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# Synergism between Histamine $H_1$- and $H_2$-Receptors in the cAMP Response in Guinea Pig Brain Slices: Effects of Phorbol Esters and Calcium

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**SUMMARY**

The synergism between $H_1$- and $H_2$-receptors in the histamine-induced stimulation of cAMP accumulation was studied in slices from guinea pig hippocampus. Since $H_1$-receptors appear to be coupled to the phosphatidylinositol cycle, the participation of the two branches of the cycle in this synergism was assessed by using phorbol esters and/or by removing $Ca^{2+}$ from the external medium. The protein kinase C activator, 4$\beta$-phorbol 12,13-dibutyrate (4$\beta$-PDB), strongly potentiated, with an $EC_{50}$ of 0.2 $\mu$M, the accumulation of cAMP elicited by dimaprit, an $H_2$-receptor agonist used at supramaximal concentration (0.3 mM). The effect of 4$\beta$-PDB was also observed in the presence of impromidine, an $H_2$-receptor agonist, and histamine. 4$\beta$-Phorbol 12-myristate, 13-acetate, another protein kinase C activator, also potentiated the effect of dimaprit in a concentration-dependent manner although less potently than 4$\beta$-PDB. In contrast, 4$\alpha$-phorbol or the phorbol esters, 4$\alpha$-phorbol 12,13-didecanoate or 4-*O*-methyl-phorbol 12-myristate, 13-acetate, all inactive on protein kinase C, had no potentiating effect. 2-Thiazolylethylamine (2-TEA), a predominantly $H_1$-receptor agonist, increased the stimulation induced by dimaprit (0.3 mM), and this response was further enhanced in the presence of 4$\beta$-PDB in maximal concentration (1 $\mu$M). Mepyramine (0.1 $\mu$M) antagonized the $H_1$-receptor-mediated effect in the absence as well as the presence of 4$\beta$-PDB. The phorbol ester did not significantly alter the $EC_{50}$ of 2-TEA or the magnitude of its effect. In the absence of phorbol esters, removal of $Ca^{2+}$ from the incubation medium did not change the response elicited by 0.3 mM dimaprit but reduced by 50% the response to a supramaximal concentration of 2-TEA. This effect was more marked when EGTA was added in the $Ca^{2+}$-free medium. The $EC_{50}$ value of 2-TEA was only slightly modified in the absence of $Ca^{2+}$ (180 ± 20 $\mu$M as compared with 70 ± 4 $\mu$M in the presence of 2.6 mM $Ca^{2+}$). In the presence of 4$\beta$-PDB (1 $\mu$M), removal of $Ca^{2+}$, particularly in the presence of EGTA, did not affect or slightly increased the response to dimaprit, but still strongly reduced the response to 2-TEA. The $Ca^{2+}$ ionophore A 23187 (10 $\mu$M) showed a tendency to mimic the potentiating effect of 2-TEA. The present data do not rule out a participation of protein kinase C in the synergistic response triggered by $H_1$-receptor stimulation but suggest a major participation of external $Ca^{2+}$, possibly mediated by inositol phosphates.

Histamine affects target cells in brain via interaction with $H_1$-receptors apparently coupled with the phosphatidylinositol cycle (1–3) and $H_2$-receptors coupled with adenylate cyclase (4). However, stimulation of $H_1$-receptors enhances cAMP formation in brain slices triggered by direct activators of the cyclase such as histamine $H_2$-, adenosine receptor agonists, or vasoactive intestinal peptide (5–9), leading to a strong amplification of the signal. For instance, the maximal increase of the histamine-sensitive adenylate cyclase in membranes (mediated by $H_2$-receptors) is by 2- to 3-fold, whereas stimulation of cAMP accumulation in slices (via both $H_1$- and $H_2$-receptor mechanisms) can be as high as 15-fold. Because $H_1$-receptors are not coupled to the cyclase, this may suggest that the intracellular signals generated by the phosphatidylinositol cycle, i.e., diacylglycerol and/or inositol phosphates, promote an increased activity of the cyclase via protein kinase C activation and/or calcium mobilization, respectively (10–13). Phorbol esters, which mimic diacylglycerol in activating intracellular protein kinase C (14), enhance cAMP accumulation elicited by various hormones in pinealocytes (15), lymphoma cells (16), anterior pituitary cells (17), brain particulate fractions (18), brain slices (19), or pheochromocytoma cells (20). Furthermore, these compounds fully mimic the effects of $H_1$-receptor activation in potentiating the cAMP response elicited by adenosine receptor stimulation in a brain particulate fraction, suggesting that the diacylglycerol branch of the phosphatidylinositol cycle is mainly, if not solely, involved in the synergism (18). However, this view was recently challenged because 4$\beta$-PMA, a phorbol ester, failed to influence in the same manner as histamine the adenosine-stimulated accumulation of cAMP in slices of guinea pig cortex (21).

