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EFFECT OF HISTAMINE ON ADENOSINE 3′, 5′-CYCLIC MONOPHOSPHATE LEVELS IN GRANULATION TISSUE

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Abstract—The effect of histamine on cyclic AMP levels in rat granulation tissue was examined *in vivo* and *in vitro*. Granulomas formed by subcutaneous implantation of formalin-soaked filter paper disks were used as granulation tissue. Histamine injection (5 mg/kg, i.p.) produced a significant increase in the cyclic AMP levels for 10–30 min after injection. Administration of either burimamide or mepyramine at 1 mg/kg, s.c., 30 min before histamine administration blocked the cyclic AMP increase induced by 5 mg/kg histamine. Burimamide was more effective than mepyramine. Neither of these histamine antagonists at 1 mg/kg blocked the cyclic AMP increase induced by adrenaline (1 mg/kg, i.p.). Propranolol at 1 mg/kg, s.c., reversed the cyclic AMP increase due to adrenaline but partially blocked a similar effect of histamine. Histamine (1 mM and less), added alone to the incubation medium in which the chopped granulation tissue was immersed, had little or no effect on cyclic AMP levels in the granulation tissue. However, when histamine (1 and 10 mM) was added concomitantly with 3-isobutyl-1-methylxanthine (IBMX) (1 mM), a significant increase in cyclic AMP occured, compared to the addition of IBMX alone. IBMX (1 mM) alone caused a significant increase in cyclic AMP compared to controls in which the drug was omitted. 2-Methylhistamine (5 mM) induced a cyclic AMP increase in the presence of IBMX (1 mM). Metiamide (0.01 mM) partially blocked the cyclic AMP increase caused by histamine (1 mM) in the presence of IBMX (1 mM). Mepyramine (0.01 mM) completely blocked this histamine effect. Propranolol (0.01 mM) markedly inhibited the effect of histamine (1 mM). These data suggest that histamine causes the cyclic AMP increase partly by the activation of H_2-receptors on the cells of granulation tissue and partly through the β-receptor action of catecholamines released, although the involvement of H_1-receptors cannot be excluded.

Although it is well known that most of the histamine in connective tissue is contained in mast cells [1], the role of histamine in the proliferation and function of the cells of this tissue is poorly understood.

In other tissues, histamine markedly stimulates gastric acid secretion and has positive inotropic and chronotropic effects on a variety of cardiac preparations [2]. Interaction of histamine with H_2-receptors in gastric mucosa [3–5] and cardiac muscle [6–8] results in increased intracellular levels of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) through the stimulation of adenylate cyclase. It has been proposed that such an increase in cyclic AMP levels leads, in turn, to the emergence of pharmacodynamic effects via intermediate steps [3–8].

Granulation tissue is the site of cell proliferation and the active synthesis of intercellular substances. It is formed as a connective tissue response to the later stages of inflammation [9]. If H_2-receptors exist on cells in this granulation tissue, activation of such receptors may be important in cellular functions because of the intermediate effect of elevated cyclic AMP levels. In this respect, we have already reported the H_2-receptor-mediated inhibition of growth of granulation tissue around subcutaneously implanted formalin-soaked filter paper disks. This inhibition was caused by exogenously administered histamine [10].

In the present study, the effect of histamine on cyclic AMP levels in granulation tissue was studied in an attempt to clarify the role of this amine in the patho-physiology of connective tissue.

MATERIALS AND METHODS

Materials

Histamine dihydrochloride, theophylline, 3-isobutyl-1-methylxanthine (IBMX), 2-mercaptoethanol and activated charcoal (80–100 mesh, for column chromatography) were purchased from Nakarai Chemicals (Kyoto, Japan); 2,5-diphenyloxazole (PPO), 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), toluene and Triton X-100 from Wako Pure Chemical Industries (Osaka, Japan); dextran T-70 (mean mol. wt = 70,000) from Pharmacia Fine Chemicals (Uppsala, Sweden); bovine serum albumin (fraction V) from the Armour Pharmaceutical Co. (Kankakee, IL); cyclic AMP from the Sigma Chemical Co. (St. Louis, MO); adrenaline hydrochloride from the Sankyo Co. (Tokyo, Japan); propranolol hydrochloride from the Sumitomo Chemical Co. (Osaka, Japan); and mepyramine maleate from ICN Pharmaceuticals (Plainview, NY). Burimamide, metiamide, 2-methylhistamine and 4-methylhistamine were gifts from Dr. W. A. M. Duncan (The Research Institute, Smith, Kline and French Laboratories, Welwyn Garden City, Herts). [8-^{3}H]Adenosine 3′,5′-cyclic monophosphate (cyclic [^{3}H]AMP; 27.5 Ci/mmole) was obtained from the Radiochemical Centre (Amersham, Bucks).

