Interaction between the two signal transduction systems of the histamine H2 receptor: desensitizing and sensitizing effects of histamine stimulation on histamine-dependent cAMP production in Chinese hamster ovary cells

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The histamine H2 receptor is a member of the family of G-protein-coupled receptors and is linked to the activation of adenylate cyclase phospholipase C (PLC). In this study we examined the effects of protein kinase C (PKC) activation in Chinese hamster ovary (CHO) cells stably expressing canine histamine H2 receptors. Pretreatment with 100 nM phorbol 12-myristate 13-acetate at 37 °C for 15 min led to significant potentiation of histamine-dependent and forskolin-dependent cAMP production, whereas the biologically inactive phorbol ester, 4α-phorbol 12,13-didecanoate, was without effect. These potentiating effects were abolished by preincubation with 0.5 μM bisindolylmaleimide, a PKC inhibitor. Thus the activation of PKCs seems to be involved in the potentiation of cAMP production by acting on a post-receptor mechanism. Preincubation of a CHO cell line, CHO-H2R, with 10 μM histamine for 30 min had two effects. Maximal histamine-dependent cAMP production and forskolin-dependent cAMP production were potentiated by 36% and 105.2% respectively. The other effect was a desensitization of the histamine-dependent adenylate cyclase response as demonstrated by a three-fold increase in EC_{50}. Administration of 0.5 μM bisindolylmaleimide before preincubation of CHO-H2R with 10 μM histamine did not alter the desensitizing effect on cAMP production, but did abolish the sensitizing effect. Preincubation of CHO-H2R cells with 10 nM histamine resulted in moderate potentiation, which was also abolished by bisindolylmaleimide, but not in desensitization of the histamine-dependent cAMP production. Thus these results suggest that preincubation with histamine had a sensitizing effect on cAMP production mediated by PLC and PKC activation, as well as a desensitizing effect on the H2 receptor. The former effect is dependent on the intensity of PLC and PKC signals delivered by H2 receptors. The latter effect requires a higher concentration of histamine.

INTRODUCTION

Desensitization has been recognized in a number of G-protein-coupled receptors, among which the β2 adrenergic receptor has been the most intensively studied. The β2 adrenergic receptor contains, in the third intracellular loop, consensus phosphorylation sites for protein kinase A and protein kinase C (PKC), phosphorylation of which is reported to be involved in desensitization of the receptor [1]. The histamine H2 receptors, which belong to the family of G-protein-coupled receptors, contain in the corresponding region a consensus phosphorylation site for PKC, but not for protein kinase A. In addition, they share another phosphorylation site for PKC in the C-terminal region [2–4].

PKC activation by phorbol esters has been shown to either stimulate or inhibit cAMP production via several G-protein-coupled receptors in a number of cell lines, cell types and tissues [5–11]. For the histamine H2 receptor it has been reported that in guinea-pig brain slices, which contain both histamine H1 and H2 receptors, PKC activation via the H1 receptor or by phorbol esters potentiated cAMP production via the H2 receptor [12]. In gastric parietal cells, phorbol esters decreased [13–15] or increased [16] histamine-dependent cAMP production via the H2 receptor. Thus PKC activation can lead to either an increase or a decrease in the histamine-dependent adenylate cyclase response mediated by the H2 receptor, depending on the type of cell in which the H2 receptor resides.

It has been established that the histamine H2 receptor is linked to activation of both adenylate cyclase and phospholipase C (PLC) [17,18]. PLC activation leads to subsequent activation of PKC. We have previously shown that preincubation with 10 μM histamine led to attenuation of the cAMP production that occurs in response to histamine, indicating homologous desensitization of the H2 receptor in Chinese hamster ovary (CHO) cells stably expressing the canine histamine H2 receptor [19]. However, the potential effect of stimulating PKC on histamine-dependent cAMP production via stimulation of the H2 receptor remains to be clarified.

