Histamine-Induced Cortisol Secretion from Bovine Adrenocortical Cells: Co-incubated with Bovine Adrenal Medullary Cells

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ABSTRACT—Histamine at concentrations higher than 10^{-9} M significantly elicited cortisol secretion from bovine adrenocortical (BAC) cells co-incubated with bovine adrenal medullary (BAM) cells, suggesting that BAM cells are responsible for histamine-induced cortisol secretion. Cortisol secretion from BAC cells co-incubated with BAM cells was also elicited by both an H_1 agonist, 2-methylhistamine, and an H_2 agonist, 4-methylhistamine. However, 4-methylhistamine was much less effective than 2-methylhistamine. Histamine-induced cortisol secretion was inhibited not only by H_1 antagonists (pyrilamine and diphenhydramine) but also by H_2 antagonists (cimetidine and ranitidine). Histamine effectively increased ^{45}Ca uptake and IP_3 production in BAM cells. These responses were antagonized by the H_1 antagonist but not by the H_2 antagonist. Histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells was inhibited by β-adrenoceptor antagonists, propranolol and timolol, as well as an NK_1-receptor antagonist, D-Arg^1-D-$Trp^{7,9}$-Leu^{11}-substance P. These results indicate that histamine can induce cortisol secretion from BAC cells at physiological concentrations through H_1 receptors on BAM cells, and catecholamine and substance P may participate in histamine-induced cortisol secretion.

Keywords: Histamine, Cortisol, Bovine adrenocortical cell, Bovine adrenomedullary cell, H_1 antagonist

It is well-recognized that cortisol is secreted from the adrenocortical cells by certain secretagogues such as ACTH, adrenaline and substance P (1−3). Histamine is also reported as a potent stimulant for the pituitary-adrenocortical axis, though the exact mechanism has not been clearly demonstrated (4−6). It has been demonstrated that histamine in the central nervous system plays an important role in the steroidogenesis in rats and dogs (7, 8). However, the role of peripheral histamine on the steroidogenesis is not well-understood.

Bornstein et al. (9) reported that there is an intimate relationship between chromaffin and cortical cells within the human adrenal gland. On the other hand, it is well-known that histamine stimulates adrenal medullary cells and consequently catecholamine secretion occurs via H_1 receptors (10). Since both histamine and corticosteroids are considered to participate in inflammatory responses, it is worthwhile to investigate the role of histamine in steroidogenesis with adrenocortical cells in the presence of adrenal medullary cells in in vitro. Therefore, the present study was undertaken to clarify the role of hista-

mine in steroidogenesis with bovine adrenocortical (BAC) cells co-incubated with bovine adrenal medullary (BAM) cells and also to investigate whether H_1 or H_2 receptors are responsible for the histamine-induced responses.

MATERIALS AND METHODS

Preparation of BAC cells

BAC cells were prepared as described previously (3). In brief, bovine adrenal cortex, freshly excised immediately after slaughter, was sliced (0.5- to 1.0-mm-thick slices) in an ice-cold bath. The slices were minced and incubated for 30 min at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.2% collagenase Type I and 2% bovine serum albumin (BSA). Thereafter, DMEM containing 10% fetal calf serum (FCS) was added and the cells were dispersed by repeated pipettings. To obtain the cell suspension, both incubation and dispersion were repeated under the same conditions. The dispersed cells were passed through stainless steel meshes (200 and 400 mesh) and washed 3 times with DMEM. The isolated cells were primarily cultured in DMEM supplemented with 10% FCS, 5% horse serum, 100 units/ml

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penicillin and 100 μg/ml streptomycin for 48 to 96 hr at 37°C in a CO_2 incubator with 5% CO_2 in humidified air. Approximately 5×10^7 BAC cells/g tissue were collected, and the cell viability assessed by the 0.3% Trypan blue dye exclusion test was higher than 90%.

Preparation of BAM cells

BAM cells were isolated from the bovine adrenal medulla according to the method of Keogh and Marley (11). Briefly, the bovine adrenal glands were perfused retrogradely with Ca^{2+}-free DMEM containing collagenase Type I and 0.5% BSA. The cells were then dissociated by filtration through a nylon sieve, and chromaffin cells were further purified by self-generating Percoll density gradient centrifugation. Subsequently, chromaffin cells were washed by low-speed centrifugation $(60 \times g)$ in DMEM at 4°C and suspended in the medium supplemented with 10% FCS containing 10^{-5} M cytosine arabinoside. The cells were grown on 100 mmϕ plastic plates in a 5% CO_2 incubator. A population of the cells that are at least 95% authentic chromaffin cells were obtained by this procedure, as judged by staining with neutral red.

