

## PRELIMINARY COMMUNICATION

### DESENSITIZATION OF H<sub>2</sub>-LIKE HISTAMINE RECEPTORS STIMULATING CYCLIC AMP FORMATION IN THE CHICK CEREBRAL CORTEX

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Histamine potently stimulates cyclic AMP formation in slices of the chick cerebral cortex. Pretreatment of the tissue slices with 10 μM histamine for 2–30 min led to a time-dependent attenuation (when compared with values observed in the control tissue) of the cyclic AMP response produced by subsequent re-stimulation with 1, 10 or 100 μM histamine. The observed histamine-induced desensitization appears to be specific and homologous as the increase in cyclic AMP formation evoked by both forskolin or pituitary adenylate cyclase-activating polypeptide (PACAP) in slices pretreated with 10 μM histamine for 15 min was unchanged. It is concluded that in the chick cerebral cortex, H<sub>2</sub>-like receptors linked to cyclic AMP-generating system undergo rapid homologous desensitization.

**Key words:** *histamine, H<sub>2</sub>-like histamine receptor, cyclic AMP, chick brain, desensitization, forskolin, PACAP*

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## INTRODUCTION

Histamine is an established neurotransmitter/neuromodulator in the mammalian brain [2]. Experimental evidence accumulated during the last two decades indicate that histamine also plays a role of a regulator in the avian central nervous system (CNS) [6]. It has been shown that histamine potently stimulates cyclic AMP synthesis in the pineal gland of chick, duck and goose [7, 8], as well as in the chick cerebral cortical slices [15], yet displaying only poor activity in the duck cerebrum [8]. Using a selective (according to mammalian criteria) H<sub>2</sub>-type histamine receptor radioligand [<sup>3</sup>H]tiotidine, a single class of high affinity and high capacity receptor binding sites has recently been demonstrated in cortical membranes of the chick and duck [15, 16]. A close similarity in the pharmacological profile of the histamine-evoked cyclic AMP production and [<sup>3</sup>H]tiotidine binding sites in the chick cerebrum led us to suggest that these two parameters are functionally related and characteristic of a specific histamine receptor [16]. However, as the pharmacological profile of this receptor is different from that of the H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub> subtypes described for mammalian tissues, it has been proposed that it represents either an avian-specific H<sub>2</sub>-like histamine receptor or a novel histamine receptor subtype [15].

Histamine receptors belong to a large family of G protein-coupled receptors (GPCRs). One of the characteristic features of GPCRs is that, when overstimulated, they undergo a complex process termed desensitization, resulting in a reduction of cellular response to a given compound [3]. Homologous desensitization of both H<sub>1</sub> and H<sub>2</sub> histamine receptors in various mammalian cell lines and tissues has been described (e.g. [1, 4, 10, 12, 14]). On the contrary, little is known about regulation of histamine receptors in non-mammalian species, including birds. The aim of this work was to investigate whether H<sub>2</sub>-like receptor in the chick cerebral cortex, by analogy to its mammalian counterpart, can undergo a process of homologous desensitization.

## MATERIALS and METHODS

### Animals

Experiments were carried out on 2–3 weeks old white male leghorn chicks (*Gallus domesticus*). The animals, purchased on the day of hatching,

were kept in warmed brooders with standard food and tap water available *ad libitum*, under a 12 h light/12 h dark lighting schedule (150 lx; lights on between 21.30 and 09.30). The experiments were performed in accordance with the Polish governmental regulations concerning experiments on animals (Dz.U. 97.111.724) and rules followed at the Department of Biogenic Amines.

### Assay of cyclic AMP formation

On the day of an experiment, the chicks were killed by decapitation, with lights on, between 09.00–09.30. Each experiment was conducted on the cerebral cortex rapidly isolated from two birds and cross-sliced (0.25 mm) with the aid of McIlwain tissue chopper. The tissue slices were suspended in O<sub>2</sub>/CO<sub>2</sub> (95:5)-gassed, glucose-containing modified Krebs-Henseleit medium (KHM; mmol/l): 118 NaCl, 5 KCl, 1.3 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 11.7 D-glucose, pH 7.4.

The formation of [<sup>3</sup>H]cyclic AMP in [<sup>3</sup>H]adenine-prelabeled tissues was assayed according to Shimizu et al. [13]. Tissue slices were preincubated with 10 μM histamine for 2, 5, 15 and 30 min at 37°C, and then washed five times with an excess of ice-cold O<sub>2</sub>/CO<sub>2</sub>-gassed KHM in order to remove the amine. Next, the cerebral cortical slices were stimulated again for 15 min with histamine, forskolin or pituitary adenylate cyclase-activating polypeptide (PACAP38). The reaction was stopped by adding 0.55 ml of ice-cold 10% trichloroacetic acid to test tubes; the resulting mixture was then homogenized and centrifuged, and the formed cyclic AMP was quantified in a supernatant fraction.