In this study we used CHO cells expressing histamine H2 receptors to examine the effects of a phorbol ester, stimulating PKC, on histamine-dependent cAMP production via the histamine H2 receptor. We also investigated the effect of PKC activation via the H2 receptor on histamine-dependent cAMP production via the same receptor. Here we present evidence that stimulation of the H2 receptor with histamine produces both sensitization and homologous desensitization of adenylate cyc-

The abbreviations used are: $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CHO, Chinese hamster ovary; PKC, protein kinase C; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate.

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lase activity, by different mechanisms, and that the latter requires a high concentration of histamine.

EXPERIMENTAL

Materials

Phorbol 12-myristate 13-acetate (PMA) and 4α-phorbol 12,13-didecanoate were purchased from Sigma (St. Louis, MO, U.S.A.). [^{3}H]Tiotidine was purchased from Du Pont–NEN (Boston, MA, U.S.A.). The acetoxymethyl ester of fura 2 was obtained from Dojin Chemicals (Kumamoto, Japan). Bisindolylmaleimide was purchased from Calbiochem-Novabiochem (La Jolla, CA, U.S.A.).

Cell culture

CHO-K1 cells were grown in Ham's F-12 medium containing 10% (v/v) fetal calf serum, 100 i.u./ml penicillin G and 0.1 mg/ml streptomycin sulphate, and were maintained in an atmosphere of 95% air/5% CO_2.

Construction of a cDNA for a mutant H2 receptor

The cloning and sequencing of the canine histamine H2 receptor were as previously described [19]. Substitution mutations were introduced into the gene encoding the canine histamine H2 receptor by oligonucleotide-directed mutagenenesis. A cDNA for a mutant H2 receptor, in which potential phosphorylation sites for PKC, Ser-221 and Ser-316, were replaced with alanine, was constructed and termed $A^{221}A^{316}$.

Expression of the histamine H2 receptor in CHO cells

Wild-type and $A^{221}A^{316}$ H2 receptor cDNAs were then cloned into the expression vector pMTHneo and subsequently transfected into CHO-K1 cells by the calcium phosphate precipitation method [19]. Clones resistant to 600 μg/ml of the neomycin derivative G418 were isolated and used in the following experiments.

Ligand-binding assay on intact cells

CHO cells, grown in 24-well plates, were assayed at a density of 10^5 cells per well. The cells were incubated for 2 h at 37 °C in 200 μl of Hepes/Tyrode buffer containing 1 nM [^{3}H]tiotidine and increasing concentrations of unlabelled tiotidine [20]. All samples were analysed in triplicate. After incubation, cells were washed three times with ice-cold PBS, then removed from the wells in 0.1% SDS; radioactivity was determined by liquid-scintillation counting. Specific binding was calculated by subtracting the non-specific binding, which was determined in the presence of 100 μM cimetidine. No specific binding was observed in either parental CHO cells or CHO cells transfected with the expression vector alone.

cAMP production

CHO cells, grown in 24-well plates, were assayed at a density of 10^5 cells per well, as described previously [21]. The cells were incubated for 30 min at 37 °C in 450 μl of Hepes/Tyrode buffer containing 0.1% BSA and 0.1 mM 3-isobutyl-1-methylxanthine; 50 μl of histamine was then added to initiate the reaction. After 10 min of incubation the reaction was terminated by the addition of 500 μl of 12% (w/v) trichloroacetic acid. The samples were centrifuged for 5 min at 3000 ***g*** at 4 °C. After extraction of the supernatants three times with diethyl ether, cAMP contents in the samples were measured with a radioimmunoassay [22]. Histamine-dependent and forskolin-dependent cAMP productions were determined by subtraction of basal cAMP production. It should be noted that basal cAMP production was not affected by preincubation with histamine or phorbol esters.

Fluorimetry of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in CHO cells

Measurement of $[Ca^{2+}]_i$ was performed essentially as described previously [23]. CHO cells were plated on glass coverslips in Ham's F-12 medium with 10% (v/v) fetal calf serum. The culture was incubated in the presence of 6 μM fura 2 AM for 2 h and was suspended in Hepes/Tyrode buffer containing 0.1% BSA. Then the culture was observed with an Olympus inverted fluorescence microscope equipped with a silicon-intensified target camera/image analysis system (ARGUS-50; Hamamatsu Photonics, Hamamatsu, Japan). Various concentrations of histamine were applied. The stored fluoromicroscopic images were processed and analysed with software supplied with the ARGUS system, in accordance with the manufacturer's instructions.