Measurement of cortisol secretion

The cultured BAC cells were washed in serum-free DMEM and preincubated for 2 hr at 37°C in a 24-well plastic plate. The cell density in each well was adjusted to 10^5 cells/ml/well. Thereafter, BAM cells were washed with serum-free DMEM in Intercell membranes (Intercell®; Kurashiki-Bouseki, Tokyo) and preincubated for 1 hr at 37°C. Subsequently, compounds were added into each well and incubation was continued for another 1 hr at 37°C in a 5% CO_2 incubator. Test drugs were added to the medium simultaneously with the stimulants. The reaction was terminated by the addition of ice-cold DMEM to the medium, and the cells were sedimented by centrifugation. The cortisol content of the supernatant was measured by means of a radioimmunoassay kit (Nippon DPC Corp., Tokyo).

Measurement of ^{45}Ca uptake

BAM cells (10^6 cells/ml/well) were preincubated in Ca^{2+}-free DMEM containing 20 mM HEPES (pH 7.2) at 37°C for 30 min. After that, 0.84 μM (18.5 kBq/ml) of ^{45}CaCl$_2$ was added and incubation was continued for a further 10 min. The cells were then stimulated with 10^{-7} M histamine for 10 min. After the reaction was terminated, the medium was aspirated and the cells were washed 3 times with ice-cold DMEM and dissolved in a solution containing 0.5 N NaOH. The cells were solubilized in 0.2 ml of 0.5 N NaOH. Thereafter, the lysate was neutralized with 0.5 N HCl, and the radioactivity of ^{45}Ca was deter-

mined by means of a liquid scintillation counter (LSC-700; Aloka, Tokyo).

Measurement of IP$_3$ content

BAM cells (10^6 cells/ml/well) were preincubated in Ca^{2+}-free DMEM containing 20 mM HEPES (pH 7.2) at 37°C for 30 min. Thereafter, the cells were stimulated by histamine for 5 sec at 37°C. The reaction was terminated by an addition of ice-cold 10% trichloroacetic acid (TCA). The reaction mixture was placed on ice for 30 min and then centrifuged at $10,000 \times g$ for 10 min at 4°C. The supernatant was neutralized with an equal volume of ice-cold Freon-amine (12). The inositol 1,4,5-trisphosphate (IP$_3$) contents in the aqueous phase were measured by means of an IP$_3$ assay kit (Amersham International, Amersham, UK).

Chemicals

The following compounds were used in this study (sources are as indicated): Dulbecco's modified Eagle's medium (Nissui, Tokyo); collagenase Type I, bovine serum albumin, cytosine arabinoside, ranitidine hydrochloride, propranolol hydrochloride, timolol maleate, adrenaline hydrochloride, noradrenaline, prazosine hydrochloride (Sigma, St. Louis, MO, USA); fetal calf serum, horse serum (Hezelton, Lenaxa, KS, USA); penicillin (Toyo Jozo, Shizuoka); streptomycin (Meiji, Tokyo); substance P, D-Arg^1-D-Trp^{7,9}-Leu^{11}-substance P, D-Pro^2-D-Trp^{7,9}-substance P, D-Pro^4-D-Trp^{7,9}-substance P (Peptide Institute, Osaka); histamine dihydrochloride (Wako, Osaka); 2-methylhistamine dihydrochloride, 4-methylhistamine dihydrochloride, cimetidine (SK&F, Welwyn, UK); pyrilamine maleate (ICN Laboratories, Covina, CA, USA); diphenhydramine hydrochloride (Iwaki, Tokyo); dibenamine hydrochloride (Tokyo Kasei, Tokyo). Other chemicals used were all reagent grade and purchased from commercial sources.

Statistical analysis

A one-way analysis of variance with Dunnett's test was used to determine the statistical significance.

