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HISTAMINE H_1 RECEPTOR ACTIVATION MEDIATES THE PREFERENTIAL RELEASE OF ADRENALINE IN THE RAT ADRENAL GLAND

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Summary

Histamine elicited the release of catecholamines from "in vitro" perfused rat adrenals with an EC_{50} of 3 μM. This concentration was in the same range as those which caused a fall in the arterial blood pressure when infused intravenously in anaesthetized rats. Histamine stimulation was potently blocked by dexclorfeniramine (IC_{50} = 300 pM), but unaffected by ranitidine, suggesting the involvement of H_1 receptors. Histamine released preferentially adrenaline. Mast cells were not detected within adrenal medulla by histochemical techniques. Compound 48/80 did not trigger catecholamine release. Catecholamine secretion evoked by splanchnic nerves stimulation was not modified by a combination of H_1 and H_2 antagonists. In conclusion, the histamine that elicited adrenaline release from rat adrenals comes from blood circulation not from local mast cells or splanchnic nerves. These effects are mediated through the activation of H_1 receptors.

Histamine is the main mediator released in anaphylactic processes. Many of its cardiovascular and respiratory actions are physiologically antagonized by adrenaline secreted from the adrenal medulla (1).

Histamine induces catecholamine release from adreno-medullary chromaffin cells in many species including dog (2), cat (3), rat (4) and cow (5). It also induces, the synthesis and release of opioid peptides from these cells (6).

Histamine could reach the adrenal medulla from three possible sources: **i**) histaminergic splanchnic fibres; **ii**) In situ mast cells and **iii**) circulating histamine. Although histaminergic fibres have been found in guinea pig adrenals they are not apparent in the rat (7, 8). Mast cells have been identified in the adrenal gland of rats, but they are mainly localized

in the cortex (9). Moreover, very low histamine concentrations have been found in the rat adrenal (10). Nevertheless, inmunohistochemical studies have found histamine associated with noradrenaline-containing chromaffin cells (8).

Histamine may act through the activation of at least three different receptors: H_1 coupled to phosphoinositoside hydrolysis (11), H_2 linked to an adenylate cyclase and H_3 which appears to be a presynaptic receptor. There is also evidence of intracellular histamine receptors (12). Histamine H_1 receptors have been described in cow (13) and in cat (3), and are associated with catecholamine secretion and the accumulation of inositol phosphates (14). A functional role of an H_2 receptor in bovine chromaffin cells has recently been described (15) and it is associated with the accumulation of cellular cAMP.

In cultured bovine chromaffin cells, histamine evokes the secretion of adrenaline and noradrenaline in the same proportions as found in the cells (5). In other species, however, the preferential secretion evoked by histamine has not been studied. In the cat, for example, muscarinic agonists preferentially release adrenaline (16). Khalil, et al., (17) detected an increase in the blood levels of both adrenaline and noradrenaline in the rat after a treatment with high doses of histamine, but they could not determine if the noradrenaline came from adrenal gland or from sympathetic nerves.

This paper therefore describes a series of experiments designed to determine: (i) the origin of histamine causing the secretion of catecholamines; (ii) the subtype of histamine receptor mediating such response and (iii) the preferential release of adrenaline or noradrenaline.

Materials and Methods

Female Sprague-Dawley rats, weighing 200-300 g, were anaesthetized with sodium pentobarbitone (50 mg / Kg i.p.). All animal procedures were in strict accordance with the NIH Guide for the care and use of laboratory animals and were approved by The Ethical Committee of La Laguna University.

Blood pressure studies. The left femoral vein was cannulated with a polyethylene tubing and used to inject histamine. Arterial blood pressure was measured via a cannula inserted into the common carotid artery, and connected through a pressure transducer to a Harvard polygraph. Body temperature was monitored with a rectal probe and maintained at 37 °C by means of a heat lamp. Adrenal removal was carried out, in a group of animals, by the tying of both adrenal glands prior to histamine injection. Data were quantified, by the planimetric analysis of the areas under the initial pressure values and expressed in cm^2 .

