involved in the transmission of noxious information also possesses local regulatory or "sensory efferent" functions. Sensory nerve-mediated neurogenic inflammatory responses such as involving vasodilatation and plasma protein extravasation are the most prominent features of sensory efferent functions. These tissue reactions are mediated primarily by tachykinins and calcitonin gene-related peptide (CGRP) released from stimulated sensory nerve terminals. In addition, a modulatory, pro-inflammatory effect of these sensory neuropeptides has also been documented [6]. In contrast to mammals, neurogenic inflammatory responses are only moderately expressed in avian species [3, 8]. This species difference cannot be explained by an inappropriate level of sensory neuropeptides. Immuno-histochemical studies revealed that avian dorsal root ganglion neurones contain tachykinins and CGRP similar to mammalian sensory cells [5, 8]. However, in pigeon dorsal root ganglia the proportion of galanin-immunoreactive neurones is considerably higher than in mammals [5]. Previous studies have shown that exogenous galanin inhibited neurogenic inflammatory reactions in the rat by inhibition of peptide release from afferent nerves [4, 10]. We hypothesised that in the pigeon endogenous galanin may interfere with the effect of sensory neuropeptides or inflammatory mediators leading to a reduction of their vascular actions. To test this hypothesis, in the present experiments we studied the effect of a specific galanin antagonist on sensory nerve-mediated cutaneous vascular responses.

Materials and methods

Experiments were performed on 76 domestic pigeons anaesthetised with an isoflurane-oxygen gas mixture. The dorsal surface of the wing was defeathered one day prior to the experiments. The arterial blood pressure and the body temperature were continuously monitored. In 30 animals the ulnar artery was canulated for close arterial injection of galanin antagonist (galanin (1–13) – bradykinin (2–9) amide, M35, Neosystem) dissolved in physiological saline. Skin blood flux (SBF) was measured on the defeathered wing skin by a Laser Doppler Imager (LDI). In the first series of experiments different doses of M35 and its vehicle were administered into the ulnar artery (50 µl/min). Sixteen consecutive LDI images were recorded from a standardised skin area at 1 min intervals. After determination of the baseline perfusion a piece of filter paper moistened with 5% mustard oil dissolved in liquid paraffin was placed onto the skin. In 6 cases the radial nerve innervating the investigated skin area was transected 4–6 days before the experiments (chronic denervation). In the second series of experiments antidromic nerve stimulation was used to elicit cutaneous vascular responses in guanethidin pretreated animals (20 mg/kg s.c.). The nervus cutaneous

antebrachii lateralis was exposed for electrical nerve stimulation. Following an intra-arterial infusion of M35 or its vehicle sequential LDI images were taken with a frequency of 8/min for 8 min. Nerve stimulation was started after the 1st min with rectangular pulses of 10 Hz, 20 V and 0.5 ms for 20 s. Values of SBF were determined by calculation of the average flux values of single images. For a detailed description of the data analysis see reference [9]. Statistical analysis of the experimental data was performed with ANOVA.

In 46 animals plasmapheresis capillaries (diameter 0.4 mms, cut off size 300 kDa, Asahi Japan) were inserted intracutaneously with the aid of a guiding cannula and connected through fine plastic tubes (Tygon) to a perfusion pump. The capillaries were perfused with Ringer's solution at a flow rate of 3.25 μl/min. The effluent fractions were collected every 20 min for photometric determination of their protein content using Bradford's method [2]. After a 60 min equilibration period histamine or bradykinin dissolved in Ringer's solution were perfused for 40 min. To study a possible modulatory role of galanin on cutaneous vasodilatation and plasma extravasation the galanin antagonist M35 was co-perfused with histamine and bradykinin. SBF was measured with LDI as described above. To study the contribution of nervous elements to the mechanism of inflammatory responses chronic cutaneous denervation was produced by sectioning the radial and ulnar nerves 5–7 days prior to the experiments.

