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H_1-HISTAMINERGIC ACTIVATION OF CATECHOLAMINE RELEASE BY CHROMAFFIN CELLS

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Abstract—Bovine adrenal medullary chromaffin cells, prelabeled with [^{3}H]norepinephrine, released a large proportion of cellular ^{3}H-labeled catecholamines (CAs) when stimulated with nicotine, K^+, histamine, γ-aminobutyric acid (GABA) and several peptidic hormones [bradykinin, angiotensin II, thyrotropin releasing hormone (TRH) and neurotensin]. The histamine-induced response was dose dependent and occurred through H_1 histaminergic receptors. Quantitatively and temporally the histamine- and nicotine-induced responses differed. Nicotine, during the first minutes, induced a large increase of [^{3}H]CAs, but this response was desensitized rapidly. In contrast, histamine initially provoked a smaller release of [^{3}H]CAs than nicotine but, with prolonged exposure (hours), a much greater response was found with histamine. Moreover, little desensitization was observed with histamine even during extended stimulation. External Ca^{2+} was obligatory for the histamine response, and both inorganic (Co^{2+} and Ni^{2+}) and organic (verapamil, nifedipine and D-600) Ca^{2+} channel blockers significantly reduced release of [^{3}H]CAs. These studies suggest that histamine as well as certain other neuroactive substances could play an important role in the physiology and biochemistry of adrenal medullary chromaffin cells.

The availability of primary dissociated monolayer cultures of adrenal medullary chromaffin cells has permitted extensive studies on the biochemistry and physiology of this model neural cell system. Since chromaffin cells contain cholinergic–nicotinic receptors which, when activated, induce the release of neuractive substances such as catecholamines and opioid peptides, particular attention has been devoted to understanding the role played by the cholinergic system in the exocytotic process (for review see Ref. 1). However, there is a paucity of information on how activation of other secretagogues are involved in this process.

Considerable evidence is now available which indicates that histamine functions as a neurotransmitter in both the central and peripheral nervous system (for review see Ref. 2). As early as 1926, Burn and Dale [3] found that histamine stimulates the adrenal medulla of cats to liberate catecholamines. Later, similar observations were made on other animal species [4–7]. More recently, Noble *et al.* [8], using bovine adrenal medullary chromaffin (BAMC§) cells maintained in culture, have shown the presence of H_1 histaminergic receptors which, when activated, stimulate phosphotidylinositide hydrolysis, a process known to liberate internally sequestered Ca^{2+} [9, 10].

In this study, we report that histamine as well as other neuroactive substances stimulated the release of catecholamines from BAMC cells. Histamine stimulation was found to be dose dependent and occurred through activation of H_1 histaminergic receptors. In contrast to cholinergic–nicotinic activation, histamine initially induced a small release of catecholamines, but with prolonged exposure it produced a much greater response than nicotine and, in addition, the H_1 receptor showed little desensitization over time. Moreover, this histamine-induced release was found to be Ca^{2+} dependent and was attenuated by Ca^{2+} channel blockers.

MATERIALS AND METHODS

Materials. Dulbecco's Modified Eagle's Medium (DMEM), phosphate-buffered saline (PBS) pH 7.4, F12, fetal calf serum, penicillin G, kanamycin, amphotericin, fluorodeoxyuridine and arabinosylcytosine were from GIBCO Laboratories (Karlsruhe, FRG). Collagenase was from Worthington. The rest of the chemicals were purchased either from Sigma (Taufkirchen, FRG) or Merck (Darmstadt, FRG). DL-[7-^{3}H(N)]Norepinephrine (11.7 Ci/mmol, 99.7% pure) and $^{45}CaCl_2$ (21.88 mCi/mg Ca^{2+}) were from New England Nuclear.

Cell culture. Chromaffin cells were isolated from fresh bovine adrenal glands as described by Wilson and Kirshner [11]. To reduce the number of non-chromaffin cells, the differential plating method of Waymire *et al.* [12] was used. Adrenal medullary cells were suspended in a solution containing 45%

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§ Abbreviations: BAMC, bovine adrenal medullary chromaffin; CA, catecholamine; PBS, phosphate-buffered saline; SP, Substance P; GABA, γ-aminobutyric acid; TRH, thyrotropin releasing hormone; and F12, Ham's F12 tissue culture medium.