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Formalin-soaked filter paper granuloma

Male Wistar rats weighing about 210 g were used. Granulomas were induced by subcutaneous implantation of formalin-soaked filter paper disks, according to a method reported previously [10].

In vivo *effect of drugs*

The drugs to be tested were administered to rats 7 days after implantation of filter paper disks. At a given time after drug administration, the animals were exposed to 40-sec whole body microwave irradiation to rapidly inactivate adenylate cyclase and phosphodiesterase, according to the procedure of Schmidt *et al.* [11]. For this purpose, a microwave oven (Sharp, model R-1501; 1.3 kW microwave output) was used. The temperature of the subcutaneous tissue rose to above 80° during this procedure.

Immediately after microwave irradiation, granulomas were excised, and the weight was measured after removing the enclosed filter paper disks. Each granuloma was homogenized in a glass homogenizer with cold 7% (w/v) trichloroacetic acid added in the ratio of 0.9 ml to 100 mg tissue. Two ml of the homogenate were pipetted into each of two test tubes and used for the cyclic AMP assay. A portion of the remainder was saved for protein determination.

Drugs were dissolved in 0.9% saline. Histamine and adrenaline were injected i.p. and the other drugs s.c. The injection volume was 0.1 ml/100 g body wt. When testing the influence of other drugs on either the histamine or the adrenaline effect, the relevant drug was administered 30 min before histamine or adrenaline. The dose of histamine was expressed as the base, and the dose of adrenaline, propranolol or mepyramine as the salt. Control animals received 0.9% saline.

In vitro *effects of drugs*

Preparation of tissue pieces. Rats were bled to death by decapitation on day 7 after implantation of filter paper. Granulomas were removed immediately and cut, in the cold, into cuboid pieces, each side of which was less than 2 mm.

Incubation of tissue pieces. Pieces of granulation tissue (granuloma) were incubated in 10-ml glass homogenizers to speed up later processing. About 500 mg of tissue were suspended in 4.5 ml of modified Krebs–Henseleit Ringer bicarbonate solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 25 mM; and glucose, 11.7 mM) and incubated for 30 min at 37° with adequate oxygenation achieved by bubbling a mixture of 95% O_2 and 5% CO_2.

At the end of the 30-min preincubation, the suspension medium was discarded and replaced by 4.5 ml of fresh 37° modified Krebs–Henseleit solution, which contained the test drugs and which had been aerated with 95% O_2–5% CO_2. Incubation at 37° with adequate bubbling was recommenced. At the end of incubation, the suspension medium was removed, 4.5 ml of cold 7% trichloroacetic acid were added, and the mixture was homogenized in an ice bath. Further procedures were the same as those described for the *in vivo* experiments.

Preparation of cyclic AMP samples

To each of two 2-ml aliquots of homogenate, 0.045 pmole (about 2700 dis./min) of cyclic [^{3}H]AMP was added for a recovery check. Each sample was centrifuged at 2000 *g* for 15 min at 4°, following the addition of 0.2 ml of 1 N HCl. The supernatant fraction was transferred into a 10-ml glass-stoppered centrifuge tube, shaken for 30 sec with 6 ml of water-saturated ether, and centrifuged. The ether layer was removed by aspiration. The extraction with ether was repeated another four times. Any ether remaining in the aqueous phase was removed by immersing the centrifuge tube in 90° water for 3 min.

Using the method of Otten *et al.* [12], the entire aqueous phase was applied to a column (0.7 × 3 cm) of Dowex 50W-X8 (200–400 mesh, H^+-form) which had been equilibrated with 0.1 N HCl. After the column had been washed with 3 ml of 0.1 N HCl and then with 2 ml of deionized water, cyclic AMP was eluted with 4 ml of deionized water. The eluate was lyophilized and stored in a refrigerator until use in the cyclic AMP assay.

