An activator of protein kinase C (phorbol-12-myristate-13-acetate) augments 2-chloroadenosine-elicited accumulation of cyclic AMP in guinea pig cerebral cortical particulate preparations

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Norepinephrine and histamine markedly augment accumulations of cyclic AMP elicited by 2-chloroadenosine in a guinea pig cerebral cortical vesicular preparation. In addition, these biogenic amines stimulate phosphatidylinositol turnover. Phosphatidylinositol turnover is associated with mobilization of internal calcium and with stimulation of protein kinase C. Phorbol-12-myristate-13-acetate (PMA), a known activator of protein kinase C, has no effect on cyclic AMP levels alone, but in a concentration-dependent manner enhances accumulations of cyclic AMP elicited by 2-chloroadenosine. PMA, like norepinephrine, also enhances accumulations of cyclic AMP elicited by histamine. PMA has no effect on the synergistic accumulations of cyclic AMP elicited by combinations of amines and 2-chloroadenosine. PMA also augments accumulations of cyclic AMP elicited by forskolin. The results suggest that activation of phosphatidylinositol turnover by biogenic amines may lead via stimulation of protein kinase C to enhanced responsiveness of cyclic AMP-generating systems.

Protein kinase C *Phosphatidylinositol turnover* *Phorbol-12-myristate-13-acetate* *2-Chloroadenosine* *Cyclic AMP* *Guinea pig cerebral cortex*

1. INTRODUCTION

Biogenic amines, such as norepinephrine and histamine, can markedly augment accumulations of cyclic AMP elicited by adenosines in rodent brain slices and particulate preparations ([1,2] and references therein). The mechanism(s) involved in these calcium-dependent synergisms [3] is unknown. However, the α_1-adrenergic and H_1-histaminergic receptors that subserve synergistic increases in cyclic AMP in brain slices also stimulate phosphatidylinositol turnover in brain slices [4–6]. Receptor-mediated hydrolysis of phosphatidylinositol forms both inositol phosphates and diacylglycerols [7]. The inositol phosphates stimulate release of calcium from intracellular stores, while diacylglycerol activates protein kinase C. Thus, either calcium release or activation of protein kinase C, initiated by α_1-adrenergic or H_1-histaminergic receptors, might be involved in synergistic cyclic AMP accumulations in brain preparations. Phorbol-12-myristate-13-acetate (PMA), a naturally occurring activator of protein kinase C [8], has now been found to augment 2-chloroadenosine-elicited accumulations of cyclic AMP in a particulate preparation from guinea pig cerebral cortex.

2. MATERIALS AND METHODS

2.1. *Particulate preparations*

The brain particulate preparation [2,9,10] was obtained as follows: brains from two male guinea pigs (210–300 g) were excised after decapitation. Slices of cerebral cortical grey matter were homo-

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genized in 7 ml Krebs-Henseleit (KRBS) buffer which contains 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$ and 20 mM glucose at pH 7.4 and pregassed with 95% O_2–5% CO_2. The homogenate was centrifuged at 2000 × *g* for 10 min. The supernatant was discarded and the pellet resuspended in 20 ml KRBS buffer containing 10 μg/ml adenosine deaminase.

2.2. *Cyclic AMP accumulation*

The resuspended particulate preparation was incubated in 10 ml KRBS buffer in the presence of 0.3 μM [^{3}H]adenine (100 μCi) for 30 min, while being shaken gently and gassed with 95% O_2–5% CO_2. After centrifugation at 1000 × *g* for 10 min and washing, the pellet (~50 mg protein) was resuspended in 50 ml KRBS buffer containing 10 μg/ml adenosine deaminase. After incubation for 30 min, 1 ml aliquots were placed in scintillation vials and incubated an additional 10 min. Agonists were added and after incubation at 37°C for 10 min, each suspension was transferred to a microfuge tube and centrifuged at 1000 × *g* for 5 s. After the supernatant was removed, the pellet was vortex-mixed with 1.2 ml of 6% trichloroacetic acid containing 230 μl of 1 mM cyclic AMP. Isolation of [^{3}H]cyclic AMP was as described [1]. Results are expressed as percentage of total [^{3}H]-adenine nucleotides present as [^{3}H]cyclic AMP.