Catecholamine release studies. The abdomen was opened and the left adrenal gland was exposed. The adrenolumbar vein was cannulated using a PE10 Portex (800-100-100) tube after all the branches were ligated. The glands were then removed and perfused retrogradely in vitro at 1 ml/min, by a peristaltic pump, with a Krebs-bicarbonate solution containing (in mM): NaCl, 119; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; and glucose 11; the pH was kept at 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. The glands were cleaned of the surrounding fat and several punctures made with a needle to facilitate the efflux of perfusate. All secretion experiments were carried out at room temperature.

Catecholamine detection was carried out by following the technique described by Borges, et al., (18) and adapted for the rat. Briefly, glands were placed into individual hermetic plastic chambers, and the emanating perfusate was passed through an electrochemical detector Bio Analytical System LC4B (West Lafayete, IN, USA.). An oxidation potential of +650 mV was maintained between a glassy carbon working electrode and a Ag/AgCl reference electrode. The oxidation current was continuously recorded on a ABB SE110 chart recorder. Adrenaline and noradrenaline standards were passed through the detector cell by the end of the experiments in order to calibrate the oxidation currents caused by the catecholamines secreted. Under these conditions the glands could maintain secretory responses for over 10 hours. No damaged or oedematous cells were found in histological analysis. HPLC separation of the perfusate showed that at least 95% of the total oxidation currents came from catecholamines. (19, 18).

Electronically-driven three-way valves (General Valve Co. IL. USA) were placed closely to the entry of the peristaltic pump in order to apply the drug with precision and to reduce the dead space.

Two silver electrodes placed in the chambers allowed the application of electrical field stimulation to the gland. Square pulses of 10 Hz, 1 ms, for 5 s at supramaximal voltage (40-50 V) were applied. This kind of stimulation caused the secretion of catecholamines only as a response to the acetylcholine released from splanchnic nerves (20, 21).

In experiments, where electrochemical detection could not be used, catecholamines were measured by a fluorimetric trihydroxyindole method of Anton and Sayre, (22) without the intermediate purification on alumina. In order to analyze the secretion of the three catecholamines: adrenaline, noradrenaline and dopamine, samples of the perfusate were collected for its analysis by HPLC-ED (18).

Histological analysis. Adrenal glands were perfused with Krebs solution for 10 min, then fixed by perfusion with 5% formalin phosphate buffer. Mast cell staining with toluidine was carried out following the technique described by Wolman (23).

Statistical analysis. Data were expresed as a representative traces or as the Mean ± SEM. Calculations were performed using a Student's *t*-test.

Chemicals. Ranitidine was a kind gift of Dr. C. Sunkel, Lab. Alter S.A., Madrid, Spain. All other drugs were purchased from Sigma (USA). All salts used in the preparation of buffers were reagent grade.

Results

The effect of histamine on arterial blood pressure. The aim of these initial experiments were: (i) to test the role of the adrenal medulla in the cardiovascular response to histamine and (ii) to compare the estimated concentrations of histamine causing a decrease in arterial pressure with those causing the "in vitro" catecholamine release. Figure 1a shows typical traces obtained by the i.v. injection of histamine 1, 10 and 100 µg/kg body weight (100 µl bolus). Histamine caused a rapid decrease in the arterial blood pressure followed by a quick recovery. When histamine was administrated in adrenalectomized animals, the amplitude of the blood pressure fall was similar to the control group, but the time lasted longer (figure 1b). The effect of histamine on arterial blood pressure was largely prolonged by adrenal removal.

