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Regulation of substance P release mediated via prejunctional histamine H_3 receptors

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Abstract

The involvement of the histamine H_3 receptor in the regulation of substance P release in neurogenic inflammation was studied by using rat hindpaw skin. *R*-(−)-α-Methylhistamine, a specific histamine H_3 receptor agonist, significantly inhibited the increased vascular permeability induced by antidromic electrical stimulation of the sciatic nerve in a dose-dependent manner at doses of 0.5–3 mg/kg (i.v.), and thioperamide (2 mg/kg i.p.), a specific histamine H_3 receptor antagonist, prevented the inhibitory effect of *R*-(−)-α-methylhistamine. The antidromic stimulation also caused a significant increase in immunoreactive substance P release in the subcutaneous (s.c.) perfusate in the rat hindpaw. *R*-(−)-α-Methylhistamine (0.25–2 mg/kg) dose dependently inhibited the increase in release of immunoreactive substance P, and thioperamide (2 mg/mg i.p.) antagonized it. Perfusion of histamine (10^{-3} M) elicited a significant increase of immunoreactive substance P release in the perfusate, which was reduced by *R*-(−)-α-methylhistamine and the antagonism of thioperamide was also observed. Histamine (in the presence of histamine H_1 and H_2 receptor antagonists) had an inhibitory effect on the electrically evoked release of immunoreactive substance P. These results strongly support the hypothesis that histamine regulates substance P release via prejunctional histamine H_3 receptors that are located on peripheral endings of sensory nerves.

Keywords: Substance P; Histamine H_3 receptor; Neurogenic inflammation

1. Introduction

In neurogenic inflammation, it has been generally accepted that a neuropeptide, substance P, is released from capsaicin-sensitive sensory nerves by noxious stimuli as an inflammatory chemical mediator. After being released substance P causes, besides acute vascular effects, the release of histamine from mast cells, which are in close proximity to nerves and blood vessels (Heine and Foster, 1975; Wiesner-Menzel et al., 1981), and conversely histamine stimulates the release of substance P. Through this positive feedback loop between sensory nerve and mast cells, inflammatory responses such as increased vascular permeability and vasodilatation proceed. Although there is some histological and functional evidence for a close relationship between substance P and histamine, it is not yet fully defined, e.g., the modulation of substance P release via presynaptic histamine receptors has to be examined in more detail.

The histamine H_3 receptor was found by Arrang et al. (1983) as a third histamine receptor subtype and shown to be located in the central nervous system. The histamine H_3 receptor exists as an autoreceptor in presynaptic terminals of histaminergic neurons and mediates inhibition of histamine release and synthesis (Arrang et al., 1987a,b). In addition, it has been reported that the histamine H_3 receptor acts in the presynaptic regulation of the release of other neurotransmitters such as noradrenaline (Schlicker et al., 1989) and serotonin (Fink et al., 1990) in the central nervous system. In the periphery, it has been suggested that the histamine H_3 receptor may exist in perivascu-

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lar nerve terminals distributed in lung, airway and pupil, and that it regulates acetylcholine release from parasympathetic nerves, or noradrenaline release from sympathetic nerve endings (Ishikawa and Sperelakis, 1987; Ichinose et al., 1989; Koss and Hey, 1993). Furthermore, it was shown that the histamine H_3 receptor modulates nonadrenergic noncholinergic (NANC) neural bronchoconstriction in guinea-pig in vivo (Ichinose and Barnes, 1989). The evidence led to a hypothesis that the histamine H_3 receptor is involved in the prejunctional regulation of the release of sensory neuropeptides including substance P. However, this evidence is based on physiological responses such as muscle contraction and vascular permeability.

In order to demonstrate more clearly that the histamine H_3 receptor is involved in the regulation of substance P release from afferent sensory nerve endings, we determined the amount of immunoreactive substance P released into the s.c. perfusate in response to antidromic electrical and histamine stimulation, and investigated the effects of a specific histamine H_3-receptor agonist, *R*-(−)-α-methylhistamine, and a specific antagonist, thioperamide.

2. Materials and methods

2.1. Double coaxial perfusion in the rat hindpaw

Male Wistar rats (250–300 g) were anesthetized with pentobarbital (50 mg/kg i.p.), and a double polyethylene tube was introduced into the subcutaneous (s.c.) space of the rat hindpaw according to the methods of Rocha e Silva and Antonio (1960). Perfusion was carried out at a rate of 1 ml/10 min with saline containing 20 mM bacitracin and 100 mM captopril (both of which were added to prevent degradation of substance P), using a peristaltic pump (Microperpex 2132; LKB). Perfusates were collected in the test tubes in an ice bath every 10 min, using a fraction collector. From 1 h after starting the perfusion, antidromic electrical or histamine stimulation was carried out for 20 min.