In the present study we have assessed the participation of

**ABBREVIATIONS:** 4$\beta$-PMA, 4$\beta$-phorbol 12-myristate, 13-acetate; 2-TEA, 2-thiazolylethylamine; 4$\beta$-PDB, 4$\beta$-phorbol 12,13-dibutyrate; 4$\alpha$-PDD, 4$\alpha$-phorbol 12,13-didecanoate; EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-*N,N,N,′,N′*-tetraacetic acid.

the two branches of the cycle in the synergism between $H_1$- and $H_2$-receptors in the histamine-induced stimulation of cAMP accumulation in hippocampal slices, using phorbol esters and/or removing $Ca^{2+}$ from the external medium.

## Materials and Methods

Hippocampal slices from male Hartley guinea pigs (300–350 g) (Iffa-Credo, France) were prepared and used as described (7). Briefly, pooled slices (250 μm thick) prepared with a McIlwain tissue slicer were first incubated for 30 min at 37° in a modified Krebs-Ringer bicarbonate medium under a constant stream of $O_2/CO_2$ (95:5) with agitation. After twice washing the slices with fresh medium, 200-μl aliquots of the tissue suspension (corresponding to 0.5 mg of protein) were distributed to incubation tubes and preincubated for 15 min at 37° (alone or with phorbol esters when required) before addition of various test agents. Fifteen min later, incubations were stopped by sonication and the homogenates were heated at 95° for 8 min. cAMP concentration was determined by the protein binding assay method (22). Proteins were assayed by the Folin procedure (23) using bovine serum albumin as the standard. Results are expressed as pmol of cAMP/mg of protein, accumulated during the incubation time or as percentages of stimulation over basal level.

$^3$H-cAMP (8-[$^3$H]adenosine 3′,5′-cyclic phosphate, 30 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, U.K.). Dimaprit, impromidine, 2-TEA, and cimetidine were kindly provided by Smith Kline and French Laboratories (U.K.) and mepyramine by Specia (France). Phorbol esters, obtained from Sigma Chemical Co. (St. Louis, MO), were diluted from a stock solution in ethanol and kept at −20°. In the routine experiments where 4β-PDB was used at 1 μM, ethanol was present in the medium at 0.05% concentration (v/v) and had no measurable effect on basal or stimulated cAMP levels. In experiments with the ionophore A 23187 (Sigma), this agent and 4β-PDB were dissolved in dimethyl sulfoxide. At its maximal concentration in the incubation medium (0.1%) this solvent did not modify either basal or stimulated cAMP levels. $EC_{50}$ values were obtained by fitting the data with an iterative computer least squares method (24). Statistical evaluation of the results was by Student's *t* test.

## Results

**Effect of phorbol esters on the stimulation of cAMP accumulation mediated by histamine receptors.** 4β-PDB strongly enhanced the maximal stimulation of cAMP accumulation elicited by the selective $H_2$-receptor agonist dimaprit in a concentration-dependent manner over the range of 0.1–1 μM with an $EC_{50}$ of 0.2 μM (Fig. 1). Another phorbol ester, 4β-PMA, also caused significant increases in cAMP accumulation over dimaprit-stimulated levels at concentrations of 1–10 μM. Because of a solvent effect, it was not possible to use 4β-PMA at concentrations higher than 10 μM, but at this concentration, the effect of 4β-PMA was not far from the maximal effect of 4β-PDB, suggesting that these two phorbol esters induce the same maximal response. In contrast, the inactive phorbol ester 4α-PDD had no stimulatory effect at 10 μM (Fig. 1). In addition, 4α-phorbol or 4-*O*-methyl phorbol 12-myristate, 13-acetate, two other agents inactive on protein kinase C, were also without effect at 10 μM, the per cent stimulation over basal level being 312 ± 16% and 267 ± 17%, respectively, as compared to 292 ± 20% for dimaprit alone after a 40-min preincubation. When the preincubation in the presence of phorbol esters was increased from 15 to 40 min, the potentiating effect of 4β-PDB (0.1 μM) remained unchanged, but 4β-PMA (1 μM) became more effective (Table 1).

