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Possible participation of histamine H_3 receptors in the modulation of noradrenaline release from rat spinal cord slices

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Abstract

Rat spinal cord slices prelabelled with [^{3}H]noradrenaline were superfused with a medium containing 1 μM desipramine plus 0.3 μM phentolamine. Histamine (0.01–10 μM) and the selective histamine H_3 receptor agonist *R*-(−)-α-methylhistamine (0.001–10 μM) caused a concentration-dependent decrease in the release of radioactivity evoked by electrical field stimulation (0.8 Hz, 20 mA, 2 min). The inhibitory effect of histamine was not modified by either pyrilamine (1 μM) or ranitidine (10 μM), but it was antagonized by burimamide (1 μM). The inhibitory action of histamine (1 μM) was attenuated by pertussis toxin (3 μg/ml) and was abolished by *N*-ethylmaleimide (30 μM). Neither forskolin (10 μM) nor rolipram (100 μM), nor the combination of both drugs, modified the inhibitory effect of histamine. Histamine (1 μM) did not modify the overflow of tritium induced by electrical stimulation in the absence of phentolamine. The present results suggest that in the rat spinal cord the release of noradrenaline elicited by electrical stimulation is negatively modulated by histamine, probably through the activation of histamine H_3 receptors. This modulatory mechanism is likely to involve the participation of regulatory G_o/G_i proteins.

Keywords: Spinal cord, rat; [^{3}H]Noradrenaline; Electrical stimulation; Histamine; Histamine H_3 receptor

1. Introduction

A good deal of evidence suggests that the activation of presynaptic histamine H_3 receptors inhibits the evoked release of noradrenaline in peripheral tissues (Ishikawa and Sperelakis, 1987; Schlicker et al., 1990; Koss and Hey, 1992, 1993; Malinowska and Schlicker, 1991, 1993; Luo et al., 1991; Molderings et al., 1992; McLeod et al., 1993; Endou et al., 1994) and in some regions of the central nervous system such as the rat and mouse brain cortex (Schlicker et al., 1989, 1992a,b) and the rat hypothalamus (Smits and Mulder, 1991).

In the central nervous system the histaminergic neuronal pathways arise from the posterior hypothalamus and project to most of the cerebral areas. Moreover, a descending pathway reaching the spinal cord has been described (Wahlestedt et al., 1985; Airaksinen and Panula, 1988; Inagaki et al., 1988). The spinal cord also possesses noradrenergic descending axons that are involved in the control of nociception, preganglionic sympathetic outflow and voluntary muscle contraction (Phillis et al., 1968; Reddy et al., 1980; Coote, 1988).

Since both noradrenaline and histamine are present in the spinal cord, this study was undertaken to investigate whether the activation of histamine H_3 receptors modify the electrically evoked release of noradrenaline in rat spinal cord slices. The participation of G proteins and cyclic AMP in this modulatory mechanism was also investigated.

2. Materials and methods

2.1. Experimental procedure

Wistar rats of either sex (180–220 g body weight) were anaesthetized with urethane (1.2 g/kg, i.p.) and killed by decapitation. In a cold room (4°C) the rat vertebral column was carefully opened and the spinal cord was excised. Slices 0.4 mm thick were cut from the thoraco-lumbar segment with a Sorvall TC-2 tissue sectioner adapted to manual use and immediately submerged in cold Krebs solution until the collection of