RESULTS

Expression of the histamine H2 receptor

We have obtained several CHO clones stably expressing canine histamine H2 receptors. One clone, termed CHO-H2R, was selected for this study. Scatchard plot analyses of tiotidine

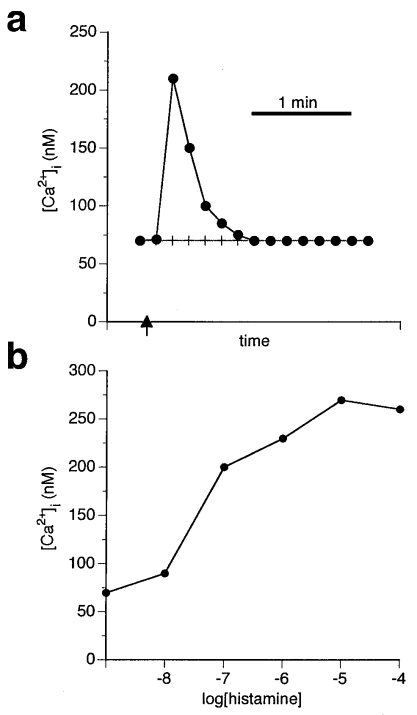

Figure 1 Histamine-dependent $[Ca^{2+}]_i$ elevation in CHO cells

(**a**) Time course of histamine-dependent intracellular Ca^{2+} mobilization in a CHO-H2R cell (●) and a control CHO cell (+). CHO-H2R cells and control CHO cells, loaded with fura 2 AM, were challenged with 1 μM histamine; $[Ca^{2+}]$ in single cells was measured with an ARGUS-50. A representative experiment is shown. (**b**) Dose–response curve for histamine-induced elevation of $[Ca^{2+}]_i$ in CHO-H2R cells.

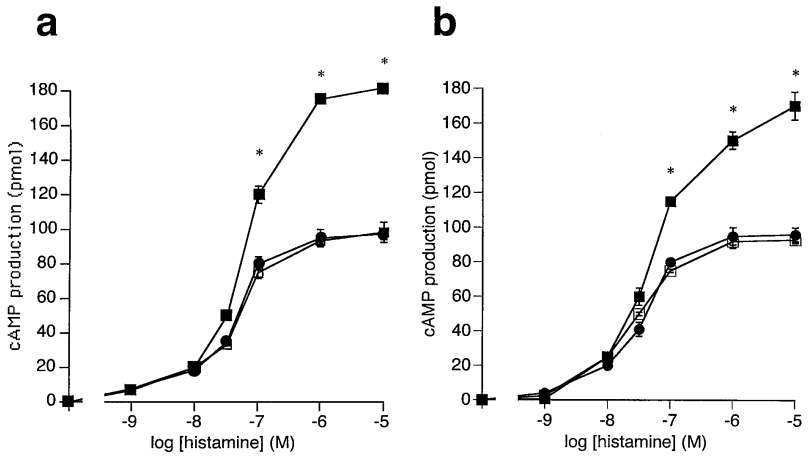

Figure 2 Effects of preincubation with phorbol esters on histamine-dependent cAMP production in CHO cells

CHO-H2R cells (**a**) and CHO-$A^{221}A^{316}$ (**b**) cells (10^5 of each) were incubated on 24-well plates in the presence or absence of phorbol esters, PMA and 4α-phorbol 12,13-didecanoate at 37 °C for 15 min. After being washed three times with the incubation buffer, the cells were incubated with histamine (0 M, 1 nM to 10 μM) at 37 °C for 10 min. cAMP contents were measured by radioimmunoassay. Symbols: ●, preincubation with vehicle; ■, PMA; □, 4α-phorbol 12,13-didecanoate. Results are expressed as means ± S.E.M. for three experiments performed in triplicate. * Significantly different from the value in control cells (●) ($P < 0.05$).

