Biochemical Pharmacology, Vol. 54, pp. 501–507, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00203-7

Histamine-Stimulated Cyclic AMP Formation in the Chick Pineal Gland: Role of Protein Kinase C

Jolanta B. Zawilska,† Agata Woldan-Tambor and Jerzy Z. Nowak*
INSTITUTE OF BIOGENIC AMINES, POLISH ACADEMY OF SCIENCES, ŁÓDŹ, POLAND

ABSTRACT. The role of protein kinase C (PKC) in histamine (HA)-stimulated cyclic AMP formation in intact chick pineal glands was investigated. In the pineal gland of chick HA, 2-methylHA, 4-methylHA, and Nα,Nα-dimethylHA potently increased cyclic AMP accumulation in a concentration-dependent manner. Treatment of intact glands with PKC inhibitors, i.e. chelerythrine and staurosporine, reduced the stimulatory effect of the HA-ergic compounds on cyclic AMP formation. HA, 2-methylHA, 4-methylHA, and Nα,Nα-dimethylHA significantly increased inositol-1,4,5-trisphosphate (IP_3) levels in intact chick pineal glands, indicating their activities on phospholipase C and 1,2-diacylglycerol formation. The stimulatory effect of HA on IP_3 levels was antagonized by aminopotentidine, a potent blocker of H_2-like HA receptors in avian pineal gland. Preincubation of chick pineal glands with a PKC activator, 4β-phorbol 12,13-dibutyrate (4β-PDB), enhanced the accumulation of cyclic AMP elicited by HA, 2-methylHA, 4-methylHA, and Nα,Nα-dimethylHA. On the other hand, 4β-phorbol, inactive on the PKC, was ineffective. Our results point to the possibility that PKC is involved in the regulation by HA of cyclic AMP synthesis in the pineal gland of chick. Furthermore, the cyclic AMP response to pineal HA receptor stimulation can be positively modulated by a concomitant activation of the PKC pathway. BIOCHEM PHARMACOL 54;4:501–507, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. cyclic AMP; histamine; protein kinase C; phorbol esters; pineal gland; chick

Histamine (HA) is an established neurotransmitter/neuromodulator in the central nervous system of mammals [1] and some nonmammalian species [2, 3]. One of the widely reported actions of HA is stimulation of cyclic AMP production, with the effect observed in, among other tissues, brains of the guinea pig, rabbit, and chick [4–8]. Recently, we have shown that HA is a powerful stimulator of cyclic AMP synthesis in intact pineal glands of the chick and duck, but not the rat [7, 9, 10]. Interestingly, the HA-evoked cyclic AMP response in the avian pineal gland was much stronger than that observed in other brain tissues tested (including mammalian tissues), and resulted from stimulation of the HA receptor, whose pharmacological profile is different from that of the H_1-, H_2-, and H_3-subtype of HA receptors [1, 11]. It has been suggested that this pineal HA receptor represents either an avian-specific H_2-like HA receptor or a novel HA receptor subtype [7, 9, 10]. The molecular mechanism underlying HA action on cyclic AMP formation in the pineal gland of birds is unknown in nature, because HA appears to be a weak stimulator of adenylate cyclase activity in membrane preparations of the chick pineal gland§ as well as those of chick cerebral cortex [12]. It is not unlikely that HA may activate the cyclic AMP generating system of the avian pineal gland indirectly, and such a mode of HA action would be in line with the results of electrophysiological experiments demonstrating the ability of the amine to evoke an inward current in dissociated chick pineal cells [10].

Calcium/phospholipid-dependent protein kinase (PKC)‡ is present in a vast majority of different cells [13, 14], including pinealocytes [15]. Activation of PKC results in phosphorylation of numerous protein substrates, and such an action has been linked to a wide variety of biological responses [13, 14]. There is growing evidence that PKC stimulation can significantly influence (either potentiate or inhibit) the formation of cyclic AMP [15–21]. A relevant (in the context of the present study) example of a highly synergistic interaction between PKC and cyclic AMP generation is that occurring in the rat pinealocyte, where α_1-adrenoceptor-related PKC activation strongly amplifies β-adrenoceptor–stimulated cyclic AMP production [15, 19, 22]. It is unknown, at present, whether a similar interaction takes place in the pineal gland of animals other than rat.

This work is an extension of our earlier studies on the nature of the HA-evoked cyclic AMP response in the chick pineal gland, and its aim was: (1) to assess whether the

* Permanent address: Department of Pharmacodynamics, Medical University of Łódź, Muszyńskiego 1, Łódź, 90-151 Poland.
† Corresponding author: Dr. Jolanta B. Zawilska, Institute of Biogenic Amines, Polish Academy of Sciences, P.O. Box-225, Łódź-1, 90-950 Poland. FAX: (48)(42) 815-283.
§ Zawilska JB, Sęk B, Nowak JZ, unpublished observation.
Received 12 August 1996; accepted 7 March 1997.

