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ATP-sensitive K^+ channels mediate regulation of substance P release via the prejunctional histamine H_3 receptor

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Abstract

Perfusion of histamine (10^{-3} M) elicited a significant increase of immunoreactive substance P release in the subcutaneous perfusate in the rat hindpaw. The active L-enantiomer of cromakalim, lemakalim (50 μg/kg, i.v.), a selective K^+ channel activator, significantly inhibited the immunoreactive substance P release. Glibenclamide (10 mg/kg, i.v.), an ATP-sensitive K^+ channel blocker, abolished the response to lemakalim on the release of immunoreactive substance P. *R*(−)-α-methylhistamine (1 mg/kg, i.v.), a specific histamine H_3 receptor agonist, significantly inhibited the release of immunoreactive substance P. Glibenclamide (10 mg/kg, i.v.) antagonized the inhibitory effect of *R*(−)-α-methylhistamine. Tetraethylammonium (10 mg/kg, i.p.), a K^+ channel blocker, also reduced the inhibitory effect significantly. These results suggest that the inhibition of substance P release from sensory nerve endings via prejunctional histamine H_3 receptors may be achieved by activating the ATP-sensitive K^+ channel coupled to the histamine H_3 receptor in the rat skin.

Keywords: K^+ channel, ATP-sensitive; Histamine H_3 receptor; Prejunctional inhibition; Substance P

1. Introduction

A third histamine receptor subtype, the histamine H_3 receptor, was described in 1983 (Arrang et al., 1983) from work with rat brain tissue. Histamine inhibits its own release from histaminergic nerve endings via histamine H_3 receptors. Recently, the histamine H_3 receptor has also been found in peripheral tissues. We had already demonstrated that histamine regulates substance P release via prejunctional histamine H_3 receptors that are located on peripheral endings of sensory nerves in the rat hind paw (Ohkubo et al., 1995). It has been demonstrated or postulated that there are also several prejunctional receptors for inhibitory modulators such as μ-opioids, γ-aminobutyric acid ($GABA_B$), somatostatin, galanin and neuropeptide Y 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 (Gazelius et al., 1981; Lembeck and Donnerer, 1985; Belvisi et al., 1989; Giuliani et al., 1989; Maggi 1991; Ray et al., 1991). Furthermore, it has been suggested that these prejunctional receptors share a common mechanism linking K^+ channels, because these receptor agonists, such as opioids, increase K^+ conductance (North and Williams, 1985; Christie and North, 1988; North, 1989), and the effects of some are inhibited by a K^+ channel blocker (Stretton et al., 1992) and are mimicked by a K^+ channel activator (Ichinose and Barnes, 1990; Burka et al., 1991). However, it is still unclear which type of K^+ channel is involved. It has been postulated that the histamine H_3 receptor may be coupled to K^+ channels (Barnes et al., 1990). The ATP-sensitive K^+ channel is involved in inhibition of excitatory non-adrenergic non-cholinergic (NANC) neural bronchoconstrictor responses (Ichinose and Barnes, 1990), which is regulated by histamine via the histamine H_3 receptor, as well as other modulators (Ichinose and Barnes, 1989).

The purpose of this study was to determine whether activation of ATP-sensitive K^+ channels is involved in the regulation of substance P release via the prejunctional histamine H_3 receptor on sensory nerve endings.

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2. Materials and methods

2.1. Double coaxial perfusion in the rat hind paw

Male Wistar rats (250–300 g) were anesthetized with pentobarbital (50 mg/kg, i.p.), and a double polyethylene tube about 5 cm length (the inner tube was 5 mm longer than the outer one), was introduced into the subcutaneous (s.c.) space of the rat hind paw according to the methods of Rocha e Silva and Antonio (1960) and Yonehara et al. (1987). 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 dual peristaltic pump (Microperpex 2132; LKB). Perfusates were collected through the outer tube in the test tubes in an ice bath using a fraction collector every 10 min. From 1 h after starting the perfusion, histamine stimulation was carried out for 20 min.