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slices was completed. After a preincubation period of 30 min at 37°C, the slices were incubated for 30 min in Krebs solution containing 0.05 μM levo-[ring-2,5,6-^{3}H]noradrenaline (specific activity 52.3 Ci/ mmol; DuPont-NEN, Boston, MA). The composition of the Krebs solution was (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, NaH_2PO_4 1.0, glucose 11.1, EDTA 0.004, ascorbic acid, 0.11. The Krebs solution was equilibrated with a mixture of 95% O_2-5% CO_2. After labelling with [^{3}H]noradrenaline, aliquots of the slice suspension were transferred to four superfusion chambers (0.2 ml capacity) equipped with two wire electrodes 5 mm apart. The tissue (approximately 270 μg protein) was placed on a nylon mesh between the electrodes. Superfusion was performed at 37°C, at a flow rate of 0.5 ml/min. The Krebs solution contained 1 μM desipramine to prevent the reuptake of the noradrenaline and 0.3 μM phentolamine to prevent activation of presynaptic α_2-adrenoceptors by noradrenaline. Phentolamine was omitted in some experimental groups. After a washout period of 60 min, superfusate samples were collected at 5 min intervals directly into vials containing scintillation liquid. Two periods of electrical stimulation (rectangular pulses of 2 ms duration, 20 mA, during 2 min) were applied after 70 and 110 min of superfusion (S_1 and S_2, respectively). Otherwise indicated, the frequency of stimulation was 0.8 Hz.

Histamine, *R*-(−)-α-methylhistamine and clonidine were added to the perfusion medium 15 min before S_2. Forskolin, rolipram and histamine antagonists were added to the medium at the beginning of the superfusion. These drugs remained in the perfusion medium until the end of the experiment. Some tissues were incubated in the presence of either *N*-ethylmaleimide (30 μM, 30 min) or pertussis toxin (3 μg/ml, 4 h) and then washed 3 times with fresh Krebs solution before labelling with [^{3}H]noradrenaline.

At the end of the experiment, the slices were homogenized in 1 ml of 0.4 N $HClO_4$ and centrifuged at $1000 \times g$ for 10 min. The radioactivity in the supernatant and in the superfusate samples was determined by liquid scintillation spectrometry. Proteins in the pellet were determined by the method of Lowry et al. (1951).

2.2. Calculations

The total overflow of radioactivity evoked by electrical stimulation was used as a measure of [^{3}H]noradrenaline release. It was calculated by subtracting the radioactivity in the sample prior to the stimulation period, from the radioactivity in the stimulation and post-stimulation samples. The result was expressed as a percentage of the total radioactivity present in the tissue at the onset of the stimulation. The spontaneous outflow of tritium (Sp) in the samples preceeding the stimulation periods was expressed as a percentage of the radioactivity remaining in the tissue at the beginning of the respective collection period. The effects of drugs on the evoked and on the spontaneous release of radioactivity were evaluated by the ratios S_2/S_1 and Sp_2/Sp_1, respectively.

The apparent dissociation constant for the antagonist burimamide (K_b) was calculated from the equation $[A']/[A] - 1 = [B]/K_b$, where $[A']/[A]$ is the ratio of concentrations of the agonist giving an equal response in the presence (A') and in the absence (A) of the antagonist; $[B]$: concentration of the antagonist (Furchgott, 1972).

2.3. Statistics

Data were expressed as means ± S.E.M. Number of experiments denotes the number of animals used per group. Statistical significance was determined by the two tailed Student's *t*-test. When more than one group was compared with a control, significance was assessed by one way analysis of variance followed by Dunnett's test.

2.4. Drugs

N-Ethylmaleimide, forskolin, histamine dihydrochloride, pertussis toxin and ranitidine hydrochloride (Sigma Chemical Co, USA); *R*-(−)-α-methylhistamine dihydrochloride (RBI, USA). The following drugs were kindly donated: burimamide (Smith, Klein and French, UK); clonidine hydrochloride (Boehringer Ingelheim, Germany); desipramine hydrochloride, phentolamine methanesulfonate, pyrilamine maleate (Ciba-Geigy, Argentina); rolipram (Schering AG, Germany). Rolipram was dissolved in Krebs solution. Pertussis toxin was dissolved in 0.1 M sodium phosphate buffer (pH 7) with 0.5 M sodium chloride. Forskolin was dissolved in absolute ethanol; the final concentration of ethanol in the perfusion medium (0.1% v/v) had no effects on the basal or evoked tritium overflow. Other drugs were dissolved in distilled water.