‡ *Abbreviations:* DAG, 1,2-diacylglycerol; IP_3, inositol-1,4,5,-trisphosphate; HA, histamine; KHM, Krebs-Henseleit medium; 4β-PDB, 4β-phorbol 12,13-dibutyrate; PGE, prostaglandin E; PKC, protein kinase C; PLC, phospholipase C.

stimulatory effect of HA on cyclic AMP accumulation involves PKC; and (2) to see whether the HA response can be modulated by a concomitant activation of PKC. Recently, a strong stimulatory effect of prostaglandin E (PGE) on cyclic AMP formation in chick pinealocytes has been reported [23], inciting us to investigate whether there is a PGE component in the described cAMP effect of HA in chick pineal gland.

MATERIALS AND METHODS

Animals

Male chicks (*Gallus domesticus*, white leghorn) were purchased locally on the day of hatching, and kept in warmed brooders with *ad libi* standard food and water. The animals were raised under a photoperiodic regime of 12 hr light:12 hr dark (lights on between 2300 and 1100 hr) for 2–3 weeks prior to the study. Light intensity near the floor of the animals' room was about 150 lux. The experiments were carried out in strict accordance with the Polish governmental regulations concerning experiments on animals.

On the day of an experiment, the animals were killed by decapitation (always between 1000 and 1100 hr), and their pineal glands were quickly removed and stored (until completion of the tissue collection) in cold, O_2/CO_2 (95:5)-gassed, glucose-containing modified Krebs-Henseleit medium (KHM; containing 118 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11.7 mM D-glucose; pH 7.4).

Assay of Cyclic AMP Formation

The synthesis of [^{3}H]cyclic AMP in isolated pineal glands prelabeled with [^{3}H]adenine was determined by the method of Shimizu *et al.* [24]. In brief, the collected glands were thoroughly washed in an O_2/CO_2 (95:5)-gassed KHM, suspended in 15 mL of fresh KHM, and kept at 37°C for a 20-min adaptation period. Then, the KHM was changed, and [^{3}H]adenine was added to the incubation mixture. After a 60-min incubation at 37°C, the pineals were washed, individually distributed into Eppendorf tubes (HTL, Warsaw, Poland) containing 480 μL of freshly gassed O_2/CO_2 KHM, and preincubated for 10 min (experiments with phorbol derivatives) or 15 min (experiments with inhibitors of protein kinase C) at 37°C. Subsequently, HA and other HA agonists were added, and the incubation continued for 10 min. The reaction was terminated by adding 550 μL of 10% trichloroacetic acid. The glands were sonicated and centrifuged, and the supernatants (of which 50 μL aliquots were saved for determination of total radioactivity) were transferred into test tubes. The [^{3}H]cyclic AMP formed was isolated by a sequential cochromatography using Dowex 50Wx4 and aluminum oxide columns (Bio-Rad Laboratories, Hercules, CA), with a tracer [^{14}C]cyclic AMP used for measurement of the recovery of each assay (the mean recovery was 48–56%) according to the method of Salomon *et al.* [25]. The Dowex column system was eluted with deionized water, and the alumina column was then eluted with 0.1 M imidazole solution. The final eluate was tested for radioactivity in a liquid scintillation counter (Wallac 1410, Pharmacia-LKB, Turku, Finland; ^{3}H/^{14}C channel). Data presented have been corrected for recovery and expressed as percent conversion ([^{3}H]cyclic AMP × 100/ total [^{3}H]).

Determination of Inositol-1,4,5-Trisphosphate (IP_3) Content

The collected pineal glands were thoroughly washed in an O_2/CO_2 (95:5)-gassed KHM, suspended in 15 mL of fresh KHM, and kept at 37°C for a 20-min adaptation period. Then, the glands were washed, individually distributed into Eppendorf tubes containing 480 μL of freshly gassed O_2/CO_2 KHM, and preincubated for 10 min at 37°C. In some experiments, aminopotentidine was added to the preincubation 5 min prior to HA. Subsequently, HA and methylated derivatives of HA were added, and following a 1-min incubation, pineals were quickly removed from the incubation medium and frozen on dry ice. The glands were sonicated in 15% trichloroacetic acid in a proportion of 1 mL/gland and centrifuged. IP_3 levels were measured in extracts of tissue supernatants using a specific IP_3 [^{3}H] radioreceptor assay kit (Du Pont de Numerous-NEN, Boston, MA).

Chemicals

Radioactive compounds were [2,8-^{3}H]adenine (specific activity 26.9 Ci/mmol) and [^{14}C]cyclic AMP (specific activity 52.3 mCi/mmol); both from Du Pont de Numerous-NEN, Bad Homburg, Germany).

