Chick retinal pigment epithelium

A new culture system for studying H_2-histamine receptors

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Histamine elevates the intracellular cyclic AMP levels in cultured embryonic chick retinal pigment epithelium. The half-maximal activity is 6×10^{-6} M. The effect of histamine is mediated by H_2 receptors, i.e., inhibited by the H_2 antagonist cimetidine and not affected by the H_1 antagonists diphenhydramine and pyrilamine. The inhibition constant (K_I) of cimetidine is 1.3×10^{-8} M. Thus, the present system offers the opportunity of studying the nature of coupling between histamine receptors and adenylate cyclase under controlled conditions in a homogeneous cell type.

Histamine *Cyclic AMP* *Cimetidine* *Diphenhydramine* *Pyrilamine*
Retinal pigment epithelium

1. INTRODUCTION

The nature of coupling between cellular histamine receptors and the cyclic AMP system is not as well understood as that for most other hormonal and neuromodulatory receptors, e.g., catecholamine and glucagon receptors. Partly, this is due to the unavailability of cultured cell lines and easily isolatable pure cell types responsive to histamine [1].

The retinal pigment epithelium (RPE) is a single layer of specialized neuroepithelial cells juxtaposed between the choroid and the neurosensory retina of the eye. RPE from chick embryo of 7–10 days of development can easily be isolated free from contaminating cell types and grown in culture. This culture system has been studied for some time and is quite well defined [2]. These cultured cells retain many of the differentiated characteristics seen in cell in vivo [3,4] and are capable of performing several specialized functions including producing melanin, and phagocytizing isolated bovine rod outer segment [5].

Histamine has been postulated to be involved in the control of capillary permeability [6] and thus of potential importance in maintaining the blood-brain barrier. Since the RPE is the actual site of the blood-retina barrier [7,8], the histamine response in these cells may be of particular interest. Our preliminary work has shown that several hormones and neurotransmitters, including histamine, elevated the intracellular cyclic AMP concentration of cultured RPE [9]. Here, we have characterized the histamine effect on the intracellular cyclic AMP concentration in cultured chick embryonic RPE and found that the histamine effect is mediated by the H_2 receptor; i.e., inhibited by H_2 antagonist and not affected by H_1 antagonists.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Sheets of RPE cells were dissected cleanly from eyes of 9-day old chicken embryo. After incubation of RPE sheets in an enzyme solution containing 0.6 units/ml collagenase, 0.01% trypsin, 0.2% chicken serum and 0.4 mM EDTA in Dulbecco's phosphate-buffered saline (pH 7.4) for 5 min at 37°C, cells were seeded at 2×10^4 cells/ml in

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35 mm Costar culture dishes. Monolayer cultures were maintained in 2 ml of Eagle's minimal essential medium (with Earl's salts) containing 5% heat-inactivated fetal bovine serum with 50 units/ml penicillin and 50 μg/ml streptomycin. The cultures were incubated at 37°C under humidified 5% CO_2/95% air for 3–4 weeks before use. These cultures retained many of the differentiated characteristics, i.e., pigmentation and phagocytic capacity, seen in cells in vivo [3–5]. When the procedure of isolating cells was carried out carefully, no contaminating cell types were detected under the light microscope.

2.2. *Intracellular cyclic AMP determination*

The procedures used were very similar to those reported earlier [10]. The culture medium was changed to 2 ml serum-free Eagle's minimum essential medium containing 20 mM Hepes buffer (pH 7.2) and 20 μM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro20-1724), a phosphodiesterase inhibitor. After 40 min at 25°C, histamine solution (20 μl) was added to the culture. The incubation was continued for an additional 3 min, before removing the incubation medium and extracting intracellular cyclic AMP with 2 ml of boiling water. The reaction period was kept at 3 min to allow linear production of intracellular cyclic AMP without leakage into the medium. When applicable, histamine antagonists were added to the incubation medium 12 min before application of histamine. Each dish was scraped with a rubber policeman and its content was boiled for 15 min. The concentration of cyclic AMP in the extract was determined by radioimmunoassay using ^{125}I-cyclic AMP and cyclic AMP antiserum complex.

2.3. *Protein quantitation*

The protein concentration was determined as in [11].

2.4. *Materials*

Dulbecco's phosphate-buffered saline and ingredients used in the culture media were obtained from Grand Island Biological Co. (Grand Island, NY). ^{125}I-cyclic AMP and cyclic AMP antiserum complex were from New England Nuclear (Boston, MA). Histamine 2HCl, cimetidine, diphenhydramine HCl, pyrilamine–maleate were from Sigma (St Louis, MO). Ro-20-1724 was a kind gift from Hoffman LaRoche Inc.

3. RESULTS

The effect of increasing concentrations of histamine on the intracellular cyclic AMP concentration of cultured RPE cells is shown in fig.1. The concentration required for a half-maximal effect is about 5.8×10^{-6} M, while the maximal effect is observed at about 10^{-4} M. The maximal cyclic AMP concentration achieved by histamine is about 40-fold over the basal level of 9.3 pmol/mg protein. Also shown in fig.1 is that cimetidine, a specific H_2-antagonist, at 3×10^{-6} M, completely abolishes the effect of histamine.

