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HISTAMINE, A NEUROMODULATOR OF NORADRENERGIC TRANSMISSION IN UTERINE HORNS FROM MICE IN DIESTRUS

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Abstract—The effect of histamine on [^{3}H]norepinephrine ([^{3}H]NE) release in uterine horns from mice in estrous and diestrous states was studied under two different experimental conditions: resting NE release and stimulus-evoked NE release. It was found that [^{3}H]NE release was higher for the diestrous state under both resting and stimulus-evoked (100 mM K^+ and electrical stimulus) conditions. Histamine only potentiated the stimulus-evoked [^{3}H]NE outflow in uterine horns from mice in the diestrous state and from ovariectomized mice treated with progesterone. This effect was dose dependent and was antagonized by H_1 but not by H_2 or H_3 antagonists. *R*-α-Methylhistamine, a H_3 agonist, had no effect on stimulus-evoked [^{3}H]NE release. According to these results, it could be concluded that: (a) histamine regulates the NE release from noradrenergic nerve terminals in uterine tissues; (b) this heterologous regulation depends on progesterone predominance and on terminal depolarization; and (c) presynaptic H_1 receptors located on noradrenergic terminals could be responsible for such an effect.

Key words: norepinephrine release; histamine receptors; uterine adrenergic innervation

For many years NE† has been known to be involved in the regulation of uterine contractility. Evidence for this fact has been provided by the use of β_2-adrenergic drugs to inhibit uterine contractions [1, 2] and by studies that show that uterine tissue is densely innervated by noradrenergic fibers [3]. These noradrenergic nerves, which run along smooth muscle fibers of the myometrium and blood vessels, have been described as short and sex steroid-sensitive neurones. It has also been demonstrated that estrogens and progesterone regulate adrenergic responses in the myometrium, acting by opposing their effects at multiple sites including both pre- and postsynaptic levels [3, 4]. As a consequence, myometrium contractility is modified. Thus, the relaxation promoted by progesterone could be due, in part, to its stimulant effects on released NE, on NE concentration at the synaptic cleft, and on the efficacy of β-adrenergic receptors (for a review, see Refs. 3 and 5).

It has also been demonstrated that high levels of histamine are present in mouse uterine tissue [6]. Mast cells, which seem to be the main source of histamine in the uterus, are distributed in a similar way as noradrenergic nerves, i.e. in close apposition to smooth muscle myometrium and around blood vessels [6]. We are unaware of any published information on the microanatomical association of mast cells and adrenergic nerves in the myometrium, but a functional interaction has been postulated in human tissue due to the inhibition of histamine release caused by a β_2-adrenergic agonist in human uterine mast cells [7].

The role of histamine in uterine contractility is not as well known as the role of NE and is rather contradictory, since histamine relaxes the rat uterus [8] but contracts mouse and human uteri [9, 10]. Another contradictory situation has been reported where NE release can be inhibited or stimulated depending on the presynaptic histamine receptors expressed in noradrenergic terminals. Inhibition is mediated through H_3 receptors in brain [11] and in vascular adrenergic terminals [12], whereas stimulation of NE release has been postulated to be mediated through H_2 receptors in rat uterine tissue [13] and through H_1 receptors in bovine chromaffin cells and guinea pig atria [14, 15].

The present study was undertaken to investigate the effect of histamine on NE release from mouse uterine adrenergic terminals. The reason for choosing mice is the similarities observed in the uterine contractility (due to histamine effects) of this species and of human beings. A pharmacological characterization of the possible histaminergic receptors involved is also presented.