The effect of 4β-PDB (1 μM) was also observed in the presence of impromidine, another $H_2$-receptor agonist, or histamine itself, both used at supramaximal concentrations (Table 2). In this series of experiments (as well as in experiments for which data are reported in Table 3), 4β-PDB slightly increased basal cAMP levels. However, this small increase was inconsistent among various experiments, and the effect of 4β-PDB on

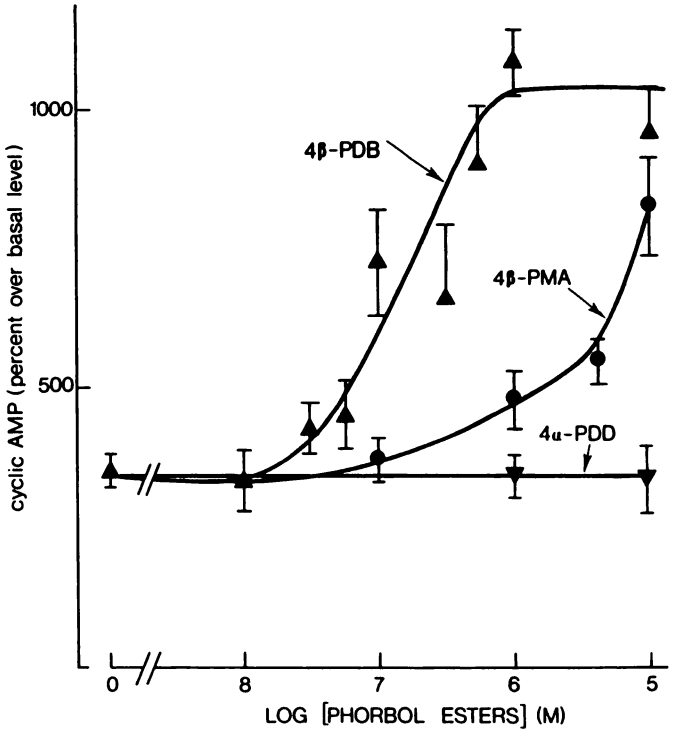


**Fig. 1.** Effect of various phorbol esters on cAMP accumulation elicited by dimaprit, an $H_2$-receptor agonist, in slices of guinea pig hippocampus. Slices were preincubated for 15 min in the presence of phorbol esters or their diluent, and dimaprit (0.3 mM) was then added for a 15-min incubation. Data are expressed as per cent stimulation over basal level (without dimaprit). Each *point* represents the mean ± standard error of values from six to nine incubations. The mean basal cAMP levels (in pmol/mg of protein) were: 5.5 ± 0.8 without phorbol esters, 6.2 ± 0.6 with 1 μM 4β-PDB, 5.7 ± 0.9 with 10 μM 4β-PMA, and 4.4 ± 0.5 with 10 μM 4β-PDD.

TABLE 1

**Influence of preincubation time with phorbol esters on the stimulation of cAMP accumulation elicited by dimaprit in slices of guinea pig hippocampus**

Slices were preincubated for 15 or 40 min with the diluent or phorbol esters before addition of dimaprit and then incubated for 15 min. Mean basal cAMP levels were: in the presence of 4β-PDB, 3.7 ± 0.5 and 3.1 ± 0.4 pmol/mg of protein after 15 and 40 min preincubation, respectively; in the presence of 4β-PMA, 3.1 ± 0.4 pmol/mg of protein and after 15 and 40 min preincubation. Values represent means ± standard errors of data from 11–22 incubations.