Histamine-2HCl was purchased from Serva (Heidelberg, Germany). 2-methylhistamine-2HCl (2-MeHA), 4-methylhistamine-2HCl (4-MeHA), and Nα,Nα-dimethylhistamine-HCl (Nα,Nα-DiMeHA) were generously donated by Profs. R. C. Ganellin and M. E. Parsons (SmithKline & Beecham, Herts, UK). Aminopotentidine was a generous gift from Prof. H. Timmerman (Vrije Universiteit, Amsterdam, The Netherlands). Staurosporine and chelerythrine chloride were purchased from Research Biochemicals Incorporated (Natick, MA). 4β-Phorbol, 4β-phorbol 12,13-dibutyrate (4β-PDB), cyclic AMP, and Dowex 50Wx4 were obtained from Sigma Chemical Co. (St. Louis, MO); and aluminum oxide was from Aldrich (Steinheim, Germany). Indomethacin was supplied by Polfa Pharmaceuticals S.A. (Rzeszów, Poland). Other chemicals were of analytical purity and were purchased from commercial sources. All drug solutions were prepared immediately before use.

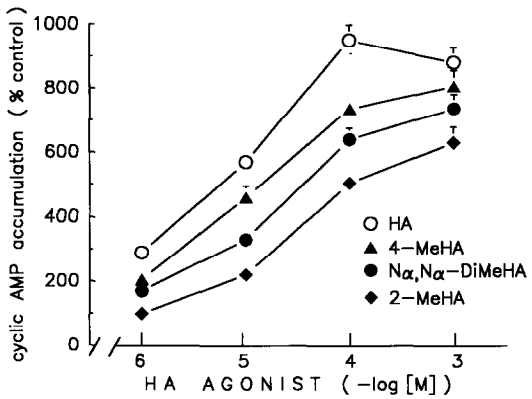

FIG. 1. Effects of HA, 2-methylhistamine (2-MeHA), 4-methylhistamine (4-MeHA), and Nα,Nα-dimethylhistamine (Nα, Nα-DimeHA) on [^{3}H]cyclic AMP synthesis in [^{3}H]adenine-prelabeled chick pineal gland. Results are expressed as percentages of basal [^{3}H]cyclic AMP accumulation. The mean levels of basal [^{3}H]cyclic AMP accumulation (expressed as percent conversion) were: *experiments with HA*, 0.31 ± 0.03 (n = 12); *experiments with 2-MeHA*, 0.28 ± 0.04 (n = 10); *experiments with 4-MeHA*, 0.50 ± 0.04 (n = 10); *and experiments with Nα,Nα-DiMeHA*, 0.46 ± 0.07 (n = 11). Data are mean ± SEM values (n = 4–12).

Data Analysis

All data are expressed as mean ± SEM values. For statistical evaluation of results, ANOVA was used followed by the Newman-Keuls test.

RESULTS

In agreement with earlier reports [7, 10], HA (0.1–1000 μM) potently stimulated cyclic AMP formation in the chick pineal gland in a concentration-dependent manner, showing maximal effects at 100 μM (Fig. 1). The action of HA was mimicked by several methylated derivatives of HA, with the following rank order of the magnitude of the cyclic AMP response to the different agonists: HA > 4-methylhistamine (4-MeHA) > Nα,Nα-dimethylhistamine (Nα,Nα-DiMeHA) > 2-methylhistamine (2-MeHA) (Fig. 1). Indomethacin, a potent cyclooxygenase inhibitor, did not significantly modify the stimulatory effect of HA on cyclic AMP synthesis. The results (expressed in percent conversion) were: control, 0.67 ± 0.08(10); HA 100 μM, 3.35 ± 0.27(12); HA + indomethacin 1 and 10 μM, 2.67 ± 0.36(12), and 3.07 ± 0.43(11); indomethacin alone did not affect the basal activity.

Two inhibitors of PKC, i.e. staurosporine and chelerythrine, were tested for their ability to modulate the stimulatory effect of HA and methylated HAs on cyclic AMP accumulation in chick pineal glands prelabeled with [^{3}H]adenine. Staurosporine markedly inhibited the response to HA; at 0.1 μM it decreased the effect of 10 and 100 μM of HA by 18% (*nonsignificant*) and 37% (P < 0.05), respectively, while a 10-fold higher concentration of this inhibitor (i.e. 1 μM) significantly reduced the respective effects of 10 and 100 μM of HA by 43% and 60% (Table 1). Similarly, 1 μM staurosporine reduced the stimulatory effects of 2-MeHA (1,000 μM) and Nα,Nα-DiMeHA (100 μM) on cyclic AMP formation in chick pineal gland by approximately 56%, and that of 4-MeHA (100 μM) by 48% (Table 1). Chelerythrine, another tested PKC inhibitor, potently antagonized increases in [^{3}H]cyclic cAMP synthesis evoked by both HA and HA derivatives, producing near complete abolishment of the observed effects at a 50 μM concentration (Fig. 2, Table 2).