3. Results

3.1. Effects of histamine and R-(−)-α-methylhistamine on the evoked release of radioactivity in spinal cord slices prelabelled with [^{3}H]noradrenaline

In slices superfused with a medium containing 0.3 μM phentolamine and stimulated at 0.8 Hz during 2 min, the fraction of tissue radioactivity released by the first period of electrical stimulation (S_1) was 0.95 ± 0.16%. The spontaneous outflow of tritium during the

5-min period previous to S_1 (Sp_1) was 2.09 ± 0.01%. The corresponding S_2/S_1 and Sp_2/Sp_1 ratios were 0.97 ± 0.04 and 0.90 ± 0.01, respectively ($n = 8$). When Ca^{2+} was omitted in the perfusion medium from 30 min before S_2, the S_2/S_1 ratio was 0.03 ± 0.03 ($n = 3$).

Histamine and the selective histamine H_3 receptor agonist, *R*-(−)-α-methylhistamine, caused a concentration-dependent inhibition in the stimulation-evoked tritium overflow. The maximal effect, about 40% decrease, was reached at 0.1 μM *R*-(−)-α-methylhistamine and at 1.0–10 μM histamine (Fig. 1). The inhibitory effect of histamine was not modified by either the histamine H_1 receptor antagonist pyrilamine (1 μM) or by the histamine H_2 receptor antagonist ranitidine (10 μM), but it was significantly reduced by 1 μM burimamide (Fig. 2). The apparent dissociation constant (K_b) for burimamide, 30 nM, was estimated on the basis of the concentrations of histamine that produced 20% inhibition in the evoked release of tritium, both in the absence and in the presence of burimamide (Fig. 2C). Burimamide, added to the perfusion medium 20 min before S_2, did not modify by itself the evoked release of radioactivity (S_2/S_1: 1.05 ± 0.08, $n = 6$). The spontaneous outflow of tritium was not modified by either histamine receptor agonists or histamine receptor antagonists (data not shown).

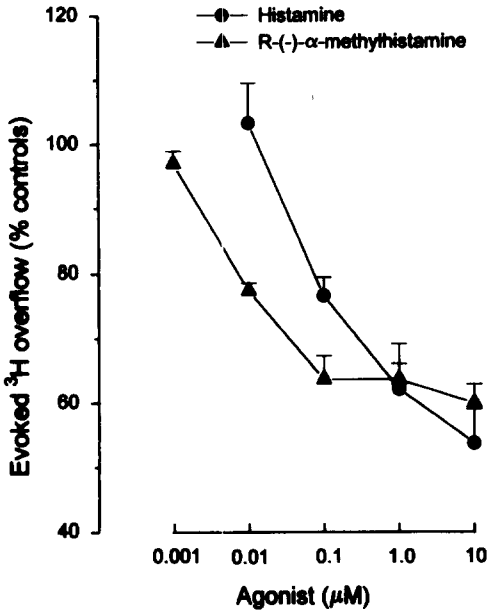

Fig. 1. Inhibition by histamine and *R*-(−)-α-methylhistamine of the evoked overflow of tritium in rat spinal cord slices prelabelled with [^{3}H]noradrenaline. The slices were superfused with a medium containing 0.3 μM phentolamine plus 1 μM desipramine and were electrically stimulated (S_1 and S_2) at 0.8 Hz (20 mA, 2 ms, 2 min). Histamine and *R*-(−)-α-methylhistamine were added to the superfusion medium 15 min before S_2. The ratio between the overflow of tritium evoked by S_2 and by S_1 (S_2/S_1 ratio) was expressed as a percentage of the S_2/S_1 value in the control group (0.97 ± 0.04, $n = 8$; both stimulations in the absence of agonists). Each point represents the mean ± S.E.M. of five to seven experiments.