Several histamine antagonists were tested for their effects on the histamine-elevated intracellular cyclic AMP levels. As is shown in fig.2, the H_1-antagonists, diphenhydramine and pyrilamine, have no effect on the intracellular cyclic AMP elevated by 1×10^{-6} M histamine. On the other hand, cimetidine, an H_2-antagonist very effectively inhibits the histamine effect in a dose-dependent manner (fig.2). The half-maximal inhibition (IC_{50})

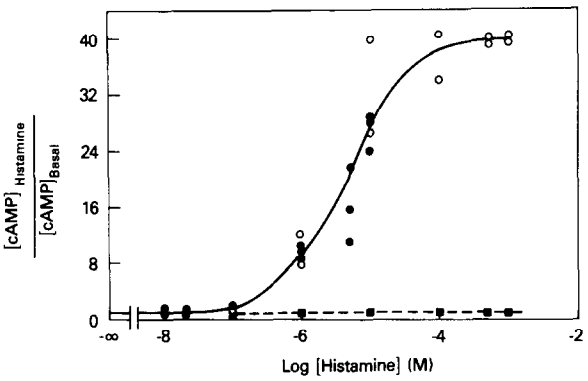

Fig.1. Concentration dependency of the histamine effect on the intracellular cyclic AMP concentration in RPE cells. RPE cells from 9-day old embryos were grown for 4 weeks before testing. In the absence of histamine, the basal cyclic AMP levels averaged 9.0 (●) and 9.8 (○) pmol/mg RPE protein, as determined from 3 and 2 culture dishes, respectively. In the presence of 3×10^{-6} M cimetidine (■), histamine had no effect on the cyclic AMP level. The basal cyclic AMP level was not affected by the presence of 3×10^{-6} M cimetidine. Each point represents one (●,○) and two (■) culture dishes, each with duplicate radioimmunoassay for cyclic AMP.

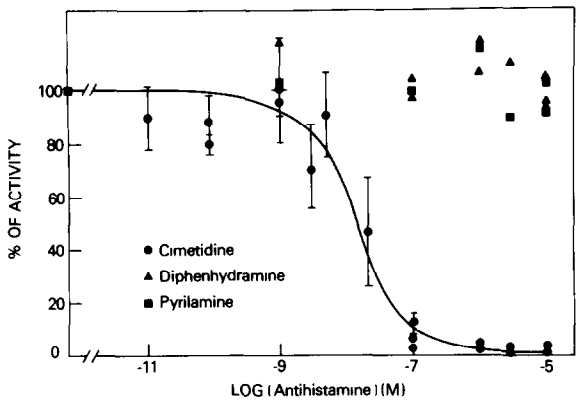

Fig.2. Inhibition by histamine antagonists of intracellular cyclic AMP concentration elevated by histamine in RPE cells. RPE cells from 9-day old embryos grown for 3 weeks in culture were reacted with 1×10^{-6} M histamine in the presence of increasing concentrations of cimetidine (●), diphenhydramine (▲) and pyrilamine (■). Maximal (100%) activity was defined as the cyclic AMP level in the presence of 1×10^{-6} M histamine alone and was 131.3 ± 17.8 pmol/mg RPE protein ($\bar{X}$ ± SEM; N = 3). Histamine-elevated cyclic AMP levels obtained in the presence of antagonists were expressed as percentages of the mean of the maximal activity. Each point represents the average value of 3 culture dishes and the bar represents the SEM.

by cimetidine occurs at 1.5×10^{-8} M. Assuming a competitive inhibition, the inhibition constant (K_I) of cimetidine can be calculated according to the equation [12]:

$$K_I = IC_{50}/(1 + S/K_a)$$

where K_a is the concentration of histamine (5.8×10^{-6} M, from fig.1) required to give half-maximal elevation of intracellular cyclic AMP concentration; IC_{50} (1.5×10^{-8} M) is the cimetidine concentration which produces half-maximal inhibition of the histamine effect and S (1×10^{-6} M) is the histamine concentration used in the inhibition study. The K_I of cimetidine so calculated is 1.3×10^{-8} M. The basal cyclic AMP concentration is not affected by the presence of these antagonists.

4. DISCUSSION

Our study shows that cultured chick embryonic RPE is responsive to histamine. The effect is very significant in that a 40-fold stimulation of intracellular cyclic AMP production can be observed with 100 μM histamine within 3 min of addition of the agonist (fig.1). Elevation of intracellular cyclic AMP by histamine shows a half-maximal activity (K_a) of 5.8×10^{-6} M. This K_a value is similar to that observed in the histamine-stimulated adenylate cyclase in a capillary-rich fraction from brain [13], and in guinea pig brain homogenate [14–16]. In the presence of 3×10^{-6} M cimetidine, the histamine effect in elevating the RPE intracellular cyclic AMP concentration is completely abolished. Therefore it appears that cimetidine is a more potent inhibitor in RPE than in the capillary-rich fraction from brain, in which even in the presence of 5×10^{-6} M cimetidine, a half-maximal activity of 4×10^{-5} M of histamine in stimulation of adenylate cyclase was observed [13]. The histamine effect is mediated through an H_2-type of receptor, since neither of the H_1-antagonists (pyrilamine and diphenhydramine) show any effect on the histamine-elevated intracellular cyclic AMP concentration, while the H_2 antagonist, cimetidine, effectively inhibits the histamine effect in a dose-dependent manner (fig.2) with an inhibition constant (K_I) of 1.3×10^{-8} M. Cimetidine is thus about 60 times more potent in the present system than in the inhibition of histamine-stimulated adenylate cyclase in the capillary-rich fraction from brain (K_I = 7.5×10^{-7} M) [13].

The physiological function of H_2 receptors in the RPE and the sources of the histamine input in vivo are not known at the present time. Nevertheless, the chick embryonic RPE appears to be the only homogeneous cell type that can be studied in culture to ultimately determine the nature of coupling between histamine receptors and adenylate cyclase.

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