HA (10–1000 μM), 2-MeHA, 4-MeHA, and Nα,Nα-DiMeHA (all at 10 and 1000 μM), concentration dependently increased the level of IP_3 in chick pineal gland (Fig. 3). The tested HA-ergic compounds showed similar activity on IP_3 formation, and at 1000 μM the response was in the range of 337.6–391.0% of the control value. An H_2-HA receptor blocker, aminopotentidine, antagonized the effect of HA on IP_3 formation. The results (expressed in pmol formed IP_3/pineal) were: control, 5.96 ± 1.47(4); HA 100 μM, 15.10 ± 2.11(4); HA 100 μM + aminopotentidine 3 μM, 6.36 ± 1.82(4); aminopotentidine 3 μM, 5.69 ± 1.11(3); *P < 0.05 versus HA.

In another series of experiments, effects of a 4β-phorbol ester—an activator of PKC—on the cyclic AMP responses to HA and HA agonists in the chick pineal gland were examined. Treatment of pineal glands with 1 μM of 4β-PDB significantly enhanced the stimulatory effect of HA, 2-MeHA, 4-MeHA, and Nα,Nα-DiMeHA (each used at a 10 μM concentration) on cyclic AMP formation (Fig. 4). Addition of 1 μM of 4β-PDB alone produced small elevations in basal cyclic AMP accumulation, with the maximal observed percent increase over basal level being approximately 48% (P < 0.05). The effect of 4β-PDB on the HA-evoked increase in cyclic AMP formation in chick pineal glands was blocked by PKC inhibitors, i.e. chelerythrine (in % conversion: control, 0.31 ± 0.05; HA 10 μM, 2.51 ± 0.17; 4β-PDB 1 μM + HA 10 μM, 3.52 ± 0.32; chelerythrine 50 μM + 4β-PDB 1 μM + HA 10 μM, 0.77 ± 0.08*. *P < 0.05 versus 4β-PDB + HA, n = 5–7/group) and staurosporine (data not shown).

4β-phorbol (10 μM), an inactive phorbol compound on PKC, did not markedly modify the HA-evoked elevation of cyclic AMP formation (in % conversion: control, 0.28 ± 0.06; 4β-phorbol 10 μM, 0.33 ± 0.07; HA 10 μM, 3.09 ± 0.46; 4β-phorbol + HA, 2.72 ± 0.28. n = 6–8/group).

DISCUSSION

The present work confirms that HA and some of its methylated derivatives (with the exception of *tele*-methylhistamine, however; [7]) markedly stimulate the process of cyclic AMP formation in organ-cultured chick pineal glands prelabeled with [^{3}H]adenine. This HA-related effect may be mediated via HA H_2-like receptor, as selective H_2-blockers, e.g. tiotidine or aminopotentidine, showed antagonistic activity [7, 10]. However, a coupling of this supposed avian H_2-like receptor with adenylate cyclase

TABLE 1. Effects of staurosporine on [^{3}H]cyclic AMP accumulation elicited by agonists of HA receptors in pineal glands prelabeled with [^{3}H]adenine

Group		Cyclic AMP accumulation (% conversion)	Cyclic AMP accumulation (% control)
Experiment 1			
Control		0.24 ± 0.04 (14)	100
Staurosporine	1 μM	0.27 ± 0.08 (11)	113
Histamine	10 μM	2.52 ± 0.24 (13)	1059
	100 μM	3.73 ± 0.42 (15)	1567
Staurosporine + Histamine	10 μM	1.58 ± 0.09 (10)*	664
	100 μM	1.97 ± 0.12 (11)†	828
Experiment 2			
Control		0.20 ± 0.03 (9)	100
Staurosporine	1 μM	0.23 ± 0.04 (11)	113
2-Methylhistamine	1,000 μM	2.05 ± 0.17 (6)	1000
4-Methylhistamine	100 μM	2.72 ± 0.32 (6)	1327
Nα,Nα-Dimethylhistamine	100 μM	2.36 ± 0.23 (5)	1153
Staurosporine + 2-Methylhistamine		1.05 ± 0.09 (6)‡	513
Staurosporine + 4-Methylhistamine		1.64 ± 0.18 (7)§	800
Staurosporine + Nα,Nα-Dimethylhistamine		1.18 ± 0.06 (5)#	576

Data are mean ± SEM values from 5–14 separate determinations.
* $P < 0.05$ when compared with histamine 10 μM.
† $P < 0.05$ when compared with histamine 100 μM.
‡ $P < 0.05$ when compared with 2-methylhistamine.
§ $P < 0.05$ when compared with 4-methylhistamine.
# $P < 0.05$ when compared with Nα,Nα-dimethylhistamine.

seems uncertain, as in broken-cell or membrane preparations of the chick pineal§ and cerebral cortex [12] HA only weakly stimulated the cyclic AMP-synthesizing enzyme. This may suggest the existance of a more complex mode of action of HA on cyclic AMP generation in the bird pineal gland. The possibility of a role of PGE (which strongly stimulates cyclic AMP accumulation in chick pineal cells in culture; [23]) as an intermediate in HA action can be ruled out on the basis of the ineffectiveness of indomethacin, a potent inhibitor of cyclooxygenase, a prostaglandin-synthesizing enzyme, to significantly prevent HA activity. Therefore, a prostaglandin-independent mechanism(s) seems to underlie the HA effects in the chick pineal gland.