Cyclic AMP assay

The method of Brown *et al.* [13] was modified slightly. The lyophilized sample was dissolved in 0.5 ml of 50 mM Tris–HCl buffer, pH 7.4, containing 8 mM theophylline and 6 mM 2-mercaptoethanol. This buffer was used for all subsequent procedures except for the preparation of the charcoal suspension. This redissolved sample was pipetted into two 12 × 105 mm glass test tubes (0.05 ml in each tube) and used for the cyclic AMP assay. Of the remainder, 0.25 ml was used for the recovery check.

Cyclic AMP was assayed in the following way. Each 0.05-ml sample was mixed with 8 nCi cyclic [^{3}H]AMP, contained in 0.05 ml buffer. To this mixture, 0.1 ml of a 1:24 dilution of cyclic AMP binding protein was added, and the final volume was adjusted to 0.35 ml with the buffer. The test tubes were left at 0° for 90 min. Subsequently, a 1 ml ice-cold suspension of activated charcoal was pipetted into each tube. The contents were mixed well and centrifuged at 2000 *g* for 15 min at 4°. The charcoal suspension was prepared as follows. Immediately before use, 10 mg/ml activated charcoal, 1.5 mg/ml dextran T-70 and 2.0 mg/ml bovine serum albumin were thoroughly suspended or dissolved in 50 mM Tris–HCl buffer, pH 7.4, without theophylline and 2-mercaptoethanol.

One ml of the supernatant fluid was pipetted into a vial containing 10 ml scintillator, which had the following composition: PPO (4 g) and POPOP (0.1 g) [in a mixture of toluene (667 ml) and Triton X-100 (333 ml)]. After shaking the vial, the radioactivity was counted in a liquid scintillation spectrometer (Aloka, model LSC-653).

Each value was corrected against a blank lacking binding protein. Standard curves were obtained with authentic cyclic AMP in the concentration range of 0.5 to 20.0 pmoles/tube. Each 0.25-ml sample for the recovery check was pipetted into a vial containing 0.75 ml water and 10 ml scintillator. The cyclic AMP contents of original samples were calculated by the direct isotope dilution method.

Table 1. Effects of intraperitoneally injected histamine on cyclic AMP levels in formalin-soaked filter paper granulomas in the rat*

Expt.	Histamine (mg/kg)	Time of injection (min before microwave irradiation)	N	Cyclic AMP concentration (pmoles/mg protein)
1	Control		10	1.70 ± 0.08
	0.05	20	8	1.89 ± 0.09
	0.5	20	10	2.01 ± 0.16
	0.5	30	7	1.81 ± 0.17
2	Control		7	1.49 ± 0.15
	5	10	11	2.36 ± 0.02†
	5	15	10	2.08 ± 0.13†
	5	20	13	2.41 ± 0.12†
	5	30	8	2.41 ± 0.13†
3	Control		10	1.70 ± 0.08
	5	60	8	1.57 ± 0.11

* On day 7 after subcutaneous implantation of filter paper, histamine was administered to the animals and they were exposed to microwave irradiation at different periods thereafter. Histamine level is expressed as the base. Control groups received 0.9% saline. Immediately after microwave irradiation, two to four granulomas were removed from each rat and duplicate determinations of the cyclic AMP content of each granuloma were made. The numbers of such determinations are given in the table. Values represent the means ± S.E.M.

† Significantly different from respective control groups by *t*-test, $P < 0.01$.

Protein determination

The protein content of a homogenate was determined by the method of Lowry *et al.* [14] using bovine serum albumin as standard.

RESULTS

In vivo *effects of drugs on the cyclic AMP levels in granulomas*

Effect of histamine. Histamine injected intraperitoneally caused increased cyclic AMP levels in the granulomas. The effect was dose-dependent (Table 1). At a level of 0.05 and 0.5 mg/kg, the effects were not significant, compared to the cyclic AMP levels in granulomas of control animals. At a level of 5 mg/kg, histamine produced a significant increase in cyclic AMP levels for 10–30 min after injection. This effect reached a maximum at 20 min after injection, but the cyclic AMP value had returned to control levels by 60 min after injection.