DMEM, 45% F12 and 10% fetal calf serum to which were added penicillin G (100 units/ml), kanamycin (50 μg/ml), amphotericin (5 μg/ml) fluorodeoxyuridine (20 μmol/ml) and arabinosylcytosine (10 μmol/ml). The cell suspension was placed in a 75 mm^2 Falcon flask for 2–3 hr at 37° to allow attachment of non-chromaffin cells. The supernatant fraction was then decanted and plated at a concentration of 4×10^5 cells/ml of the above medium in Costar 24 multiwell dishes pretreated with collagen (50 μg/ml). The cultures were maintained at 37° in a humidified atmosphere of 7.5% CO_2 in air. Within 6–12 hr after plating the cells became firmly attached and, by 48 hr, they had lost their rounded appearance and process outgrowth and cell-to-cell contact had become evident. Medium was changed twice a week, and the cells were used after a minimum of 4 days and a maximum of 10 days in culture.

For cultivation of non-chromaffin cells, the adhered cells in the above preplating procedure were washed with 45% DMEM, 45% F12 and 10% fetal calf serum containing the three antibiotics indicated above, but no antimitotics were added. The flask was then placed in the CO_2 incubator and the cells remained undisturbed in this medium for 5 days during which time they achieved confluency [13]. The attached cells were then passaged by trypsinization (0.25% trypsin), seeded 4.5×10^5 cells/ml in Costar multiwell dishes, placed in the CO_2 incubator, and used when they achieved confluency. Examination by light microscopy revealed the non-chromaffin cells to consist primarily of endothelial cells with few, if any, chromaffin cells [13].

Catecholamine secretion studies. Experiments were initiated by discarding the old medium from each well and replacing it with 1 ml of fresh medium to which was added 1 mM ascorbic acid and 1 μCi [^{3}H]norepinephrine. After a 6-hr loading period, the cultures were washed twice with PBS, and any unbound [^{3}H]norepinephrine and metabolites were then washed out by incubating the cells in DMEM for a subsequent 2 hr in the CO_2 incubator. The DMEM medium was removed, and the cells were washed once with PBS and adapted to this medium for 15 min at 25°. Secretion was initiated by removing the preincubation buffer and replacing it with PBS to which various substances were added.

At various time periods, aliquots were removed from the medium and centrifuged for 2 min at 10,000 *g* to remove any unattached cells, and the supernatant fractions were added to EP Ready Solve for ^{3}H determination in a Beckman scintillation counter. Remaining cellular ^{3}H was determined by discarding the rest of the medium and solubilizing the cells with a 1 N NaOH for 2 hr at 37°. An aliquot of this solution was used to determine cellular protein content by the method of Lowry *et al.* [14]; and the remaining solution was neutralized with 1 N HCl and ^{3}H activity was measured.

$^{45}Ca^{2+}$ uptake studies. Before initiating $^{45}Ca^{2+}$ uptake experiments, the culture medium was removed from each well and the cells were washed three times with 0.5 ml of standard buffer [15] and preincubated for 10 min in standard buffer at 25°. The preincubation buffer was discarded and replaced with 0.5 ml of standard buffer containing 1 μCi $^{45}CaCl_2$ and various secretagogues, and the mixtures were incubated for 5 min. The radioactive medium was removed and the cultures were washed rapidly five times (<1 min) with 0.75 ml of standard buffer and incubated for 1 hr with 0.25 ml of 1% Triton X-100 and 1 mM EDTA. The entire 0.25 ml of Triton–EDTA solution was added to a scintillation vial, the wells were washed with 0.25 ml of the same solution, and the wash was added to the same vial [15]. Radioactivity in the cells was measured by liquid scintillation spectrometry.

HPLC analysis. ^{3}H-Labeled catecholamines ([^{3}H]CAs) released into the medium were separated using ion-pair HPLC as described [16]. The elution buffer consisting of 64% 0.02 M K_2HPO_4 containing 1 g/liter heptanesulfonic acid and 36% of methanol–water (3:2) was passed through two 5 μm C18 columns (Altex ultrasphere ODS, 4.6 mm i.d.) connected in line at a flow rate of 0.8 ml/min, and 0.2-ml fractions were collected. Authentic [^{3}H]CAs were used for standardization and identification.