MATERIALS AND METHODS

Four-month-old female albino mice, weighing 30–40 g, were obtained from the animal holding unit of the Department of Pharmacology, University of Concepción. Temperature, humidity and standard light conditions were controlled in the housing. The classical vaginal examination method of smears was

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† Abbreviation: NE, norepinephrine.

used to determine the estrous cycle state [16]. The results of this exam were ratified by observing the macroscopic physical aspect of the uterine horns after they were dissected, since hyperemia and water content and weight increase are characteristics of predominance of estrogens [17]. Experiments were performed only with uterine horns in which both parameters coincided in the diagnosis of the estrous cycle state. The pharmacological determination of the histamine receptors involved in [^{3}H]NE release was done by using uterine horns of 7-day ovariectomized mice treated with progesterone (5 mg/kg/day for 4 days) [5].

Drugs and chemicals. L[ring-2,5,6-^{3}H]Norepinephrine, sp. act. 14.2 and 10.1 Ci/mmol, was purchased from the New England Nuclear Corp., Boston, MA, U.S.A., and was used, respectively, for electrical stimulation and high K^+ studies. Histamine, pyrilamine and cimetidine were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Thioperamide and *R*-α-methylhistamine were purchased from Cookson Chem., England. Astemizole was a gift from Laboratorios Andrómaco S.A. Chile.

The Ringer-bicarbonate solution used in these experiments had the following composition: 153 mM NaCl, 5.6 mM KCl, 0.88 mM $CaCl_2$, 1.13 mM $MgCl_2$, 2.7 mM glucose. It was gassed continuously with 95% O_2 and 5% CO_2. A high K^+ solution (100 mM KCl) was made by replacing a portion of the NaCl with equimolar amounts of KCl.

Experimental procedure. After killing the mice, uterine horns were isolated, and the excess fat and connective tissue were removed. Afterward, they were transferred to an organ bath at 30° containing Ringer-bicarbonate solution bubbled with 95% O_2 and 5% CO_2.

Outflow of tritium from mouse uterine horns after preincubation with [^{3}H]NE. Horns from the same uterus were incubated with [^{3}H]NE (0.1 μM) for 60 min at 30°. Then they were washed and subjected to an experimental protocol that included the transfer of the uterine horns through a series of 32 tubes containing 4 mL of oxygenated Ringer-bicarbonate solution at 30°. The incubation of the uterine horns continued for 1 min in each tube. Each uterine horn was stimulated for 1 min with high K^+ solution in tube No. 4 and No. 24 (S_1 and S_2, respectively) (Fig. 1). When electrical stimulation was applied to the uterine horns, a similar sequence was followed, but instead of transferring the horns through the tubes, they were superfused at a constant flow of 4 mL/min, and the superfusate was collected each minute during the experiment [18]. At the end of the experiment, the horns were weighed and homogenized in 3 mL of 10% perchloric acid. The resulting suspension was centrifuged at 600 *g* for 5 min. Aliquots (100 μL) from each tube and from the supernatant resulting from centrifugation were analyzed for tritium. Two milliliters of leftover supernatant was chromatographed on an alumina column [19] in order to analyze the proportion of unmetabolized [^{3}H]NE remaining in the tissues at the end of the experiments. The resulting proportion was 87%.

Both resting and stimulus-evoked tritium outflow

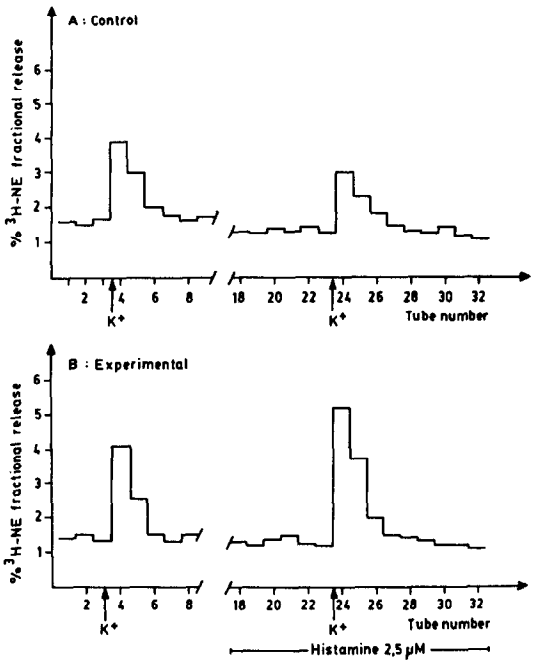

Fig. 1. Example of an experiment developed to study the effect of histamine on the outflow of tritium from uterine horns from a mouse in diestrus. The horns were preincubated with [^{3}H]NE. Arrows indicate the tubes containing high K^+ solution (S_1 and S_2, respectively). Upper tracing: "control." Bottom tracing: "experimental" preparation (2.5 μM histamine was added).