binding showed that CHO-H2R expressed 60.3 ± 2.2 fmol per 10^5 cells (results not shown). H2-receptor-specific cAMP production was observed in CHO-H2R cells but not in control CHO cells (results not shown). Histamine-dependent elevation of $[Ca^{2+}]_i$ was observed in CHO-H2R, but not in control CHO cells (Figure 1). Histamine-dependent calcium mobilizations were also inhibited by cimetidine (results not shown). These results indicate that CHO cells transfected with the vector alone do not express endogenous H2 receptors, whereas CHO-H2R cells do express functional canine H2 receptors that are coupled to adenylate cyclase and PLC.

Effects of PMA on forskolin- and histamine-dependent cAMP production

After preincubation in the presence or absence of 100 nM PMA for 15 min at 37 °C, CHO-H2R cells were incubated with various concentrations of either histamine or 100 μM forskolin for 10 min and cAMP contents were measured. Preincubation with PMA significantly enhanced histamine-dependent, as well as forskolin-dependent, cAMP production, whereas the biologically inactive phorbol ester 4α-phorbol 12,13-didecanoate was without effect (Figure 2a; Table 1). The potentiating effects of PMA were abolished by preincubation with 0.5 μM bisindolylmaleimide, a PKC inhibitor [24] (results not shown). Potentiations of cAMP production in response to higher concentrations (1 or 10 μM) of histamine were marked compared with those in response to lower concentrations (10 M or 100 nM). PMA treatment also enhanced cAMP production in response to 100 μM forskolin, a direct stimulator of adenylate cyclase, in CHO cells transfected with the vectors alone (Table 1). Thus the activation of PKC by PMA increased histamine-dependent cAMP production, which is mediated by the H2 receptor, and forskolin-dependent cAMP production, which is not mediated by the H2 receptor.

To confirm that the potentiating effect of PMA on cAMP production via the H2 receptor operated downstream from, rather than on, the H2 receptor, we used a mutant H2 receptor, termed $A^{221}A^{316}$, in which the potential phosphorylation sites for PKC had been mutated. We used a CHO clone, CHO-$A^{221}A^{316}$R, expressing 52.4 ± 3.5 fmol of $A^{221}A^{316}$ receptors per 10^5 cells. CHO-$A^{221}A^{316}$R cells were capable of binding to tiotidine, producing cAMP and elevating $[Ca^{2+}]_i$ in a manner similar to that of CHO-H2R cells. As shown in Figure 2b, treatment of CHO-$A^{221}A^{316}$R cells with 100 nM PMA resulted in increased histamine-dependent cAMP productions. This finding also suggests that the potentiating effect of PMA on cAMP production via the H2 receptor occurred via post-receptor systems.

Table 1 Effects of preincubation with PMA or histamine on forskolin-dependent cAMP production

After preincubation of CHO-H2R cells and control CHO cells in the presence or absence of 100 nM PMA (15 min), 10 μM histamine (30 min) or 10 nM histamine (30 min), the cells were washed three times with the incubation buffer then incubated with 10 mM forskolin at 37 °C for 10 min. cAMP contents were measured by radioimmunoassay. Results are shown as the percentage of cAMP production observed after preincubation with only Hepes/Tyrode buffer, and are expressed as the means ± S.E.M. for three experiments each performed in duplicate. Abbreviation: n.d., not determined.

Preincubation with	CHO-H2R (%)	Control CHO (%)
100 nM PMA	140.8 ± 5.1	155.9 ± 6.4
4α-phorbol 12,13-didecanoate	97.7 ± 3.6	100.8 ± 2.1
10 μM histamine	217.2 ± 5.5	n.d.
0.5 μM bisindolylmaleimide + 10 μM histamine	104.7 ± 2.3	n.d.
10 nM histamine	125.6 ± 4.2	n.d.