Data analysis. All experiments were conducted using triplicate samples for each condition, repeated on at least two different culture preparations. Data are expressed as the mean ± SEM, and significance was determined by Student's *t*-test. When SEM was less than 5%, data were represented as single point values.

RESULTS

HPLC analyses showed that at least 80% of the basal released ^{3}H was norepinephrine, whereas with histamine (10^{-5} M) stimulation norepinephrine represented more than 88% of the ^{3}H released into the medium. Since [^{3}H]norepinephrine and [^{3}H]epinephrine constitute practically the bulk of ^{3}H found in the medium, data are expressed on the basis of [^{3}H]CAs released. Table 1 shows that a variety of substances induced substantial releases of [^{3}H]CAs by chromaffin cells. Six- to seven-fold increases over basal release (control) were found 30 min after chromaffin cells were exposed to nicotine, K^+ and histamine. Bradykinin, GABA, TRH, angiotensin II and neurotensin enhanced [^{3}H]CA release 2- to 3-fold, whereas no significant increases were observed with several other neuroactive agents including norepinephrine. When the most active stimulators of [^{3}H]CAs in chromaffin cells were tested with endothelial cell preparations (Table 2), no significant increases were found with nicotine (10^{-4} M), acetylcholine (10^{-4} M), histamine (10^{-5} M), bradykinin (10^{-6} M) and angiotensin II (10^{-6} M) compared to controls over a 30-min period. GABA, K^+ and neurotensin showed small (17–31%), but significant, increases over controls. Moreover, basal release of [^{3}H]CAs over 30 min showed large differences between chromaffin and endothelial cells, with the former releasing only about 2%, whereas the latter released approximately 30% of their cellular [^{3}H] content.

To determine whether histamine-induced [^{3}H]CA release is mediated through H_1 or H_2 histaminergic receptors, various antagonists of these receptors were used. As Fig. 1 shows, the two H_2 receptor antagonists cimetidine and ranitidine did not affect histamine-induced [^{3}H]CA release, whereas cle-

Table 1. Secretagogue-induced ^{3}H-labeled catecholamine release by chromaffin cells

Additions	[^{3}H]CA release (% of control)	Additions	[^{3}H]CA release (% of control)
None (control)	100 ± 10*	Vasopressin (10^{-6} M)	113 ± 2
Norepinephrine (10^{-5} M)	117 ± 10	Angiotensin II (10^{-6} M)	278 ± 23†
GABA (10^{-5} M)	278 ± 28†	Bombesin (10^{-6} M)	113 ± 3
Glutamate (10^{-4} M)	145 ± 20	Atrial naturitic	
Nicotine (10^{-4} M)	617 ± 44†	Factor (10^{-6} M)	112 ± 8
Muscarine (10^{-4} M)	121 ± 27	Substance P (10^{-5} M)	114 ± 8
Histamine (10^{-5} M)	707 ± 55†	TRH (10^{-6} M)	173 ± 8†
K^+ (50 mM)	689 ± 87†	ACTH (10^{-6} M)	121 ± 3
Bradykinin (10^{-6} M)	278 ± 5†	Neurotensin (10^{-6} M)	340 ± 11†

* Represents 1.91 ± 0.20% of total cellular [^{3}H]CA released in 30 min under basal conditions. This value is the average ± SEM of triplicate wells from nineteen different preparations. The rest of the values are results from triplicate wells obtained from at least two different cell preparations.
† $P < 0.001$ compared to control.

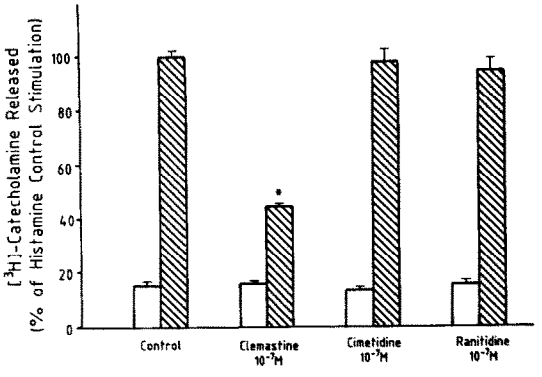

Fig. 1. Histamine receptor antagonists and histamine-induced [^{3}H]CA release. BAMC cells were prelabeled with [^{3}H]norepinephrine and exposed in PBS for 30 min to histamine (10^{-5} M) and various histamine receptor antagonists. [^{3}H]CAs released into the medium were measured as described under Materials and Methods. Open and crossed bars are responses in the absence and presence of histamine respectively. Values represent means ± SEM for three determinations, as percent of histamine (10^{-5} M) control stimulation. Key: (*) $P < 0.001$.