RESULTS

Histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells

As shown in Fig. 1, when histamine was added into the medium containing cultured BAC cells, histamine caused no cortisol secretion even at a concentration of 10^{-6} M. However, when BAC cells were co-incubated with BAM cells for 1 hr, a significant cortisol secretion from BAC cells was observed dose-dependently by histamine at concentrations higher than 10^{-9} M.
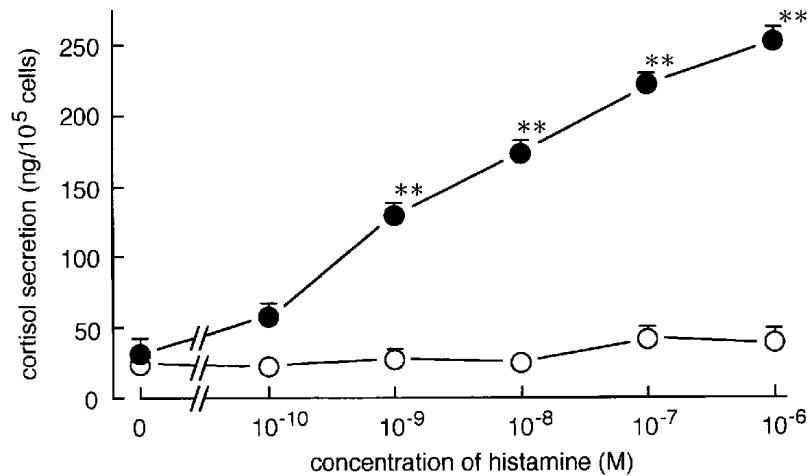
Fig. 1. Effect of histamine on the cortisol secretion from either BAC cells alone or BAC cells co-incubated with BAM cells. Each point represents the mean±S.E.M. obtained from 5 separate experiments. ** indicates a significant difference from the control at $P < 0.01$. ○: BAC cells alone, ●: BAC cells co-incubated with BAM cells.

Effects of histamine agonists on the cortisol secretion from BAC cells co-incubated with BAM cells

To clarify which histamine receptor is involved in the histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells, the cells were stimulated with histamine receptor agonists. As shown in Fig. 2, 2-methylhistamine, an H_1 agonist caused cortisol secretion from BAC cells co-incubated with BAM cells in a dose-dependent manner, and a significant increase was observed at concentrations higher than 10^{-8} M. On the other hand, 4-methylhistamine, an H_2 agonist showed a weak increasing effect on cortisol secretion. A significant effect was observed at a concentration of 10^{-6} M.

Effects of histamine antagonists on the histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells

When H_1 antagonists, pyrilamine and diphenhydramine, were simultaneously added with histamine to BAC cells co-incubated with BAM cells, the histamine (10^{-7} M)-induced cortisol secretion from BAC cells was inhibited dose-dependently. A significant inhibition was observed at concentrations higher than 10^{-8} M for pyrilamine and 10^{-7} M for diphenhydramine (Fig. 3).

H_2 antagonists, such as cimetidine and ranitidine, also inhibited histamine (10^{-7} M)-induced cortisol secretions from BAC cells co-incubated with BAM cells, at concen-
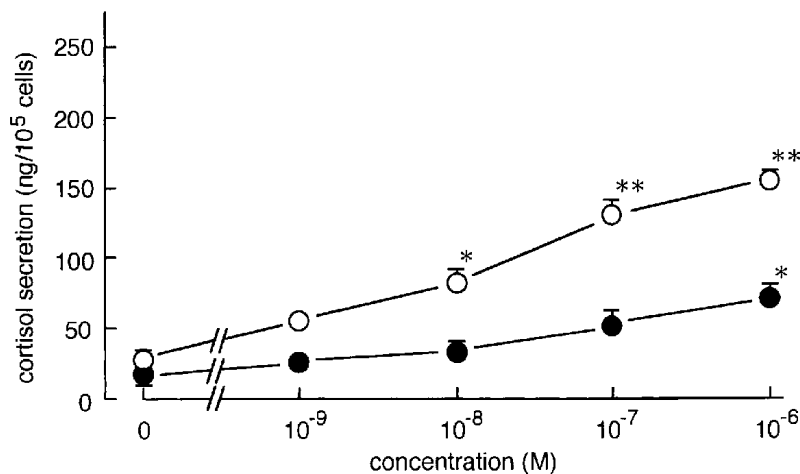

