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The effect of histamine receptor antagonists on stress-induced catecholamine secretion: an adrenomedullary microdialysis study in the rat

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Abstract

The effects of pretreatment with selective histamine receptor antagonists on changes in sympathoadrenal activity and haemodynamics, induced by 60-min immobilization stress, were studied in conscious rats. Using adrenomedullary microdialysis, it was shown that ranitidine (5 mg/kg, i.v.), a histamine H_2 receptor antagonist, selectively suppressed stress-stimulated noradrenaline secretion without affecting adrenaline response, whereas triprolidine (10 mg/kg, i.v.), a histamine H_1 receptor antagonist, had little effect on stress-induced secretion of both catecholamines. Neither triprolidine nor ranitidine changed the pressor response to 60-min stress. The stress-induced increase in heart rate was not altered by triprolidine, whereas ranitidine reduced it after 30 min of stress. To test whether the anti-secretory effect of ranitidine could be of peripheral origin, in a separate experimental series, a local catecholamine secretion was stimulated by histamine (0.5 mM) perfused through the adrenomedullary dialysis probe. It appeared that triprolidine, but not ranitidine, reduced this effect of histamine. Thus, the present results suggest that during stress, the activity of the central histaminergic system, via histamine H_2-receptors, may selectively modulate noradrenaline secretion by the adrenal gland.

Keywords: Adrenal medulla; Microdialysis; Catecholamine secretion; Haemodynamics; Stress; Triprolidine; Ranitidine

1. Introduction

Central histamine administration is known to result in increases in arterial blood pressure and heart rate (Brezenoff and Jenden, 1969; Martin et al., 1988) and in activation of sympathetic adrenomedullary system (Donoso and Barontini, 1986; Nishibori et al., 1987; Knigge et al., 1990) leading to hyperglycemia (Nishibori et al., 1987). It is interesting that stress, in particular immobilization, evokes similar responses (Kvetnansky et al., 1979; Sun et al., 1979; Kuzmin et al., 1995). On the other hand, stress was found to alter histamine content in the hypothalamus (Taylor and Snyder, 1971), a brain area with the highest density of histaminergic fibers (Watanabe et al., 1984; Inagaki et al., 1988). Taken together, these findings suggest that central histaminergic neurons may be involved in the expression of haemodynamic and sympathoadrenal responses to stress. To test this hypothesis, in rats exposed to immobilization stress, we studied the effects of a selective histamine H_1 and H_2 receptor antagonists, triprolidine and ranitidine, respectively, on changes in haemodynamics, and, using adrenal gland microdialysis (Vaupel et al., 1988; Kuzmin et al., 1990; Kuzmin et al., 1995), on changes in catecholamine secretion.

2. Materials and methods

2.1. Surgery and microdialysis procedure

Male Wistar rats (280–350 g) were used. Animals were housed in groups of five with free access to food and

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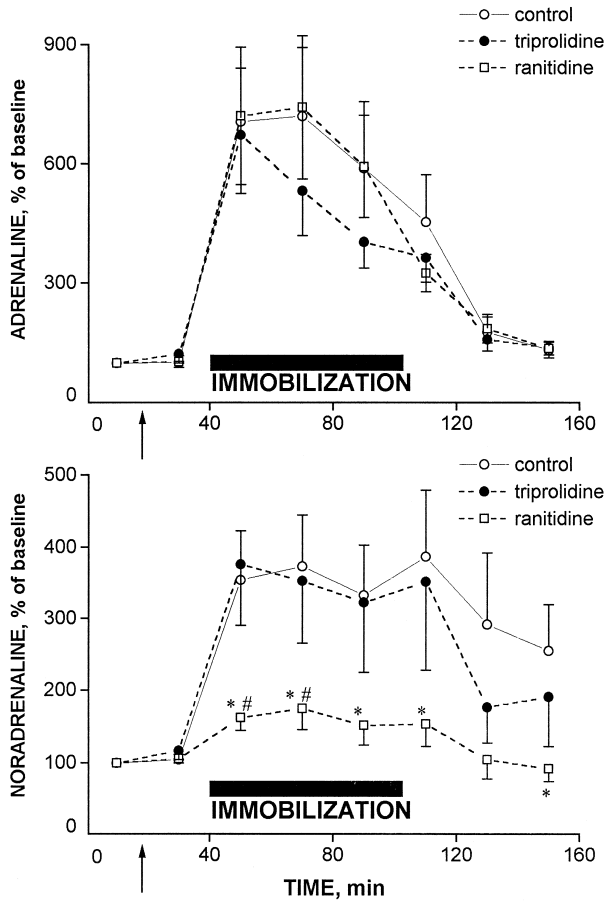