Table 2. Secretagogue-induced ^{3}H-labeled catecholamine release by endothelial cells

Additions	[^{3}H]CA release (% of control)
None (control)	100 ± 6*
Histamine (10^{-5} M)	109 ± 7
Nicotine (10^{-4} M)	112 ± 2
Acetylcholine (10^{-4} M)	110 ± 7
Bradykinin (10^{-6} M)	109 ± 3
Neurotensin (10^{-5} M)	131 ± 3†
Angiotensin II (10^{-6} M)	103 ± 3
GABA (10^{-4} M)	117 ± 1†
K^+ (50 mM)	121 ± 5‡

* Represents 29.0 ± 1.7% of total cellular [^{3}H]CA released in 30 min under basal conditions. This value is the average ± SEM of triplicate wells containing confluent endothelial cells from two different preparations.
† $P < 0.02$ compared to control.
‡ $P < 0.05$ compared to control.

mastine, an H_1 receptor antagonist, markedly attenuated this response.

The response of chromaffin cells to histamine was found to be dose dependent. Moreover, clemastine caused a parallel shift of the histamine dose–response curve to higher agonist concentrations, consistent with competitive antagonism (Fig. 2). Using the ALLFIT computer fit program [17], the EC_{50} of histamine-induced [^{3}H]CA release was 1.91×10^{-6} M and the EC_{50} of histamine + clemastine was

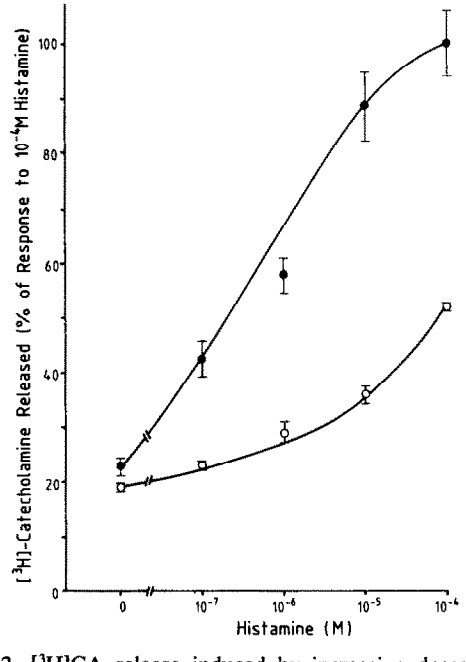

Fig. 2. [^{3}H]CA release induced by increasing doses of histamine in the presence (○) and absence (●) of clemastine (10^{-7} M). BMAC cells were prelabeled with [^{3}H]norepinephrine and exposed in PBS for 30 min to histamine ± clemastine; the percentage of intracellular [^{3}H]CAs released into the medium was measured as described under Materials and Methods. The response to 10^{-4} M histamine was set at 100% after subtracting basal release. Points are means ± SEM for three determinations, as percent of response to 10^{-4} M histamine.

4.55×10^{-4} M, resulting in EC_{50} (histamine + clemastine)/EC_{50} (histamine) of 238.

The time–course characteristics of the histamine response by chromaffin cells are shown in Fig. 3, A and B. In long-term incubations, nicotine and K^+ produced a maximal release of [^{3}H]CAs in 1 hr and 2 hr, respectively, with no further net releases up to 6 hr of exposure to these substances (Fig. 3A). On the other hand, histamine at both concentrations (10^{-5} M and 10^{-6} M) used, produced a steady increase throughout the 6-hr period of exposure. Moreover, at the 6-hr time point, 10^{-5} M histamine produced about a 3-fold, and 10^{-6} M histamine an approximately 2.5-fold, greater increase of [^{3}H]CA release than 10^{-5} M nicotine.