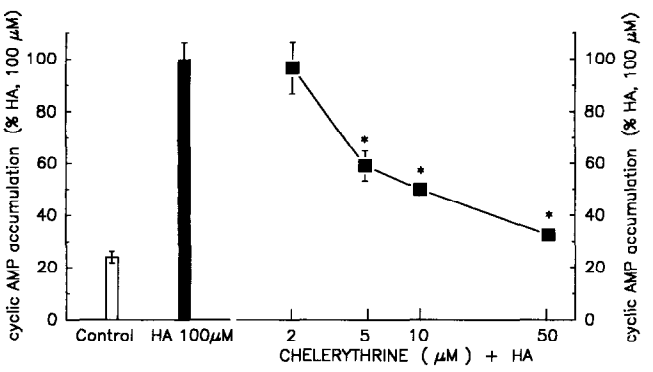

FIG. 2. Effect of chelerythrine on the HA-evoked increase in [^{3}H]cyclic AMP formation in [^{3}H]adenine-prelabeled chick pineal gland. Glands were preincubated for 15 min in the presence of various concentrations of chelerythrine or its dilutent, and HA (100 μM) was then added for a 10-min incubation. Results are expressed as percentages of the HA response at 100 μM [2.93 ± 0.26% (n = 15) conversion, defined as 100%]. Data are mean ± SEM values (n = 6–15). *$P < 0.05$ versus HA.

A growing number of reports in the literature indicate that two enzymes of central importance in receptor-mediated transmembrane signaling, adenylate cyclase and Ca^{2+}/phospholipid-dependent protein kinase (PKC), may interact in both a synergistic or antagonistic manner [13, 14]. Generally, the mode of such an interaction is dependent on the type of cell/tissue studied, reflecting, primarily, the expression of various types of adenylate cyclase as well as isozymes of PKC. In the brain and neurally derived tissues, PKC activation is frequently reported to increase cyclic AMP formation [13–15, 26].

To investigate the participation of PKC in the stimulatory action of HA on cyclic AMP formation in the chick pineal gland, we have employed two inhibitors of PKC, staurosporine and chelerythrine [26, 27]. Prior treatment of the cultured pineal glands with staurosporine or chelerythrine did not affect the basal cyclic AMP accumulation. On the other hand, such a treatment resulted in a marked reduction (or even abolishment in the case of chelerythrine) of increases in cyclic AMP formation evoked by both HA and the studied methylated derivatives of HA. These data indicate that PKC may contribute in a significant manner to the HA-dependent regulation of cyclic AMP synthesis in the chick pineal gland. By analogy to earlier observations in guinea pig brain with HA [18] and rat pineal with NA [15], two examples of a highly synergistic

TABLE 2. Effects of chelerythrine on [^{3}H]cyclic AMP accumulation elicited by agonists of HA receptors in chick pineal glands prelabeled with [^{3}H]adenine

Group		Cyclic AMP accumulation (% conversion)	(% control)
Control		0.45 ± 0.05 (9)	100
Chelerythrine	50 μM	0.51 ± 0.08 (7)	113
2-Methylhistamine	1,000 μM	2.34 ± 0.16 (6)	520
4-Methylhistamine	100 μM	2.82 ± 0.17 (8)	626
Nα,Nα-Dimethylhistamine	100 μM	2.48 ± 0.39 (3)	551
Chelerythrine			
+ 2-Methylhistamine		0.73 ± 0.08 (7)*	162
+ 4-Methylhistamine		0.97 ± 0.08 (8)†	215
+ Nα,Nα-Dimethylhistamine		0.74 ± 0.02 (4)‡	164

Data are mean ± SEM values from three to eight separate determinations.
* $P < 0.05$ when compared with 2-methylhistamine.
† $P < 0.05$ when compared with 4-methylhistamine.
‡ $P < 0.05$ when compared with Nα,Nα-dimethylhistamine.

interaction between receptor-related cyclic AMP generation and PKC, it is not unlikely that the PKC-dependent phosphorylation of the chick pineal adenylyl cyclase may sensitize the enzyme in such a way that even a weak signal provided by HA may be sufficient to substantially stimulate cyclic AMP formation.

The concentrations of staurosporine and chelerythrine effective against the HA- and methylated HA-evoked increases in cyclic AMP formation were, 10–30-fold and 10-fold, respectively, those required to inhibit partially purified PKC [27–29]. Such a discrepancy may reflect, among other factors, the rate of penetration of these compounds into target cells within the pineal gland. In line with this assumption is the observation of Vesegna *et al.* [29] that staurosporine inhibited partially purified PKC from rat basophilic leukemia cells with an IC_{50} value of 3 nM, while it reduced the 4β-PMA–induced activation of PKC in intact cells with an IC_{50} of 0.9 μM. Furthermore, in human embryonic kidney cells transfected with adenylate cyclase type II, 0.1 μM of staurosporine inhibited an increase in cyclic AMP formation produced by 4β-PDB by only 52% [26].