Results

Epicutaneous application of mustard oil induced a slight increase in SBF (15.27± 2.39%). Intraarterial infusion of 1 or 5 nM M35 significantly and dose-dependently increased mustard oil-induced enhancement of SBF (1 nM : 29.74±4.33% and 5 nM : 40.4±5.85%) calculated by the area under the curve method (Fig. 1). Neither infusion of vehicle nor infusion of 5 nM M35 alone altered significantly the SBF. In the chronically denervated skin, mustard oil evoked responses similar to that was seen in intact skin (16.12±3.56%). In contrast, administration of M35 failed to result in an enhancement of mustard oil-induced cutaneous vasodilatation (Fig. 1).

Antidromic electrical stimulation of the cutaneous antebrachii lateralis nerve with a short train of suprathreshold impulses elicited a transient increase in SBF (max. 5.15± 2.51%) in guanethidin pretreated animals. Intraarterial infusion of 1 nM M35 resulted in an increase of the immediate vasodilatatory response (9.91±1.22%) followed by a further increase in the SBF (10.99±1.24%) after a latency of 60s. Infusion of 5 nM M35 produced a further enhancement (13.79±0.71%) of these vasodilatatory responses.

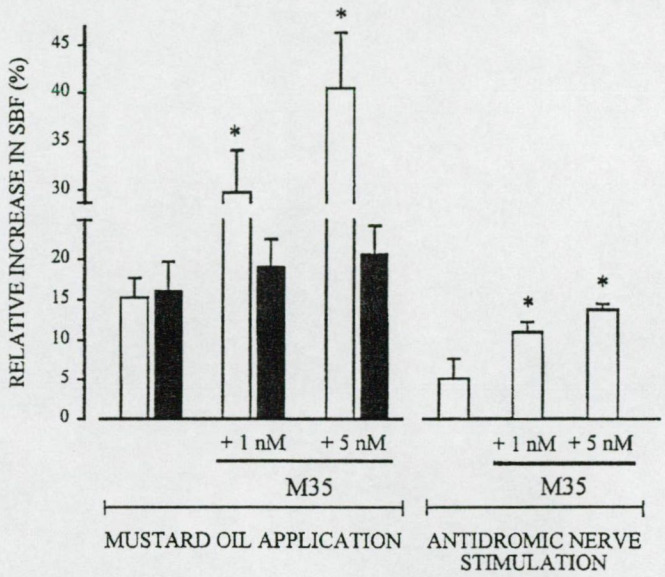

Fig. 1. Effect of M35, a galanin antagonist on relative increases in skin blood flow (mean ± S.E.M.) induced by mustard oil application or antidromic nerve stimulation in intact (open columns) and chronically denervated (black columns) skin. *: significantly different from corresponding control value, $p<0.05$

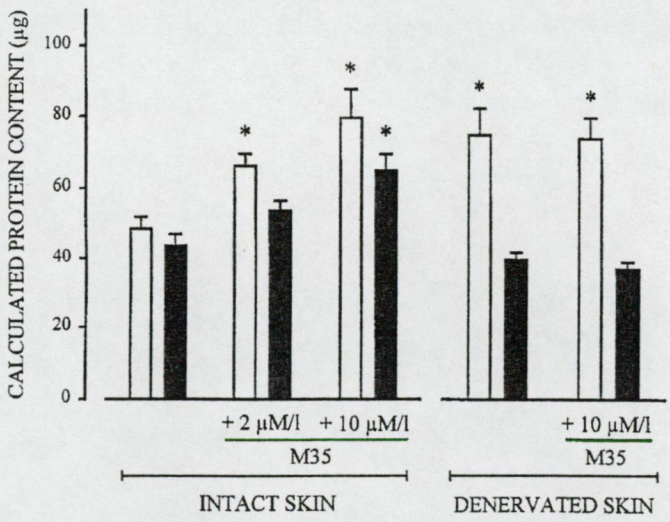

Fig. 2. Effect of M35, a galanin antagonist on histamine- (500 µM/l, open columns) and bradykinin- (200 µM/l, black columns) induced cutaneous plasma protein extravasation in intact and denervated skin. Values represent the total protein content of the perfusate (mean ± S.E.M.). *: significantly different from corresponding control value, $p<0.05$