Fig. 1. Effects of ranitidine (5 mg/kg, i.v., $n = 7$), triprolidine (10 mg/kg, i.v., $n = 9$) or saline ($n = 9$) on stress-induced stimulation of catecholamine secretion measured by adrenomedullary microdialysis. The time of ranitidine, triprolidine or saline injections is indicated by an arrow. $^{*}P < 0.05$ vs. control group at the same time; $^{\#}P < 0.05$ vs. triprolidine-treated group at the same time.

water, and kept on a 12-h light/dark cycle. All experiments were performed between 1200 and 1800 h.

The animals were anaesthetized 24 h before experimentation with ketamine (40 mg/kg, i.p.). For blood pressure and heart rate measurements and for blood sampling, a polyethylene catheter (PE-10 + PE-50, Portex, USA) was inserted into the abdominal aorta through the femoral artery. For drug administration, a similar catheter was inserted into the femoral vein.

The left adrenal gland was exposed via laparotomy. The dialysis system was prepared by gluing a dialysis fibre (Cordis Dow, Belgium; 0.25 mm OD; molecular weight cutoff 5000) into the end of a silicone tube. With the aid of a needle, the fiber was then passed through the centre of the adrenal gland and its free end was glued into another piece of silicone tubing. The two silicone tubes were connected to PE-10 tubings which were subcutaneously extended and exteriorized together with catheters through the skin at the nape of the neck.

2.2. Experiment 1

On the day of the experiment, the arterial catheter was attached to an ISOTEC blood pressure transducer (Hugo Sachs Electronic, Germany) for continuous measurement of pulsatile arterial pressure and heart rate. Analog signals of blood pressure were sampled by a physiological data acquisition system 'BioShell' (National Cardiology Research Center, Moscow, Russia) on a personal computer. One end of the dialysis system was connected to a perfusion pump (CMA/100 Carnegie Medicin, Sweden), and physiological Ringer solution (mM: 147 NaCl, 4.0 KCl, 2.3 $CaCl_2$) was pumped through the system at a flow rate of 2.5 μl/min. An initial 60-min period of dialysis equilibration elapsed prior to the collection of two 20-min baseline dialysate samples. Then, ranitidine (5 mg/kg, $n = 7$; RBI, USA), triprolidine (10 mg/kg, $n = 9$; RBI) or saline ($n = 9$) was injected 20 min before restraint stress. The stress consisted of immobilizing the rats by taping their limbs to a board for 60 min. The dialysate samples were collected every 20 min during stress and after its cessation. In series with vehicle and ranitidine treatment, blood samples (0.7 ml) were obtained 10 min before and after drug administration and at 5 and 55 min after the start of stress.

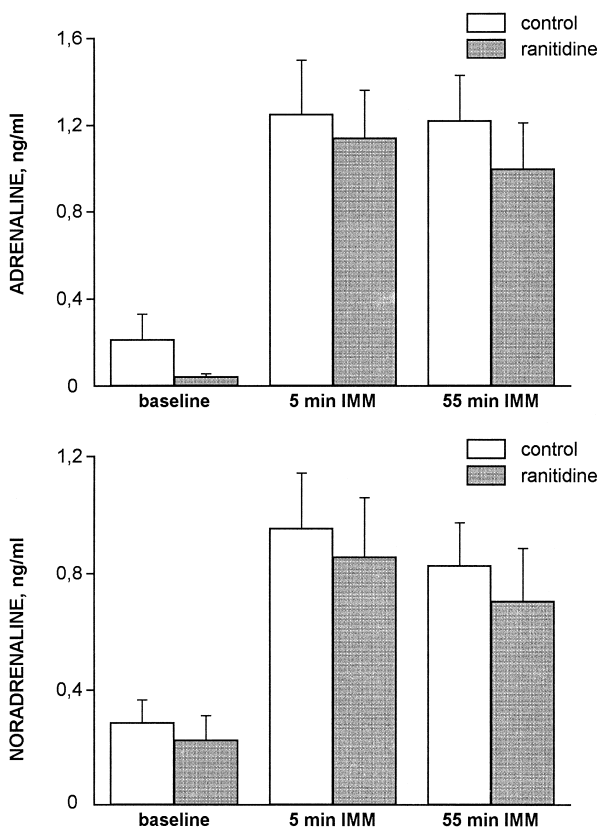

Fig. 2. Effect of ranitidine (5 mg/kg, i.v., $n = 7$) or saline ($n = 9$) on stress-induced increase in plasma catecholamine levels. IMM: immobilization.

