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Ionic mechanisms involved in the secretory effects of histamine in the rat adrenal medulla

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Histamine activation of H_1 receptors elicited the release of adrenaline from in vitro perfused rat adrenal with an EC_{50} of 3 μM. Neither Na^+ deprivation nor complete membrane depolarization abolished the histamine-mediated secretory response but it was partially dependent on extracellular Ca^{2+}. Nitrendipine and BAY-K-8644 affected the release induced by histamine concentrations at over 3 μM. Delayed application of histamine pulses, after external Ca^{2+} removal, led to a decline in to a plateau at 50% of the initial release. Pretreatment with ionomycin abolished this Ca^{2+} deprivation-resistant component. These data suggest that secretion evoked by low concentrations of histamine occurs by mobilization of Ca^{2+} from internal stores whereas higher concentrations use Ca^{2+} from both intracellular and extracellular sources.

Adrenal medulla; Chromaffin cell; Histamine; Catecholamine release; Ca^{2+} channels; Ca^{2+} (intracellular)

1. Introduction

Histamine induces catecholamine release from the adrenomedullary chromaffin cells of many species including dog (Wada et al., 1940), cat (Robinson, 1982), rat (Yoshizaki, 1973) and cow (Livett and Marley, 1986). Histamine H_1 subtype receptors have been described in the cow (Wan et al., 1989) and the cat (Robinson, 1982), and are associated with catecholamine secretion and the accumulation of inositol phosphates (Noble et al., 1988; Plevin and Boarder, 1988; Stauderman and Pruss, 1990). A functional role of an H_2 receptor in bovine chromaffin cells has been recently described (Marley et al., 1991) and is associated with the accumulation of cellular cAMP. Recently, Pender and Burgoyne (1992) have reported that only an undefined subtype of bovine adrenal chromaffin cell secreted catecholamines in response to histamine.

Histamine increases intracellular free Ca^{2+} levels following two different patterns, an initial component independent of extracellular Ca^{2+}, followed by a steady state that disappears when the cation is removed from the extracellular fluid. However, conflicting reports concerning the source of the Ca^{2+} needed to trigger the secretory response to histamine stimulation have been published (Stauderman et al., 1990; Ito et al., 1991; Bunn and Boyd, 1992).

This study was performed to determine: (i) the ion requirements for sustained catecholamine secretion and (ii) the source of Ca^{2+} that triggers histamine-evoked catecholamine secretion in the rat.

2. Materials and methods

Female Sprague-Dawley rats, weighing 200–300 g, were anaesthetized with sodium pentobarbitone 50 mg/kg i.p.

2.1. Catecholamine release studies

The left adrenal gland was exposed and the adrenolumbar vein was cannulated using a PE10 (Portex 800-100-100) tube. The glands were then removed and perfused retrogradely in vitro at 1 ml/min, using 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 were made with a needle to facilitate the efflux of perfusate. All secretion experiments were carried out at room temperature.

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Catecholamine detection was carried out as described by Borges et al. (1986) but 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 recorded continuously on a ABB SE110 chart recorder. Adrenaline and noradrenaline standards were passed through the detector cell at 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 h. No damaged or oedematous cells were found on histological analysis. HPLC separation of the perfusate showed that at least 95% of the total oxidation currents were caused by catecholamines (Herrera et al., 1985; Borges et al., 1986).

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

High potassium solutions were prepared by isosmotically reducing the amount of NaCl in the Krebs solution. When solutions containing low Ca^{2+} were used, $CaCl_2$ was replaced isosmolarly by $MgCl_2$ in order to keep the divalent cation concentration constant.

2.2. *Statistical analysis*

Data are expressed as representative traces or as the means ± S.E.M. Statistical analyses were performed with a Student's t-test.

2.3. *Chemicals*

Nitrendipine and BAY-K-8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) were gifts from Professor A.G. García, Universidad Autónoma de Madrid, Spain. All other drugs were purchased from Sigma (USA). All salts used in the preparation of buffers were reagent grade.

3. Results

3.1. *The release of catecholamines evoked by histamine in the adrenal medulla in vitro*

In order to compare the time course of secretion evoked by histamine with that induced by membrane depolarization, glands were perfused for 5 min with histamine (1, 10 and 100 μM) and catecholamine secretion monitored continuously. Histamine induced an increase in catecholamine output. The time course of secretion exhibited a rapid rise followed by a decrease to a steady state (fig. 1a). This secretory profile differs from that evoked by high K^+ depolarizing solutions (fig. 1b).