were calculated by dividing the tritium content in each incubation tube by the tritium content remaining in the uterine horn when incubated in the corresponding tube, and are expressed as percentages of fractional release [20]. The resting tritium outflow was monitored in the three tubes preceding the stimulus. The stimulus-evoked tritium outflow was calculated as the net fractional release above resting levels.

To analyze the effect of histamine in both resting and stimulus-evoked tritium release, histamine was added 6 min before S_2, i.e. into experimental tubes No. 18–32 (Fig. 1). The difference in the relation of S_2/S_1 from both control and histamine-treated preparations was considered for statistical analysis. To study the interaction between histamine and the corresponding H_1, H_2 and H_3 antagonists, both compounds were added simultaneously into tubes No. 18–32. The same protocol was followed to analyze the effect of the H_3 agonist *R*-α-methylhistamine, but this time histamine was omitted.

Statistics. All values are expressed as means ± SEM. Statistical differences were determined by Student's *t*-test. A probability level below 0.05 was considered significant. All experiments were performed with at least 3 mice.

RESULTS

Tritium outflow from mouse uterine horns

Table 1. Resting, high K^+, and electrically stimulated outflow of tritium from mouse uterine horns preincubated with [^{3}H]NE

Experimental conditions	Resting outflow	High K^+	Electrical stimulation
Estrous state	1.32 ± 0.07 (5)	1.95 ± 0.21 (4)	7.07 ± 0.56 (5)
Diestrous state	1.64 ± 0.10*(4)	4.18 ± 0.32†(8)	11.87 ± 0.81†(4)
Ovariectomized + progesterone	1.60 ± 0.08*(8)	5.05 ± 0.45†(8)	

Resting outflow was measured as the percentage of spontaneous outflow per minute in each experiment. Stimulus-evoked release was evaluated as the net fractional outflow over resting levels, prior to the application of the high K^+ or electrical stimulus. Values are means ± SEM from the number of experiments indicated in parentheses.

*,†Significantly different as compared with the estrous state: *$P < 0.05$, and †$P < 0.005$.

Table 2. Effect of histamine on stimulus-evoked tritium outflow in mouse uterine horns preincubated with [^{3}H]NE

Experimental conditions	High K^+ (S_2/S_1)	Electrical stimulation (S_2/S_1)
Estrous state		
control	0.79 ± 0.01 (3)	0.60 ± 0.10 (5)
histamine (2.5 μM)	0.78 ± 0.05 (3)	0.55 ± 0.06 (5)
Diestrous state		
control	0.59 ± 0.08 (9)	0.56 ± 0.10 (4)
histamine (2.5 μM)	1.45 ± 0.10* (9)	1.65 ± 0.34* (4)

Histamine given at S_2 was added to the incubation medium 6 min prior to the onset of stimulation. S_1 and S_2 were evaluated as the net tritium outflow over resting levels prior to the application of the corresponding stimulus. Values are means ± SEM from the number of experiments indicated in parentheses.

* $P < 0.005$ as compared with control in the diestrous state.