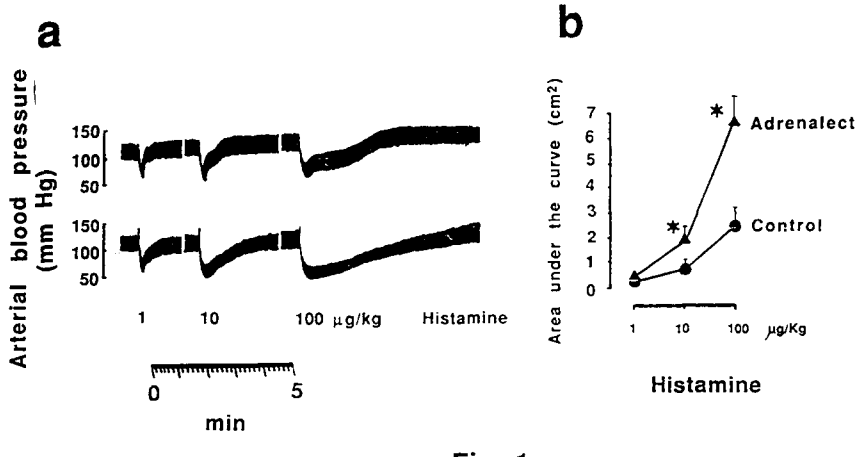


Fig 1.

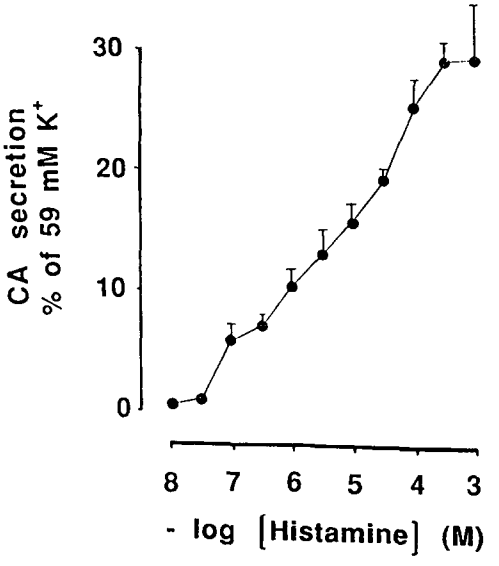
Effect of adrenalectomy on blood pressure responses to histamine. Histamine 1-100 µg/kg were injected iv. **a)** Upper panel shows hypotensive responses in a typical control rat. Lower panel shows responses in an adrenalectomized animal. Large marks in time calibration show minutes. **b)** Shows the planimetric measurement of the area under the mean blood pressure record as it fall below the baseline. The means ± S.E.M. are from 4 different animals. Circles represent the control group, triangles represent adrenalectomized animals. *P< 0.05 compared to control.

Histamine-evoked secretion. Histamine is a potent agent eliciting the catecholamine release from rat adrenals. Increasing concentrations of histamine were applied in 15 seconds pulses every 8 minutes. The results were normalized by the comparison with an initial pulse of an isotonic solution containing 59 mM of K^+ (figure 2). The estimated EC_{50} was around 3 μM. Maximal secretion was obtained at 300 μM, higher concentrations of the drug caused erratic responses probably due to toxic effects

Histamine releases mainly adrenaline. In order to test if histamine can preferentially release any catecholamine, increasing concentrations of histamine were applied to perfused glands. The amount of noradrenaline, expressed as a percentage of the total catecholamine secreted, was compared with the secretory response obtained by the infusion of high potassium depolarizing solutions. Table I shows the amount of adrenaline and noradrenaline in the perfusate, analyzed by HPLC,

Fig 2.

Catecholamine secretion from perfused rat adrenals in response to histamine. Each gland was first stimulated for 15 s with a Krebs solution containig 59 mM of K^+ and 15 min later with increasing concentrations of histamine for 15 s at 8 min intervals. The histamine-evoked secretion data were normalized to the responses obtained in the same gland with high potassium solution. Means ± S.E.M. are from 4-6 different glands.



when they were stimulated for 1 min with 1, 10 and 100 μM histamine and 59 mM of high K^+ solution. Histamine caused the preferential release of adrenaline and had little effect on the noradrenaline output. However high K^+ stimulation, which depolarized both populations of chromaffin cells, elicited the secretion of both catecholamines in similar proportions to the total catecholamine content. Traces of dopamine (<1%) were also detected.