Effects of preincubation with 10 μM histamine on forskolin- and histamine-dependent cAMP production

CHO-H2R cells were preincubated with or without 10 μM histamine for 30 min, followed by thorough washing with the incubation buffer. cAMP produced in response to histamine and to 100 μM forskolin for 10 min was measured. Interestingly,

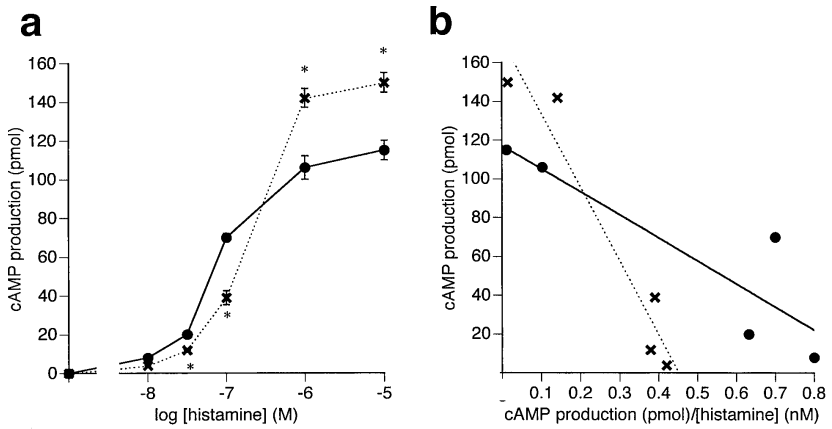

Figure 3 Effects of preincubation with 10 μM histamine on histamine-dependent cAMP production in CHO cells

(**a**) CHO-H2R cells (10^5) on 24-well plates were preincubated in the presence or absence of 10 μM histamine for 30 min. After the cells had been washed with the incubation buffer three times, they were incubated in the presence of histamine. Symbols: ●, preincubation without histamine; ×, preincubation with histamine (0 M, 10 nM to 10 μM) at 37 °C for 10 min. cAMP contents were measured by radioimmunoassay. Results represent the means ± S.E.M. for three experiments performed in triplicate. * Significantly different from the value in control cells (●) ($P < 0.05$). (**b**) Eadie–Hofstee plot analysis of the cAMP production data. Symbols: ●, preincubation without histamine; ×, preincubation with histamine.

preincubation of CHO-H2R cells with 10 μM histamine led to potentiation of the maximal cAMP production in response to histamine (1 μM or more) by approx. 30 % (Figure 3a). Forskolin-dependent cAMP production, which represents endogenous adenylate cyclase activity, was enhanced by 117.2 % by histamine preincubation (Table 1). However, cAMP production at lower histamine concentrations (100 nM or less) was attenuated by preincubation with 10 μM histamine (Figure 3a). An Eadie–Hofstee analysis of the results revealed a 3-fold increase in EC_{50} from 118 to 380 nM (Figure 3b), indicating that the H2 receptor was desensitized by preincubation with 10 μM histamine. In light of these findings, preincubation of CHO-H2R cells with 10 μM histamine seems to have dual effects on cAMP production in response to histamine: one is desensitization of the H2 receptor itself, and the other is an enhancing effect on cAMP production through one or more as yet unknown post-receptor signalling mechanism(s).

The potentiating effects of histamine preincubation lasted for more than 2 h after cessation of preincubation with 10 μM histamine (results not shown).

Effect of bisindolylmaleimide on the response to preincubation with 10 μM histamine

To investigate the possibility that activation of PKC via the H2 receptor is involved in the potentiating effect of preincubation with 10 μM histamine, the effect of bisindolylmaleimide, a PKC inhibitor, on histamine-induced potentiation of cAMP production via the H2 receptor was examined. CHO-H2R cells were incubated with 0.5 μM bisindolylmaleimide at 37 °C for 10 min, followed by preincubation in the presence or absence of 10 μM histamine at 37 °C for 30 min. After three washings with incubation buffer, the cAMP produced in response to histamine for 10 min was measured. Figure 4 shows that bisindolylmaleimide pretreatment resulted in decreased histamine-dependent cAMP production after preincubation with 10 μM histamine. The potentiation of forskolin-dependent cAMP production after histamine preincubation was also abolished in the presence of bisindolylmaleimide (Table 1). These results suggest that treatment with bisindolylmaleimide abolished the enhancing effect on