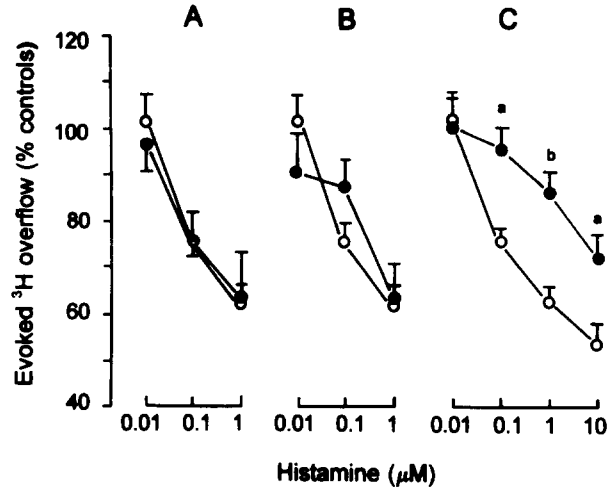

Fig. 2. Inhibitory effect of histamine in the presence of histamine receptor antagonists. Spinal cord slices prelabelled with [^{3}H]noradrenaline and then superfused with a medium containing 0.3 μM phentolamine plus 1 μM desipramine, were electrically stimulated (S_1 and S_2) at 0.8 Hz (20 mA, 2 ms, 2 min). Histamine was added to the superfusion medium 15 min before S_2. Open circles: no antagonist. Closed circles: 1 μM pyrilamine (A), 10 μM ranitidine (B) and 1 μM burimamide (C) were added to the medium at the beginning of the superfusion period. The ratio between the overflow of tritium evoked by S_2 (in the presence of histamine) and that evoked by S_1 (S_2/S_1) was expressed as a percentage of the S_2/S_1 value in the corresponding control group (both stimulations in the absence of histamine). The control S_2/S_1 values were: 0.97 ± 0.04 (no antagonist, $n = 8$); 1.04 ± 0.08 (pyrilamine, $n = 4$); 0.90 ± 0.03 (ranitidine, $n = 4$); 0.99 ± 0.04 (burimamide, $n = 6$). Each point represents the mean ± S.E.M. of four to seven experiments. [a] $P < 0.05$; [b] $P < 0.005$ when compared with histamine alone (Student's *t*-test).

3.2. Effects of histamine in the presence of forskolin and rolipram

To evaluate the possibility that cyclic AMP was involved in the inhibitory effect of histamine, some experiments were performed with a superfusion medium containing either the activator of adenylate cyclase forskolin (10 μM) or the inhibitor of phosphodiesterase rolipram (100 μM). As shown in Fig. 3, neither forskolin nor rolipram nor the combination of both, modified the effect of 1 μM histamine on the evoked release of radioactivity. Forskolin per se increased by about 40% the release of tritium induced by electrical stimulation. A similar effect was observed when both forskolin and rolipram were present in the superfusion medium. These compounds did not modify the spontaneous outflow of radiactivity (Table 1).

3.3. Effects of histamine in spinal cord slices pretreated with N-ethylmaleimide and pertussis toxin

To investigate whether the inhibitory effect of histamine involved the activation of G_o/G_i proteins, the slices were pretreated either with pertussis toxin (3 μg/ml) or with the sulfhydryl alkylating agent *N*-ethyl-