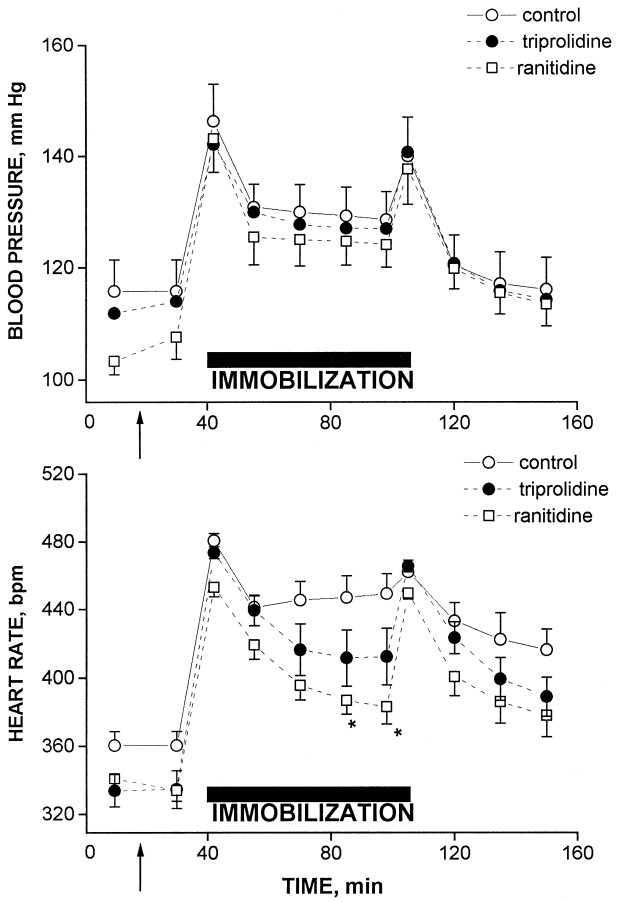

Fig. 3. Effects of ranitidine (5 mg/kg, i.v., $n = 7$), triprolidine (10 mg/kg, i.v., $n = 9$) or saline ($n = 9$) on haemodynamic response to stress. The time of ranitidine, triprolidine or saline injections is indicated by an arrow. $^{*}P < 0.05$ vs. control group at the same time.

2.3. Experiment 2

This experiment was performed to evaluate possible peripheral effects of histamine receptor antagonists on catecholamine secretion from the adrenal medulla in conscious rats. After collection of two 20-min baseline dialysate samples, the dialysis probe perfusion medium was switched to the Ringer solution containing 0.5 mM histamine (RBI) which has been shown in in vitro experiments to stimulate catecholamine secretion (Owen et al., 1989; Wan et al., 1989; Borges, 1993). After 40-min perfusion with histamine, the perfusion medium was switched back to Ringer solution, and 80 min later, saline ($n = 9$), ranitidine (5 mg/kg, $n = 7$) or triprolidine (10 mg/kg, $n = 6$) was intravenously injected. Then, after collection of baseline dialysate sample, histamine stimulation was repeated.

2.4. Analytical procedure

Dialysate samples, as well as blood plasma samples after partial purification by absorption on alumina, were analysed for noradrenaline and adrenaline using high-performance liquid chromatography with electrochemical detection (Kuzmin et al., 1995).

2.5. Statistics

The results are presented as means ± S.E.M. Statistical comparisons between groups were carried out by Student's *t*-analysis of variance (ANOVA) followed by Bonferroni method for multiple comparisons (Wallenstein et al., 1980).

3. Results

No differences were noted between noradrenaline and adrenaline baseline adrenal dialysate levels for the various experimental groups. Mean basal concentrations in all rats tested ($n = 47$) were 2.1 ± 0.4 for noradrenaline and 3.9 ± 0.8 ng/ml for adrenaline. In both protocols performed, neither ranitidine nor triprolidine changed baseline dialysate catecholamine levels.