To elicit plasma extravasation, histamine and bradykinin were perfused at constant doses of 500 and 200 μM/l, respectively, which have been shown to produce similar and reproducible increases in vascular permeability. The calculated protein content of the fractions collected during a 60 min period following the start of the perfusion of histamine and bradykinin amounted to 48.65±3.36 μg and 43.73±3.36 μg, respectively, which were significantly higher than compared to the control values obtained after perfusion of Ringer's solution (20.91±1.29 μg). Co-administration of increasing doses (2 and 10 μM/l) of M35 dose-dependently enhanced the permeability increasing effects both of histamine and bradykinin by 63% and 48%, respectively (Fig. 2).

Chronic denervation *per se* significantly enhanced the permeability increasing effect of histamine but was without effect on the action of bradykinin. Co-perfusion of galanin (10 μM/l) reduced this augmented response following histamine application. Co-administration of M35 failed to affect significantly the permeability increasing effect of either histamine or bradykinin (Fig. 2).

Perfusion of both histamine and bradykinin increased cutaneous blood flow by 28.43±4.19% and 22.04±2.9%. Co-administration of M35 significantly augmented the vasodilatatory effect of histamine (SBF increase: 40.92±6.25%) but not that of bradykinin (26.41±4.82%). Chronic denervation reduced the vasodilatation elicited by histamine (17.81±5.33%) but failed to affect the response to bradykinin (21.2±3.7%). However, in the chronically denervated skin M35 was without any effect on irritant induced vasodilatation (histamine: 19.32±3.1%; bradykinin: 22.62±3.23%).

Discussion

Neurogenic cutaneous vascular responses elicited by chemical irritants and mediated by activation of afferent nerves are common in mammalian species. In contrast, in the pigeon only moderate vascular responses were evoked by epicutaneous application of mustard oil in the present study. This weak vasodilatatory response was greatly augmented by a prior close arterial injection of M35, a high affinity galanin antagonist. Similarly, the modest cutaneous vasodilatation produced by antidromic nerve stimulation was also significantly enhanced after the administration of the galanin antagonist. These findings strongly indicate that endogenous galanin exerts a tonic inhibitory action on sensory nerve-mediated cutaneous vasodilatation. Furthermore, the findings showing an abolition of the pro-inflammatory effect of the galanin antagonist in the chronically denervated skin suggest that inhibitory modulation of the vasodilatatory response by galanin is neurogenic in nature.

Histamine and bradykinin produced distinct plasma protein extravasation in the pigeon skin. The amount of extravasated plasma protein was greatly increased after a prior administration of M35. These results suggest that endogenous galanin may exert an inhibitory effect on histamine- and bradykinin-evoked increases in vascular permeability. In the chronically denervated skin administration of M35 was without effect on histamine or bradykinin elicited vascular responses. This suggests that the inhibitory modulation of the permeability enhancing effects of these vasoactive agents involves a neurogenic link. It is worthy to mention that chronic denervation resulted in a marked augmentation of the vascular permeability enhancing effect of histamine but not bradykinin. This may be explained by a possible difference in the (neural) regulation of vascular histamine and bradykinin receptors, respectively. Alternatively, these changes may be related to denervation-induced alterations in the prejunctional modulation of histamine-induced neuropeptide release from sensory nerves [7].

In conclusion, the present experiments revealed a marked inhibitory modulation of cutaneous vascular responses involving neurogenic vasodilatation and plasma extravasation by endogenous galanin. The results provide evidence for a new, hitherto unrecognized inhibitory local efferent function of peptidergic cutaneous sensory nerves. Inhibition of peptide release from sensory nerves by endogenous agents, e.g. by galanin, may explain the inability of chemical irritants or antidromic nerve stimulation to evoke sensory nerve-mediated neurogenic vascular responses in species other than small laboratory rodents.