Figure 3B shows the time-course of [^{3}H]CA release for short periods of chromaffin cell exposure to various substances. Nicotine produced a prompt response of [^{3}H]CA secretion as early as 30 sec after its addition. This response ceased after 3 min of exposure to this agent. Tubocurarine markedly blocked the nicotine-induced [^{3}H]CA release throughout the time–course of the study (data not shown). Histamine, on the other hand, produced a much smaller initial release than nicotine but the response increased gradually over the 5-min incubation period. When chromaffin cells were exposed to histamine and nicotine simultaneously, [^{3}H]CA release was additive, particularly at the 5-min time point. This additivity was also evident for longer periods of exposure (1 and 2 hr) (data not shown).

Substance P (SP) has been shown to enhance nicotine-induced receptor desensitization in chromaffin cells [18, 19]. Using conditions similar to those

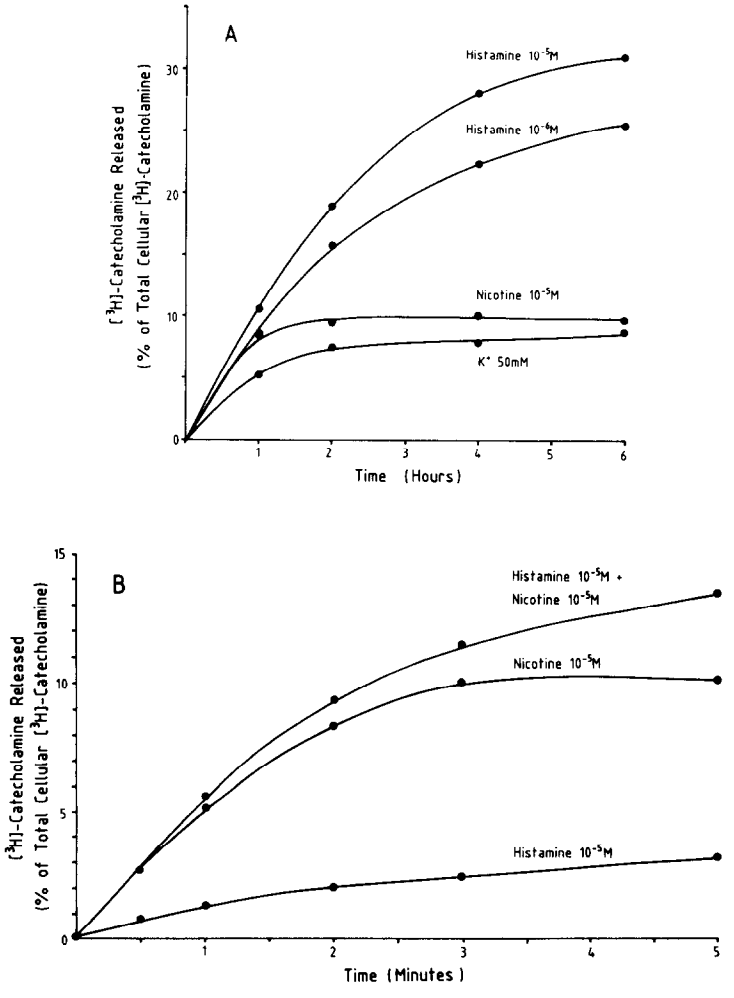

Fig. 3. Time–course of [^{3}H]CA release induced by various secretagogues. BMAC cells were prelabelled with [^{3}H]norepinephrine and received in PBS prolonged (A) or brief (B) exposures to various agents as described under Materials and Methods. Values are means ± SEM for three determinations and represent percent of intracellular [^{3}H]CA released into the medium minus basal release.

in Fig. 3B, chromaffin cells were exposed for only 5 min to histamine (10^{-5} M), nicotine (10^{-5} M), SP (10^{-5} M), histamine (10^{-5} M) + SP (10^{-5} M) and nicotine (10^{-5} M) + SP(10^{-5} M). The [^{3}H]CA release minus basal release as percent of total cellular ^{3}H content was calculated and gave the following results: histamine = 3.91 ± 0.11%, nicotine = 11.0 ± 0.2%, SP = 0%, histamine + SP = 4.22 ± 0.81%, and nicotine + SP = 2.45 ± 0.30%. The results show that the previously noted SP effect on desensitizing nicotinic receptor activation was clearly observed; however, SP did not affect histaminergic activation of [^{3}H]CA release.