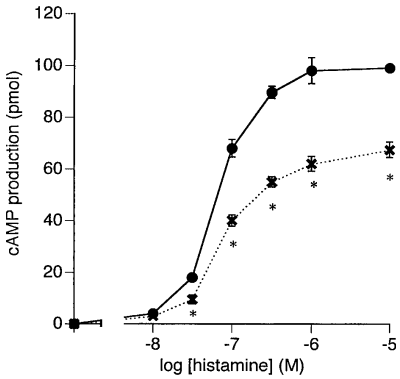

Figure 4 Effects of bisindolylmaleimide on the response to preincubation with 10 μM histamine

CHO-H2R cells (10^5) on 24-well plates were treated with 0.5 μM bisindolylmaleimide at 37 °C for 10 min, followed by preincubation with or without 10 μM histamine. Then, after the cells had been washed with the incubation buffer three times, they were incubated in the presence of histamine (0 M, 10 nM to 10 μM) at 37 °C for 10 min. cAMP contents were measured by radioimmunoassay. Symbols: ●, preincubation without histamine; ×, preincubation with histamine. Results are expressed as means ± S.E.M. for three experiments.* Significantly different from the value in control cells (●) ($P < 0.05$).

cAMP production induced by preincubation with 10 μM histamine, without affecting the desensitization induced by preincubation with histamine. Thus it is likely that PKC activation is involved in the enhancing effect on cAMP production induced by preincubation with 10 μM histamine, whereas concomitant desensitization of the H2 receptor is mediated by a bisindolylmaleimide-insensitive, i.e. PKC-independent, mechanism.

Effects of preincubation with 10 nM histamine on forskolin- and histamine-dependent cAMP production

CHO-H2R cells were preincubated with or without 10 nM histamine for 30 min followed by thorough washing with the incubation buffer. Next, cAMP production in response to histamine or 100 μM forskolin at 37 °C for 10 min was measured.

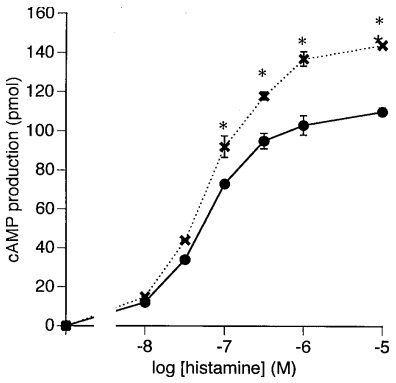

Figure 5 Effects of preincubation with 10 nM histamine on histamine-dependent cAMP production

CHO-H2R cells (10^5) on 24-well plates were preincubated in the absence or presence of 10 nM histamine for 30 min. After being washed three times with incubation buffer, the cells were incubated in the presence of histamine (0 M, 10 nM to 10 μM) at 37 °C for 10 min. cAMP contents were measured by radioimmunoassay. Symbols: ●, preincubation without histamine; ×, preincubation with histamine. Results are expressed as means ± S.E.M. for three experiments. * Significantly different from the value in control cells (●) ($P < 0.05$).

As shown in Figure 5, preincubation with 10 nM histamine led to the potentiation of cAMP production in response to lower, as well as higher, concentrations of histamine. This potentiation was abolished in the presence of 0.5 μM bisindolylmaleimide (results not shown). It should be noted that no desensitization took place in the presence of bisindolylmaleimide, indicating that desensitization by a PKC-independent mechanism did not occur after preincubation with 10 nM histamine. Forskolin-dependent cAMP production was also enhanced by 26 % after preincubation with 10 nM histamine (Table 1). Taken together, these results indicate that preincubation with unsaturating concentrations of histamine led to sensitization, but not desensitization, of cAMP production and that activation of PKC is involved in this sensitization.