| Preincubation time | Agent | cAMP accumulation | |
|---|---|---|---|
| | | pmol/mg of Protein | % Over basal level |
| *min* | | | |
| 15 | None | 4.0 ± 0.7 | |
| | Dimaprit (0.3 mM) | 21.7 ± 2.0 | 442 ± 26 |
| | +4β-PDB (0.1 μM) | 43.3 ± 5.5 | 982 ± 104 |
| | +4β-PMA (1 μM) | 27.7 ± 1.7 | 592 ± 39 |
| 40 | None | 2.9 ± 0.3 | 483 ± 32 |
| | Dimaprit (0.3 mM) | 16.9 ± 1.2 | 900 ± 87 |
| | +4β-PDB (0.1 μM) | 29.0 ± 2.9 | 807 ± 90* |
| | +4β-PMA (1 μM) | 26.3 ± 3.1 | |

*$p < 0.004$ as compared with corresponding value for 15-min preincubation.

TABLE 2

**Effect of 4β-PDB on the stimulation of cAMP accumulation mediated by histamine $H_1$- and $H_2$-receptors**

Hippocampal slices were preincubated for 15 min with 4β-PDB or its diluent and, when required, the histamine receptor antagonists (cimetidine or mepyramine). A further 15-min incubation was performed in the presence of histamine receptor agonists, either alone (histamine and impromidine or dimaprit, two $H_2$-receptor agonists) or in combination (dimaprit together with 2-TEA, an $H_1$-receptor agonist). Mean basal cAMP levels were 5.3 ± 0.8 and 6.3 ± 0.6 pmol/mg of protein in the absence and presence of 4β-PDB, respectively. The percentage over basal level was calculated taking into account the basal level of cAMP in the absence of 4β-PDB. Values represent means ± standard errors of 6–16 incubations.

| Agent | cAMP accumulation (% over basal level) | |
|---|---|---|
| | Without 4β-PDB | With 4β-PDB (1 μM) |
| Histamine (0.45 mM) | 1466 ± 256 | 2721 ± 436[a] |
| Impromidine (10 μM) | 250 ± 39 | 1155 ± 186[a] |
| Dimaprit (0.3 mM) | 281 ± 50 | 1049 ± 106[a] |
| +Cimetidine (0.45 mM) | −27 ± 16[b] | 86 ± 18[b] |
| +2-TEA (1 mM) | 684 ± 76 | 1614 ± 110[a] |
| +2-TEA + mepyramine (0.1 μM) | 352 ± 41[b] | 1121 ± 47[b] |

[a] $p < 0.001$ as compared with corresponding values obtained in the absence of 4β-PDB.

[b] $p < 0.002$ as compared with the corresponding values obtained in the absence of antagonist (cimetidine or mepyramine).

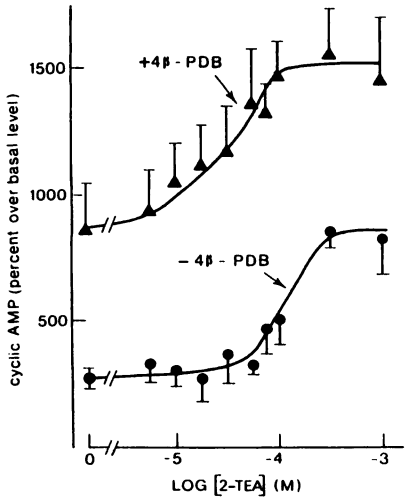


**Fig. 2.** Effect of 4β-PDB on cAMP accumulation elicited by 2-TEA, an $H_1$-receptor agonist, in the presence of dimaprit, an $H_2$-receptor agonist. After a 15-min preincubation with 4β-PDB (1 μM) or its diluent, slices were incubated for 15 min in the presence of dimaprit together with 2-TEA in increasing concentrations. Data are expressed as per cent stimulation over basal level (in the absence of any agent). Each *point* represents the mean ± standard error of values from three to nine incubations. The mean basal cAMP level was 2.6 ± 0.2 pmol/mg of protein.

dimaprit-treated slices was much larger. Cimetidine, an $H_2$-receptor antagonist, blocked the effects of dimaprit used alone or in combination with 4β-PDB (Table 2). 2-TEA, a predominantly $H_1$-receptor agonist, enhanced the dimaprit-induced stimulation, and this response was further enhanced in the presence of 4β-PDB. Thus, the 2-TEA-induced increase in cAMP levels over levels in the presence of dimaprit alone represented 21.0 ± 5.4 and 29.4 ± 7.9 pmol/mg of protein without and with 4β-PDB, respectively.