2.2. Histamine stimulation

Histamine dissolved in saline was injected into the s.c. space as described above, and then perfusates were collected.

2.3. Antidromic electrical stimulation of the sciatic nerve

The sciatic nerve was exposed unilaterally at the thigh site and cut. The distal stump of the nerve was placed on platinum electrodes and stimulated (15 V, 10 Hz, 1 ms) for 20 min.

2.4. Radioimmunoassay of substance P

Acetic acid was added to the samples to give a final concentration of 0.2 N. After centrifugation, the supernatant was lyophilized and then assayed for substance P, using the corresponding antiserum, by the method of Yanaihara et al. (1976). The antiserum to substance

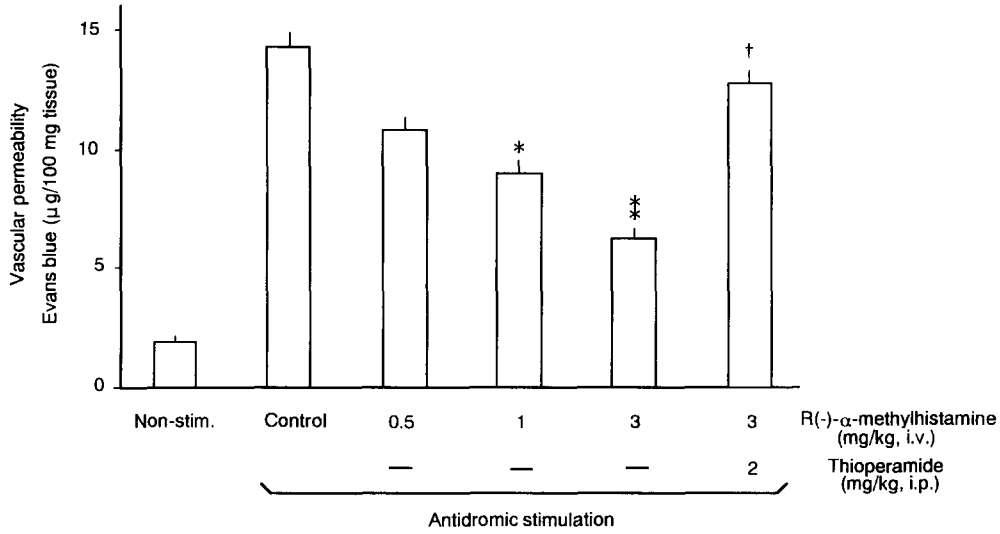

Fig. 1. Effects of *R*-(−)-α-methylhistamine and thioperamide on the increased vascular permeability induced by antidromic electrical stimulation of the sciatic nerve in rats. 30 min before the stimulation *R*-(−)-α-methylhistamine and thioperamide were administered i.v. and i.p., respectively. Each value is the mean ± S.E. ($n = 6$). * $P < 0.05$, ** $P < 0.01$ when compared with control animals. † $P < 0.05$ when compared with *R*-(−)-α-methylhistamine-treated animals.

P showed less than 0.1% cross-reactivity with neurokinin A and B.

2.5. Measurement of vascular permeability in rat hindpaw

Permeability was measured by dye extravasation. Under pentobarbital anesthesia Evans blue was injected intravenously at a dose of 50 mg/kg, and then electrical stimulation was administered unilaterally for 5 min. Immediately afterwards the rats were killed by cervical dislocation, and the paw skin was removed. Dye extraction was carried out by the method of Gamse et al. (1980). Tissue was chopped and immersed in formamide maintained at 60°C for 24 h. The quantity of the extracted dye was measured with a spectrophotometer (Shimadzu, UV-160) at 610 nm. To block cholinergic and adrenergic functions, atropine (1 mg/kg) and propranolol (1 mg/kg) were administered i.v. before the experiment.

2.6. Drugs

R-(−)-α-Methylhistamine dihydrochloride (Research Biochemicals International, Natick, MA, USA); Evans blue; histamine dihydrochloride (Wako Pure Chemical Industries, Osaka, Japan); and ^{125}I-labeled substance P (Amersham International, Amersham, Buckinghamshire, UK) were used. Thioperamide was kindly donated by Bioprojet (Paris, France) and Eisai Co. (Tokyo, Japan).

2.7. Statistical analysis

Data were examined by analysis of variance (ANOVA) and Scheffé's test for multiple comparisons with a single control group.