DISCUSSION

The histamine H2 receptor is a member of the family of G-protein-coupled receptors and activates both adenylate cyclase and PLC in CHO cells. The activation of PLC leads to subsequent activation of PKC. In this study we have shown that preincubation with a phorbol ester, which activates PKC, enhances cAMP production in response to forskolin and histamine in CHO cells expressing the canine histamine H2 receptor. Although histamine receptors contain two potential phosphorylation sites for PKC [2–4], two important findings suggest that phosphorylation of the H2 receptor by PKC is not likely to be involved in this enhancing effect. First, a similar enhancement was also observed in a mutant H2 receptor in which both potential phosphorylation sites for PKC are mutated. Secondly, cAMP production in response to forskolin, as well as that in response to histamine, was also enhanced by preincubation with PMA, indicating that this enhancement is not mediated by direct activation of the H2 receptor, but rather through activation mechanisms operating on post-receptor signalling pathways. Thus the activation of PKC seems to be essential for the sensitization of cAMP production induced by preincubation with histamine. To demonstrate clearly that histamine binding to the H2 receptor sensitized cAMP production via activation of PKC, the effect of a PKC inhibitor, bisindolylmaleimide, was examined. As discussed in detail in the Results section, treatment with bisindolylmaleimide completely abolished the enhancement of cAMP production induced by preincubation with 10 μM or 10 nM histamine. Thus the activation of PKC seems to be essential for the sensitization of cAMP production induced by preincubation with histamine. We have confirmed that treatment of CHO cells expressing rat H2 receptors with PMA also resulted in potentiation of histamine-dependent and forskolin-dependent cAMP production (results not shown). Therefore although histamine-stimulated acid secretory activity was enhanced by phorbol esters in canine parietal cells [16] but was attenuated in rat [13] and rabbit [14] cells, we presume that the different responses were due to differences in the cells in which H2 receptors were expressed.

In CHO-H2R, stimulating adenylate cyclase via the H2 receptor at concentrations of histamine below saturation (100 nM or less) resulted in a dose-dependent increase in cAMP production, whereas high concentrations of histamine (1 μM or more) resulted in the stimulus delivered via the H2 receptor being saturated. We analysed the effect of preincubation with 10 μM histamine on cAMP production in response to various concentrations of histamine by using an Eadie–Hofstee plot. This plot revealed homologous desensitization of the H2 receptor as demonstrated by a 3-fold increase in EC_{50}, even when cAMP production was enhanced through PKC activation by preincubation with histamine. In other words, preincubation with 10 μM histamine induced homologous desensitization of the H2 receptor simultaneously with sensitization of cAMP production via a post-receptor signalling pathway. As a result of these two distinct effects, after preincubation with 10 μM histamine, cAMP production was slightly suppressed at non-saturating histamine concentrations but was enhanced at saturating histamine concentrations. It is likely that cAMP production in response to non-saturating histamine concentrations was attenuated more markedly by the desensitizing effect. In contrast, at saturating histamine concentrations, stimulation via the H2 receptor is more than adequate even after desensitization and potentiation of the post-receptor mechanism has led to potentiation of cAMP production. This might account for the biphasic response to preincubation with 10 μM histamine.

In contrast, preincubation of CHO-H2R cells with 10 nM histamine for 30 min led to sensitization of both histamine- and forskolin-dependent cAMP production, but not to desensitization. This indicates that homologous desensitization of the H2 receptor requires preincubation with high concentrations of histamine. In addition, the finding that desensitization after preincubation with 10 μM histamine occurred in the presence of a PKC inhibitor suggests that there is a form of desensitization that is not mediated by PKC. As reported for other G-protein-coupled receptors, it is possible that phosphorylation of the H2 receptor by kinases such as β-adrenergic receptor kinases and/or internalization of the receptor are involved in this desensitization.

In conclusion, we used CHO cell lines expressing histamine H2 receptors to demonstrate that preincubation with 10 μM and 10 nM histamine leads to sensitization of histamine-dependent cAMP production via PLC and PKC activation, which occurs through the H2 receptor. In addition, preincubation with 10 μM, but not 10 nM, histamine simultaneously led to homologous desensitization of the adenylate cyclase response via the H2 receptor. These observations point to an interaction between two distinct signalling pathways originating with the histamine H2 receptor. Further studies are needed to clarify the mechanisms underlining histamine H2 receptor desensitization.