The effectiveness of PKC inhibitors in counteracting the HA-evoked stimulation of cyclic AMP production in the chick pineal implies that HA may activate PKC in this tissue. 1,2-Diacylglycerol (DAG) is a physiological activator of PKC [13, 14], and its origin is mainly (although not exclusively) linked to cell-surface receptor-activated phospholipase C (PLC), which splits cell membrane phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP_3; an intracellular Ca^{2+} mobilizer) and DAG [30]. Thus, these two second messengers, i.e. IP_3 and DAG, are

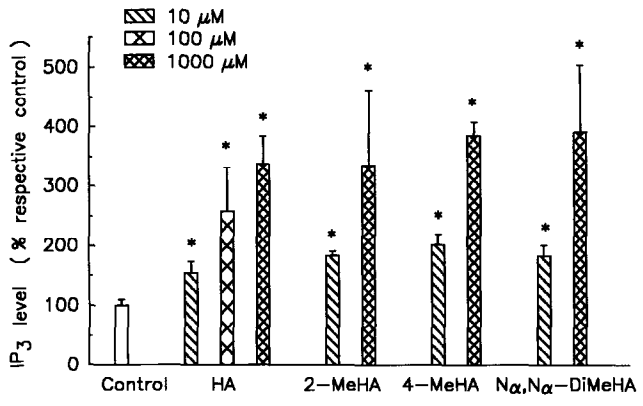

FIG. 3. Effects of HA, 2-methylhistamine (2-MeHA), 4-methylhistamine (4-MeHA), and Nα,Nα-dimethylhistamine (Nα, Nα-DiMeHA) on inositol-1,4,5-trisphosphate (IP_3) accumulation in chick pineal gland. Data are mean ± SEM values (n = 3–11), and are expressed in percent of the respective control value. The mean IP_3 level in the control tissues was 6.85 ± 1.84 (n = 9) pmol/pineal. *$P < 0.05$ versus control.

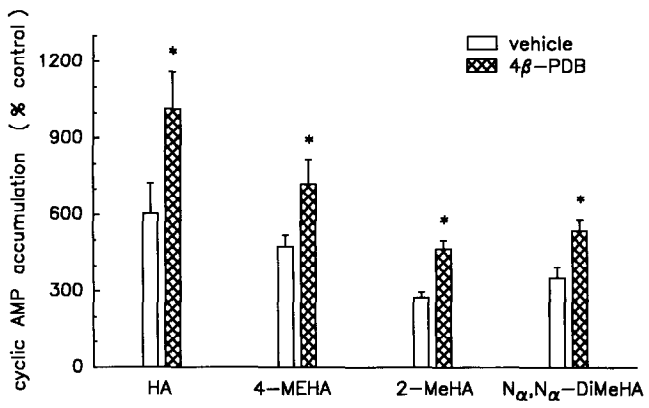

FIG. 4. Effect of 4β-phorbol 12,13-dibutyrate (4β-PDB) on [^{3}H]cyclic AMP accumulation elicited by HA, 2-methylhistamine (2-MeHA), 4-methylhistamine (4-MeHA), and Nα,Nα-dimethylhistamine (Nα,Nα-DiMeHA) in [^{3}H]adenine-prelabeled chick pineal gland. Glands were preincubated for 10 min in the presence of 4β-PDB (1 μM) or its dilutent, and HA or the methylated derivative of HA (10 μM, each) was then added for a 10-min incubation. The mean basal levels of cyclic AMP accumulation (in percent conversion) were in the range of 0.23 ± 0.05—*control*; 0.34 ± 0.09—*4β-PDB* (experiments with 2-MeHA), and 0.50 ± 0.05—*control*; and 0.61 ± 0.09—*4β-PDB* (experiments with 4-MeHA). Data are mean ± SEM values (n = 6–16/group). *$P < 0.05$ versus vehicle.

produced in parallel, and by measuring the accumulation of IP_3 in a given tissue one can assume a concomitant formation of DAG. In the chick pineal gland, HA (and methylated HA derivatives) significantly, and in a concentration-dependent manner, increased the IP_3 level (see Fig. 3), suggesting that the amine has the capability of stimulating PLC activity, and thus DAG production. It is noteworthy that all of the tested compounds, including 2-MeHA and 4-MeHA (selective H_1- and H_2-HA receptor agonists, respectively; [1, 11]), were equally potent in stimulating IP_3 accumulation. The HA-evoked increase in IP_3 levels in the chick pineal gland was a receptor-mediated phenomenon, as it was antagonized by aminopotentidine, a potent blocker of the H_2-like HA receptor in avian pineal gland [7, 9, 10]. In mammalian tissues, activation of phosphoinositide metabolism is specifically linked with the H_1-subtype HA receptor [1, 11]; thus, our "chick" data may further point out an atypical pharmacological characteristic of HA receptor(s) present in avian pineal gland [7–9].