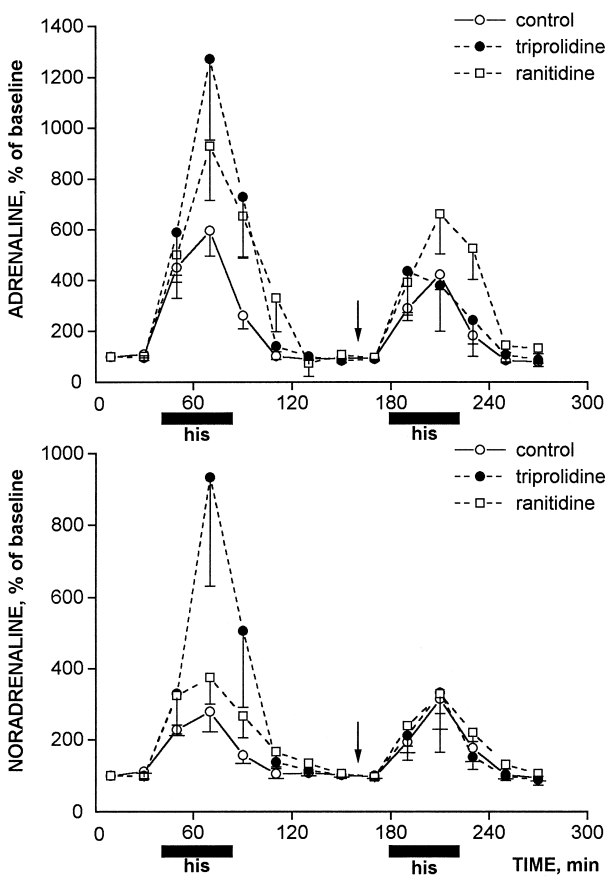

Fig. 4. Changes in catecholamine concentrations in the adrenal dialysate during the two subsequent stimulations of a local catecholamine secretion by 0.5 mM histamine (his) perfused via the dialysis probe in rats which received saline ($n = 9$), ranitidine (5 mg/kg, i.v., $n = 7$) or triprolidine (10 mg/kg, i.v., $n = 6$) 20 min before the start of the second stimulation. The time of drug injections is indicated by an arrow.

Immobilization evoked a significant fourfold and sevenfold increases ($P < 0.05$) in renal dialysate concentrations of noradrenaline and adrenaline, respectively (Fig. 1). After cessation of stress, these concentrations returned slowly to basal values. Ranitidine significantly reduced stress-stimulated secretion of noradrenaline, but not that of adrenaline, whereas triprolidine had little or no effect on stress-induced secretion of both catecholamines.

At 5 and 55 min after the start of stress, a significant increase ($P < 0.05$) in plasma concentrations of noradrenaline and adrenaline compared with baseline values was observed (Fig. 2). Ranitidine did not change either basal levels of circulating catecholamines or their response to stress. (Because preliminary studies with adrenomedullary dialysis have shown that triprolidine (5 and 10 mg/kg) was not able to change secretory response to immobilization, in the experimental series with this drug, plasma catecholamines were not measured).

Immobilization evoked marked and sustained increases in blood pressure and heart rate (Fig. 3). The pressor and tachycardiac responses reached their peaks within the first 3 min after the onset of stress ($+30 \pm 2$ mm Hg, $+120 \pm 7$ bpm, $P < 0.05$). The second, less pronounced peaks observed might be caused by freeing of the animals. Both histamine receptor antagonists had no effect on the pressor response to stress. The stress-induced increase in heart rate was only slightly altered by triprolidine, whereas ranitidine significantly reduced it after 30 min of stress.

In the second experimental series, the adrenal dialysis probe was perfused with 0.5 mM histamine. To estimate the concentration of histamine delivered into the interstitial space, the recovery of the dialysis probe should be taken into account. For the dialysis probe used, approximately 2 mm, the recovery value may be estimated to be approximately 5%, and thus the interstitial histamine concentration should be approximately 25 μM. This value is quite comparable with histamine concentrations used in the in vitro studies to stimulate catecholamine secretion (10–30 μM) (Owen et al., 1989; Wan et al., 1989; Borges, 1993).