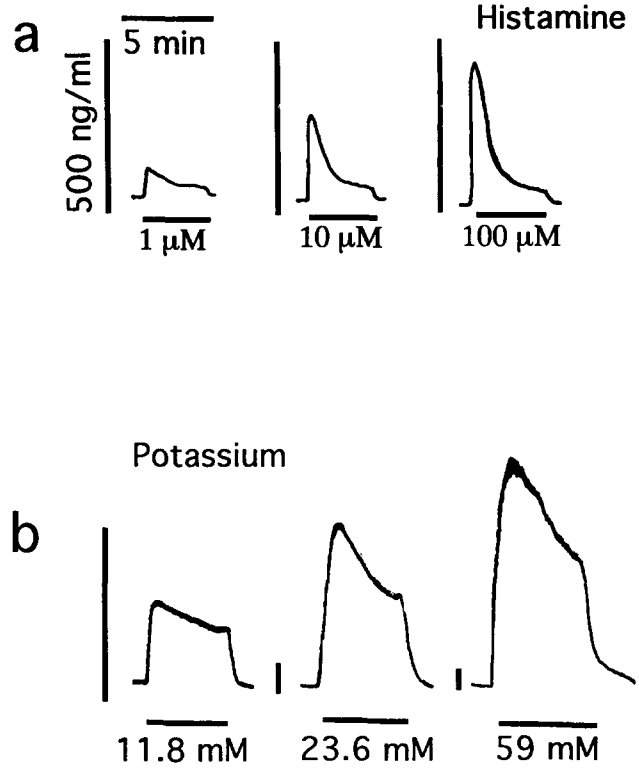

Fig. 1. Time course of catecholamine secretion evoked by different agents. Histamine (a) and high K^+ containing solutions (b) were applied for 4 min as shown by the solid horizontal line and at the concentrations indicated. Solids vertical lines indicate the oxidation current corresponding to that elicit by 500 ng/ml of adrenaline. Notice the different size of these bars depending of the range of sensitivities used. Traces are representative of 10–15 typical experiments.

When histamine (30 μM) was applied in short pulses of 15 s every 8 min, only the initial component was observed. Under these conditions the amount of catecholamines secreted in each pulse became very reproducible lasting for 2–3 h.

3.2. *The effect of drugs that modulate calcium channels*

The following experiments were carried out to determine the role of L-type Ca^{2+} channels in histamine-mediated secretory responses. Increasing concentrations of histamine were applied in pulses of 15 s every 8 min in the absence or presence of 10^{-7} M BAY-K-8644, a Ca^{2+} channel activator in chromaffin cells (García et al., 1984; López et al., 1992) or nitrendipine, a potent Ca^{2+} channel blocker in chromaffin cells (Ceña et al., 1983). Higher concentrations of

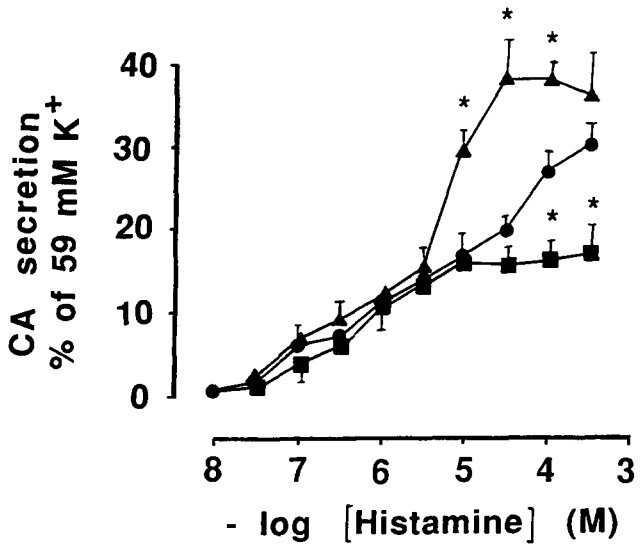

Fig. 2. Secretory responses evoked by increasing concentrations of histamine. The effect of calcium channel agonists and antagonists. Increasing concentrations of histamine were applied as 15-s pulses every 8 min in the absence (circles, control group) or in the presence of 10^{-7} M nitrendipine (squares) or BAY-K-8644 (triangles). The results were normalized by comparison with an initial pulse, without dihydropyridines, of an isotonic solution containing 59 mM of K^+. Experiments were carried out in the dark to avoid the photodegradation of dihydropyridines. Means ± S.E.M. from four to six different glands, *P < 0.05 to control group.