An interaction between intracellular signaling systems linked to adenylate cyclase and PKC is a subject of increasing interest. The tumor-promoting phorbol esters, which activate PKC by substituting for the endogenous activator DAG [12], enhance cyclic AMP accumulation elicited by various hormones and neurotransmitters in a variety of tissues. For example, these compounds have been shown to potentiate increases in cyclic AMP accumulation mediated by HA H_2-receptors in slices of guinea pig hippocampus [18], and by β-adrenoceptors or by vasoactive intestinal peptide in rat pinealocytes [15, 19, 31]. Hence, it can be hypothesized that the HA-evoked stimulation of cyclic AMP formation in the chick pineal gland may also be a subject of modulation by PKC. To test this assumption, we examined the effects of 4β-phorbol ester, i.e. 4β-PDB, known to enter cells and activate PKC (e.g. [32, 33]), on the action of HA and methylated HA derivatives. 4β-PDB enhanced the cyclic AMP response to the tested HA receptor agonists. The specificity of this phenomenon is indicated by two lines of evidence, i.e. the ineffectiveness of 4β-phorbol (a phorbol compound inactive on PKC), and abolishment by the PKC inhibitors (staurosporine and chelerythrine) of the 4β-PDB action on the HA-evoked increase in cyclic AMP formation.

In summary, in this study we have shown that the HA-related increase in cyclic AMP formation in the chick pineal gland can be strongly affected by drugs acting on PKC: (1) PKC inhibitors, such as staurosporine and chelerythrine attenuated (or even prevented in the case of the latter compound) the HA effect, and (2) the PKC activator (β-phorbol ester) enhanced this effect. Thus, these findings may suggest on the one hand the importance of PKC in the HA-triggered cascade leading to stimulation of cyclic AMP formation and on the other, a potential of the PKC pathway in reinforcing the HA-dependent cyclic AMP effect. Although the physiological significance of the HA-stimulated cyclic AMP-generating system in the avian pineal gland remains to be established [34], the present data point to a complexity of biochemical events in this directly photosensitive organ that may follow its exposure to HA.

We thank Professor Hendrik Timmerman for fruitful discussion and critical comments. The skilful assistance of B. Krzemieniewska (M.Sc.) in measurements of IP_3 is highly appreciated. This work was supported in part by grant No. 6 P04A 026 12 from the State Committee for Scientific Research (KBN) in Poland.

References

1. Leurs R, Smit MJ and Timmerman H, Molecular and pharmacological aspects of histamine receptors. *Pharmacol Ther* **66:** 413–463, 1995.
2. Nowak JZ, Histamine in the retina and some other components of the visual system. In: *Progress in Retinal Research*, Vol. 12 (Eds. Osborne NN and Chader GJ), pp. 41–74. Pergamon Press, Oxford, 1993.
3. Nowak JZ, Histamine in the central nervous system: Its role in circadian rhythmicity. *Acta Neurobiol Exp (Suppl)* **54:** 65–82, 1994.
4. Nahorski SR, Rogers KJ and Smith BM, Stimulation of cyclic adenosine 3',5'-monophosphate in chick cerebral hemisphere slices: effects of H_1 and H_2 histaminergic agonists and antagonists. *Brain Res* **126:** 387–390, 1977.
5. Palacios JM, Garbarg M, Barbin G and Schwartz J-C, Pharmacological characterization of histamine receptors mediating the stimulation of cyclic AMP accumulation in slices from guinea pig hippocampus. *Mol Pharmacol* **14:** 971–982, 1978.
6. Al-Gadi M and Hill SJ, Characterization of histamine receptors mediating the stimulation of cyclic AMP accumulation in rabbit cerebral cortical slices. *Br J Pharmacol* **85:** 877–888, 1985.
7. Nowak JZ and Sęk B, Stimulatory effect of histamine on cyclic AMP formation in chick pineal gland. *J Neurochem* **63:** 1338–1345, 1994.
8. Nowak JZ and Zawilska JB, Histamine- and isoprenaline-evoked stimulation of cAMP formation in chick cerebral cortex. *Pol J Pharmacol* **47:** 541–544, 1995.
9. Nowak JZ, Sęk B and Zawilska JB, A bizarre receptor mediating stimulatory effect of histamine on cyclic AMP formation in duck pineal gland. *Neurosci Lett* **202:** 65–68, 1995.
10. Nowak JZ, Sęk B, D'Souza T and Dryer SE, Does histamine stimulate cyclic AMP formation in the avian pineal gland via a novel (non-H_1, non-H_2, non-H_3) histamine receptor subtype. *Neurochem Int* **27:** 519–526, 1995.
11. Hill SJ, Distribution, properties and functional characteristics of three classes of histamine receptor. *Pharmacol Rev* **42:**45–83, 1990.
12. Zawilska JB, Kolodziejczyk M and Nowak JZ. Effects of compounds affecting protein kinase C on histamine-evoked stimulation of cAMP formation in chick cerebral cortex. *Pol J Pharmacol* **48:** 589–594, 1996.
13. Nishizuka Y, Studies and perspectives of protein kinase C. *Science* **233:** 305–312, 1986.
14. Nishizuka Y, Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J* **9:** 484–496, 1995.
15. Sugden D, Vanecek J, Klein DC, Thomas TP and Anderson WB, Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes. *Nature* **314:** 359–361, 1985.
16. Garcia-Sainz JA, Mendlovic F and Martinez-Olmedo MA, Effects of phorbol esters on alpha$_1$-adrenergic-mediated and glucagon-mediated actions in isolated rat hepatocytes. *Biochem J* **228:** 277–280, 1985.