Using Ca^{2+}-free PBS, histamine (10^{-5} M) did not evoke any release of [^{3}H]CAs over a 30-min period. However, when 0.1, 0.5 and 2.0 mM Ca^{2+} were added to this medium, 5.30 ± 0.09%, 5.61 ± 0.37% and 7.00 ± 0.41% of total cellular ^{3}H, respectively, were released into the medium following histamine stimulation.

The effects of various Ca^{2+} channel blockers and stimulators on the histamine response are shown in Fig. 4. In Ca^{2+}-containing medium, the histamine-induced [^{3}H]CA release was reduced significantly in the presence of 1 mM each of EDTA, Co^{2+} or Ni^{2+}, with Ni^{2+} showing the greatest effect (Fig. 4A). The Ca^{2+} channel blockers, D-600, verapamil and nifedipine, had no effects on basal [^{3}H]CA release; however, they significantly blocked the histamine response (Fig. 4B). Bay K 8644, a Ca^{2+} channel stimulator, significantly stimulated both basal and histamine-induced [^{3}H]CA release. However, when chromaffin cells were simultaneously exposed to both Bay K 8644 and nifedipine, the Bay K 8644 stimulation of [^{3}H]CA release was entirely blocked in both the basal and histamine-induced responses (Fig. 4B).

$^{45}Ca^{2+}$ uptake was not affected by muscarine; however, significant increases were found following a 5-min exposure of chromaffin cells to histamine and nicotine, with nicotine showing the greater effect (Fig. 5).

DISCUSSION

There is evidence in the older literature that certain substances, besides those related to acetylcholine, induce CA release from the intact adrenal gland. Studies using various animal species have

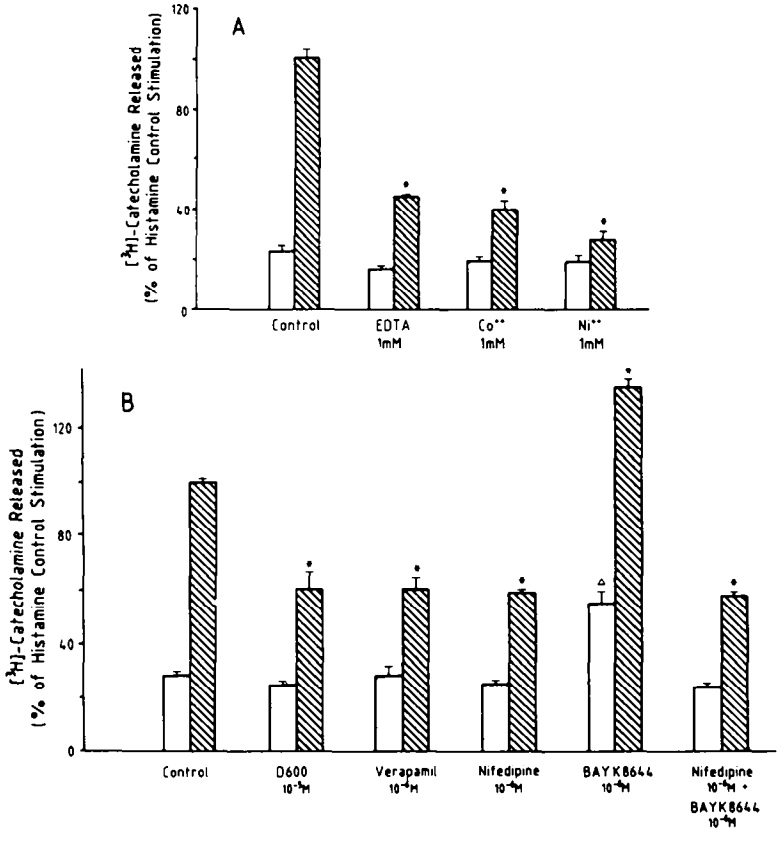

Fig. 4. Modifiers of Ca^{2+} channel activity and histamine-induced [^{3}H]CA release. BAMC cells were prelabeled with [^{3}H]norepinephrine and exposed in PBS for 30 min to histamine (10^{-5} M) and inorganic (A) or organic (B) modifiers of Ca^{2+} channels. [^{3}H]CAs released into the medium were measured as described under Materials and Methods. Open and crossed bars are responses in the absence and presence of histamine respectively. Values represent means ± SEM for three determinations, as percent of histamine (10^{-5} M) control stimulation. Key: (*) $P < 0.01$ compared to histamine-stimulated control; and (△) $P < 0.01$ compared to unstimulated control.

