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Histamine-induced edema in the rat paw – effect of capsaicin denervation and a CGRP receptor antagonist

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Abstract

Histamine is known to cause edema and excitation of small-diameter primary afferent neurons. In the present study we wanted to investigate to which extent afferent neurons participate in histamine-induced edema and, subsequently, determine possible inhibitory effects of a tachykinin NK_1 receptor and CGRP receptor antagonist on the histamine response. Intraplantar injection of histamine (0.5 μmol) into the rat hind paw caused a 34% increase of paw volume. In capsaicin-denervated rats, this effect of histamine was nearly abolished. The calcitonin gene-related peptide (CGRP) receptor antagonist CGRP-(8–37), but not the tachykinin NK_1 receptor antagonist SR140333, caused significant inhibition of the edema response. Further indication that CGRP can promote the histamine action was obtained in capsaicin-denervated rats, where co-injection of CGRP (0.3 pmol) increased the edema response to intraplantar histamine. In additional experiments, plasma protein extravasation in the paw skin was evaluated after close arterial infusion of histamine. Also in these experiments CGRP-(8–37), but not SR140333, significantly reduced the histamine effect. The observation that in the rat hind paw a CGRP receptor antagonist, but not a tachykinin NK_1 receptor antagonist, attenuates histamine-induced vascular leakage raises the possibility that in some tissues CGRP receptor antagonists may be superior to tachykinin NK_1 receptor antagonists in reducing histamine-induced neurogenic inflammatory responses.

Keywords: Histamine; Paw edema; Neurogenic inflammation; CGRP (calcitonin gene-related peptide)

1. Introduction

Histamine is known to increase vascular permeability mainly by acting on postcapillary venules where the opening of endothelial gaps leads to increased extravasation of plasma proteins (cf. Grega et al., 1981). In addition to its vascular effects, histamine excites small diameter afferent neurons and evokes the release of vasoactive mediators from local nerve endings (Saria et al., 1988). Therefore, histamine-induced edema formation may be the consequence of vascular as well as of neuronal actions of histamine. In agreement with this view, it has been shown that in rats which had been treated with capsaicin to destroy small diameter afferents (capsaicin-denervated rats), histamine-induced plasma protein extravasation was attenuated (Arvier et al., 1977; Jancsó et al., 1980).

Mediator release from afferent terminals may interact with the vascular effects of histamine in several ways. On the one hand, neurokinins may act on vascular tachykinin NK_1 receptors, thus by themselves increasing vascular leakage (Eglezos et al., 1991; Xu et al., 1992) and adding to the histamine effect. On the other hand, CGRP may cause vasodilation which can be expected to promote the effect of compounds which increase vascular permeability (Gamse and Saria, 1985; Cambridge and Brain, 1992; Newbold and Brain, 1993).

In the present study, we used the rat hind paw to determine the contribution of capsaicin-sensitive afferents to histamine-induced vascular leakage and edema formation. Furthermore, we wanted to determine to which extent blockade of tachykinin NK_1 and CGRP receptors is able to modify the histamine response.

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2. Materials and methods

2.1. Animals

Male Sprague-Dawely rats (300–350 g body weight; Himberg, Austria) were anesthetized with pentobarbital sodium (Sanofi). After tracheostomy one jugular vein was cannulated for drug administration.

2.2. Capsaicin denervation

Rats were treated on the second day of life with capsaicin (50 mg/kg s.c.) or the corresponding volume of vehicle under ether anesthesia (Jancsó, 1968).

2.3. Paw volume measurement

Anesthetized rats received an intraplantar injection (50 μl) of histamine (0.5 μmol) or 5-hydroxytryptamine (5-HT, 5 nmol). The contralateral paw was injected with 50 μl saline and served as control. 20 min after intraplantar injection, the paw volume was measured using a plethysmometer (Ugo Basile, Varese, Italy). Values were calculated as percent difference between saline and histamine (5-HT)-injected paw. In another series of experiments, rats received intraplantar injections (50 μl) of CGRP (0.3 pmol) alone or of histamine (0.5 μmol) together with CGRP (0.3 pmol).

2.4. Plasma protein extravasation

One superficial epigastric artery was cannulated for close arterial infusion into the femoral artery. 5 min after Evans Blue (20 mg/kg i.v.) injection, histamine (6 μmol/min) or saline was infused into the femoral artery at a rate of 60 μl/min. After 5 min, infusion was stopped, the animals were killed by an overdose of sodium pentobarbital, and the dorsal and plantar skin removed from the ipsi- and contralateral hind paws. Dye content was measured after formamide extraction. In another series of experiments, mustard oil (2.5% in liquid paraffin) was applied on the dorsal surface of the paws for 15 min.