17. Mukhopadhyay AK and Schumacher M, Inhibition of hGC-stimulated adenylate cyclase in purified mouse Leydig cells by the phorbol ester PMA. *FEBS Lett* **187:** 56–60, 1985.
18. Garbarg M and Schwartz J-C, Synergism between histamine H_1 and H_2 receptors in the cAMP response in guinea pig brain slices: Effects of phorbol esters and calcium. *Mol Pharmacol* **33:** 38–43, 1988.
19. Sugden D and Klein DC, Activators of protein kinase C act at a postreceptor site to amplify cyclic AMP production in rat pinealocytes. *J Neurochem* **50:** 149–155, 1988.
20. Gusovsky F and Gutkind JS, Selective effects of activation of protein kinase C isozymes on cyclic AMP accumulation. *Mol Pharmacol* **39:** 124–129, 1991.
21. McKenna JP, Williams JM and Hanson PJ, The α isoform of protein kinase C inhibits histamine-stimulated adenylate cyclase activity in a particulate fraction of the human gastric cancer cell line HGT-1. *Inflamm Res* **44:** 66–69, 1995.
22. Sugden AL, Sugden D and Klein DC, Essential role of calcium influx in the adrenergic regulation of cAMP and cGMP in rat pinealocytes. *J Biol Chem* **261:** 11608–11612, 1986.
23. Voisin P, Van Camp G, Pontoire C and Dollin JP, Prostaglandins stimulate serotonin acetylation in chick pineal cell: Involvement of cyclic AMP-dependent and calcium/calmodulin-dependent mechanisms. *J Neurochem* **60:** 666–670, 1993.
24. Shimizu H, Daly JW and Creveling CR, A radioisotopic method for measuring the formation of adenosine 3′,5′-cyclic monophosphate in incubated slices of brain. *J Neurochem* **16:** 1609–1619, 1969.
25. Salomon Y, Londos C and Rodbell M, A highly sensitive adenylate cyclase assay. *Anal Biochem* **58:** 541–548, 1974.
26. Yoshimura M and Cooper DMF, Type-specific stimulation of adenyl cyclase by protein kinase C. *J Biol Chem* **268:** 4604–4607, 1993.
27. Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M and Tomita F, Staurosporine, a potent inhibitor of phospholipid/Ca^{++}-dependent protein kinase. *Biochem Biophys Res Commun* **135:** 397–402, 1986.
28. Herbert JM, Augereau JM, Gleye J and Maffrand JP, Chelerythrine is a potent and specific inhibitor of protein kinase C. *Biochem Biophys Res Commun* **172:** 993–999, 1990.
29. Vegesna RVK, Wu H-L, Mong S and Crooke ST, Staurosporine inhibits protein kinase C and prevents phorbol ester-mediated leukotriene D_4 receptor desensitization in RBL-1 cells. *Mol Pharmacol* **33:** 537–542, 1988.
30. Berridge MJ and Irvine RF, Inositol phosphates and cell signalling. *Nature* **341:** 197–205, 1989.
31. Chick CI, Ho AK and Klein DC, α_1-Adrenergic potentiation of vasoactive intestinal peptide stimulation of rat pinealocyte adenosine 3′,5′-monophosphate and guanosine 3′,5′-monophosphate: evidence for a role of calcium and protein kinase C. *Endocrinology* **122:** 702–708, 1988.
32. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa Y and Nishizuka Y, Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257:** 7847–7851, 1982.
33. Kraft AS and Anderson WB, Phorbol esters increase the amount of Ca^{++}, phospholipid-dependent protein kinase associated with plasma membrane. *Nature* **301:** 621–623, 1983.
34. Nowak JZ, Zawilska JB, Woldan-Tambor A, Sęk B, Voisin P, Lintunen M and Panula P, Histamine in the chick pineal gland: Origin, metabolism, and effects on the pineal function. *J Pineal Res*, **22:**26–32, 1996.