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Inhibitory neurogenic modulation of histamine-induced cutaneous plasma extravasation in the pigeon

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Abstract

The neurohumoral modulation of the permeability increasing effect of histamine was studied in pigeon skin. Substances were administered through plasmapheresis capillaries inserted into the dorsal wing skin and the protein contents of the perfusates were determined by a quantitative method. The vascular labelling technique was also utilized to histologically identify leaky blood vessels. In the innervated skin histamine evoked a significant, dose-dependent plasma extravasation which was markedly augmented by the coadministration of a specific galanin receptor antagonist, galanin-1-16-bradykinin-2-9-amide (M35). Chronic cutaneous denervation per se resulted in a significant elevation of the permeability-enhancing effect of histamine. In the denervated skin this response was not affected by M35 but was significantly inhibited by galanin. It is concluded that in the normally innervated skin endogenous galanin may exert a neurogenic tonic inhibitory effect on histamine-induced plasma leakage. It is suggested that sensory nerves possess not only pro-inflammatory, but also anti-inflammatory (inhibitory) sensory-efferent functions.

Keywords: Skin; Inflammation; Galanin; Sensory; Vascular labelling

1. Introduction

Histamine is a major mediator of inflammatory reactions and plays an important role in cutaneous vascular responses. Humoral and neural influences may effectively modulate the permeability enhancing effects of a variety of vasoactive agents involving histamine. In particular, selective sensory denervation has been shown to markedly and significantly inhibit cutaneous dye leakage responses evoked by histamine [1–4]. The pro-inflammatory action of sensory nerves has been attributed to a release of vasoactive peptides from sensory nerve endings upon stimulation by inflammatory mediators resulting in an amplification of the primary response [2,3,5,6]. The sensory-efferent or local regulatory function of sensory nerves accomplished by the release of vasoactive sensory neuropeptides is well established in several mammalian species [7–9]. Similar sensory nerve-mediated cutaneous responses could not be demonstrated in avian species [10]. However, recent findings have shown that following the administration of a specific galanin receptor antagonist, electrical or chemical stimulation of cutaneous sensory nerves also elicited neurogenic vasodilatation and plasma extravasation in the pigeon [11,12]. The available experimental evidence indicated that the release of sensory neuropeptides may be under a tonic galaninergic inhibition in this species. This galanin-mediated modulation is neurogenic in nature, since chronic cutaneous denervation completely eliminated the effect of the galanin antagonist. Therefore, it has been concluded that sensory nerves may exert not only pro-inflammatory (stimulatory) but also inhibitory sensory-efferent functions [12]. The present experiments were initiated in an attempt to characterize the

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(inhibitory) modulatory action of cutaneous sensory nerves on histamine-induced plasma extravasation in the pigeon.

2. Materials and methods

Forty-six domestic pigeons (*Columba livia*) anaesthetised with an isoflurane–oxygen gas mixture were used in these experiments. The feathers covering the dorsal surface of the wing were removed the day before the experiment. The femoral artery and vein were cannulated for recording of the mean arterial blood pressure and intravenous injections. Body temperature was continuously monitored and maintained at 39±1°C. Plasmapheresis capillaries (diameter: 0.4 mm, cut-off size: 3000 kDa, Asahi, Japan) were inserted intracutaneously with the aid of a guiding cannula and connected through a fine tubing (Tygon, Novodirekt, Germany) to a perfusion pump. The capillaries were perfused with Ringer's solution at a flow-rate of 3.25 ml/min. Fractions of the effluent were collected every 20 min for the determination of their protein content with Bradford's method [13] using an MRX microplate reader (Dynatech, Germany). After an equilibration period of 60 min, histamine (Sigma) dissolved in Ringer's solution was perfused through the capillaries for 40 min. Porcine galanin (Neosystem) or the specific galanin antagonist, M35 (galanin-1-16-bradykinin-2-9-amide, Neosystem [14]) were co-perfused with histamine to examine its effect on plasma protein extravasation. To study the role of cutaneous nerves in the mechanism of histamine-induced plasma extravasation and of the effects of the galanin antagonist, cutaneous denervation was performed by sectioning the ulnar and radial nerves 5–7 days before the experiment.