Histamine exerts its action via H_1 subtype receptor. If histamine (30 μM) was applied in short pulses of 15 s every 8 min, the extent of secretory responses became very reproducible lasting for 2-3 h (Fig 3a

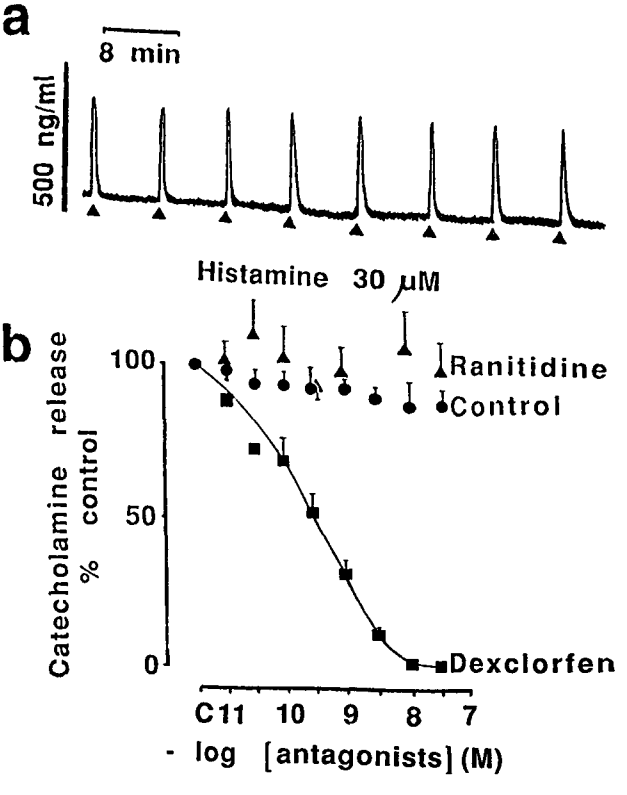
TABLE I.

Agent	* Noradrenaline	* Adrenaline	#% NA
None	0.9± 0.1	9.1±1.8	9
Histamine 1 μM	1.1± 0.7	17.2± 3.1	6
Histamine 10 μM	2.3± 0.9	29.5± 7.3	7
Histamine 100 μM	4.7± 0.9	49.7± 9.0	9
K^+ 59 mM	53.6± 8.3	89.0± 8.3	38

Adrenaline and noradrenaline secretion (ng/ml) evoked by histamine and high K^+ depolarizing solutions. Glands were stimulated with histamine 1, 10, 100 μM and K^+ 59 mM for 1 min and samples of the perfusate analized by HPLC-ED. Secretagogues were administrated at 30 min intervals. (*) In ng/ml. Means ± S.E.M. from 4-7 different glands. (#) %NA, percentage of noradrenaline from the total catecholamines secreted.

Fig 3.

Secretory responses to short pulses of histamine in perfused rat adrenals. Histamine 30 μM was applied in short pulses of 15 s every 8 min and the secretory response continuously monitored. **a)** Traces of the oxidation peaks. **b)** The extent of the height of the oxidation peaks was measured in the absence (circles) or in the presence of cumulative concentrations of histamine antagonists H_1 dexclorfeniramine (squares) or H_2 ranitidine (triangles). Values were normalized as a percentage of the mean of the initial five histamine stimulation pulses (C) prior to the antagonists administration. (Means ± S.E.M. of 4-6 different glands). * $P < 0.05$ to control group



and b). In order to determine the histamine receptor subtype involved in triggering the secretion, dexclorfeniramine (an H_1 receptor antagonist) or ranitidine (an H_2 receptor blocker) was applied 5 min before each pulse of histamine and increased cumulatively from 10^{-11} to $3x10^{-8}$ M. Dexclorfeniramine potently inhibited catecholamine secretion with an IC_{50} close

to $3x10^{-10}$ M. Secretion was unaffected however by the H_2 receptor antagonist ranitidine. These data suggest strongly that the histaminergic receptor associated with secretion is of the H_1 subtype (Figure 3b).

Histamine is not released from splanchnic nerves nor from local mast cells. In order to test if splanchnic nerves secrete histamine, histamine receptor blockers were applied to the perfused glands. Dexclorfeniramine and ranitidine (1μM) did not modify the release of catecholamines evoked by splanchnic nerve stimulation (data not shown).