The extent of the effect of 2-TEA was not significantly changed in the presence of 4β-PDB when varying the incubation time with the agonist from 5 to 25 min (not shown). The $H_1$-receptor antagonist mepyramine (0.1 μM) prevented the potentiation elicited by 2-TEA without or with 4β-PDB being present (Table 2). The 2-TEA concentrations at which half-maximal stimulation ($EC_{50}$) was achieved were 40 ± 7 μM and 122 ± 25 μM in the presence and absence of 4β-PDB, respectively (Fig. 2).

**Influence of external $Ca^{2+}$ on the cAMP response mediated by histamine receptors in the absence or presence of 4β-PDB.** In the absence of phorbol esters, removal of $Ca^{2+}$ from the incubation medium caused only a slight increase in basal cAMP levels, but a further change in the same direction was observed in the presence of EGTA, a calcium-chelating agent (Table 3). Under the same conditions, the response elicited by dimaprit in supramaximal concentration (expressed either in pmol/mg of protein or as per cent stimulation over basal level) was not markedly modified (Table 3). When expressed in pmol/mg of protein, the dimaprit-induced increase was enhanced from 10.1 ± 1.6 (2.6 mM $Ca^{2+}$) to 13.0 ± 1.7 (0 $Ca^{2+}$) and 15.4 ± 1.8 (0 $Ca^{2+}$ + EGTA).

In contrast, the response to 2-TEA in supramaximal concentration was reduced by about 50% (using either mode of expression of data). Thus, at 2.6 mM $Ca^{2+}$, the response to 2-TEA over that to dimaprit was about 3 times higher than that elicited by dimaprit alone, whereas it was almost equivalent to the latter in the absence of $Ca^{2+}$ and inferior to the latter in the absence of $Ca^{2+}$ and the presence of EGTA (Table 3). The response to 2-TEA was progressively restored upon addition of $Ca^{2+}$ at mM concentrations (not shown).

In the presence of 1 μM 4β-PDB, removal of $Ca^{2+}$, particularly in the presence of EGTA, resulted in markedly enhanced basal levels of cAMP (Table 3). Under the same conditions, the response to dimaprit was enhanced when considering the increases in cAMP levels in pmol/mg of protein (49.6 ± 3.9 and 63.4 ± 6.8 in Ca-free and Ca-free + EGTA media, respectively, as compared to 34.1 ± 2.8 in the corresponding control medium), but was decreased when expressed as per cent stimulation over basal level. In contrast, the response to 2-TEA was decreased by at least 50% whatever the mode of expression. For instance, the increase in cAMP levels elicited by 2-TEA over that elicited by dimaprit (28.6 ± 5.5 pmol/mg of protein) was almost equivalent to that elicited by dimaprit alone in the presence of 2.6 mM $Ca^{2+}$ but represented less than 25% of the latter in the absence of $Ca^{2+}$ (with or without EGTA).

The $EC_{50}$ value of 2-TEA in stimulating cAMP accumulation over dimaprit was only slightly modified in the absence of $Ca^{2+}$ (180 ± 20 μM as compared to 70 ± 4 μM in the presence of 2.6 mM $Ca^{2+}$) (Fig. 3).

The $Ca^{2+}$ ionophore A 23187 (10 μM) was ineffective by itself but showed a tendency to mimic the potentiating effect of 2-TEA when the concentration of $Ca^{2+}$ in the incubation medium was increased from 2.6 mM to 5.2 mM and the preincubation time extended from 15 to 40 min (Table 4). Under these conditions, the effect of dimaprit, alone or together with either 4β-PDB or 2-TEA, on cAMP accumulation was still present, although less pronounced. The increase in cAMP level elicited by the ionophore was not additive with the response to 4β-PDB or to 2-TEA.