3. Results

3.1. Effects of R-(−)-α-methylhistamine and thioperamide on the neurogenic plasma leakage

Antidromic electrical stimulation of the transected sciatic nerve resulted in a marked increase in dye extravasation in the ipsilateral hindpaw of the rat. As can be seen in Fig. 1, *R*-(−)-α-methylhistamine (0.5, 1, 3 mg/kg i.v.) dose dependently inhibited the increase in vascular permeability. Thioperamide (2 mg/kg i.p.) antagonized the inhibition induced by 3 mg/kg of *R*-(−)-α-methylhistamine. At this dose thioperamide itself had no significant effect on the dye extravasation.

3.2. Release of immunoreactive substance P by antidromic electrical stimulation and effect of R-(−)-α-methylhistamine

A significant increase in immunoreactive substance P release in the s.c. perfusate was observed in response to antidromic stimulation of the sciatic nerve. The average amount of immunoreactive substance P released was 0.036 ± 0.0035 pmol in the 10-min fraction

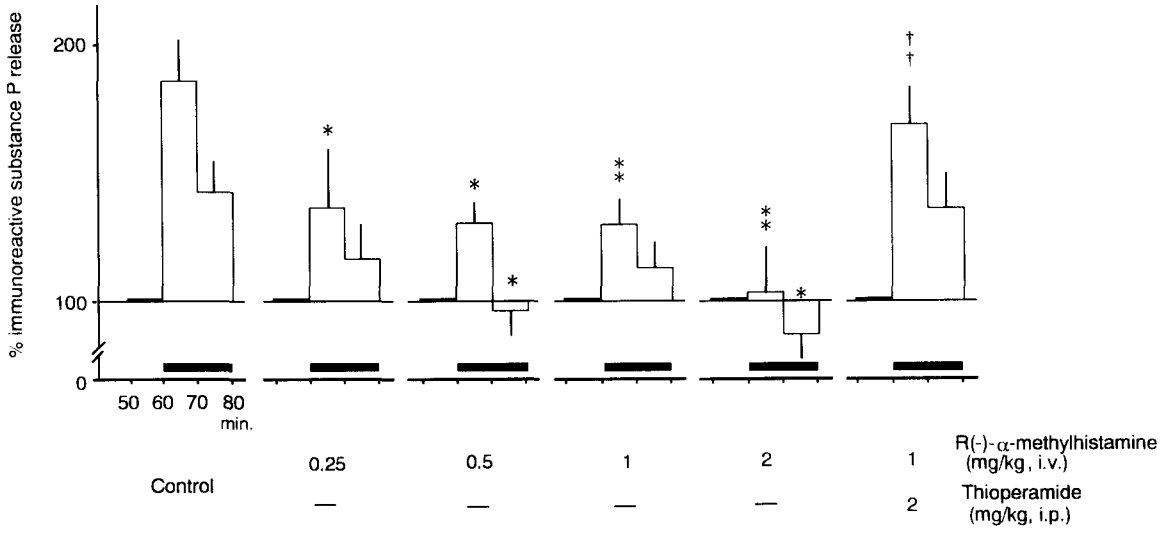

Fig. 2. Effect of *R*-(−)-α-methylhistamine on the release of immunoreactive substance P induced by antidromic electrical stimulation, and the interaction with thioperamide. Antidromic stimulation was given for 20 min (black bar). 30 min before the stimulation *R*-(−)-α-methylhistamine and thioperamide were administered i.v. and i.p., respectively. Ordinate: percent immunoreactive substance P release = substance P content of each 10-min fraction/substance P content of pre-stimulation fraction (50–60 min) × 100. Each value is the mean ± S.E. (control group: $n = 15$, drug-treated groups: $n = 6$–11). * $P < 0.05$, ** $P < 0.01$ when compared with each fraction of control animals. †† $P < 0.01$ when compared with *R*-(−)-α-methylhistamine-treated animals.