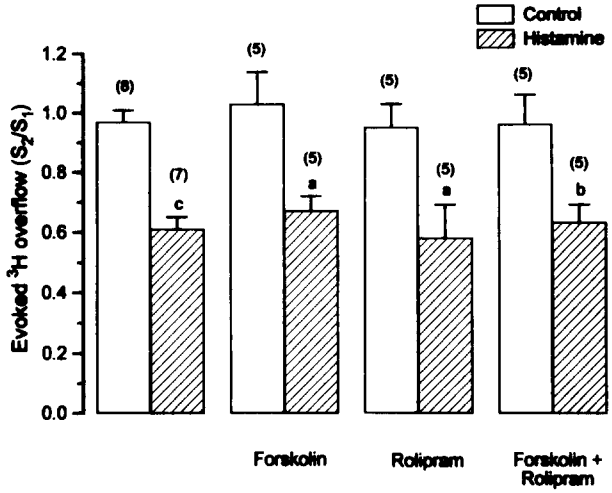

Fig. 3. Inhibitory effect of histamine on the evoked release of tritium in spinal cord slices exposed to forskolin and rolipram. Spinal cord slices prelabelled with [^{3}H]noradrenaline and then superfused with a medium containing 0.3 μM phentolamine plus 1 μM desipramine, were electrically stimulated (S_1 and S_2) at 0.8 Hz (20 mA, 2 ms, 2 min). The tritium overflow evoked by S_2 was expressed as a ratio of that evoked by S_1 (S_2/S_1 ratio). Forskolin (10 μM), rolipram (100 μM) or both drugs combined were added to the medium at the beginning of the superfusion period. Histamine (1 μM) was added to the medium 15 min before S_2. Shown are mean values ± S.E.M.. Number above bars: number of experiments. [a] $P < 0.025$; [b] $P < 0.05$; [c] $P < 0.001$ when compared to the corresponding control value (Student's t-test).

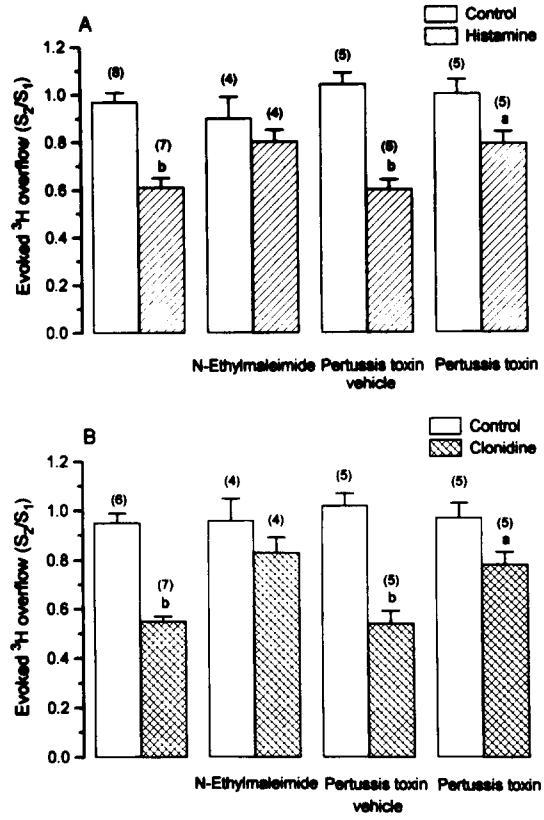

Fig. 4. Inhibitory effects of histamine and clonidine on the evoked release of tritium in spinal cord slices exposed to either N-ethylmaleimide or pertussis toxin. Spinal cord slices prelabelled with [^{3}H]noradrenaline were electrically stimulated (S_1 and S_2: 2 min, 20 mA, 2 ms). The tritium overflow evoked by S_2 was expressed as a ratio of that evoked by S_1 (S_2/S_1 ratio). (A) Frequency of stimulation 0.8 Hz; the superfusion medium contained 1 μM desipramine plus 0.3 μM phentolamine. (B) Frequency of stimulation 3 Hz; the superfusion medium contained 1 μM desipramine, but not phentolamine. Histamine (1 μM) and clonidine (1 μM) were added to the superfusion medium 15 min before S_2. Incubation in the presence of either N-ethylmaleimide (30 μM; 30 min), pertussis toxin (3 μg/ml, 4 h) or pertussis toxin vehicle solution (12 μl/ml, 4 h) was performed before labelling with [^{3}H]noradrenaline. Shown are mean values ± S.E.M. Number above bars: number of experiments. [a] $P < 0.05$; [b] $P < 0.001$ when compared to the corresponding control value (Student's t-test).

maleimide (30 μM). To verify the effectiveness of the pretreatments, the α_2-adrenoceptor agonist clonidine was assayed in some experiments. For this purpose, phentolamine was omitted in the perfusion medium and the slices were electrically stimulated at 3 Hz.