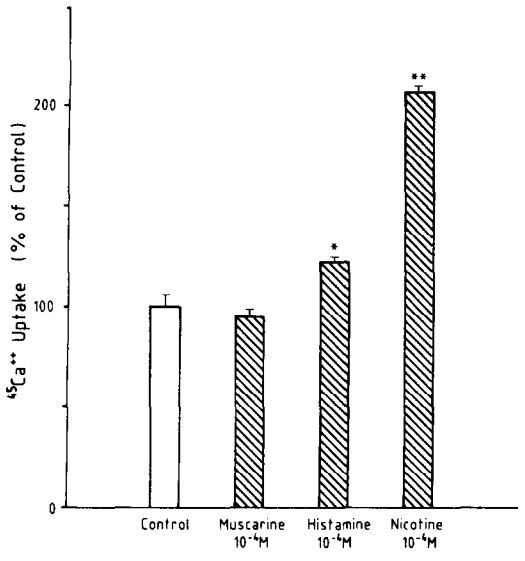

Fig. 5. Effects of various substances on $^{45}Ca^{2+}$ uptake. BMAC cells were adapted for 10 min to standard buffer and then stimulated for 5 min in the presence of $^{45}Ca^{2+}$ and various secretagogues as described under Materials and Methods. Controls represented 100% response, and values are means ± SEM for three determinations. Key: (*) $P < 0.05$, and (**) $P < 0.01$ compared to controls.

shown that histamine stimulates CA output by this gland, whereas antihistaminics block this response [5, 7, 20, 21]. Similar stimulatory effects have been described for bradykinin and angiotensin [5, 7, 22] and neurotensin.*

The present studies on cultured bovine adrenal medullary cells show that it is indeed the chromaffin and not the endothelial cell that is the target of secretagogue-induced CA release. They also affirm previous investigations showing that the cholinergic-induced release occurs through activation of nicotinic rather than muscarinic receptors (for review see Ref. 1). Of particular significance is the observation that histamine was a strong stimulator of chromaffin cell CA release. Moreover, GABA and various peptidic hormones including bradykinin, angiotensin II, TRH and neurotensin were also found to be strong activators of CA release.

Since histamine was found to be a powerful stimulator of CA release, a more detailed characterization of this response was obtained. Histamine-induced CA secretion was found to be manifested through activation of H_1 rather than H_2 receptors (Fig. 2 and [8]), supporting previous studies which demonstrated the presence of H_1 receptors in intact adrenal glands of rats and cats [4, 5, 7, 23, 24]. Moreover, clemastine, a specific H_1 receptor antagonist, caused a parallel shift to the right of the histamine dose–response curve (Fig. 2), similar to its effect on inositol-1-phosphate accumulation [8], suggesting a competitive mechanism of inhibition.

The present observations support numerous studies which describe desensitization of nicotinic receptors in chromaffin cells exposed to nicotinic agonists over periods that range from 3 to 30 min [25–28], dependent on particular cell preparations and other experimental conditions [1]. Similar desensitizing effects of K^+ on [^{3}H]CA secretion have also been noted [29–31]. In contrast, histamine induced little desensitization of its receptor, and the relative slight slowing of [^{3}H]CA release following prolonged exposure to this agent is probably more a function of depletion of releasable CA pools. Moreover, an additive effect of histamine and nicotine was obtained during the period of nicotine desensitization (Fig. 3B); and SP-induced desensitization of the nicotinic response was not observed with histamine stimulation. Put together, these data suggest that H_1 stimulation of exocytotic release, in contrast to stimulation induced by nicotine and K^+, is a process that is not appreciably desensitized, and it is independent of nicotinic activation. Furthermore, over extended periods of exposure, histamine was a substantially more potent releaser of CAs than either nicotine or K^+.