2.2. Histamine stimulation

Histamine (10^{-3} M) dissolved with saline was injected into the s.c. space as described above, and perfusates were collected. Details are described elsewhere (Ohkubo et al., 1995).

2.3. Radioimmunoassay of substance P

Acetic acid was added to the samples to give a final concentration of 0.2 N. After centrifugation (3000 rpm $\times$ 20 min), the supernatant was lyophilized then assayed for substance P using the corresponding antiserum by the method of Yanaihara et al. (1976). The lyophilized sample was redissolved in 1 ml of substance P assay buffer (0.05 M citric buffer, 0.025 M EDTA, 0.5% bovine albumin, 0.5% dithiothreitol, pH 4.8). ^{125}I-labeled substance P (10000 cpm) 100 μl per tube, 100 μl of standard substance P or sample, 100 μl of antiserum and 400 μl of assay buffer were successively added into a test tube. The tube was incubated at 4° C for 48 h. Bound and free radiolabeled peptide were separated by means of 0.25% dextran-2.5% charcoal. The antiserum (Otsuka Pharmaceuticals, Tokyo, Japan) to substance P showed less than 0.1% cross-reactivity with neurokinin A and B. The minimum detectable concentration was 7.5 pg/ml.

2.4. Drugs

R(−)-α-methylhistamine dihydrochloride (Research Biochemicals, Natick, MA, USA); glibenclamide (Sigma, Poole, UK); tetraethylammonium chloride; histamine dihydrochloride (Wako Pure Chemical Industries, Osaka, Japan); ^{125}I-labeled substance P (Amersham International, Amersham, Buckinghamshire, UK) were used. Lemakalim was kindly donated by Dr. Saito (Osaka University, Osaka, Japan). Glibenclamide was dissolved by using a small amount of NaOH (0.1 N) and then a slow addition of distilled water containing glucose (50 g/l) under continuous sonication (Cavero et al., 1989). Other drugs were dissolved with saline, and lemakalim was dissolved under sonication.

2.5. Statistical analysis

The data were evaluated by analysis of variance (ANOVA) and Scheffé's test for multiple comparisons.

3. Results

3.1. Effects of lemakalim and glibenclamide on immunoreactive substance P release induced by histamine stimulation

Perfusion of histamine (10^{-3} M) elicited a significant increase of immunoreactive substance P release in the s.c. perfusate. Lemakalim (50 μg/kg, i.v.), a K^+ channel activator, significantly inhibited the immunoreactive substance P release. Glibenclamide (10 mg/kg, i.v.), an ATP-sensitive K^+ channel blocker, abolished the response to lemakalim on the release of immunoreactive substance P (Fig. 1). At this dose, glibenclamide itself produced no significant increase in the immunoreactive substance P release (data not shown).

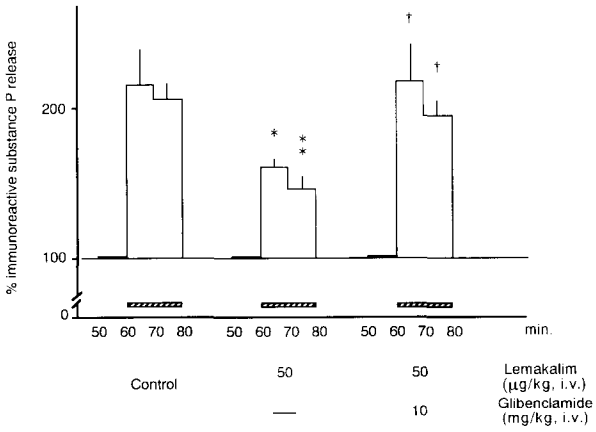

Fig. 1. Effects of lemakalim and glibenclamide on immunoreactive substance P release induced by histamine stimulation. Histamine (10^{-3} M) was given for 20 min (hatched bars). Lemakalim and glibenclamide were administered i.v. 30 min before the stimulation. Ordinate: % immunoreactive substance P release = substance P content of each 10-min fraction/substance P content of pre-stimulation fraction (50–60 min)$\times$100. Each value is the mean $\pm$ S.E. (n = 6–7). * $P < 0.05$, ** $P < 0.01$ when compared with control animals. † $P <$ 0.05 when compared with lemakalim-treated animals.