Pretreatment of the slices with N-ethylmaleimide completely prevented the inhibitory effects of both histamine (1 μM, Fig. 4A) and clonidine (1 μM, Fig. 4B). Exposure of the slices to pertussis toxin attenuated the inhibitory effects of both agonists (Fig. 4A,B).

Pretreatment with N-ethylmaleimide per se did not alter significantly the evoked overflow of tritium, although there was a tendency towards an increase, either in the presence or in the absence of phentolamine. N-Ethylmaleimide increased significantly the spontaneous outflow of radioactivity. Neither the evoked release nor the spontaneous outflow of tritium was modified by pretreatment with pertussis toxin (Table 2).

Table 1
Effects of forskolin and rolipram on the evoked and spontaneous outflow of tritium

	n	S_2/S_1	Sp_2/Sp_1
Control	8	0.97 ± 0.04	0.90 ± 0.02
Forskolin	5	1.43 ± 0.10 [a]	1.03 ± 0.07
Rolipram	5	0.99 ± 0.07	0.91 ± 0.02
Forskolin + rolipram	5	1.39 ± 0.06 [a]	1.00 ± 0.08

Spinal cord slices prelabelled with [^{3}H]noradrenaline were electrically stimulated (S_1 and S_2) at 0.8 Hz (20 mA, 2 ms, 2 min). S_2/S_1: overflow of tritium evoked by S_2 expressed as a ratio of the tritium overflow elicited by S_1. Sp_2/Sp_1: ratio of the spontaneous outflow of tritium in the 5-min superfusate samples immediately preceding the periods of stimulation. The Krebs solution contained 1 μM desipramine and 0.3 μM phentolamine. Forskolin (10 μM), rolipram (100 μM) or both drugs combined (forskolin + rolipram) were added to the superfusion medium 20 min before S_2. Shown are mean values ± S.E.M. n = number of experiments. [a] $P < 0.05$ when compared with the control value (Dunnett's test).

Table 2
Effects of *N*-ethylmaleimide and pertussis toxin on the evoked and spontaneous outflow of tritium

	(A) 0.8 Hz, 0.3 μM phentolamine			(B) 3 Hz, without phentolamine		
	n	S_1 (%)	Sp_1 (%)	*n*	S_1 (%)	Sp_1 (%)
Control	8	0.94 ± 0.21	2.08 ± 0.21	7	0.92 ± 0.22	1.94 ± 0.12
N-Ethylmaleimide	8	1.31 ± 0.20	3.73 ± 0.26 [a]	8	1.65 ± 0.32	3.76 ± 0.21 [a]
Pertussis vehicle	4	0.86 ± 0.15	2.11 ± 0.13	4	0.79 ± 0.13	2.36 ± 0.10
Pertussis toxin	5	0.74 ± 0.15	2.50 ± 0.36	5	0.95 ± 0.14	2.20 ± 0.28

Spinal cord slices prelabelled with [^{3}H]noradrenaline were electrically stimulated (20 mA, 2 ms, 2 min). (A) Frequency of stimulation 0.8 Hz; the superfusion medium contained 1 μM desipramine plus 0.3 μM phentolamine; (B) frequency of stimulation 3 Hz; the superfusion medium contained 1 μM desipramine, but not phentolamine. S_1 (%): percentage of tissue radioactivity released by the first period of stimulation; Sp_1 (%): percentage of tissue radioactivity spontaneously released during the 5-min period before the first period of stimulation. Incubation in the presence of either *N*-ethylmaleimide (30 μM, 30 min), pertussis toxin (3 μg/ml, 4 h) or pertussis toxin vehicle solution (12 μl/ml, 4 h) was performed before labelling with [^{3}H]noradrenaline. Shown are mean values ± S.E.M. *n* = number of experiments. [a] $P < 0.01$ when compared with the control values (Student's *t*-test).