Antagonism of histamine effect by burimamide and mepyramine. To study the influence of other drugs on the effect of histamine, a 20-min interval was chosen because at this time the histamine effect was maximal. As shown in Table 2, the effect of 5 mg/kg histamine was almost completely suppressed by pretreatment of the rats with 1 mg/kg burimamide, a histamine H_2-receptor antagonist [15]. When 5 mg/kg histamine was administered to rats which had been treated with 5 or 10 mg/kg burimamide, granuloma cyclic AMP levels decreased to below those of the animals who received burimamide alone. This reverse histamine effect was more marked at a level of 10 mg/kg than at 5 mg/kg.

Table 2. Antagonism by drugs of histamine action on cyclic AMP levels in formalin-soaked filter paper granulomas in the rat*

Drug pretreatment (mg/kg, s.c.)	Cyclic AMP concentration (pmoles/mg protein)		
	Control A	Histamine (5 mg/kg, i.p.) B	B − A
Control	1.49 ± 0.15 (7)	2.41 ± 0.12 (13)	0.92†
Burimamide (1)	1.76 ± 0.13 (8)	1.85 ± 0.16 (8)	0.09
Burimamide (5)	1.57 ± 0.13 (8)	1.36 ± 0.12 (10)	−0.21
Burimamide (10)	1.49 ± 0.08 (10)	1.11 ± 0.17 (10)	−0.38
Mepyramine (1)	1.42 ± 0.15 (8)	1.92 ± 0.20 (7)	0.50
Mepyramine (5)	1.36 ± 0.08 (10)	1.12 ± 0.05 (10)	−0.24
Propranolol (1)	1.39 ± 0.07 (8)	1.75 ± 0.09 (8)	0.36†
Propranolol (5)	1.34 ± 0.06 (8)	1.10 ± 0.05 (8)	−0.24

* On day 7 after subcutaneous implantation of filter paper, histamine was injected 20 min before microwave irradiation, and test drugs were administered 30 min before histamine injection. Control groups received 0.9% saline. Histamine level is expressed as the base and those of mepyramine and propranolol as the salts. Values are the means ± S.E.M. for the numbers of granulomas indicated in parentheses.

† Significant difference between control (A) and histamine-treated (B) group, $P < 0.01$.

Table 3. Antagonism by drugs of adrenaline action on cyclic AMP levels in formalin-soaked filter paper granulomas in the rat*

Drug pretreatment (mg/kg, s.c.)	Cyclic AMP concentration (pmoles/mg protein) Control A	Adrenaline (1 mg/kg, i.p.) B	B − A
Control	1.49 ± 0.15 (7)	2.46 ± 0.16 (4)	0.97†
Propranolol (1)	1.39 ± 0.07 (8)	1.10 ± 0.09 (8)	−0.29
Propranolol (5)	1.34 ± 0.06 (8)	1.07 ± 0.08 (8)	−0.27
Burimamide (1)	1.76 ± 0.13 (8)	2.51 ± 0.15 (8)	0.75‡
Burimamide (5)	1.57 ± 0.13 (8)	2.32 ± 0.23 (7)	0.75§
Mepyramine (1)	1.42 ± 0.15 (8)	2.15 ± 0.17 (8)	0.73‡
Mepyramine (5)	1.36 ± 0.08 (10)	2.49 ± 0.19 (7)	1.13‡

* On day 7 after subcutaneous implantation of filter paper, adrenaline was injected 20 min before microwave irradiation; test drugs were administered 30 min before adrenaline injection. Control groups received 0.9% saline. Adrenaline, propranolol and mepyramine are expressed as the salts. Values are the means ± S.E.M. for the numbers of granulomas indicated in parentheses.

† Significant difference between control (A) and adrenaline-treated (B) group, $P < 0.05$.

‡ Significant difference between control (A) and adrenaline-treated (B) group, $P < 0.01$.

§ Significant difference between control (A) and adrenaline-treated (B) group, $P < 0.02$.