2.3. *Phosphatidylinositol turnover*

The resuspended guinea pig cerebral cortical particulate preparations were incubated in 10 ml KRBS at 37°C for 30 min with 0.5 μM [^{3}H]inositol (0.85 μCi) with shaking and gassing with 95% O_2–5% CO_2. After centrifugation at 1000 × *g* for 10 min and washing, the pellet (~50 mg protein) was resuspended in 50 ml KRBS buffer and 1 ml aliquots were incubated with various agents in the presence of 10 mM LiCl. The inositol phosphates, after removal of lipids with a chloroform:methanol mixture, were isolated by ion-exchange chromatography essentially as described in [11].

3. RESULTS

The phorbol ester (PMA) has no effect on basal accumulation of cyclic AMP, but augments the 2-chloroadenosine response by 60% in a dose-dependent manner with an apparent EC_{50} of about 5 μM (fig.1). This EC_{50} is comparable to the EC_{50} of 2 μM reported for the effects of PMA in parotid slices [12], but is much higher than the concentrations required in cell-free systems to activate protein kinase C [8]. PMA (10 μM) also augments the accumulation of cyclic AMP elicited by 2-chloroadenosine by about 40% in brain slices (not shown). PMA augments the accumulations of cyclic AMP elicited by another agonist, histamine, and by a direct activator of adenylate cyclase, forskolin (fig.2). Synergistic accumulations of cyclic AMP elicited by combinations of norepinephrine and 2-chloroadenosine, histamine and 2-chloroadenosine, or norepinephrine and histamine are not further augmented by the presence of PMA (fig.2). 4-*O*-Methyl-PMA (10 μM), a phorbol ester that is inactive with respect to protein kinase C [8], has no effect on the 2-chloroadenosine-elicited accumulation of cyclic AMP (not shown).

Norepinephrine and histamine elicit significant accumulations of inositol phosphates in guinea pig cerebral cortical vesicular preparations: during 60 min incubations of [^{3}H]inositol-labeled pre-

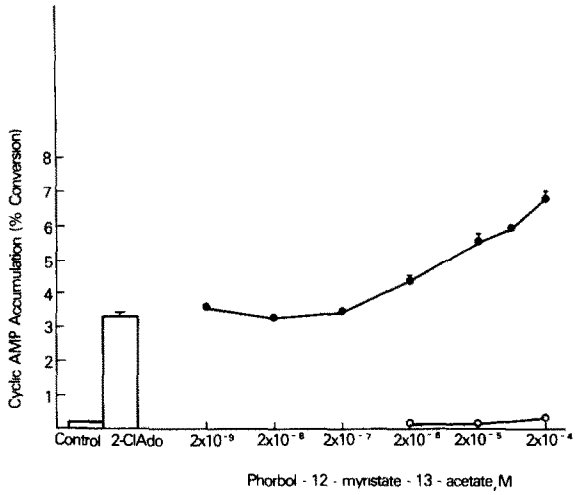

Fig.1. Accumulations of radioactive cyclic AMP elicited by phorbol-12-myristate-13-acetate (PMA, 2×10^{-9} to 2×10^{-4} M) alone (○) and in the presence of 2-chloroadenosine (●) in [^{3}H]adenine-labeled Krebs-Henseleit particulate preparations from guinea pig cerebral cortex. Incubations were for 10 min. Values are means ± SE of a typical experiment performed in triplicate. Two other experiments gave similar results.