To demonstrate the vascular changes associated with increased permeability, the vascular labelling technique [15–17] was used for the histological visualization of the distribution and type(s) of leaky blood vessels in the intact (innervated) and denervated wing skin. Briefly, different doses of histamine (1 or 10 μg per site) were injected intracutaneously 10 min after injections (100 μl) of Tyrode's solution or M35 (5 or 10 μM) at the same sites. Immediately after the injection of histamine, infusion of a 1% solution of colloidal silver (1 ml/100 g b.w.) was started at a rate of 0.5 ml/min. The animals were sacrificed 20 min later by an overdose of the anaesthetic and skin samples were removed and processed for histological examination as whole mounts [2,16]. The density of leaky blood vessels was determined by measuring the lengths of silver-labelled vessels with the aid of a camera lucida and a digitizing tablet using a computer program (SIGMA-SCAN). Values are expressed as the length of the labelled blood vessels per unit skin area (mm/mm^2). Diameters of silver-labelled blood vessels were measured using an ocularmicrometer. All values are expressed as mean±S.E.M. Statistical evaluation of the experimental data was performed using ANOVA.

3. Results

Perfusion of Ringer's solution through intracutaneously placed plasmapheresis capillaries resulted in a gradual decrease of protein concentration of the perfusate reaching a low constant level after 40 min. The effective doses of histamine in inducing plasma protein extravasation were determined in pilot experiments. Increasing doses of histamine evoked a dose-dependent increase in vascular permeability as determined on the basis of the maximal (peak) increases in the protein content of the perfusates (data not shown) or by calculating the total amount of extravasated protein following a 40-min period of histamine application (Table 1).

In further experiments a histamine concentration of 500 μM was chosen which produced marked plasma protein extravasation. The calculated total protein content of the fractions collected for 60 min after the start of the perfusion were significantly higher after the administration of histamine (48.65±3.36 μg) as compared to the control i.e. infusion of Ringer's solution (20.91±1.29 μg). Coadministration of increasing doses of the galanin antagonist, M35 caused dose-dependent increases in histamine-induced plasma protein extravasation. Calculation of the total protein content of the perfusates collected for a 60-min period after coadministration of histamine and M35 showed significant dose- and drug-dependent increases (Table 2). Perfusion of the galanin antagonist alone failed to affect the protein concentration of the perfusate (data not shown).

In the chronically denervated skin the permeability increasing effect of histamine was greatly augmented; the total protein content of the perfusate increased by 49±3% as compared to the control (Fig. 1, $P < 0.05$, $n = 6$). This enhanced response to histamine was not affected by coadministration of M35 (Fig. 1). However, in the chroni-

Table 1
Dose-dependent effect of histamine on the protein content of the skin perfusates

Histamine (μM)	Protein content[a] (μg)	Percent increase
Control	19.71±1.18	
10	27.95±4.19*	41.81±21.26*
100	39.02±5.76*	97.97±29.22*
500	48.64±3.35*	146.78±16.98*
1000	61.51±9.37*	212.07±47.53*

[a] Data represent the total protein content of skin perfusates collected for 40 min during the administration of histamine at the concentrations indicated.

*, Values are significantly different from the control and from each other, $P < 0.05$, $n = 5$.

Table 2
Dose-dependent effect of the galanin antagonist, M35 on the histamine-induced changes in the protein concentration of skin perfusates

	Protein content[a] (μg)	Increase (%)
Control	20.91±1.29	
Histamine	48.64±3.35*	132.62±16.02*
+ 0.2 μM M35	54.05±2.61*	158.49±12.48*
+ 2 μM M35	66.09±3.34*	216.07±15.97*
+ 10 μM M35	79.43±8.07*	279.87±38.59*
10 μM M35	21.59±1.75	3.25±8.37

[a] Data represent the total protein content of skin perfusates collected for 40 min during the coadministration of histamine and M35. Histamine was used at a concentration of 500 μM.