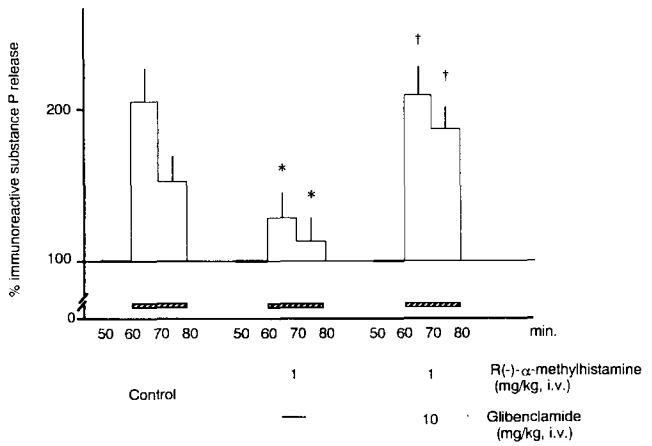

Fig. 2. Effect of glibenclamide on the inhibition of immunoreactive substance P release by $R(-)$-α-methylhistamine. Histamine (10^{-3} M) was given for 20 min (hatched bars). 30 min before the stimulation, $R(-)$-α-methylhistamine and glibenclamide were administered. Each value is the mean ± S.E. (control group: $n = 15$, drug-treated groups: $n = 6$–7). * $P < 0.05$ when compared with control animals. † $P < 0.05$ when compared with $R(-)$-α-methylhistamine-treated animals.

3.2. Effect of $R(-)$-α-methylhistamine and interaction with glibenclamide and tetraethylammonium

$R(-)$-α-Methylhistamine (1 mg/kg, i.v.), a specific histamine H_3 receptor agonist, significantly inhibited the immunoreactive substance P release induced by histamine stimulation. Glibenclamide (10 mg/kg, i.v.) abolished the inhibitory effect of $R(-)$-α-methylhistamine (Fig. 2). A K^+ channel blocker, tetraethylammonium (10 mg/kg, i.p.), also blocked the effect of $R(-)$-α-methylhistamine (Fig. 3). Tetraethylammonium itself had no significant effect on the immunoreactive substance P release (data not shown).

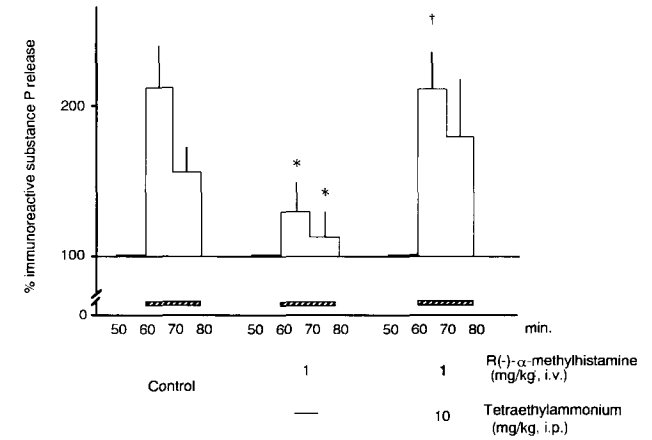

Fig. 3. Effect of tetraethylammonium on the inhibition of immunoreactive substance P release by $R(-)$-α-methylhistamine. Histamine (10^{-3} M) was given for 20 min (hatched bars). 30 min before the stimulation $R(-)$-α-methylhistamine and tetraethylammonim were administered. Each value is the mean ± S.E. (control group: $n = 15$, drug-treated groups: $n = 6$–7). * $P < 0.05$ when compared with control animals. † $P < 0.05$ when compared with $R(-)$-α-methylhistamine-treated animals.