Fig. 2. Effects of histamine receptor agonists on the cortisol secretion from BAC cells co-incubated with BAM cells. Each point represents the mean±S.E.M. obtained from 5 separate experiments. * and ** indicate a significant difference from the control at $P < 0.05$ and $P < 0.01$, respectively. ○: 2-methylhistamine, ●: 4-methylhistamine.
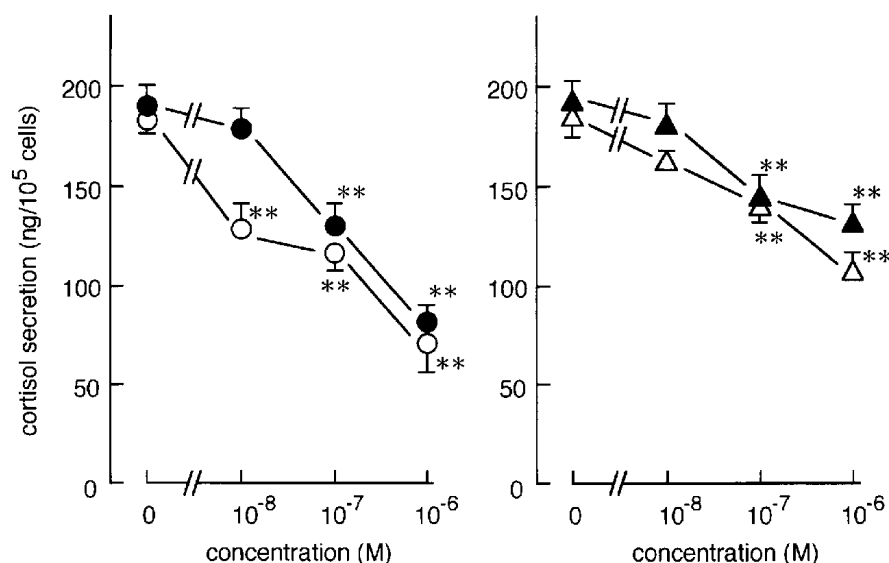
Fig. 3. Effects of H_1 and H_2 antagonists on histamine (10^{-7} M)-induced cortisol secretion from BAC cells co-incubated with BAM cells. Each point represents the mean±S.E.M. obtained from 5 separate experiments. ** indicates a significant difference from the control at P<0.01. ○: pyrilamine, ●: diphenhydramine, △: cimetidine, ▲: ranitidine.

trations higher than 10^{-7} M for both drugs.

^{45}Ca uptake and IP_3 production in BAM cells induced by histamine

From the results indicated above, it was suggested that BAM cells are responsible for histamine-induced cortisol secretion from BAC cells. In addition, cortisol release induced by histamine is mainly related to H_1 receptors. Therefore, the effect of histamine on ^{45}Ca uptake and

IP_3 production was determined with BAM cells. When BAM cells were stimulated by histamine at concentrations ranging from 10^{-9} M to 10^{-7} M, both ^{45}Ca uptake and IP_3 production were increased significantly in a dose-dependent manner (Fig. 4). Pyrilamine at 10^{-7} M significantly inhibited both ^{45}Ca uptake and IP_3 production in BAM cells induced by histamine (10^{-7} M). On the contrary, cimetidine (10^{-7} M) showed no significant effect on these histamine-induced responses.
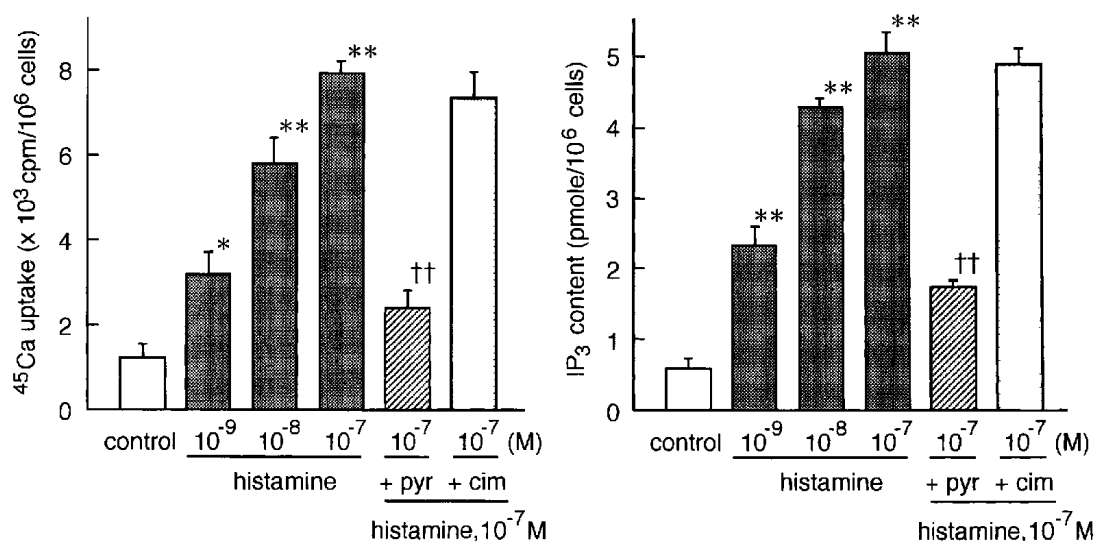