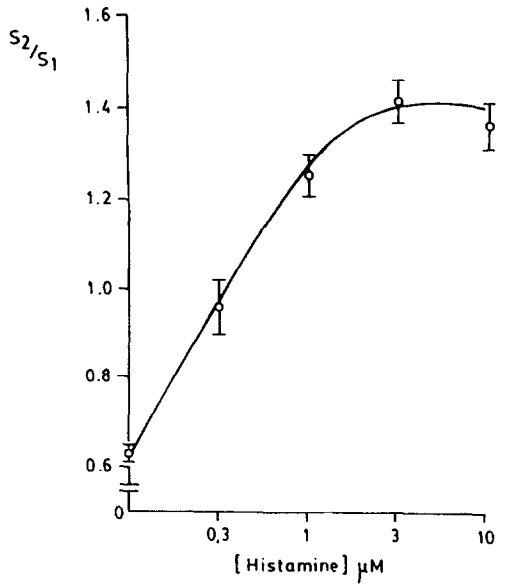

Fig. 2. Concentration–response curve showing the effect of histamine on high K^+-evoked tritium outflow from uterine horns from mice in diestrus. The horns were preincubated with [^{3}H]NE. S_2/S_1 corresponds to the ratio between the stimuli in the presence and in the absence of histamine. Each point is the mean ± SEM from at least four experiments.

preincubated with [^{3}H]*NE*. Table 1 shows the results of the tritium outflow from uterine horns from mice in the estrous or diestrous state and from ovariectomized mice treated with progesterone. Resting tritium outflow was the lowest in the estrous state. Depolarization-evoked stimuli (100 mM K^+ or electrical stimulus) enhanced the tritium outflow. The outflow was greater after electrical stimulus, and it was significantly higher in the uterine horns from mice in the diestrous state and in uterine horns from ovariectomized mice treated with progesterone (Table 1). Stimulation at S_2 evoked a lower tritium outflow, as shown by the ratio of S_2/S_1 in Table 2.

Effect of histamine on resting and evoked tritium outflow from mouse uterine horns preincubated with [^{3}H]*NE*. Histamine (2.5 μM), added to the superfusion system 6 min prior to S_2, enhanced the stimulus-evoked tritium outflow by 245% after high K^+ and 294% after electrical stimuli. This stimulatory effect was dependent on the concentration of the amine (Fig. 2) and on the estrous state of the animal, since it was observed only in uterine horns from mice in the diestrous state (Table 2) and in ovariectomized plus progesterone-treated mice (Table 3). Histamine and the drugs added 6 min before S_2 had no effect on the resting tritium outflow.

As also shown in Table 3, H_1 antagonists [pyrilamine (0.1 μM) and astemizole (10 μM)] had no effect on the high K^+-evoked tritium outflow but antagonized the stimulatory effect of histamine. H_2 and H_3 antagonists [cimetidine (1 and 50 μM) and thioperamide (1 μM)] had no effect on the high K^+- and histamine-evoked tritium outflow. *R*-α-Methylhistamine (5 μM) did not simulate the action of histamine.

DISCUSSION

Release of radiolabeled neurotransmitters from isolated tissues is a commonly used model for evaluating compounds that act at presynaptic

Table 3. Effect of histamine analogues on histamine high K^+-evoked tritium outflow in uterine horns from ovariectomized mice treated with progesterone

Experimental conditions	High K^+ (S_2/S_1)
Control	0.61 ± 0.12 (9)
Histamine (2.5 μM)	1.48 ± 0.08 (9)
Histamine (2.5 μM) + pyrilamine (0.1 μM)	0.57 ± 0.17*(4)
Histamine (2.5 μM) + astemizole (10 μM)	0.79 ± 0.06†(4)
Histamine (2.5 μM) + cimetidine (1 μM)	1.36 ± 0.24 (4)
Histamine (2.5 μM) + cimetidine (50 μM)	1.26 ± 0.07 (4)
Histamine (2.5 μM) + thioperamide (1 μM)	1.46 ± 0.13 (4)
R-α-Methylhistamine (5 μM)	0.71 ± 0.09‡(4)
Pyrilamine (0.1 μM)	0.79 ± 0.05‡(4)
Astemizole (10 μM)	0.65 ± 0.14‡(4)

After preincubation with [^{3}H]NE, the uterine horns were incubated for 1 min each in tubes containing Ringer-bicarbonate solution with histamine or the drugs indicated, and were stimulated for two periods by transferring them to tubes containing high K^+ (100 mM) (S_1 and S_2). Histamine and the corresponding drugs, given at S_2, were added to the incubation medium 6 min prior to S_2. S_1 and S_2 were evaluated as the net tritium outflow over resting levels prior to the application of the stimulus (see Fig. 1). Values are means ± SEM from the number of experiments indicated in parentheses.