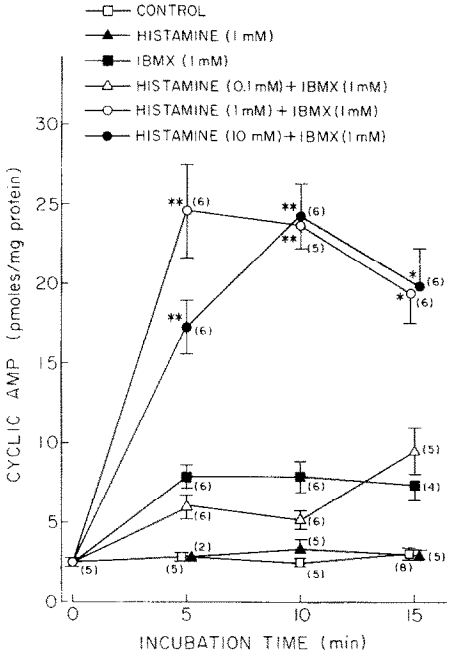

Fig. 1. Effects of histamine and IBMX on cyclic AMP levels in chopped granulation tissue. Granulomas were removed from rats on day 7 after subcutaneous implantation of filter paper and immediately chopped into small pieces. Granulation tissue was incubated for periods indicated on the abscissa in modified Krebs–Henseleit Ringer bicarbonate solution with or without test drugs. Each point is the mean for the number of experiments given in parentheses, and the vertical bar represents the S.E.M. The asterisk indicates values significantly different from corresponding values in the presence of IBMX alone: *$P < 0.005$, and **$P < 0.001$.

Burimamide administered alone had no significant effect on granuloma cyclic AMP levels at any level tested.

Pretreatment with 1 mg/kg mepyramine, a classical H_1-receptor antagonist, inhibited the cyclic AMP-increasing effect of 5 mg/kg of histamine. The increase was 45.7 per cent less than the increase obtained without mepyramine pretreatment, but this inhibition was weaker than the inhibitory effect of the same dose of burimamide. When the level of mepyramine used for pretreatment was increased to 5 mg/kg, granuloma cyclic AMP levels of animals receiving the combined treatment decreased to below the levels of the group given 5 mg/kg mepyramine alone.

Mepyramine administered alone had no significant effect on granuloma cyclic AMP levels at 1 and 5 mg/kg.

Influence of propranolol on the effect of histamine. The cyclic AMP-increasing effect of 5 mg/kg histamine was inhibited by pretreatment with 1 mg/kg propranolol, a β-adrenergic blocker. The increase was 60.9 per cent less than the increase obtained without propranolol pretreatment. When 5 mg/kg propranolol was used for pretreatment, granuloma cyclic AMP levels in the propranolol and histamine-treated group were lower than those in the group which received only propranolol (Table 2).

Propranolol administered alone at 1 and 5 mg/kg had no significant effect on granuloma cyclic AMP levels.

Effect of adrenaline and the influences of a β-blocker and histamine-receptor antagonists on it. As shown in Table 3, 1 mg/kg adrenaline administered i.p. had a cyclic AMP-increasing action in granulomas comparable to that of 5 mg/kg histamine when these effects were determined 20 min after injection. This adrenaline effect was completely blocked by pretreatment with 1 mg/kg propranolol. Moreover, the group receiving the combined treatment of 1 mg/kg propranolol and 1 mg/kg adrenaline had

Table 4. Effects of metiamide, mepyramine and propranolol on the increase in cyclic AMP levels in chopped granulation tissue induced by histamine and its analogues in the presence of IBMX *in vitro**

Expt.	Drugs (mM)	N	Cyclic AMP concentration (pmoles/mg protein)	P (vs. Expt. group)
a	Control	6	7.90 ± 0.73	
b	His (1)	6	24.61 ± 2.92	< 0.001 (a)
c	4-MeH (5)	6	15.89 ± 4.43	NS† (a)
d	2-MeH (5)	6	13.50 ± 1.82	< 0.02 (a)
e	Met (0.01)	6	9.98 ± 0.91	
f	His (1) + Met (0.01)	6	14.61 ± 2.08	<0.02 (b), NS (e)
g	Mep (0.01)	6	8.51 ± 0.73	
h	His (1) + Mep (0.01)	6	6.84 ± 0.37	< 0.001 (b)
i	Pro (0.01)	6	8.16 ± 1.03	
j	His (1) + Pro (0.01)	10	13.25 ± 1.09	< 0.01 (i), <0.001 (b)
k	4-MeH (5) + Pro (0.01)	6	11.34 ± 0.88	< 0.05 (i), NS (c)
l	2-MeH (5) + Pro (0.01)	7	13.32 ± 0.56	< 0.001 (i), NS (d)
m	Pro (0.01) + Met (0.01)	6	7.40 ± 0.62	
n	His (1) + Pro (0.01) + Met (0.01)	6	9.85 ± 0.70	<0.05 (j), <0.05 (m)