histamine were not tested because of the loss of specificity of the drug in the millimolar range. The dose-response curve did not exhibit saturation within the concentration range tested. The results were normalized with respect to the response obtained with an initial stimulation with high K^+ solution (59 mM). Histamine increased catecholamine release, in a concentration-dependent manner, with an EC_{50} of about 3 μM. The effects of dihydropyridine were seen at higher concentrations of histamine. Neither BAY-K-8644 nor nitrendipine affected the response to histamine at concentrations below 3 μM. However, BAY-K-8644 and nitrendipine increased and inhibited, respectively, the secretion evoked by concentrations of histamine of 10 μM or higher (fig. 2). These data indicate that Ca^{2+} entry through L-type voltage-dependent channels is only important for the secretion evoked by high histamine concentrations.

3.3. The effect of sodium deprivation

Na^+ plays a crucial role in nicotinic-mediated secretory responses (Ceña et al., 1983). The role of Na^+ and of the state of polarization of the membrane were tested in the experiments described in fig. 4. Neither the isotonic replacement of NaCl by sucrose nor the total suppression of Na^+ (bicarbonate replaced by HEPES) abolished the secretory response to histamine (see the amplitude of oxidation peaks over the initial and final basal levels). Na^+ removal caused an increase in the rate of secretion. Under these conditions, histamine could trigger the release of catecholamines (fig. 3a and b). Moreover, the complete replacement of external Na^+ by K^+, which depolarizes the cell membrane, did not reduce the release of catecholamines evoked by histamine. In fact, the secretory signal was increased (fig. 3c). These data indicate that Na^+ is not essential for secretion elicited by histamine, and that the secretory action of histamine can also occur under conditions of complete depolarization of chromaffin cells.

3.4. The effect of calcium deprivation

Figure 4a shows the effect of different levels of Ca^{2+} in the Krebs solution on the response to repeated stimulation with histamine. These experiments were carried out to determine the sensitivity to external Ca^{2+}. Ca^{2+} removal reduced but did not abolish

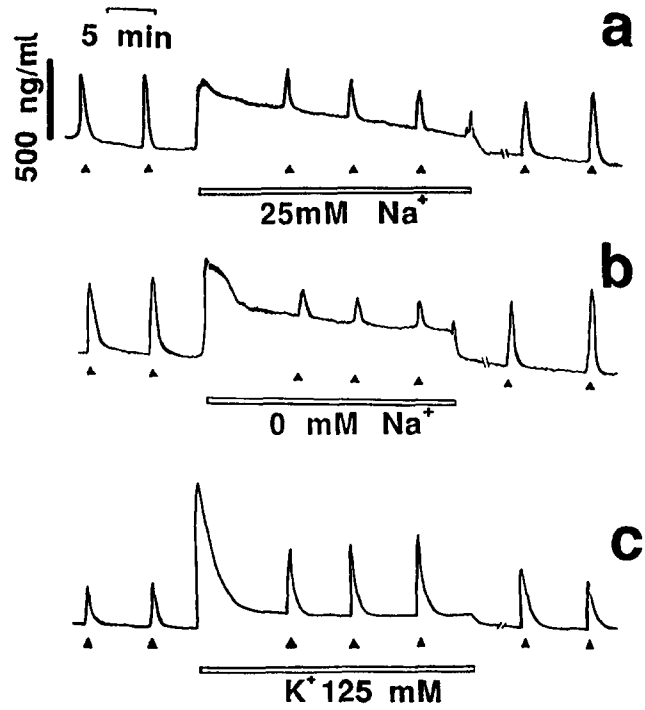

Fig. 3. Effect of sodium deprivation and full depolarization on the secretory response of histamine. Glands were perfused with 30 μM histamine for 15 s every 8 min. The histamine effect was tested under the following conditions: (a) NaCl was replaced by an equiosmolar amount of sucrose. (b) NaCl and sodium bicarbonate were replaced by an equiosmolar amount of sucrose and the solution buffered with HEPES and (c) NaCl was replaced by KCl. Histamine application is shown by the filled triangles. Horizontal open lines indicate the period of Na^+ deprivation. Traces show typical experiments from three to four rats.

the secretory response. Catecholamine release could only be suppressed if Ca^{2+} removal was accompanied by addition of EGTA (1 mM). Ca^{2+} reintroduction caused a gradual restoration of secretion. Thus in nominal Ca^{2+}-free solution a small response was already appreciable (14% of the initial control responses). At 0.25 mM Ca^{2+}, histamine-evoked secretion was fully reestablished and responses over control values were often observed at Ca^{2+} concentrations over 1 mM. These data indicate that even if extracellular Ca^{2+} is essential for histamine-evoked catecholamine release, this requirement is far less critical than for other secretagogues such as depolarizing solutions. figure 4b compares the secretion evoked by histamine with that caused by K^+-enriched solution (23 mM). High K^+ solution did not cause appreciable catecholamine secretion when the external Ca^{2+} concentration was below 1 mM, whereas histamine caused catecholamine release similar to the initial control release when external Ca^{2+} was 0.25 mM.