*, Significantly different from the control, $P < 0.05$, $n = 5$ for each treatment group.

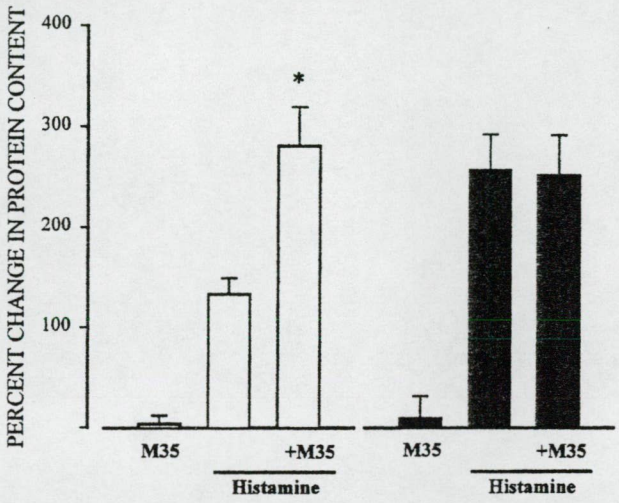


Fig. 1. Effect of the galanin antagonist M35 and of chronic cutaneous denervation on histamine-induced cutaneous plasma extravasation. Data represent changes in the total protein content of skin perfusates collected during the administration of histamine (500 μM), M35 (10 μM), or both. Open and shaded bars represent data obtained in the intact, innervated and in the denervated skin, respectively. *, Significantly different from control, $P < 0.05$, $n = 6$. For further details see text.

cally denervated skin, administration of galanin (10 μM) strongly inhibited the enhanced plasma leakage induced by histamine resulting in values similar to those obtained in the intact (innervated) skin ($P < 0.05$, $n = 5$).