* Granulomas were removed from rats on day 7 after subcutaneous implantation of filter paper and immediately chopped into small pieces. Granulation tissue was incubated for 5 min in modified Krebş–Henseleit Ringer bicarbonate solution containing IBMX (1 mM) and test drugs. Abbreviations: Histamine (His), 4-methylhistamine (4-MeH), 2-methylhistamine (2-MeH), metiamide (Met), mepyramine (Mep) and propranolol (Pro). The numbers of duplicate determinations are given in the table and each value represents the mean ± S.E.M.

† NS = not significant.

lower granuloma cyclic AMP levels than the group given 1 mg/kg propranolol only. When 5 mg/kg propranolol was used for pretreatment, a similar result was obtained. Neither burimamide nor mepyramine at 1 and 5 mg/kg had any marked influence on the effect of 1 mg/kg adrenaline.

In vitro *effects of drugs on the cyclic AMP levels in granulation tissue*

Effects of histamine and IBMX. As shown in Fig. 1, when pieces of granulation tissue were incubated in the presence of 1 mM histamine alone, no significant increase in the cyclic AMP levels in this tissue was observed 5–15 min after the start of incubation. However, in the presence of 1 or 10 mM histamine and 1 mM IBMX (a potent phosphodiesterase inhibitor [16]), the cyclic AMP levels were always significantly higher than those in the presence of IBMX alone. IBMX (1 mM) alone always significantly increased the cyclic AMP levels above those of the control without drug ($P < 0.001$). Histamine at concentrations of up to 0.1 mM was without effect, regardless of whether IBMX (1 mM) was present.

Effects of 2-methylhistamine and 4-methylhistamine. As shown in Table 4, in the presence of 1 mM IBMX, 2-methylhistamine (5 mM) increased the cyclic AMP levels in granulation tissue (the effect of 4-methylhistamine was not statistically significant). At 1 mM, 4-methylhistamine was slightly effective, but 2-methylhistamine was completely ineffective.

Influences of metiamide and mepyramine on the effect of histamine. As shown in Table 4, the effect of 1 mM histamine in the presence of 1 mM IBMX was largely blocked by 0.01 mM metiamide, another H_2-receptor antagonist [17]. The inhibition amounted to 72 per cent. On the other hand, 0.01 mM mepyramine completely inhibited the histamine effect.

Influence of propranolol on the effects of histamine and its analogues. Propranolol at 0.01 mM blocked the effect of 1 mM histamine in the presence of 1 mM IBMX as potently as 0.01 mM metiamide (Table 4). Propranolol also showed a tendency to block the cyclic AMP increase induced by 5 mM 4-methylhistamine, but it was almost ineffective in inhibiting the effect of 5 mM 2-methylhistamine.

The combination of 0.01 mM propranolol and 0.01 mM metiamide had a more pronounced inhibitory action on the histamine effect than when each was tested separately.

DISCUSSION

An almost complete blockade by 1 mg/kg burimamide of the cyclic AMP-increasing effect of 5 mg/kg histamine in granulomas indicates that this histamine effect is produced mainly through the activation of H_2-receptors. However, 1 mg/kg of mepyramine also had a definite inhibitory effect on the histamine-induced cyclic AMP elevation, although the mepyramine effect was weaker than that of the same dose of burimamide. Therefore, it appears that the activation of H_1-receptors *in vivo* may be related to the histamine-induced cyclic AMP elevation to some extent.

The specificity of the blocking actions of H_1 and H_2-receptor antagonists on the histamine-induced effect of cyclic AMP elevation is indicated by the fact that these antagonists had no inhibitory action on the adrenaline-induced cyclic AMP elevation.

It has been shown that histamine releases catecholamines from the adrenal medulla [18] and sympathetic nerve endings [19] through the stimulation of H_1-receptors. In the present experiments, the

cyclic AMP-increasing action of histamine in granulomas was inhibited *in vivo* by both mepyramine and propranolol. Therefore, it is probable that the β-receptor action of catecholamines released by histamine may be involved in the cyclic AMP-increasing action of histamine on the cells of granulomas. This indirect component, however, does not seem to be the major factor in the histamine-induced cyclic AMP elevation *in vivo*, since propranolol, at a dose sufficient for complete blockade of the adrenaline action, only partially inhibited the histamine action.