4. Discussion

Recently it has been reported that the antinociceptive effect of the μ-opioid, morphine, can be antagonized by the ATP-sensitive K^+ channel blocker, glibenclamide (Ocana et al., 1990; Wild et al., 1991; Roane and Boyd, 1993). A possible explanation is that inhibition of neurotransmitter release through opening of the K^+ channels contributes to the analgesic effect of the μ-opioid. Glibenclamide also antagonized galanin-induced inhibition of neurotransmitter release in the rat hippocampus (Zini et al., 1993). It has also been reported that the ATP-sensitive K^+ channel activator, cromakalim, inhibits excitatory NANC responses which are conceivably mediated by the release of tachykinins from unmyelinated sensory nerves in guinea pig airways in vivo (Ichinose and Barnes, 1990). It was also confirmed, using lemakalim and cromakalim (and this inhibitory effect of lemakalim was completely blocked by inhibitors of ATP-sensitive K^+ channels, BRL 31660 and glibenclamide), that ATP-sensitive K^+ channels are able to modulate the excitatory NANC responses (Burka et al., 1991; Stretton et al., 1992). However, ATP-sensitive K^+ channels are not involved in the prejunctional neural modulation by μ-opioids, α_2-adrenoceptor agonists and neuropeptide Y, whereas charybdotoxin-sensitive K^+ channels are involved in guinea pig airways (Stretton et al., 1992). It has recently been shown that ibudilast, an antiasthma drug, inhibits neurogenic leakage by prejunctional inhibition of neuropeptide release from airway sensory nerve terminals via an ATP-sensitive K^+ channel (Ichinose et al., 1993). Thus, there is probably a modulatory mechanism mediated via ATP-sensitive K^+ channels on neuropeptide release.

In our previous study, $R(-)$-α-methylhistamine, a specific histamine H_3 receptor agonist, dose dependently inhibited the increase in immunoreactive substance P release induced by antidromic electrical stimulation of the sciatic nerve or histamine stimulation from peripheral sensory nerve endings, and thioperamide, a histamine H_3 receptor antagonist, reduced the inhibitory effect of $R(-)$-α-methylhistamine in the rat paw skin. In agreement with other authors (Ichinose et al., 1990), we concluded that histamine H_3 receptors are located on sensory nerve endings and are involved in the regulation of tachykinin release as well as prejunctional receptors of other inhibitory modulators (Ohkubo et al., 1995). In the present study, we have demonstrated that the active L-enantiomer of cromakalim, lemakalim, a selective K^+ channel activator,

inhibited immunoreactive substance P release in the s.c. perfusates. The inhibitory effect of lemakalim was blocked by glibenclamide. Since this drug has been shown to specifically block ATP-sensitive K^+ channels (Fosset et al., 1988; Amoroso et al., 1990), our data would indicate that the ATP-sensitive K^+ channel may be important in the regulation of substance P release from sensory nerve endings. That the histamine H_3 receptor is linked to K^+ channels is supported by the antagonism by tetraethylammonium, a compound thought to block various types of K^+ channels, of the inhibition of immunoreactive substance P release induced by the histamine H_3 receptor agonist $R(-)$-α-methylhistamine. Furthermore, glibenclamide abolished the inhibition of immunoreactive substance P release induced by $R(-)$-α-methylhistamine. These results suggest that the inhibition of neuropeptide release via prejunctional histamine H_3 receptors on sensory nerve endings may be achieved by activating the ATP-sensitive K^+ channel coupled to the receptor in the rat skin.

There is a close relationship between the nervous system and inflammation. In particular, positive and negative feedback loops in the release of histamine and neuropeptides may be important for the regulation of neurogenic inflammation because of their close functional and histological interrelationship. Therefore, it may be beneficial for therapy of various inflammatory diseases to demonstrate the intracellular mechanism for inhibition of substance P release from sensory nerves via the prejunctional histamine receptor.

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