Fig. 4. ^{45}Ca uptake and IP_3 production in BAM cells induced by histamine and effects of histamine antagonists on histamine-induced responses. Each column represents the mean±S.E.M. obtained from 6 separate experiments. * and ** indicate a significant difference from the control at P<0.05 and P<0.01, respectively. †† indicates a significance from the histamine (10^{-7} M)-treated group at P<0.01. pyr: pyrilamine, cim: cimetidine.

Table 1. Effects of adrenoceptor and neurokinin-receptor antagonists on the cortisol secretion induced by histamine (10^{-7} M) from BAC cells co-incubated with BAM cells

Drugs	Concentration (M)	Cortisol secretion (ng/10^5 cells)	
		−histamine	+ histamine (10^{-7} M)
Control		20.3±1.4	208.4± 7.1
α-Adrenoceptor antagonists			
Dibenamine	10^{-6}	22.8±1.1	185.8±13.7
Prazosin	10^{-6}	21.8±1.5	178.6±11.3
β-Adrenoceptor antagonists			
Propranolol	10^{-6}	21.2±2.3	113.6±10.3**
Timolol	10^{-6}	24.6±1.6	109.7± 8.8**
NK_1-receptor antagonist			
D-Arg^1-D-Trp^{7,9}-Leu^{11}-substance P	10^{-6}	23.5±1.5	110.8± 7.9**
NK_2-receptor antagonist			
D-Pro^2-D-Trp^{7,9}-substance P	10^{-6}	21.4±2.0	189.7±10.9
NK_3-receptor antagonist			
D-Pro^4-D-Trp^{7,9}-substance P	10^{-6}	23.7±1.7	197.7±12.4

Each value represents the mean±S.E.M. obtained from 6 separate experiments. ** represents a statistical significance in comparison with the control (histamine 10^{-7} M) at $P < 0.01$.

Effects of neurotransmitter antagonists on the histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells

It is well-known that adrenal medullary cells contain not only catecholamine but also several neuropeptides including substance P (13). To determine which mediator participates in the histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells, effects of several neurotransmitter antagonists on the histamine-induced cortisol secretion were investigated.

As shown in Table 1, none of antagonists used in the present study affected basal cortisol secretion from BAC cells co-incubated with BAM cells. α-Adrenoceptor antagonists, such as dibenamine (10^{-6} M) and prazosine (10^{-6} M), exhibited only a slight inhibition on the histamine (10^{-7} M)-induced cortisol secretion, but not significantly. On the other hand, β-adrenoceptor antagonists, propranolol (10^{-6} M) and timolol (10^{-6} M), significantly inhibited the histamine-induced cortisol secretion. The NK_1-receptor antagonist D-Arg^1-D-Trp^{7,9}-Leu^{11}-substance P (10^{-6} M) also significantly inhibited histamine-induced cortisol secretion, while no significant inhibition was observed with the NK_2-receptor antagonist D-Pro^2-D-Trp^{7,9}-substance P and the NK_3-receptor antagonist D-Pro^4-D-Trp^{7,9}-substance P.