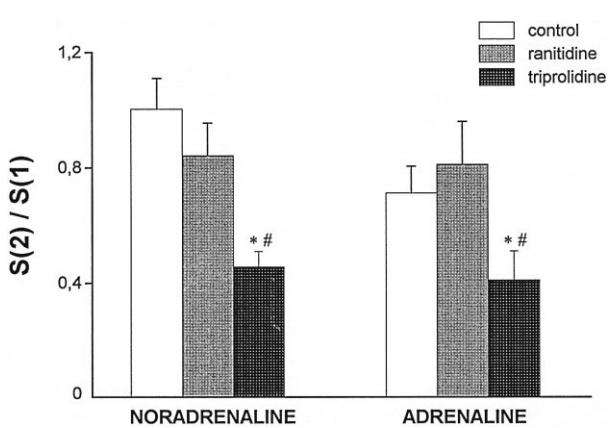

Fig. 5. Effects of ranitidine (5 mg/kg, i.v., $n = 7$), triprolidine (10 mg/kg, i.v., $n = 6$) or saline ($n = 9$) on histamine-stimulated local cumulative secretion of catecholamines from the adrenal gland. Results are given as ratio of the two subsequent stimulations, S(2)/S(1). The drugs were injected before the second histamine stimulation. $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ vs. ranitidine-treated group.

Perfusion of the adrenal dialysis probe with histamine resulted in a parallel increase in dialysate noradrenaline and adrenaline (Fig. 4) which magnitude was nearly similar to that observed during stress (Fig. 1). Comparison of dialysate catecholamine concentration profiles during the second histamine stimulation after drug injection did not reveal statistically significant differences between groups either for noradrenaline or for adrenaline. However, when the results were expressed as a cumulative catecholamine secretion during histamine stimulation and 40-min post-stimulation sampling period and then as S(2)/S(1) ratio of the two subsequent stimulations (Fig. 5), they clearly demonstrated that triprolidine, but not ranitidine, suppressed histamine-stimulated secretion of both noradrenaline and adrenaline.

4. Discussion

Recently, using microdialysis of the adrenal gland for the direct measurement of catecholamine secretion in conscious rats, it was clearly demonstrated that whereas the immobilization stress produces an increase in secretion of both adrenaline and noradrenaline, the stress of 2-deoxyglucose-induced central neuroglucopenia stimulates the release of adrenaline without affecting noradrenaline secretion (Kuzmin et al., 1995). These results closely agree with those obtained in in vitro experiments which demonstrated that vasoactive intestinal polypeptide, a peptidergic neurotransmitter, predominantly stimulated the secretion of adrenaline by the rat adrenal gland, whereas acetylcholine evoked the secretion of both catecholamines (Guo and Wakade, 1994). Taken together, these findings may suggest that the two types of adrenal chromaffin cells, adrenaline-storing and noradrenaline-storing cells, may receive different innervations, and, at least under stressful conditions, a separate central neuronal mechanisms and pathways for regulation of secretion of individual catecholamines may exist. In accordance with these suggestions, the results of the present study demonstrate that central histaminergic neurones, via activation of histamine H_2 receptors, are involved in noradrenaline, but not adrenaline, secretory response to immobilization stress: the histamine H_2 receptor antagonist ranitidine suppressed the stress-induced increase in secretion of noradrenaline, whereas the histamine H_1 receptor antagonist triprolidine did not alter secretory response.

In cultures of bovine adrenal chromaffin cells (Owen et al., 1989; Wan et al., 1989) as well as in the in vitro perfused rat adrenal gland (Borges, 1993), histamine has been found to stimulate catecholamine secretion. Although the effects of stress on circulating histamine or its tissue

release have not, to our knowledge, been investigated, it seems possible that the stress-induced increase in catecholamine secretion might be, at least in part, due to direct histamine stimulation of the adrenal chromaffin cells, and the anti-secretory effect of ranitidine observed might be caused by its peripheral action. To test this hypothesis, in the second experimental series, histamine (0.5 mM) was infused through the adrenomedullary dialysis probe. Like in vitro studies (Owen et al., 1989; Wan et al., 1989; Borges, 1993), it appeared that histamine stimulated local secretion of both adrenaline and noradrenaline via an action on histamine H_1 receptors. Taken the results of both experimental series together, it may be concluded that, although ranitidine is thought to have a relatively poor ability to cross the blood–brain barrier (Hill, 1990), it is likely that the effect of this drug on stress-induced noradrenaline secretion was centrally mediated. On the other hand, the lack of anti-secretory effect of triprolidine during stress suggests that peripheral histamine plays a minor role in activation of catecholamine secretion.