2.5. Treatment with antagonists

SR140333 was administered s.c. 3 h before testing and CGRP-(8–37) was administered i.v. 2 min before testing.

2.6. Substances and solutions

Histamine and 5-hydroxytryptamine creatinine sulfate were obtained from Sigma. CGRP-(8–37) (Bachem) was dissolved (1 mM) in distilled water and diluted in saline. SR140333 and SR140603 (generously provided by Sanofi Research) were dissolved (15 mM) in dimethyl sulfoxide (DMSO) and diluted in saline to the appropriate concentration.

2.7. Statistics

Values were calculated as means ± S.E.M. Statistically significant differences were assessed using the Kruskal-Wallis analysis of variance (ANOVA) on ranks followed by Dunn's comparison between control and treated groups.

3. Results

3.1. Effect of intraplantar histamine on paw volume

20 min after injection of histamine (0.5 μmol) the paw volume was significantly increased as compared to the vehicle-injected, contralateral side (+33.9 ± 2.9%; $n = 11$). In rats which were treated with capsaicin 2 days after birth, histamine caused only a slight increase in paw volume (+5.0 ± 2.2%; $n = 9$). In these rats, injection of 0.3 pmol CGRP alone did not produce significant edema (data not shown), but, when co-injected with histamine, increased the effect of histamine (+18.8 ± 1.6%; $n = 5$). The results of this first series of experiments are summarized in Fig. 1.

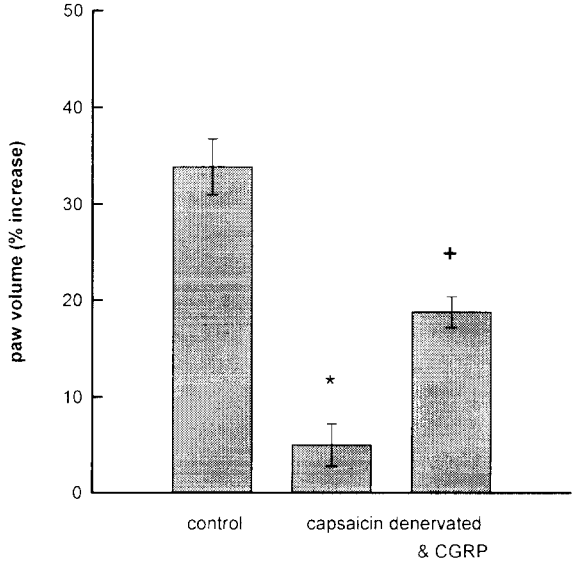

Fig. 1. Effect of intraplantar injection of 0.5 μmol histamine on paw volume (percent increase over contralateral, vehicle-injected paw). Means ± S.E.M. in controls ($n = 11$, left column), in capsaicin-denervated rats ($n = 9$, middle column), and in capsaicin-denervated rats when coinjected with 0.3 pmol CGRP ($n = 5$, right column). $^{*}P < 0.05$ as compared to control group, $^{+}P < 0.05$ as compared to histamine alone.

3.2. Effect of tachykinin NK_1 receptor and CGRP receptor antagonists on histamine-induced paw edema

Both antagonists were used at doses which show good inhibitory effects in the respective tests: (a) CGRP-(8–37) (50 nmol/kg i.v., 2 min before testing) has been shown to selectively inhibit the CGRP-induced vasodilator response in the rat paw (Holzer and Jocic, 1994). (b) In preliminary experiments we found that s.c. administration of SR140333, but not its inactive enantiomer SR140603, causes a dose-dependent inhibition of mustard oil-induced plasma protein extravasation (Table 1).

When evaluated against histamine-induced edema, SR140333 failed to show inhibitory effects. At a dose (150 nmol/kg s.c.) which prevented mustard oil-induced plasma protein extravasation (Table 1), we found no inhibition, but rather an enhancement of histamine-induced edema (Fig. 2). In contrast to the tachykinin NK_1 receptor antagonist, the CGRP receptor antagonist was clearly effective. Histamine-induced edema was significantly reduced by 30% by CGRP-(8–37) (50 nmol/kg i.v.; Fig. 2).

In a series of experiments, 5-HT (5 nmol) was used instead of histamine. 5-HT caused edema (paw volume $+29.8 \pm 1.3\%$; $n = 6$) which was not significantly reduced in capsaicin-denervated rats ($+27.8 \pm 2.9\%$; $n = 4$) or in rats which were treated with CGRP-(8–37) ($+32.9 \pm 3.2$; $n = 6$).