It is not clear why the granuloma cyclic AMP levels of groups treated with the combination of large doses of H_1- or H_2-antagonist and histamine fell below those of the groups given either antagonist alone. However, the phosphodiesterase stimulation by histamine [20] may be related to this phenomenon.

Since *in vivo* experiments failed to clarify whether there are histamine receptors on the cells of granulomas, *in vitro* examination of pieces of granulation tissue was performed. In the *in vitro* experiments, burimamide increased granuloma cyclic AMP levels in the presence of IBMX (data not shown). The burimamide effect was more marked than the histamine effect, and this may be due to a potent catecholamine-releasing action of burimamide [21]. Moreover, burimamide is less active than metiamide as an H_2-antagonist [22]. Therefore, in the *in vitro* studies we used metiamide instead of burimamide.

4-Methylhistamine is a selective H_2-receptor agonist [15]. The 4-methylhistamine-induced cyclic AMP elevation in granulation tissue in the presence of IBMX indicates that there are H_2-receptors on the cells of this tissue. This is also supported by a marked blockade by metiamide of the histamine-induced cyclic AMP elevation. Because 2-methylhistamine, a relatively selective H_1-receptor agonist [15], also produced an increase in cyclic AMP levels in granulation tissue, some degree of involvement of H_1-receptors in the *in vitro* histamine effect cannot be excluded. Therefore, the possibility must be taken into account that the specific blockade of H_1-receptors may also contribute to the *in vitro* inhibition by mepyramine of the histamine-induced increase in cyclic AMP, in addition to the non-specific blockade of the H_2-receptor action. The *in vivo* significance of these findings on the H_1-receptor action obtained with chopped tissue is not clear, because mepyramine was less effective than burimamide in intact animals, and because the H_1-receptor-mediated component of the histamine effect *in vivo* may be explained by catecholamine release.

Propranolol, a β-blocker, markedly inhibited the effects of histamine and 4-methylhistamine on cyclic AMP levels in granulation tissue. This shows that histamine and its 4-methyl analogue probably release catecholamines from sympathetic nerve endings in granulomas *in vitro* and that the histamine-induced cyclic AMP increase in the chopped tissue is partly produced by the β-receptor action of the catecholamines released.

We have found that granuloma formation in response to subcutaneously implanted formalin-soaked filter paper was markedly inhibited by daily s.c. injections of histamine [10]. There have been many findings suggesting a causal relation between the increase in intracellular cyclic AMP levels and the inhibition of cell proliferation. For example, cyclic AMP and its analogue inhibit growth of cultured tumor cells [23,24] and transformed cells [25]; prostaglandins that stimulate adenylate cyclase suppress growth of cultured tumor cells [24] and fibroblasts [26]; and there is an inverse correlation between growth rate and levels of cyclic AMP in cultured transformed and untransformed fibroblasts [27]. Therefore, the histamine-induced inhibition of granuloma growth might be mediated by an increase of cyclic AMP in the cells of this tissue [10]. In the present experiments, histamine did indeed have such an action on granulation tissue.

The histamine-induced inhibition of granuloma growth was blocked by burimamide, but not by mepyramine [10]. However, the cyclic AMP-increasing action of histamine in granulomas was inhibited not only by burimamide but also by mepyramine. If intracellular cyclic AMP accumulation is causally related to the suppression of the granuloma growth, the reason why only the H_2-receptor stimulation was effective in inhibiting granuloma growth remains unclear. One possible explanation is that two kinds of histamine receptors and β-adrenergic receptors are located on different kinds of cells in granulation tissue, but that only the growth of cell populations possessing H_2-receptors is affected by cyclic AMP accumulation. Another possibility is that the respective receptor–adenylate cyclase complexes for different agonists are located in functionally distinct cellular compartments and mediate different effects on cell growth.

The histamine-induced rise of cyclic AMP levels in granulomas may be important in the regulation of such cell functions as the synthesis and secretion of intercellular substances by fibroblasts, as well as in inhibition of cell proliferation.

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