3.4. Effect of histamine in the absence of phentolamine

In order to investigate whether histamine inhibits the evoked release of noradrenaline in the absence of an α-adrenoceptor antagonist, the frequency of stimulation was raised to 2.4 Hz (288 pulses), because the amount of radioactivity released at 0.8 Hz (96 pulses) in the absence of phentolamine was too low to obtain reliable results. The evoked overflow of tritium at 2.4 Hz in the absence of phentolamine, 0.81 ± 0.13% (S_1; $n = 12$), was increased to 1.82 ± 0.32% (S_1; $n = 11$) when phentolamine was present in the superfusion medium. Histamine (1 μM) did not modify the evoked release of tritium at 2.4 Hz when phentolamine was omitted in the perfusion medium, but produced a 40% decrease in the presence of the α-adrenoceptor antagonist (Fig. 5).

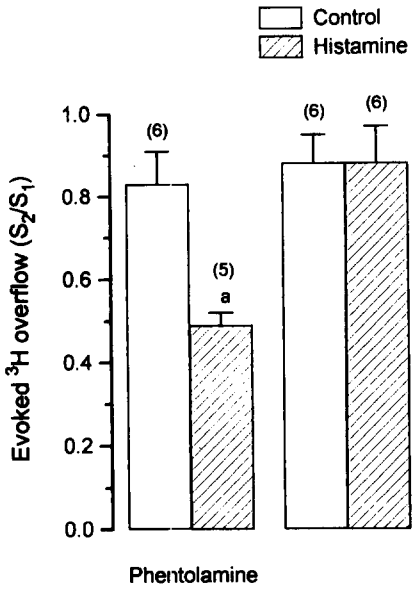

Fig. 5. Effect of histamine on the evoked release of tritium in the absence and in the presence of phentolamine. Spinal cord slices were prelabelled with [^{3}H]noradrenaline and then superfused with a medium containing 1 μM desipramine were electrically stimulated (S_1 and S_2) at 2.4 Hz (20 mA, 2 ms, 2 min). The overflow of tritium evoked by S_2 was expressed as a ratio of that evoked by S_1 (S_2/S_1 ratio). Histamine (1 μM) was added to the perfusion medium 15 min before S_2. Phentolamine (0.3 μM) was added at the beginning of the superfusion period. Shown are mean values ± S.E.M. Numbers above bars: number of experiments. [a] $p < 0.01$ when compared with the corresponding control value (Student's *t*-test).

4. Discussion

In rat spinal cord slices superfused with a medium containing desipramine and phentolamine, histamine inhibited in a concentration-dependent manner the evoked release of [^{3}H]noradrenaline elicited by electrical field stimulation. This effect was mimicked by the selective H_3 receptor agonist *R*-(−)-α-methylhistamine and was antagonized by burimamide. Burimamide was formerly known as a H_2 receptor antagonist (Black et al., 1972), but its affinity is about 100 times higher for H_3 receptors than for H_2 receptors (Arrang et al., 1983; Schwartz et al., 1986). The apparent dissociation constant for burimamide estimated in the present study (30 nM) is close to the values found for presynaptic histamine H_3 receptors in the mouse and rat brain cortex (Arrang et al., 1983; Schlicker et al., 1989, 1992a). In contrast to burimamide, neither pyrilamine, a H_1 receptor antagonist, nor ranitidine, a H_2 receptor antagonist, modified the concentration-effect curve for histamine. These results, taken together, suggest that in the rat spinal cord the activation of histamine H_3 receptors induces a decrease in the evoked release of noradrenaline. These receptors do not appear to be tonically activated by endogenous histamine in the present experimental conditions because the antagonist burimamide by itself did not increase the electrically evoked release of noradrenaline. This result, probably related to the low density of histaminergic innervation in the spinal cord (Airaksinen and Panula, 1988; Inagaki et al., 1988), does not preclude the possibility that endogenous histamine may

have a modulatory role on the release of noradrenaline when specific noradrenergic and histaminergic spinal pathways are activated in vivo.