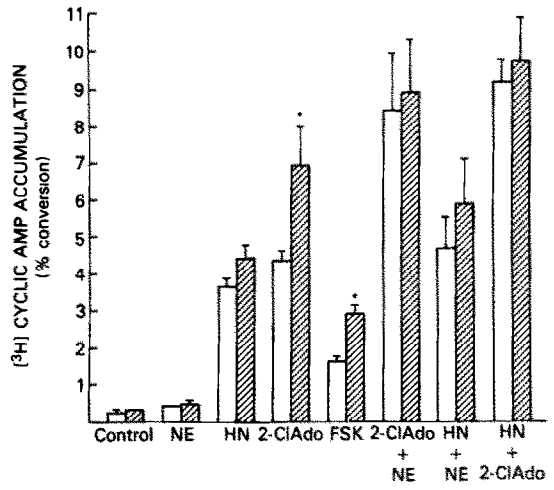

Fig.2. Accumulations of radioactive cyclic AMP elicited by various agents in the presence (hatched bars) or absence (open bars) of phorbol-12-myristate-13-acetate (PMA) (10 μM) in [^{3}H]adenine-labeled Krebs-Henseleit particulate preparations from guinea pig cerebral cortex. Incubations were for 10 min in the presence of 100 μM norepinephrine (NE), or 100 μM histamine (HN), or 100 μM 2-chloroadenosine (2-ClAdo) or 10 μM forskolin (FSK) or combinations thereof. Values are means ± SE of 2–4 experiments run in triplicate. Asterisk denotes a significant greater stimulation by the agent in the presence of PMA ($p < 0.05$).

Table 1

Accumulation of radioactive inositol phosphates in [^{3}H]inositol-labeled Krebs Henseleit particulate preparations from guinea pig cerebral cortex

Agent	[^{3}H]Inositol phosphates (% of control)
Norepinephrine (0.1 mM)	221 ± 27
Histamine (0.1 mM)	138 ± 4
Carbamylcholine (2 mM)	194 ± 14

Particulate preparations were labeled, incubated with agents for 60 min and assayed as described in section 2. Results are expressed as means ± SE of a typical experiment run in triplicate

parations in the presence of 10 mM lithium, norepinephrine increases the accumulation of inositol phosphates by 2.2-fold over basal values, while histamine increases levels by 1.4-fold (table 1). The norepinephrine-response is mediated by an α_1-adrenergic receptor; the histamine-response by an H_1-histaminergic receptor [13].

4. DISCUSSION

Biogenic amines augment the accumulations of cyclic AMP elicited by adenosine and adenosine analogs in brain preparations via interaction with α_1-adrenergic and H_1-histaminergic receptors ([1,2] and references therein). α_1-Adrenergic and H_1-histaminergic receptors have no known direct effects on adenylate cyclase systems. Activation of α_1-adrenergic and H_1-histaminergic receptors does stimulate phosphatidylinositol breakdown in guinea pig cerebral cortical preparations as assessed in the presence of 10 mM Li^+ (table 2; see also [4–6,13]), and it appeared possible that the products of phosphatidylinositol turnover might be involved in synergistic accumulations of cyclic AMP in brain preparations.

It has been proposed that receptor-mediated breakdown of phosphatidylinositol has the following sequelae: (1) calcium release from the endoplasmic reticulum through the action of inositol triphosphate; (2) stimulation of protein kinase C through the action of the diacylglycerol [7]. Diacylglycerol-elicited activation of protein kinase C can be mimicked by PMA [8]. Since PMA, like the biogenic amines, augments the accumulation of cyclic AMP elicited by 2-chloroadenosine, the data suggest that activation of α_1-adrenergic and H_1-histaminergic receptors might augment cyclic AMP accumulation in guinea pig cerebral cortical preparations through stimulation of phosphatidylinositol turnover, thereby generating diacylglycerol, which then activates protein kinase C. PMA does not further augment the synergistic responses, providing evidence supportive of the conclusion that the amines norepinephrine and histamine may act through the same pathway activated by PMA, namely protein kinase C. PMA also augments accumulations of cyclic AMP elicited by forskolin (fig.2). The effect of activation of α_1-adrenergic receptors or H_1-histaminergic receptors on forskolin-responses has not been fully defined [14]. Further studies will be needed to define the protein substrate involved in PMA-mediated augmentation of cyclic AMP-accumulation in brain tissue.

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