## Discussion

The present work confirms that stimulation of histamine $H_1$-receptors in brain slices leads to a marked enhancement of the

TABLE 3

**Influence of 4β-PDB and calcium ions on the cAMP response to dimaprit and 2-TEA**

After a 30-min preincubation at 37° in a normal Krebs-Ringer medium, the pooled slices were washed four times in either a calcium-free or a normal Krebs-Ringer solution. A further 15-min preincubation of aliquots of the slice suspension was followed by a 15-min incubation in the presence of dimaprit alone or together with 2-TEA. When required, EGTA was added 2 min before the agonists. The percentage over basal level was calculated taking into account the basal level of cAMP under the same conditions in the absence of 4β-PDB. Values represent the means ± standard errors of data from six to nine incubations.

| Conditions | cAMP accumulation | | | |
|---|---|---|---|---|
| | Without 4β-PDB | | With 4β-PDB (1 μM) | |
| | *pmol/mg protein* | *% over basal level* | *pmol/mg protein* | *% over basal level* |
| With calcium (2.6 mM) | | | | |
| Controls | 3.4 ± 0.4 | | 6.9 ± 0.9 | 103 ± 25 |
| Dimaprit (0.3 mM) | 13.5 ± 1.6 | 298 ± 47 | 41.0 ± 2.7 | 1106 ± 81 |
| Dimaprit (0.3 mM) + 2-TEA (1 mM) | 38.4 ± 2.3 | 1033 ± 68 | 69.6 ± 4.8 | 1948 ± 143 |
| Calcium-free | | | | |
| Controls | 5.5 ± 0.5* | | 19.1 ± 1.5 | 248 ± 27 |
| Dimaprit (0.3 mM) | 18.5 ± 1.6 | 236 ± 30 | 68.7 ± 3.6 | 1148 ± 66 |
| Dimaprit (0.3 mM) + 2-TEA (1 mM) | 35.1 ± 2.8 | 539 ± 50* | 80.7 ± 4.8 | 1367 ± 87* |
| Calcium-free + EGTA (0.1 mM) | | | | |
| Controls | 7.0 ± 0.9* | | 28.2 ± 4.8 | 293 ± 69 |
| Dimaprit (0.3 mM) | 22.4 ± 1.6 | 210 ± 23 | 91.6 ± 4.9 | 1198 ± 70 |
| Dimaprit (0.3 mM) + 2-TEA (1 mM) | 33.1 ± 2.2 | 362 ± 31* | 107.2 ± 7.0 | 1422 ± 101* |

*$p < 0.01$ as compared with the corresponding conditions in the 2.6 mM $Ca^{2+}$ incubation medium.

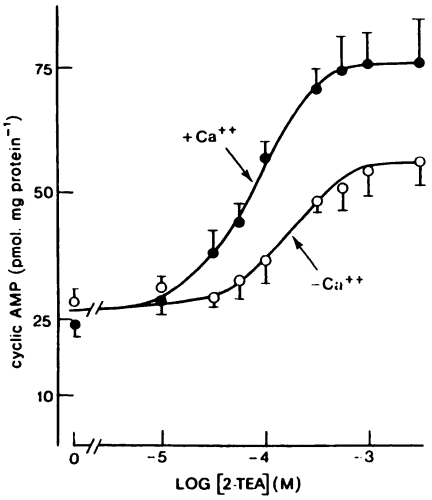


**Fig. 3.** Stimulation of cAMP accumulation elicited by 2-TEA in the presence of dimaprit: influence of calcium ions. After a 30-min preincubation in a normal Krebs-Ringer medium, the pooled slices were washed four times in a calcium free or a normal ($Ca^{2+}$ = 2.6 mM) Krebs-Ringer solution. A 15-min incubation of aliquots of slices was followed by a 15-min incubation in the presence of dimaprit (0.1 mM) and increasing concentrations of 2-TEA in the appropriate Krebs-Ringer medium. Data are expressed as pmol of accumulated cAMP/mg of protein. Each *point* is the mean ± standard error of values from four incubations. The mean basal cAMP levels (in pmol/mg of protein) were 6.0 ± 0.8 and 6.7 ± 0.8 in the absence and presence of $Ca^{2+}$, respectively.