In rat brain cortex slices (Schlicker et al., 1989, 1992b) and in the pig retina (Schlicker et al., 1990) the activation of presynaptic histamine H_3 receptors does not inhibit the evoked release of noradrenaline unless the medium contains either phentolamine or a selective α_2-adrenoceptor antagonist such as rauwolscine. Moreover, α_2-adrenoceptor blockade potentiates the inhibitory effects of histamine H_3 receptor agonists in the mouse brain (Schlicker et al., 1992b). From these findings it has been suggested that presynaptic α_2-adrenoceptors and presynaptic histamine H_3 receptors interact, either at the level of the receptors themselves or at a step beyond the receptors (Schlicker et al., 1992b). This interaction, leading to a decrease in the effect of histamine when the α_2-adrenoceptor-mediated inhibitory mechanism is activated, would also exist in the rat spinal cord (present study) because histamine did not decrease the evoked release of noradrenaline when phentolamine was omitted in the perfusion medium. It is of interest that α_2-adrenoceptor antagonists do not potentiate the effects of either histamine or *R*-(−)-α-methylhistamine on the evoked release of noradrenaline in the guinea-pig myocardium (Endou et al., 1994) and in the human saphenous vein (Luo et al., 1991; Molderings et al., 1992). This suggests that the extent of interaction between the modulatory mechanisms mediated by presynaptic histamine H_3 receptors and presynaptic α_2-receptors may depend on the species and/or tissue under study.

Since cyclic AMP has a facilitatory effect on the release of neurotransmitters (Cubeddu et al., 1975; Markstein et al., 1984; Mulder and Schoffelmeer, 1985), the histamine H_3 receptor-mediated inhibition of noradrenaline release could be related to a decrease in cyclic AMP levels in noradrenergic axons. The present study does not show evidence for this mechanism of action, because neither the adenylate cyclase activator forskolin (Daly et al., 1982) nor the phosphodiesterase inhibitor rolipram (Fredholm and Lindgren, 1987) nor the combination of both drugs, modified the inhibitory effect of histamine in the rat spinal cord. In support of these results, a study by Schlicker et al. (1994) suggests that adenylate cyclase is not involved in the inhibitory effect of *R*-(−)-α-methylhistamine on the evoked release of noradrenaline in the mouse brain cortex.

The inhibitory mechanisms of the release of noradrenaline mediated by α_2, opioid and adenosine A_1 presynaptic receptors involve the activation of G_i/G_o proteins sensitive to inactivation by either pertussin toxin or low concentrations of the sulfhydryl alkylating agent *N*-ethylmaleimide (Allgaier et al., 1985, 1986; Fredholm and Lindgren, 1987; Werling et al., 1989; Maura et al., 1992; Murphy et al., 1992). The finding that in the rat spinal cord the inhibitory effects of both histamine and clonidine were abolished by *N*-ethylmaleimide and were attenuated by pertussis toxin suggest that presynaptic histamine H_3 receptors, similarly to presynaptic α_2-adrenoceptors, are coupled to G_i/G_o proteins.

Evidence for the participation of a G protein in the histamine H_3 receptor-mediated modulatory mechanism of noradrenergic neurotransmission was previously obtained in *N*-ethylmaleimide-pretreated mouse brain cortex slices (Schlicker et al., 1994) and in pertussis toxin-pretreated guinea-pig atria (Endou et al., 1994). Moreover, there is evidence that histamine H_3 receptors are coupled to G proteins at peripheral cholinergic nerve endings (Poli et al., 1993).

It was recently reported that the inhibition of noradrenaline release mediated by the activation of presynaptic histamine H_3 receptors could be related to a decrease in Ca^{2+} influx through N-type voltage sensitive Ca^{2+} channels in nerve terminals (Endou et al., 1994; Fink et al., 1994). This mechanism may contribute to the inhibitory effect of histamine on the evoked release of noradrenaline in the rat spinal cord. In conclusion, the present study suggests that in the rat spinal cord the activation of histamine H_3 receptors induces a decrease in the electrically evoked release of noradrenaline. This mechanism could involve the activation of G_i/G_o proteins sensitive to pertussis toxin and to *N*-ethylmaleimide. There was found no evidence for the participation of adenylate cyclase in this regulatory mechanism.

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