DISCUSSION

In the present study, it was found that histamine at a concentration of 10^{-9} M caused cortisol secretion from BAC cells co-incubated with BAM cells, although it induced no cortisol secretion from BAC cells alone even at a concentration of 10^{-6} M. It has been reported that histamine stimulated steroidogenesis in isolated dog adrenocortical cells; however, the concentration of histamine used in this study was 10^{-6} M or higher (5, 6). As shown in the present study, the concentration of histamine causing steroidogenesis was 10^{-9} M, and this concentration was considered to be a physiological concentration. It is clear from the present findings that histamine-induced steroidogenesis is correlated to not only H_1 but also H_2 receptors, though the contribution of the H_2 receptor is less important than that of the H_1 receptor. We have reported that an increase in corticosterone release in rats induced by intracerebroventricular injection of histamine involved not only the H_1 but also the H_2 receptor (8). As shown in Figs. 2 and 3, the potency of cortisol secretion induced by H_2 agonist was relatively weak, whereas an inhibitory effect of H_2 antagonists on histamine-induced cortisol secretion was almost same as that of the H_1 antagonists. On the other hand, it has been reported that H_2 antagonists, cimetidine and ranitidine, showed an inhibition of cytochrome P-450, an essential enzyme in steroidogenesis (14, 15). Therefore, it was thought at the time that the inhibitory effect of H_2 antagonists observed in the present study was in part due to their direct action on cytochrome P-450.

It is generally recognized that the H_1 receptor is intimately coupled with the receptor-operated Ca^{2+}-channel and hydrolysis of inositol phospholipids (16–18) and that Ca^{2+} in the medium is necessary to induce the H_1-recep-

tor-mediated cell response (19). Therefore, we tried to determine if these two parameters are elevated by histamine or not. Our results confirmed that Ca^{2+} uptake and IP_3 content were increased significantly by histamine at a concentration of 10^{-9} M, and an increase in two parameters was inhibited by an H_1 antagonist, pyrilamine. Consistent with this, an H_2 antagonist, cimetidine, showed no inhibitory effect on an increase in Ca^{2+} uptake and IP_3 content.

In the present study, to avoid any direct contact of BAC cells and BAM cells, they were separated with a membrane filter, which allows passive diffusion of humoral factors. Therefore, histamine-induced cortical secretion from BAC cells co-incubated with BAM cells can be ascribed to humoral factors released from BAM cells under histamine stimulation. To investigate the humoral factors that exist in BAC cells, the effects of certain neurotransmitter antagonists were investigated. It is clear from the present results that histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells was inhibited by β-antagonists (propranolol and timolol), but not by α-antagonists (dibenamine and prazosin). These findings indicate that catecholamine may be released from BAM cells under stimulation with histamine. It is well-known that histamine stimulates adrenal medullary cells and consequently catecholamine release was generated (10). In addition, it was demonstrated that adrenaline can stimulate cortisol secretion from adrenocortical cells via β-receptors (20). Therefore, catecholamine seems to be one of the candidates for humoral factors. Eskay et al. (21) reported that there exist several chemical mediators in adrenal medullary cells other than catecholamine. Among such mediators, substance P was found to potently induce cortisol secretion from BAC cells by increasing the intracellular Ca^{2+} level (3). In association with this, it was also found that an NK_1 antagonist was effective in inhibiting the histamine-induced cortisol secretion from BAC cells co-incubated with BAM cells. However, both NK_2 and NK_3 antagonists did not inhibit histamine-induced cortisol secretion. Therefore, it seems likely that not only catecholamine but also substance P may be released from BAM cells by histamine.

It has been demonstrated that plasma histamine level is elevated in patients with allergic inflammations (22). The source of histamine observed in inflammatory reactions can be ascribed to mast cells and basophils, which are stimulated by the antigen-antibody reaction. On the other hand, it is well-recognized that histamine may have a direct stimulatory effect on cortisol secretions of the adrenal gland (4). Maki et al. reported that the anti-inflammatory drug prozyme-10 (proteolytic enzyme) caused a rapid elevation of plasma histamine and cortisol levels (6). These findings suggest that histamine is also one

of the possible candidates that showed an anti-inflammatory activity.

From the present study, it seems quite certain that (1) histamine stimulates BAM cells that release humoral factors, and these humoral factors induce cortical secretion from BAC cells, through both H_1 and H_2 receptors, and that (2) catecholamine as well as substance P may be responsible for this response.

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