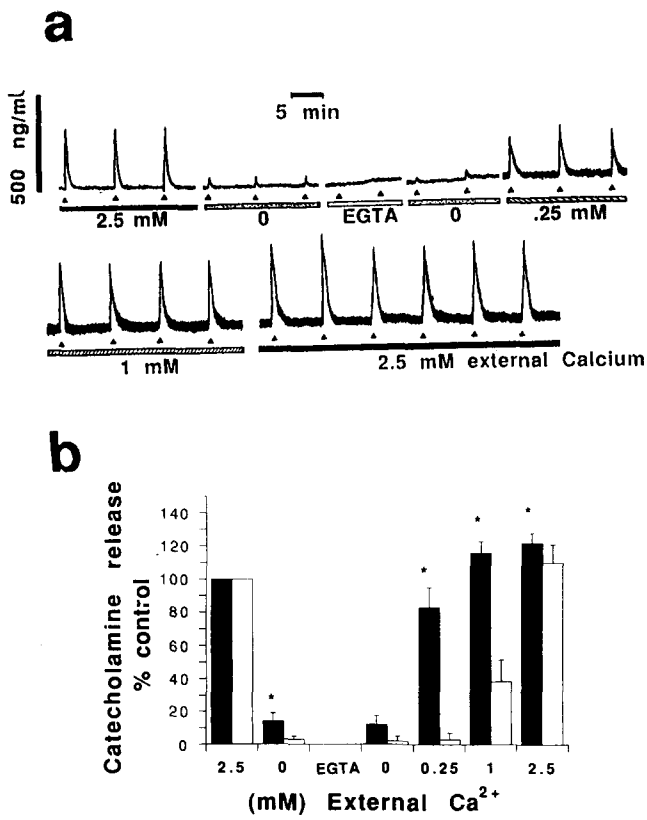

Fig. 4. Effect of calcium deprivation. Panel a shows typical traces from a typical experiment from 6. Histamine (30 μM) was applied for 15 s every 8 min as indicated by filled triangles. The Ca^{2+} content in the perfusion buffer was reduced as indicated and replaced by equiosmolar $MgCl_2$. EGTA (1 mM) was present only for the time indicated by the open horizontal bar. Panel b summarizes the effect of Ca^{2+} deprivation on the secretion evoked by histamine (30 μM) (filled columns) and the equipotent secretory solutions of K^+ 23 mM (open columns); abscissa represents external Ca^{2+} concentrations (in mM). Values were normalized as a percentage of the mean of the initial five histamine, or K^+ stimulation pulses at 2.5 mM Ca^{2+} prior to the reduction of Ca^{2+}. Data show the means ± S.E.M. from three to six different experiments. *P < 0.05 between histamine and K^+ groups.