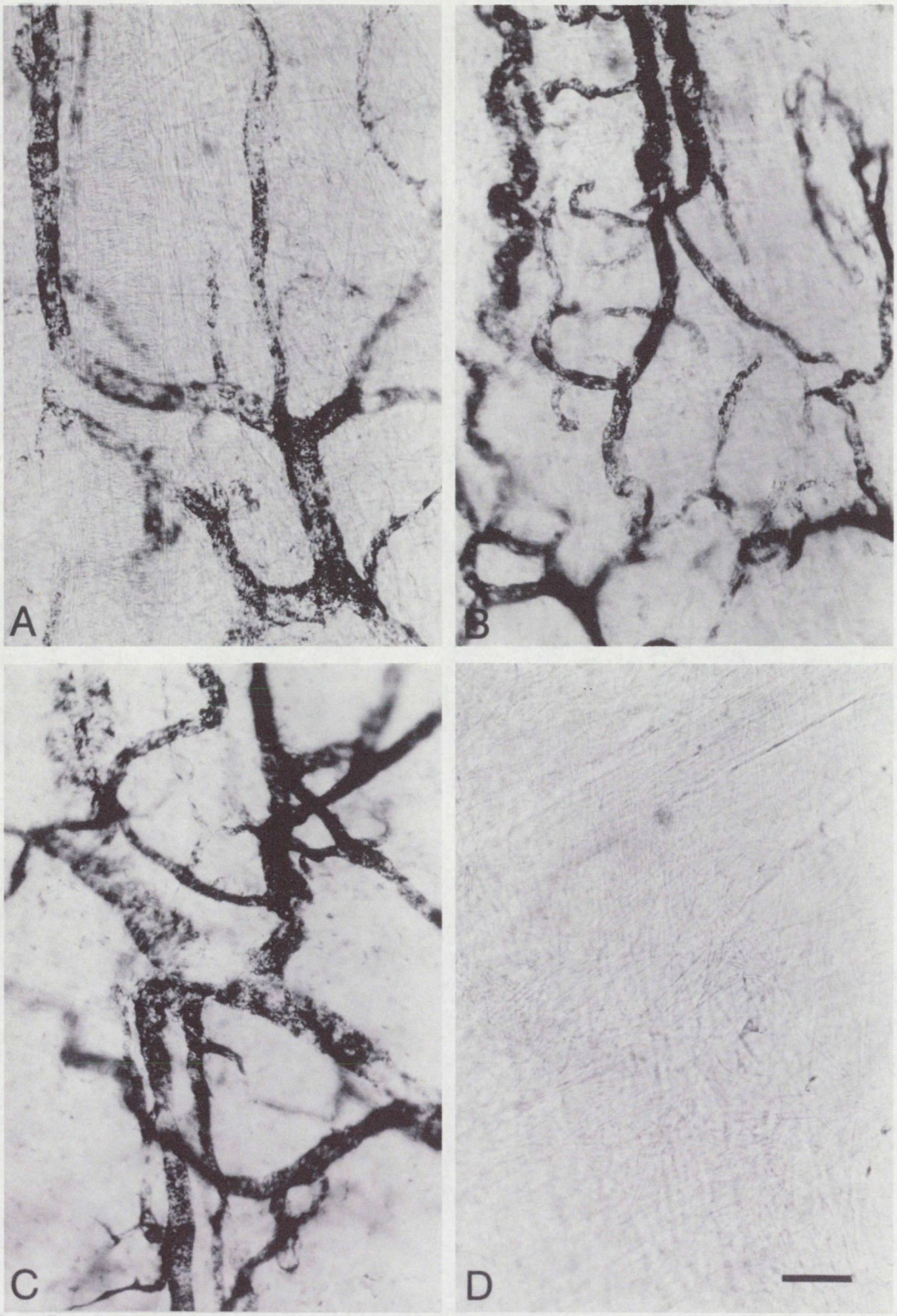
Histological examination of tissue samples obtained from skin sites injected with histamine and/or the galanin antagonist supported these findings (Fig. 2). In the innervated skin, sites of histamine injections showed marked vascular labelling: small blood vessels displayed conspicuous silver depositions in their walls, a characteristic histological feature of leaky venules [15–18]. Quantitative analysis revealed a significantly increased number of leaky blood vessels at sites of injections of the galanin antagonist and histamine as well as at sites of injections of histamine in the denervated skin (Fig. 2B and C and Table 3). Injection of the galanin antagonist on its own did not cause vascular labelling. Measurements of silver-labelled blood vessels revealed a significant increase in their diameters following the coadministration of histamine and M35 or after chronic cutaneous denervation (mean diameters of labelled vessels: histamine: 22.1±0.9, histamine plus M35: 28.5±2.4, histamine plus denervation: 28.7±1.8 μm).

4. Discussion

In recent years sensory nerves have been shown to contribute to the mechanism of a variety of vascular and inflammatory responses by the release of vasoactive sensory neuropeptides [2,5–8]. In mammalian species this particular population of afferent nerves is characterized by their sensitivity to capsaicin [3,4,19] owing to the expression of a capsaicin receptor, the type 1 vanilloid receptor, VR1 [20]. Avian species are essentially insensitive to capsaicin [10] and probably do not express such receptors. The present findings indicate that sensitivity of primary sensory neurons to capsaicin is not essential with respect to their 'sensory efferent' or local regulatory function. Hence, elimination of cutaneous nerves by chronic denervation resulted in significant changes in the inflammatory responses to histamine. This effect may be attributed to an action on sensory nerves, since pretreatment of the animals with guanethidine, an adrenergic neuron blocking agent, failed to significantly affect cutaneous inflammatory responses (unpublished observations). These findings suggest that cutaneous afferent nerves may exert an inhibitory effect on vascular responses.