Although ranitidine reduced the stress-induced increase in noradrenaline secretion, it did not change pressor response. However, the positive chronotropic response was attenuated by this drug. Since the pressor and tachycardiac effects elicited by centrally administered histamine were found to be mediated by central histamine H_1 receptors (Martin et al., 1988), it seems reasonable to assume that ranitidine exerted its bradycardiac effect during stress via the blockade of myocardial histamine H_2 receptors.

It is well known that histamine is present in relatively high concentrations in cardiac tissues in most animal species (Hill, 1990) and can be released from the myocardium such as in response to ischemia (Wolff and Levi, 1988). Moreover, the presence of histaminergic nerve fibers in the myocardium has been recently postulated (Campos and Briceno, 1992). On the other hand, perfusion of isolated hearts with histamine was shown to increase heart rate via activation of histamine H_2 receptors (Hattori and Levi, 1984; Hill, 1990). Taken together, these findings support our hypothesis. Thus, it seems likely that the stress-induced direct histamine stimulation of myocardial histamine H_2 receptors might be partially responsible for the tachycardiac effect of stress.

Although ranitidine markedly attenuated the stress-induced increase in noradrenaline secretion, plasma noradrenaline response to stress surprisingly appeared to be similar in ranitidine-treated and control groups. This might occur if the adrenal medulla contributed very little to the plasma noradrenaline level during stress. Because the noradrenaline plasma clearance was not measured in the present study, only from the dialysate and the blood plasma catecholamine concentration data, we were not able to calculate the relative contribution of the adrenal glands to circulating noradrenaline. But when it has been done, it has been shown that the plasma noradrenaline of adrenomedullary origin increases from 9 to 50% after severe blood loss in the rat (Kuzmin et al., 1990). These data suggest that the adrenal medulla may contribute greatly to the stress-induced increase in noradrenaline level in the plasma. The other possible explanation is that ranitidine reduced the plasma noradrenaline clearance during stress. But then it was to be expected that the plasma adrenaline levels in ranitidine-treated rats should be higher than those in control animals. To our mind, the most likely explanation of the lack of differences between plasma noradrenaline in ranitidine-treated and control rats seems to be a compensatory increase in the sympathetic nervous activity in ranitidine treated animals. This increase might be the reason for an unaltered pressor response observed in these rats. In accordance with this suggestion, the selective activation of adrenaline secretion evoked by 2-deoxyglucose-induced metabolic stress (Kuzmin et al., 1995), like in ranitidine-treated immobilized rats, was accompanied by an increase in the sympathetic outflow, as it was shown by plasma noradrenaline measurements (Sun et al., 1979; Medvedev et al., 1989). It should be noted that 2-deoxyglucose does not markedly alter the plasma catecholamine clearance (Medvedev et al., 1990).

In contrast to the results obtained in the present study, previous study of Knigge et al. (1990) demonstrated that both the intracerebroventricular administration of the histamine H_1 receptor antagonist mepyramine or the histamine H_2 receptor antagonist cimetidine prevented the 5-min restraint stress-induced increase in plasma adrenaline and noradrenaline in the rat. However, assuming that the volume of drug distribution is total body volume in the case of intravenous administration, whereas it is the brain volume in the case of central administration, it may be shown that estimated drug brain concentrations in the study of Knigge et al. (1990) might be more than 10 times higher than those in the present study. This may be the likely reason for the discrepancy mentioned above, because, for example, mepyramine at relatively high concentrations, additionally to blocking of histamine H_1 receptors, may also antagonise muscarinic cholinergic (Burgen and Harbird, 1983; Hill, 1990) and histamine H_2 receptors (Trendelenburg, 1960, Hill, 1990) and exhibit membrane-stabilising (Seeman and Weinstein, 1966; Hill, 1990) and sedative properties (Quach et al., 1979; Hill, 1990).

The results of the present study indicate that central histaminergic neurons, via activation of central histamine H_2 receptors, are involved in the formation of noradrenaline secretory response to immobilization stress.

Acknowledgements

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