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REFERENCES

1 Yuan, N., Friedman, J., Whaley, B. S. and Clark, R. B. (1994) J. Biol. Chem. **269**, 23032–23038
2 Gantz, I., Munzert, G., Tashiro, T., Schäffer, M., Wang, L., DelValle, J. and Yamada, T. (1991) Biochem. Biophys. Res. Commun. **178**, 1386–1392
3 Gantz, I., Schäffer, M., DelValle, J., Logsdon, C., Campbell, V., Uhler, M. and Yamada, T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 429–433
4 Ruat, M., Traiffort, E., Arrang, J. M., Luers, R. and Schwartz, J. C. (1991) Biochem. Biophys. Res. Commun. **179**, 1470–1478
5 Bell, J. D., Buxton, L. O. and Brunton, L. L. (1985) J. Biol. Chem. **260**, 2625–2628
6 Quilliam, L. A., Dobson, P. R. M. and Brown, B. L. (1985) Biochem. Biophys. Res. Commun. **129**, 898–903
7 Garcia-Sainz, J. A., Mundlovic, I. and Martinez-Olmedo, M. A. (1985) Biochem. J. **228**, 227–280
8 Karbon, E. W., Shenolikar, S. and Enna, S. J. (1986) J. Neurochem. **47**, 1566–1575
9 Jacobowitz, O., Cehn, J., Premont, R. and Iyengar, R. (1993) J. Biol. Chem. **268**, 3829–3832
10 Bascands, J.-L., Pecher, C. and Girolami, J.-P. (1993) Mol. Pharmacol. **44**, 818–826
11 Morimoto, B. H. and Koshland, Jr., D. E. (1994) J. Biol. Chem. **269**, 4065–4069
12 Garbarg, M. and Schwartz, J.-C. (1988) Mol. Pharmacol. **33**, 38–43
13 Anderson, N. G. and Hanson, P. J. (1984) Biochem. Biophys. Res. Commun. **121**, 566–572
14 Brown, M. R. and Chew, C. S. (1987) Can. J. Physiol. Pharmacol. **65**, 1840–1847
15 Hatt, J. F. and Hanson, P. J. (1989) Am. J. Physiol. **256**, G129–G138
16 Chiba, T., Fisher, S. K., Agranoff, B. W. and Yamada, T. (1989) Am. J. Physiol. **256**, G356–G363
17 Mitsuhashi, M., Mitsuhashi, T. and Payan, D. G. (1989) J. Biol. Chem. **264**, 18356–18362
18 DelValle, J., Wang, L., Gantz, I. and Yamada, T. (1992) Am. J. Physiol. **263**, G967–G972
19 Fukushima, Y., Oka, Y., Katagiri, H., Saitoh, T., Asano, T., Ishihara, H., Matsuhashi, N., Kodama, T., Yazaki, Y. and Sugano, K. (1993) Biochem. Biophys. Res. Commun. **190**, 1149–1155
20 Gajtkowski, G. A., Norris, D. B., Rising, T. J. and Wood, T. P. (1983) Nature (London) **304**, 65–67
21 Fukushima, Y., Oka, Y., Saitoh, T., Katagiri, H., Asano, T., Matsuhashi, N., Takata, K., van Breda, E., Yazaki, Y. and Sugano, K. (1995) Biochem. J. **310**, 553–558
22 Homma, M., Satoh, T., Takezawa, J. and Ui, M. (1977) Biochem. Med. **18**, 257–273
23 Bito, H., Nakamura, M., Honda, Z., Izumi, T., Iwatsubo, T., Seyama, Y., Ogura, A., Kudo, Y. and Shimizu, T. (1992) Neuron **9**, 285–294
24 Toullec, D., Pianetti, P., Coste, H., Belleverge, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boissin, P., Boursier, E., Loriollet, F., Duhamel, L., Charon, D. and Kirilovsky, J. (1991) J. Biol. Chem. **266**, 15771–15781

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