Previous studies have shown that administration of a specific galanin antagonist largely augmented cutaneous vascular responses evoked by antidromic electrical stimulation of cutaneous nerves or by direct application of chemical irritants onto the skin [11,12]. Therefore, it has been concluded that, in the intact, innervated skin, galanin may inhibit inflammatory responses. It has been suggested that galanin, at least in part, may be of neurogenic origin, since immunohistochemical studies revealed that in the pigeon a large population of sensory ganglion cells contain galanin [21]. The involvement of galanin in these responses is further supported by the present findings showing that, in the denervated skin, administration of galanin significantly reduced the augmented response to histamine.

Histamine may elicit its vascular permeability enhancing effect not only by a direct vascular action but also by an indirect action on sensory nerves. Thus, histamine has been shown to stimulate sensory nerve endings [22] and to release (proinflammatory) sensory neuropeptides from them [23]. Therefore, the most likely explanation of the present results is that in the intact skin this 'neurogenic' effect of histamine is inhibited by a tonic release of galanin. In addition, histamine may also release galanin



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68 Fig. 2. Light microscopic photographs showing details of intact (A,B,D) and denervated (C) dorsal wing skin sites injected intracutaneously with histamine
69 (A,C), with histamine and the galanin antagonist, M35 (B) or with Tyrode's solution (D). Leaky blood vessels situated mostly in the vicinity of feather
70 follicles are characterized by the intense deposition of colloidal silver in their walls. Note the marked increase of histamine-induced vascular labelling in
71 the denervated skin (C) and after the administration of M35 (B). There is no labelling after an injection of Tyrode's solution (D). Scale bar in (D)
72 represents 100 μm and applies for all microphotographs.

Table 3
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[a] (mm/mm^2)
Histamine	8.31±2.54
Histamine + M35	16.80±2.69*
Histamine + denervation	14.25±2.80*

[a] Data represent the lengths of silver-labelled cutaneous blood vessels per unit surface area of the skin. Histamine and M35 were administered at concentrations of 500 μM and 10 μM, respectively.
*, Significantly different from histamine, $P < 0.05$, $n = 6$.

which may mask the effect or result in a diminished release of pro-inflammatory peptides from the same or from different nerves. The widespread presynaptic inhibitory effect of galanin on transmitter release [24] involving the inhibition of peptide release from sensory nerves [23,25,26] is in line with this suggestion. The findings showing a marked increase in the permeability enhancing effect of histamine in the denervated skin and, in particular, in the innervated skin following a coadministration of a galanin receptor antagonist also strongly support this notion. However, the possibility that galanin may affect vascular responses by a direct action on blood vessels cannot be excluded. Chronic cutaneous denervation, by a reduction of tissue galanin level, may lead to a suppression of the inhibitory effect of galanin on vascular permeability and, consequently to an enhancement of the permeability increasing effect of histamine. This is supported by our finding showing that, in the denervated skin, administration of galanin markedly reduced the increased permeability enhancing effect of histamine. A similar postjunctional inhibitory effect of galanin on vascular permeability has been suggested also in a previous study showing inhibition of substance P-induced plasma extravasation by exogenous galanin [27].

Histological observations revealed that increase in the protein contents of skin perfusates following the coadministration of histamine and M35 is associated with an increase in the lengths of silver-labelled cutaneous blood vessels. This may 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 response. This latter possibility is supported by the quantitative histological findings which demonstrated significant increases in the diameters of labelled blood vessels after both chronic denervation and coadministration of M35 and histamine. In addition, the histological findings also disclosed that increases in the protein concentration of the perfusates resulted from the escape of plasma proteins from the circulation due to enhanced vascular permeability.

In conclusion, the present findings strongly indicate that galanin-containing nerves may fulfil a novel, inhibitory local regulatory or sensory-efferent function of cutaneous nerves. Further studies are needed to determine the factors which may influence and modify the release of peptides and/or other mediators from cutaneous nerves or non-neural elements under physiological conditions. The findings showing that galanin is the most abundant peptide among rat sensory nerve fibres [28] may indicate that galanin may be of significance in the modulation of vascular responses in mammalian species as well.

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