The apparent lack of desensitization is strikingly similar to the absence of this phenomenon when H_1 receptor-induced phosphoinositide hydrolysis was measured in guinea pig slices [32]. In contrast, in neuroblastoma cells, the H_1 receptor linked to cyclic GMP formation is desensitized by histamine, and this effect is prevented by mepyramine [33]. Furthermore, desensitization of H_2 receptors in human leukemia cells [34] and in mouse T lymphocytes [35] has also been observed and is thought to occur through structural changes in this receptor such that it is uncoupled from adenylate cyclase [34], its effector system. However, it is not readily apparent why H_1 receptors of chromaffin cells are not appreciably desensitized following even prolonged exposure to histamine.

There is little information on the ionic requirements for histamine-induced exocytosis by chromaffin cells, albeit extracellular Ca^{2+} has been shown to be an obligatory cation for stimulus–secretion coupling by nicotine and K^+ (for review see Ref. 1). The present studies show that the absence of Ca^{2+} in the medium also prevents histamine-induced [^{3}H]CA release. However, relatively small concentrations (0.1 mM) of Ca^{2+} produced an almost maximal response. Moreover, since the process of Ca^{2+} influx is the trigger that stimulates exocytosis [36, 37], histamine induced a significant increase of $^{45}Ca^{2+}$ in 5 min but less than that provoked by nicotine (Fig. 5), an observation which is in accord with the greater release of [^{3}H]CA release by nicotine than histamine during this time period (Fig. 3B). On the other hand, muscarine, which does not stimulate exocytosis in BAMC cells, had no effect on $^{45}Ca^{2+}$ influx.

One common property of all Ca^{2+} channels studied to date is that they can be blocked by multivalent cations [38]. The inorganic blocking cations appear to compete with Ca^{2+} for a common binding site [39] thus preventing ion flow through the channel.

* R. Corder, D. F. J. Mason, D. Perrett, V. Clement-Jones, L. H. Rees, G. M. Besser and P. J. Lowry, in *Molecular Neurobiology of Peripheral Catecholamine Systems*. Int. Conf., Ibiza, Spain, p. 125 (abstr.) (1982).

Organic Ca^{2+} channel blockers which appear to block the flow of Ca^{2+} through voltage-sensitive Ca^{2+} channels [40] exert their inhibiting effects on Ca^{2+} currents by affecting the probability of the channel being open and not by blocking ion flow through the open channel [41]. Histamine-induced [^{3}H]CA release was inhibited markedly by both inorganic (Co^{2+}, Ni^{2+}) and organic (D-600, verapamil and nifedipine) Ca^{2+} channel blocking agents (Fig. 4, A and B). On the other hand, Bay K 8644, a Ca^{2+} channel activator in several tissues, including BAMC cells [42], additively enhanced histamine-induced exocytosis, an effect blocked by nifedipine (Fig. 4B). Our results, therefore, show that histamine exerts at least part of its effects on exocytosis by stimulating Ca^{2+} influx through voltage-sensitive Ca^{2+} channels. However, the data also suggest that histamine stimulates other Ca^{2+} channels that contribute to exocytosis.

Histamine is widely distributed in the body. It has been identified in the adrenal gland [43, 44] and found in remarkably high concentrations in bovine splanchnic as well as other adrenergic nerves [45]. As indicated earlier, histamine infusion provokes large releases of CAs from intact adrenal glands of several animal species and histamine administration in humans produces a substantial rise in plasma CAs [46]. Circulating histamine levels in normal adults, and the high levels found in allergic conditions, certain leukemias and other pathological conditions [47], are well within the range of histamine concentrations (10^{-7} M to 10^{-5} M) found in the present studies to stimulate exocytosis. It is also interesting to note that certain antidepressants and neuroleptics are among the most powerful H_1 antagonists available [48]. These observations coupled with the clear demonstration of the powerful effects of histamine as a secretagogue suggest that future studies address the role H_1 receptors play in the homeostasis of the adrenal gland and in the possible altered function of this gland in disease states and during pharmacotherapy for mental disorders.

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