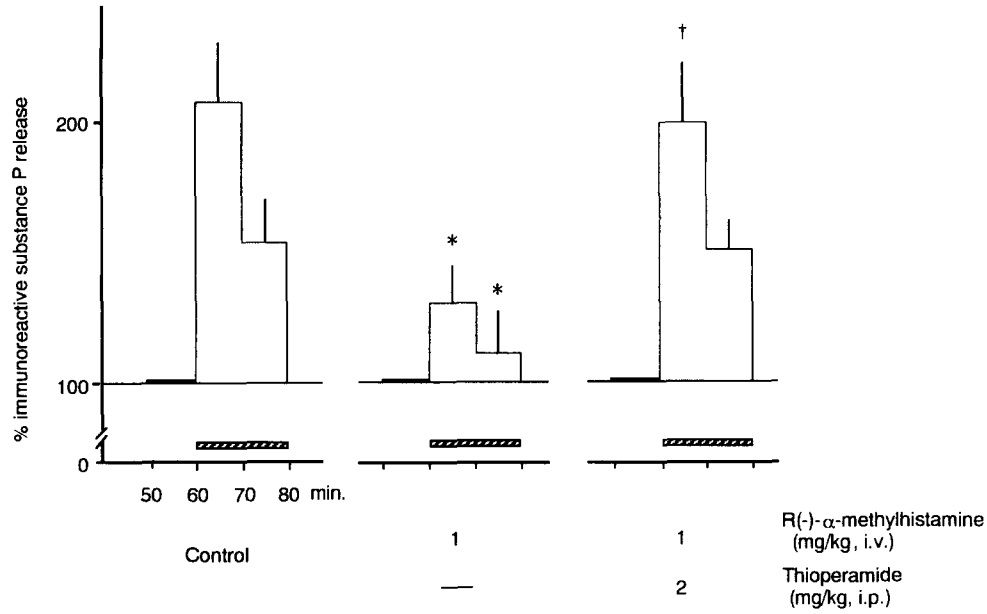

Fig. 3. Effect of *R*-(−)-α-methylhistamine on immunoreactive substance P release induced by histamine stimulation, and interaction with thioperamide. Histamine (10^{-3} M) was given for 20 min (hatched bar). Each value is the mean ± S.E. (control group: $n = 15$, drug-treated groups: $n = 6$–10). * $P < 0.05$ when compared with control animals. † $P < 0.05$ when compared with *R*-(−)-α-methylhistamine-treated animals. See Fig. 2 legend for details.

obtained before stimulation and 0.070 ± 0.0104 and 0.052 ± 0.0085 pmol in the two 10-min samples obtained during electrical stimulation ($n = 15$). As shown in Fig. 2, *R*-(−)-α-methylhistamine (0.25–2 mg/kg i.v.) inhibited the stimulus-induced increase in immunoreactive substance P release dose dependently. 2 mg/kg of thioperamide antagonized the inhibition produced by 1 mg/kg of *R*-(−)-α-methylhistamine. Although this dose of thioperamide tended to increase the release of immunoreactive substance P, this effect was not statistically significant.

3.3. Release of immunoreactive substance P by histamine stimulation and effect of R-(−)-α-methylhistamine

When perfusion of histamine (10^{-4} to 5×10^{-3} M) was carried out in the rat paw, it caused a dose-dependent release of immunoreactive substance P (data

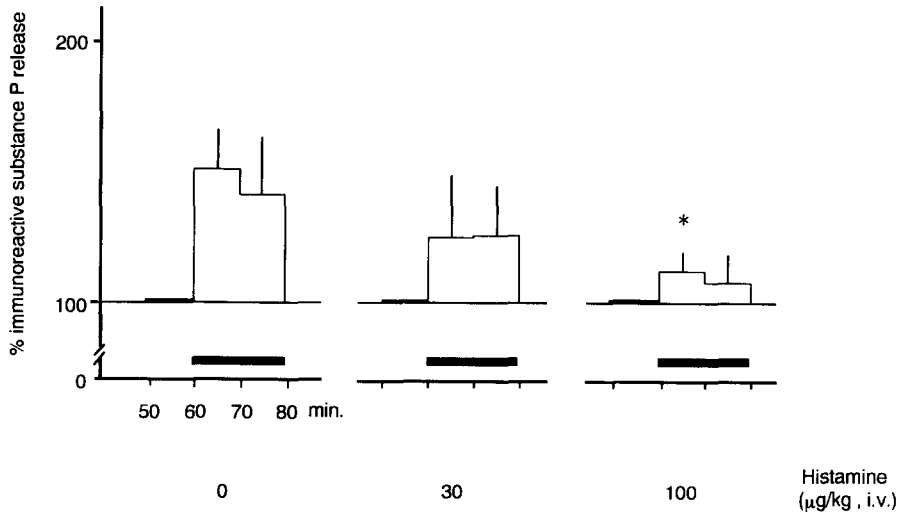

Fig. 4. Effect of histamine (in the presence of mepyramine and cimetidine) on the release of immunoreactive substance P induced by antidromic electrical stimulation. Antidromic stimulation was given for 20 min (black bar). Histamine was administered i.v. 15 min before the stimulation. Mepyramine (5 mg/kg i.p.) and cimetidine (5 mg/kg i.p.) were administered 30 min before the stimulation. Each value is the mean ± S.E. ($n = 6$–7). * $P < 0.05$.