The formed [<sup>3</sup>H]cyclic AMP was isolated by sequential Dowex-alumina column chromatography according to Salomon et al. [11]. The results were individually corrected for a percentage of recovery with the aid of [<sup>14</sup>C]cyclic AMP added to each column system prior to the nucleotide extraction. The accumulation of cyclic AMP was assessed as a percentage of the conversion of [<sup>3</sup>H]adenine to [<sup>14</sup>C]cyclic AMP.

### Chemicals

The following drugs were used: histamine (Serva, Heidelberg, Germany), forskolin and PACAP38 (Sigma Chemical Co., St. Louis, MO, USA); radioactive compounds were: [2,8-<sup>3</sup>H]adenine (specific activity 30.0 Ci/mmol) and [8-<sup>14</sup>C]cyclic AMP (spe-

cific activity 51.3 mCi/mmol), both from DuPont-NEN (Boston, MA, USA).

**Data analysis**

All data are expressed as mean ± SEM values. To calculate statistical significance between group means, one-way analysis of variance (ANOVA) followed by *post-hoc* Student-Newman-Keuls test was employed, using the GraphPad InStat software (GraphPad, San Diego, CA, USA).

**RESULTS and DISCUSSION**

In line with our earlier findings [15], histamine (1, 10 and 100 μM) produced a concentration-dependent increase in cyclic AMP production in the chick cerebral cortical slices. The obtained results (expressed in per cent of conversion) were: control, 0.45 ± 0.07 (10); histamine (data shown as net increases), 2.37 ± 0.24 (23) for 1 μM; 5.51 ± 0.34 (19) for 10 μM, and 6.09 ± 0.24 (19) for 100 μM. Pretreatment of chick cerebral cortical slices with 10 μM histamine for 2, 5, 15 and 30 min resulted in a time-dependent reductions in the ability of a subsequently applied histamine (10 μM for 15 min; re-stimulation) to activate cyclic AMP production (Tab. 1). The observed declines expressed in per cent of the respective time-point value are shown on Figure 1. The maximal reductions in the magnitude of the tissue cyclic AMP production following subsequent re-stimulation with histamine, i.e. by 51, 41 and 35 % for 1, 10 and 100 μM histamine, respectively, were observed in slices preincubated with the amine for 30 min.

The observed desensitization process seems to be homologous, as pre-exposure of the chick cerebral cortical slices to 10 μM histamine for 15 min led to significant declines only in the case of the histamine-evoked cyclic AMP responses. On the contrary, it did not significantly modify the effect of forskolin – a direct stimulator of adenylyl cyclase, or pituitary adenylyl cyclase-activating poly-

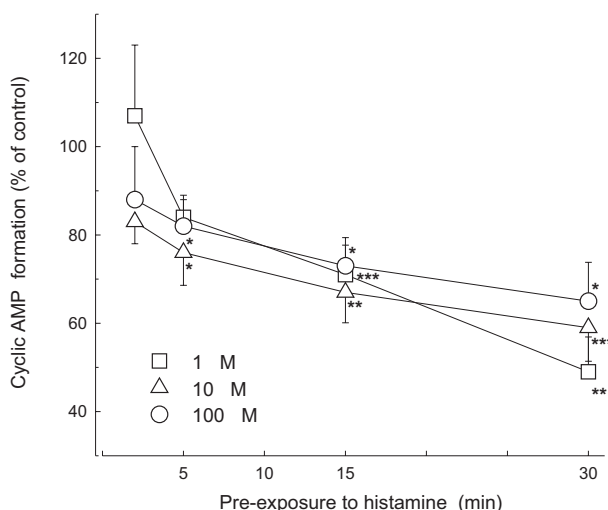
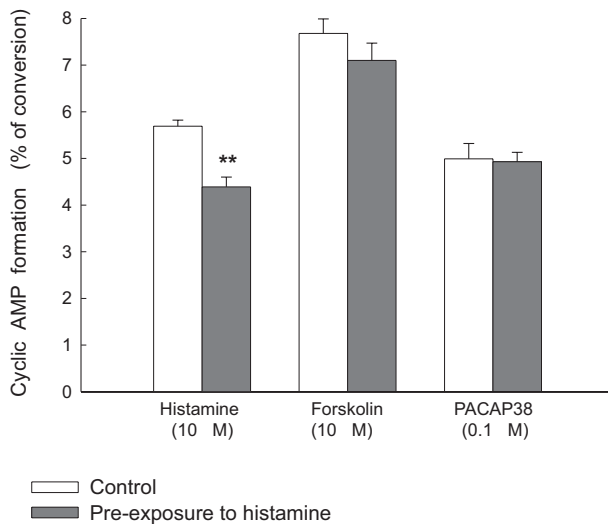