The compound 48/80, which promotes mast cell degranulation and thus histamine release, did not cause any change in the basal catecholamine output from the adrenal medulla when applied at concentrations of 1 and 10 μg/ml. The catecholamine assay for these experiments were carried out by a fluorimetric method because 48/80 covered the surface of the working electrode of the electrochemical detector causing electrical noise (data not shown). These data suggest that either mast cells are not present, or that histamine secreted from them is insufficient to stimulate chromaffin cells and promote catecholamine release.

No mast cells were found, in histological analysis of the adrenal medulla. In serial slices from 12 different glands. Neither the haematoxilin-eosin nor the toluidine blue method showed evidence of the presence of any mast cells.

Discussion

The origin of histamine in the adrenal gland. There are three possible sources of histamine in the adrenal medulla: (**i**) Histaminergic fibres in the splanchnic nerves; (**ii**) Local tissue mast cells and (**iii**) Blood-carried histamine. Our results support the idea that histamine reaches chromaffin cells from the systemic circulation.

Histaminergic fibres have not been found in rat splanchnic nerves (7) and histamine antagonist drugs do not inhibit the splanchnic nerve-mediated catecholamine release.

Although histamine-containing cells have been described within the rat adrenal gland, they are mainly located in the cortex; as the blood circulations of the cortex and medulla are independent (24), it is unlikely that these cells in the cortex can elicit the release of catecholamines from the medulla. Conversely Endo and Ogura (10) found negligible amounts of histamine in the medulla (only 2 nmoles/g tissue). Our studies support this observation as we did not find mast cells in histological rat adrenal medulla preparations. Furthermore, the compound 48/80, which promotes mast cell degranulation, failed to cause any increase in catecholamine output. On the other hand, Happola et al (8) reported the presence of his-

tamine inmunofluorescence associated with some of the noradrenaline-containing cells. Because the presence of histidine decarboxilase within the adrenal medulla has not been established, these authors suggested that histamine could be taken from the blood stream. However histamine uptake by chromaffin granules is very small (29). The real amount of histamine in these cells and its possible role regulating the adrenal medullae function remains to be established.

The concentrations of histamine needed to cause a decrease in the rat arterial blood pressure, are of the same order of magnitude to those which cause catecholamine release from in vitro perfused rat adrenals. The rat's blood volume is approximately 62 ml/kg and has a haematocrit value near 50 (25), the final concentration of histamine (MW= 111.1), after a bolus injection of 10 μg/kg, for a rat weighing 250 g, will be around 3 μM, which is the EC_{50} obtained from in vitro studies. It is therefore, plausible that the adrenal medulla secretes adrenaline in response to histamine originating in the systemic circulation (compare figures 1 and 2).

The preferential release of adrenaline. Histamine induced a preferential secretion of adrenaline in the rat adrenal medulla. This is not the case in other species like cow (5) although Pender and Burgoyne (26) have reported recently that only an undefined sub-type of bovine adrenal chromaffin cells secreted in response to histamine. Similar results can be observed with other secretagogues like muscarinic agonists in cats (27, 16). It is possible that a common transduction mechanism coupled to either cholinergic or histaminergic receptors is present in adrenaline-storing chromaffin cells. Adrenaline could contribute to the mitigation of some undesirable actions of histamine because it is not noradrenaline but adrenaline that physiologically antagonizes histamine. This is the first report describing the preferential release of adrenaline by histamine.

Histamine receptor subtypes in the adrenal medulla. Histamine triggers the rat adrenal medulla secretory response through H_1 receptors. H_1 receptors also promote the secretion in the cow (13) or the cat (3). This is the first report describing an H_1 receptor subtype in the rat. H_1 subtype is known to be coupled to a phosphoinositoside breakdown, IP_3 release promoting the release of Ca^{2+} from intracellular stores (28). Very recently there has been reported a role of the H_2 receptor on bovine chromaffin cells increasing the levels of cAMP (15).

In conclusion, the data presented in this paper suggest first, that is the histamine which activates the secretion of catecholamines in adrenal medullary chromaffin cells comes from the systemic circulation; second, that histamine elicited the preferential release of adrenaline; and third, that the histamine receptor in the rat adrenal medulla, that mediates such a response, is of the H_1 subtype.

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