TABLE 4

**Influence of the calcium ionophore A 23187 on the cAMP response to dimaprit and 2-TEA**

After a 30-min preincubation at 37° in a normal Krebs-Ringer medium, the pooled slices were washed four times in a Krebs-Ringer solution containing 5.2 mM $Ca^{2+}$. A further 40-min preincubation of aliquots of the slice suspension in the presence of ionophore (or dimethyl sulfoxide, 0.1% final concentration), together with 4β-PDB when required, was followed by a 15-min incubation in the presence of dimaprit alone or together with 2-TEA. Mean basal cAMP level was 8.3 ± 0.4 pmol/mg of protein and was not significantly modified by the ionophore (10 μM). Values represent the means ± standard errors of 4–21 determinations.

| Agent | cAMP accumulation (% over basal level) | |
|---|---|---|
| | Without ionophore | With ionophore (10 μM) |
| Dimaprit (0.3 mM) | 166 ± 15 | 223 ± 23* |
| +2-TEA (1 mM) | 377 ± 38 | 326 ± 52 |
| +4β-PDB (1 μM) | 360 ± 32 | 364 ± 85 |

*$p < 0.05$ as compared with the value obtained with dimaprit alone.

cAMP response mediated by $H_2$-receptors directly coupled to the cyclase (25, 26) and analyzes the mechanisms involved in this synergistic effect. $H_1$-receptors seem to be coupled with the phosphatidylinositol cycle (1–3, 27–29), and phorbol esters mimicking the activation of protein kinase C by diacylglycerol also enhance the cAMP responses to various direct activators of adenylate cyclase (15–20). Hence, it might have been assumed that a rise in intracellular diacylglycerol levels elicited by $H_1$-receptor stimulation was responsible for the synergism as it was proposed to be responsible for the synergism between adenosine, a direct activator of the cyclase, and histamine (18).

Indeed, phorbol esters like 4β-PDB and 4β-PMA, known to enter cells and activate protein kinase C (14, 30, 31), markedly enhanced the cAMP response to dimaprit or impromidine, two highly selective $H_2$-receptor agonists, whereas the phorbol ester 4α-PDD, inactive on the kinase, was ineffective. The observations that the $EC_{50}$ of 4 β-PDB and 4β-PMA were higher than corresponding values on the purified protein kinase (14), isolated cells (15–17), or brain particles (18), and that a reverse order of potency of the two compounds was generally found on these preparations do not contradict the hypothesis that activation of the kinase in hippocampal slices was also involved. In other slice preparations, potencies in the μM range have also been found for these phorbol esters (20, 32). Conceivably, as compared with these preparations, the slice preparation offers additional diffusion barriers to an intracellularly acting compound, as suggested by the increased effectiveness of 4β-PMA when longer lasting preincubations were used (Table 1). Also, it progressively appears that several protein kinase C isoenzymes exhibiting differing sensitivity to phorbol esters might be present in different tissues or even brain areas (33).

Nevertheless, it clearly appears that the potentiation of the $H_2$-receptor-mediated response by phorbol esters only partially mimicked the potentiation mediated by $H_1$-receptors. 2-TEA, a predominantly $H_1$-receptor agonist (34), still augmented the cAMP response to dimaprit in the presence of 4β-PDB, all

agents being used in supramaximal concentration. The maximal response to histamine, which interacts with both $H_1$- and $H_2$-receptors, was also enhanced by the phorbol ester. In fact, in the presence of 4$\beta$-PDB, the magnitude of the response attributable to $H_1$-receptor stimulation was unaffected or even slightly enhanced (Table 2), and the $EC_{50}$ of 2-TEA was not significantly modified (Fig. 2). Assuming that all effects of $H_1$-receptor stimulation result from activation of the phosphatidylinositol cycle, this suggests that, among the two intracellular signals generated by the cycle (10–13), inositol phosphates, which act by calcium mobilization, were mainly involved in the synergism. In support of this idea, the $Ca^{2+}$ ionophore A 23187, causing an influx of $Ca^{2+}$, mimicked the $H_1$-histaminergic-induced potentiation of the cAMP response, although to a lesser extent. In addition, omission of $Ca^{2+}$ from the external medium, which did not affect or even slightly increased the $H_2$-receptor-mediated response (depending on the mode of expression of data), reduced the $H_1$-receptor-mediated response without or with 4$\beta$-PDB being present (whatever the mode of data expression), particularly in the presence of EGTA, a calcium-chelating agent. Such an effect of calcium ion removal agrees with recent findings on rabbit cerebral slices (35).