*,†Significantly different from histamine (2.5 μM): *$P < 0.05$ and †$P < 0.005$.

‡ Not different from control.

receptors, either inhibiting uptake of the neurotransmitter or stimulating its release [21]. The method does not differentiate between the mechanisms, since an increased outflow is observed in both of them. An uptake inhibitor reduces the fraction of the previously released transmitter, which should be normally subject to reuptake, resulting in an increased outflow [21]. A superfusion system was used for studying the effect of histamine on [^{3}H]NE depolarization-evoked release in order to analyze the hypothesis that histamine could stimulate NE release instead of inhibiting its reuptake. The question was the following: if NE released from the tissue by depolarization could be washed out rapidly, the effect of histamine should be reduced, provided that the autocoid had a significant effect on [^{3}H]NE reuptake. Table 2 shows that the relation between the second and first stimulus was similar to that observed when the uterine horns were transferred in a series of 4-mL incubation tubes. These results support the hypothesis mentioned above that histamine could be a positive modulator on NE release in uterine horns.

Since the effect of histamine on NE release was observed only in progesterone-primed mice and when the terminal was under a depolarizing stimulus, we postulate that the consequence of this effect is a modulation in which depolarization of adrenergic nerve terminals and progesterone predominance make NE release more specific toward histamine stimulation. Molecular studies could clarify which mechanisms are involved in the modulatory effect of histamine. As it has been proposed for other depolarizing-dependent neurotransmitter effects [22], this event may involve a mechanism by which depolarization unmasks H_1 receptors that become exposed to the stimulatory action of histamine. Simultaneously, progesterone could modulate the coupling for signal transduction [23].

As already stated, histamine has been shown to stimulate NE release through the activation of either H_1 or H_2 presynaptic receptors [13–15]. A pharmacological characterization of the receptors involved in stimulus-evoked NE release was made by using pyrilamine and astemizole as H_1 and cimetidine as H_2 antagonists. Results from Table 3 demonstrate the involvement of H_1 receptors since the effect of histamine was only antagonized by pyrilamine and astemizole. A possible "anesthetic effect" of these compounds, which could interfere with the results, was discarded since they had no effect on NE-evoked release. The possibility that H_3 receptors could also be involved was discarded, since *R*-α-methylhistamine had no effect on the regulation of NE release from noradrenergic nerve terminals. Moreover, thioperamide was ineffective on the histamine-stimulatory NE-evoked release.

Over the last 20 years it has been demonstrated that estrogens stimulate histamine release from mast cells [24]. It is also known that mouse uterine horns are contracted by histamine [9], which may contribute to the increase in myometrial contractility usually observed under the predominance of estrogens [25]. On the contrary, myometrium contractility is inhibited under the predominance of progesterone. This effect has been attributed, at least in part, to the capacity of this hormone to stimulate uterine noradrenergic activity [4, 5]. The fact that histamine increases uterine contractility under the predominance of estrogens but contributes to the inhibition of the contractility, through the release of NE, under the predominance of progesterone could be considered as additional evidence that points to the prime role of sexual hormones in controlling the actions of endogenous compounds and neurotransmitters in the reproductive system.

In conclusion, our results demonstrated that histamine stimulates noradrenergic terminals in mouse uterine horns. This effect is mediated through the activation of H_1 presynaptic receptors and is dependent upon the predominance of progesterone and upon the depolarization of the noradrenergic terminal.

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