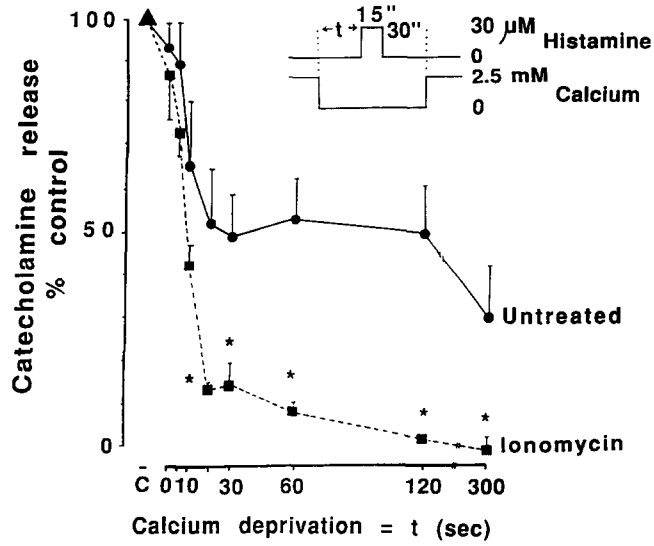

Fig. 5. Effect of the duration of wash-out of extracellular calcium on histamine secretory response. Histamine (30 μM) was applied for 15 s every 8 min, and the height of the secretory response peak was normalized as 100% (triangle). Histamine stimulation was then applied in the absence of Ca^{2+}; Ca^{2+} removal was maintained for 30 s after histamine stimuli. The duration Ca^{2+} withdrawal, prior to histamine stimulation (t), was then increased from 5 to 300 s as indicated on the abscissae (circles). Other groups of glands were pretreated with the calcium ionophore ionomycin (10 μM for 10 min at 37°C) and the same protocol was repeated (squares). Data show the means ± S.E.M. from three to four different experiments. *P < 0.05 to control group.

3.5. *The intracellular Ca^{2+} sources*

The experiments shown in fig. 5 were aimed at identifying the role of intracellular, versus extracellular Ca^{2+}, sources in the secretory response to histamine. Ca^{2+} was removed during stimulation with histamine (15 s every 8 min) and glands were perfused with Krebs solution lacking Ca^{2+} for 30 s. EGTA (100 μM) was always present during the Ca^{2+} deprivation period to prevent Ca^{2+} contamination. This stimulus was repeated until the responses became stable, and the amount of catecholamine released was considered as 100% (C). Then, the duration of Ca^{2+} deprivation prior to histamine stimulation (t) was increased from 5 to 300 s. The amplitude of the secretory peaks, decreased to 50% of their initial control values, reached a plateau after 30 s of Ca^{2+}deprivation. In glands that have been pretreated with 10 μM ionomycin (a Ca^{2+} ionophore which depletes intracellular stores), this effect was largely enhanced. These data strongly suggest a role for intracellular Ca^{2+} in the maintenance of the secretion evoked by histamine.

4. Discussion

4.1. The ionic requirements for the secretion evoked by histamine

Extracellular Ca^{2+} levels are less important for the actions of histamine than for those stimuli mediated by direct membrane depolarization, i.e. nicotinic agents or high K^+ (Douglas and Rubin, 1963). Three observations support this: (i) The data from fig. 2 show that the effects of the dihydropyridine BAY-K-8644 and nitrendipine were apparent only when high concentrations of histamine were used. The dose-response curves exhibited a different pattern from those obtained with high K^+ solutions or nicotinic agonists, where the effects of dihydropyridines are observed at low concentrations of these secretagogues (García et al., 1984; Ladona et al., 1987). These data point towards the existence of two different sources of Ca^{2+}, one source being the intracellular stores, which are activated by low concentrations of histamine, and the other being the extracellular medium used when large concentrations of the drug are applied and Ca^{2+} channels are open. (ii) When the extracellular Ca^{2+} levels were reduced, the responses to histamine were far less affected than the responses evoked by K^+-mediated depolarization (fig. 4b). (iii) When the duration of Ca^{2+} deprivation was progressively increased from 20 s to up to 2 min, the secretion of catecholamines was reduced to a steady state of 50% The observation that pretreatment of glands with ionomycin, in the absence of Ca^{2+}, which is known to deplete intracellular Ca^{2+} stores, abolished this secretory steady state (fig. 5) is consistent with the idea that histamine can mobilize an intracellular Ca^{2+} source. Recently Goh and Kurosawa (1991) reported similar data for bovine chromaffin cells.

The dose-response curve for histamine (fig. 2) did not exhibit saturation over the range of concentrations tested. Saturation usually occurs when higher concentrations of drug are used, but I did not test higher concentrations of histamine because the drug is less specific in the millimolar range and because the discriminating effects of dihydropyridines were observed at low histamine concentrations.

Histamine elicits the secretion of catecholamines by the rat adrenal medulla even in the absence of Na^+ and under conditions of complete depolarization (fig. 3). The Na^+ independence suggests that histamine stimulation does not require Na^+ channels to amplify these responses. Moreover, if histamine can promote catecholamine secretion even in the presence of K^+-mediated full depolarization, it means that Ca^{2+} channel-mediated Ca^{2+} entry is not activated by changes in voltage, but through a chemical signal generated after H_1 receptor activation.

In conclusion, the data presented in this paper suggest first that histamine-mediated responses are neither dependent on Na^+ nor on membrane potential, and second that histamine appears to promote a rise in the intracellular free Ca^{2+} concentration from at least two different sources, one by means of influx from the external medium and another through the mobilization of intracellular Ca^{2+} pools.

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