This reduction is not due to the blockade of a tetrodotoxin-sensitive release of an endogenous substance because tetrodotoxin does not prevent the cAMP response mediated by $H_1$-receptors (not shown). The calcium dependence of a large variety of $H_1$-receptor-mediated responses, such as smooth muscle contraction (36), glycogenolysis (37), or stimulation of cGMP accumulation (38), has led to the proposal that all responses triggered by $H_1$-receptor activation involve, either directly or indirectly, a $Ca^{2+}$ translocation process (39). However, in view of the established participation of $Ca^{2+}$ in multiple cellular processes, the mechanism by which $H_1$-receptor activation promotes the amplification of the $H_2$-receptor-mediated accumulation of cAMP is not entirely clear. The absolute $Ca^{2+}$ requirement for the histamine-induced phosphatidylinositol and polyphosphoinositidine breakdown is consistent with the idea that the phosphatidylinositol cycle is involved (27, 40).

It is not known whether, in the absence of extracellular $Ca^{2+}$, the key enzyme, phospholipase C [a $Ca^{2+}$-dependent enzyme (41)], would be inactivated. In such a case, the subsequent inactivation of protein kinase C might play a role in the reduction of the $H_1$-receptor-mediated responses. Among the various inositol phosphates generated by activation of the cycle, inositol 1,4,5-triphosphate mobilizes $Ca^{2+}$ from intracellular stores (41, 42), whereas there is some recent evidence that inositol 1,3,4,5-tetrakisphosphate (in conjunction with inositol 2,4,5-trisphosphate) may be responsible for initiating $Ca^{2+}$ entry from external space into the cell cytosol (42).

However, the present data, particularly the $Ca^{2+}$ requirement for the $H_1$-receptor-mediated synergism in the presence of 4$\beta$-PDB, indicate a major involvement of the inositol phosphate branch of the cycle in this synergism, presumably via a rise in intracellular $Ca^{2+}$. This may not be true for other types of amplification processes since, in a particulate fraction from cerebral cortex, phorbol esters entirely mimic the synergistic responses between adenosine and histamine without further augmenting them, suggesting that, in this system, $H_1$-receptor activation mainly operates through the protein kinase C pathway (18). Note, however, that in slices from the same brain region, a phorbol ester was inactive in this respect (21). In the same model, the ionophore A 23187 failed to potentiate the cAMP response to adenosine, suggesting that raising intracellular calcium was not the mechanism involved in the $H_1$-receptor-mediated response. However, the extent of $Ca^{2+}$ concentration increase in the involved cells is not known, and, in our study, a long preincubation with the ionophore, together with a high $Ca^{2+}$ concentration in the incubation medium, was required in order to evidence the effect of the ionophore.

Interestingly enough, in rat pinealocytes, the $\alpha_1$-adrenergic potentiation of the cAMP response to $\beta$-adrenergic stimulation was mimicked by various agents that elevate cytosolic $Ca^{2+}$, including $Ca^{2+}$ ionophores, $K^+$, or ouabain, suggesting, as in the present model, an essential role of calcium influx (43).

In the case of $H_1$-receptors, the molecular mechanisms by which the two types of signals generated by activation of the phosphatidylinositol cycle enhance the activity of the $H_2$-receptor-linked adenylate cyclase remain to be established. However, it could be postulated that activation of protein kinase C operates via phosphorylation of a guanyl nucleotide-binding protein (44–46) and that cytosolic $Ca^{2+}$ activates the cyclase via binding to the calmodulin-calmodulin-binding protein complex in the catalytic subunit of brain adenylate cyclase (47, 48). Such a $Ca^{2+}$-calmodulin interaction could be a general mechanism for the effects mediated by $H_1$-receptors as, for instance, the glycogenolytic effect of histamine could result from the interaction between intracellular $Ca^{2+}$ and the calmodulin subunit of phosphorylase kinase (49).

From a functional point of view, it should be emphasized that the participation of the two histamine receptor subtypes, each operating via two interrelated signaling systems, in the generation of a single response has, inherently, two important implications: the response occurs over a large range of amplitudes and it offers many opportunities for modulation by a variety of extracellular or intracellular messengers.

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