not shown). The average amount of immunoreactive substance P released was 0.026 ± 0.0017 pmol in the 10-min fraction obtained before stimulation with histamine and 0.057 ± 0.0082 and 0.042 ± 0.0062 pmol in the two 10-min samples obtained during administration of histamine (10^{-3} M) ($n = 15$). *R*-(−)-α-Methylhistamine (1 mg/kg i.v.) inhibited the immunoreactive substance P release produced by histamine, and thioperamide (2 mg/kg i.p.) reduced this inhibition elicited by *R*-(−)-α-methylhistamine (Fig. 3).

3.4. Inhibition by histamine

In the presence of histamine H_1 and H_2 receptor antagonists, histamine (100 μg/kg i.v.) itself significantly inhibited the immunoreactive substance P release induced by antidromic electrical stimulation of the sciatic nerve (Fig. 4).

4. Discussion

R-(−)-α-Methylhistamine inhibited the increased vascular permeability induced by antidromic electrical stimulation of the sciatic nerve under the blockade of adrenergic and cholinergic nerves, and thioperamide antagonized it. This result suggests the possibility that the histamine H_3 receptor may have an inhibitory action on the release of tachykinins in response to excitation of NANC nerves. Ichinose et al. (1990) similarly reported that the histamine H_3 receptor may exist on sensory nerve endings distributed in airways and may inhibit neurogenic microvascular leakage by prejunctional inhibition of neuropeptide release. Therefore, we tried to determine the release of substance P from sensory nerve endings in the rat hindpaw. The content of immunoreactive substance P in the s.c. perfusate was significantly increased by antidromic stimulation of the sciatic nerve. *R*-(−)-α-Methylhistamine inhibited the immunoreactive substance P release in a dose-dependent manner, and this inhibition was reduced by thioperamide. Furthermore, perfusion of histamine also caused a significant increase in immunoreactive substance P release. The inhibitory action of *R*-(−)-α-methylhistamine and the antagonism of thioperamide were also observed. This inhibitory effect was also obtained with histamine itself in the presence of mepyramine and cimetidine. These findings indicate that histamine H_3 receptors may be prejunctionally located on peripheral sensory nerve endings and regulate substance P release. It has been recently demonstrated or postulated that there are prejunctional receptors for inhibitory modulators such as μ-opioids, γ-aminobutyric acid (GABA) and somatostatin in capsaicin-sensitive sensory nerve endings, and that they have an inhibitory action on neurogenic inflammation by suppression of the release of neuropeptides, including substance P (Belvisi et al., 1989; Frossard and Barnes, 1987; Gazelius et al., 1981; Maggi, 1991). Taking into consideration the close functional and histological relationship between histamine and substance P, the involvement of the histamine H_3 receptor in the prejunctional regulation of substance P release may be very important in the control of the inflammatory response.

Stimulation of histamine H_1 receptors on endothelial cells causes increased vascular permeability, and stimulation of these receptors on sensory nerve endings causes pain and itch. Thus it seems that the histamine H_1 receptor is related to an aspect of the pro-inflammatory action of histamine. Although it is evident that histamine stimulates the release of sensory neuropeptides in the skin, the class of receptors which is involved in the interaction with peripheral sensory nervous system is less well understood. In our study, a histamine H_1 receptor antagonist, mepyramine (5, 10, 20 mg/kg i.p.), significantly inhibited the release of immunoreactive substance P into the s.c. perfusate caused by histamine stimulation in a dose-dependent manner (data not shown). Saria et al. (1988) also reported that mepyramine inhibited the release of substance P and neurokinin A elicited by histamine stimulation of sensory nerves from lung of guinea-pig. Hence, by interacting with histamine H_1 receptors, the activation of C-fibers may cause substance P release, probably via local axon reflexes, besides causing afferent transmission. Through this system a positive feedback loop between sensory nerves and mast cells in inflammation might be formed.

In conclusion, all the data from the present study support the hypothesis that histamine, like μ-opioids, GABA and somatostatin, inhibits neurogenic inflammation by interacting with prejunctional histamine H_3 receptors. It has been suggested that different inhibitory prejunctional receptors on sensory nerves share a common K^+ channel (Barnes et al., 1990; Christie and North, 1988; Stretton et al., 1992). Hence, we are now studying the involvement of the K^+ channel in the action of the histamine H_3 receptor.

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