3.3. Plasma protein extravasation induced by close arterial histamine

Close arterial infusion of histamine (6 μmol/min for 5 min) into one femoral artery caused a unilateral increase in dye content (μg Evans Blue/g tissue) of the plantar and dorsal skin of the hind paw (Fig. 3). On the contralateral side, the dye content of the paw skin (11.7 ± 1.41, plantar; 13.6 ± 1.3, dorsal skin; $n = 11$) was similar to values obtained after saline infusion (data not shown).

In capsaicin-denervated rats, histamine-induced dye leakage in the dorsal paw skin was significantly reduced (28.8 ± 3.9, $n = 6$) as compared to controls (40.3 ± 2.5, $n = 11$). In the plantar paw skin the effect of capsaicin denervation was not statistically significant (data not shown).

Table 1
Mustard oil-induced plasma protein extravasation in the dorsal skin of the rat hind paw expressed as μg Evans Blue/g tissue

Treatment	Dose	Evans Blue content
Vehicle		225 ± 17
SR140333	15 nmol/kg	124 ± 11 [a]
SR140333	45 nmol/kg	65 ± 7 [a]
SR140333	150 nmol/kg	7 ± 9 [a]
SR140603	1.5 μmol/kg	234 ± 13

Means ± S.E.M., $n = 4$–8; [a] $P < 0.05$ as compared to vehicle group.

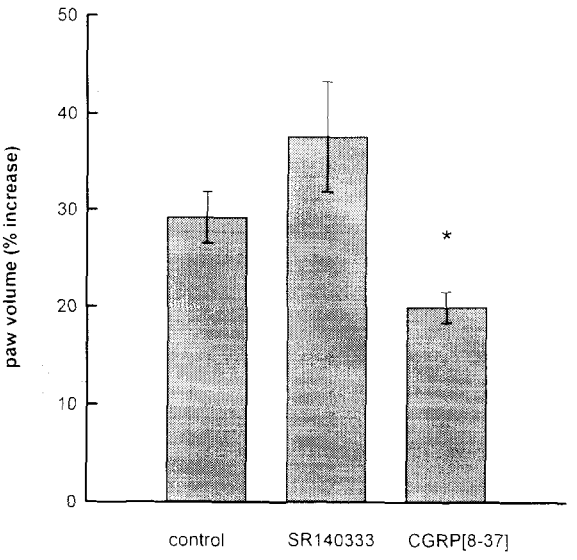

Fig. 2. Histamine-induced increase of paw volume (percent increase over contralateral, vehicle-injected paw) in control group ($n = 10$, left column), in rats treated with 150 nmol/kg SR140333 ($n = 5$, middle column) or with 50 nmol/kg CGRP-(8–37) ($n = 11$, right column). Means ± S.E.M.; $^*P < 0.05$ as compared to control group.

Treatment of rats with SR140333 (150 nmol/kg s.c.) had no significant effect on histamine-induced plasma protein extravasation while CGRP-(8–37) (50 nmol/kg i.v.) reduced histamine-induced plasma protein extravasation by 36% and 29% in the plantar and dorsal paw skin, respectively (Fig. 3).

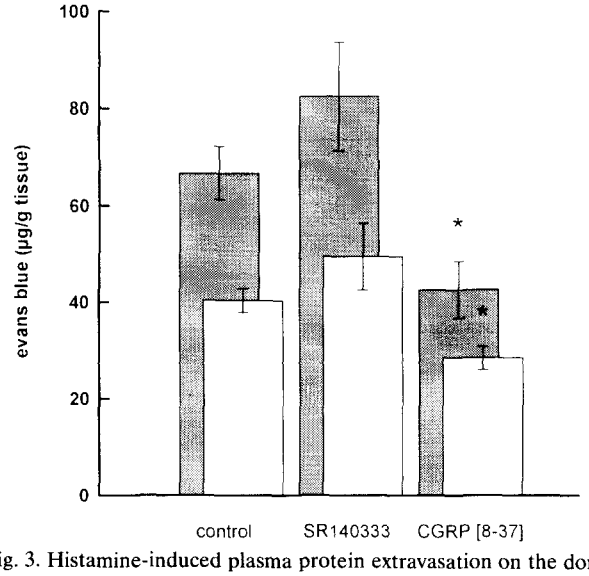

Fig. 3. Histamine-induced plasma protein extravasation on the dorsal (open columns) and plantar (hatched columns) skin of the hind paw. From left to right: control group ($n = 11$), rats treated with 150 nmol/kg SR140333 ($n = 6$) and with 50 nmol/kg CGRP-(8–37) ($n = 8$). Means ± S.E.M.; $^*P < 0.05$ as compared to corresponding control group.