Fig. 1. Time course of homologous desensitization of H<sub>2</sub>-like histamine receptor-mediated cyclic AMP response in the chick cerebral cortex. The tissue slices were pre-exposed to 10 μM histamine for 2, 5, 15, or 30 min. After extensive washing, the slices were re-stimulated with 10 μM histamine for 15 min. Data are expressed as per cent of the respective control and represent means ± SEM of 4–8 values per group. Control values were (in per cent conversion): histamine 1 μM, 2.37 ± 0.24 (n = 20); histamine 10 μM, 5.51 ± 0.34 (n = 19); histamine 100 μM, 6.09 ± 0.74 (n = 19). \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs respective control

Table 1. Time course of homologous desensitization of H<sub>2</sub>-like histamine receptor-mediated cyclic AMP response in the chick cerebral cortex

Histamine (μM)	Control	Pre-exposure to histamine (10 μM)			
		2-min	5-min	15-min	30-min
1	2.37 ± 0.24 (20)	2.58 ± 0.43 (4)	1.99 ± 0.12 (8) NS	1.71 ± 0.20 (8) p < 0.05	1.17 ± 0.19 (4) p < 0.01
10	5.51 ± 0.34 (19)	4.60 ± 0.28 (4) NS	4.21 ± 0.41 (7) p < 0.05	3.74 ± 0.38 (8) p < 0.01	3.24 ± 0.42 (4) p < 0.001
100	6.09 ± 0.74 (19)	5.35 ± 0.74 (4) NS	4.98 ± 0.37 (8) p < 0.05	4.36 ± 0.29 (8) p < 0.001	3.94 ± 0.99 (4) p < 0.05

Data represent net increases (above basal level) in cyclic AMP formation expressed as means ± SEM per cent conversion from 4–20 values per group. NS – not statistically significant



**Fig. 2.** Effect of pre-exposure to histamine on increases in cyclic AMP formation in chick cerebral cortical slices evoked by histamine, forskolin and PACAP38. The tissue slices were pre-exposed 10  $\mu$ M histamine for 15 min. After extensive washing, the slices were re-stimulated for 15 min with histamine (10  $\mu$ M), forskolin (10  $\mu$ M), or PACAP38 (0.1  $\mu$ M). Data are means  $\pm$  SEM of 6–15 values per group. \*\*  $p < 0.01$  vs control

peptide (PACAP), whose action on the cyclic AMP generating system in the chick cerebrum is mediated by specific Gs protein-coupled PAC1 receptors [9] (Fig. 2). Interestingly, we have recently found that PAC1 receptors in the chick cerebral cortex undergo rapid homologous desensitization, with a significant decline (by nearly 30%) of the 0.1  $\mu$ M PACAP38-evoked cyclic AMP response seen in the tissue slices pretreated with 30 nM PACAP38 for only 1 min. A maximal desensitizing effect has been observed after only 5 min pretreatment with the peptide [5].

Histamine receptors belong to the class I of a superfamily of GPCRs, whose members undergo autoregulatory processes leading to desensitization of a receptor-derived signal. The mechanisms triggered by an activated, agonist-bound receptor and underlying homologous desensitization may involve several levels within the cell, including such processes as phosphorylation of receptor protein catalyzed by a specific G-protein-coupled receptor kinase (GRK), internalization (sequestration) of a receptor, or – in some cases – receptor down-regulation [3]. Recent studies carried out on transiently transfected COS-7 cells expressing either rat or human adenylyl cyclase-linked  $H_2$  receptor have shown the role of both GRK-2 and GRK-3 de-

pendent phosphorylation in the desensitization of the  $H_2$  receptor evoked by histamine [10] or a selective  $H_2$ -agonist amthamine [12]. Interestingly, at least in the case of human  $H_2$  receptors, the amthamine-evoked desensitization (characterized by a decreased cyclic AMP production) occurred very rapidly ( $t_{1/2} = 0.49$  min), and the process appeared to be insensitive to both PKA and PKC inhibitors [12].

The  $H_2$ -like receptor occurring in the avian CNS is pharmacologically different from its mammalian counterpart [8, 15], and the mechanisms leading to homologous desensitization of the mammalian  $H_2$ -type and avian  $H_2$ -like receptors may vary. Thus, a question on the role of a specific GRK, including type 2 and/or type 3, or both, as well as receptor sequestration or down-regulation, in the described here desensitization of the histamine-triggered cyclic AMP response in chick cerebral cortex remains to be established.

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