4. Discussion

The present observation that edema formation after intraplantar injection of histamine is nearly absent in capsaicin-denervated rats suggests that in the rat hind paw neurogenic mechanisms are essential for histamine action. This is in apparent contrast to other reports, which describe a more moderate effect of capsaicin treatment on histamine induced edema (Arvier et al., 1977; Jancsó et al., 1980). Most likely, the anatomical region, its type of vascularization and innervation considerably influence the relative contribution of afferent neurons.

Mustard oil produces vascular leakage mainly via neurogenic mechanisms (Lembeck and Holzer, 1979). In response to mustard oil neurokinins are secreted from afferent nerve terminals, act on vascular tachykinin NK_1 receptors (Andrews et al., 1989) to increase permeability. It was therefore not surprising to find inhibition of mustard oil-induced plasma protein extravasation by SR140333, a potent non-peptide tachykinin NK_1 receptor antagonist (Edmonds-Alt et al., 1993). Less expected was the observation that neither histamine-induced increase in paw volume nor dye leakage were inhibited by SR140333, although both responses were attenuated in capsaicin-denervated rats.

It seems therefore that histamine, in contrast to mustard oil, did not induce the release of endogenous tachykinin NK_1 receptor ligands in an amount sufficiently large to gain functional relevance. This difference between mustard oil and histamine actions could be explained by the assumption that the small subpopulation of afferent C-fibres (Lang et al., 1990) which are excited by histamine contains predominantly CGRP. This seems possible, because in rat paw skin, CGRP is present in a considerably higher concentration than the tachykinin substance P (Donnerer et al., 1992) suggesting a relative surplus of CGRP-containing neurons. In addition, it is known that in some rat tissues, substance P, though being detectable in tissue extracts, is not released by capsaicin in sufficient amounts for biochemical detection although CGRP release can be easily demonstrated (Maggi et al., 1987; Amann et al., 1988). A similar situation seems to exist in the rat paw skin, since we failed to observe dye leakage after close arterial infusion of capsaicin (unpublished results) at a dose (1 nmol infused during 5 min) which was higher than necessary to elicit afferent neuron excitation (0.1 nmol infused during 30 s; Donnerer and Lembeck, 1983). It seems therefore that in the rat paw intense chemical stimuli, such as mustard oil, are necessary to produce neurokinin-mediated plasma protein extravasation.

In contrast to the tachykinin NK_1 receptor antagonist SR140333, CGRP-(8–37) produced significant inhibition of histamine-induced increase of paw volume as well as of plasma protein extravasation. It would seem possible that CGRP-(8–37) caused a decrease in basal cutaneous blood flow thus generally suppressing edema formation. However, this seems unlikely because in the rat, CGRP-(8–37) has been shown to have either no effect on basal blood flow (Escott and Brain, 1993), or to cause a small increase (Holzer and Jocic, 1994). Furthermore, in the present study, 5-HT-induced edema was not reduced by CGRP-(8–37), which is in agreement with the observation that CGRP potentiates histamine- (Gamse et al., 1987) but not 5-HT-induced vascular leakage (Gamse et al., 1987; Newbold and Brain, 1993).

At the dose of CGRP-(8–37) used in the present study, inhibition of histamine-induced edema was only about 30%, while capsaicin denervation completely prevented it. The reason for the comparatively moderate effect of CGRP-(8–37) may be that the employed dose of the antagonist was at the low end of the dose range which is reported to be effective in vivo. Alternatively, it seems possible that a vasoactive compound different from neurokinins or CGRP is released from capsaicin-sensitive afferents. Escott and Brain (1993) already have addressed this possibility when discussing the inhibitory effect of CGRP-(8–37) on vasodilatation and edema induced by antidromic stimulation of the rat saphenous nerve.

In conclusion, the present results exclude tachykinin NK_1 receptor-mediated mechanisms being involved in the capsaicin-sensitive component of histamine-induced edema formation in the rat hind paw. The observation that (a) in capsaicin-denervated rats exogenous CGRP promotes histamine-induced edema and that (b) in control rats the CGRP receptor antagonist CGRP-(8–37) attenuates histamine-induced edema as well as plasma protein extravasation, strongly suggests the participation of afferent neuron-derived CGRP in the vascular effects of histamine in the rat paw.

Acknowledgements

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