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Is histamine potentiation of adenosine-stimulated cyclic AMP accumulation in guinea-pig cerebral cortical slices mediated by products of inositol phospholipid breakdown?

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In several brain tissues the accumulation of cyclic AMP induced by directly acting stimulants, such as isoprenaline, adenosine or histamine (H_2) can be potentiated by histamine H_1-receptor agonists [1–3]. The potentiation of adenosine-coupled cyclic AMP accumulation has proved useful in quantitative studies of functional H_1-receptors in guinea-pig cerebral cortical slices [4, 5], but the recent demonstration that adenosine deaminase and histidine decarboxylase may be present in the same nerve fibres in rat brain [6] has given studies of the nature of the adenosine–histamine H_1-agonist interaction a particular functional relevance.

The mechanism of the potentiating effect of histamine has been generally assumed to be mediated by a rise in free intracellular Ca^{2+} and there is some experimental support for this proposition [7]. However, evidence has been presented recently that in S49 lymphoma cells [8] and in rat pinealocytes [9] the accumulation of cyclic AMP induced by isoprenaline can be stimulated by a phorbol ester, PMA (phorbol 12-myristate-13-acetate), an activator of protein kinase C [10, 11]. The activation of protein kinase C would normally be expected to be the result of agonist-induced inositol phospholipid breakdown, with the consequent formation of 1,2-diacylglycerol [12]. Of particular interest with respect to the mechanism of the H_1-agonist potentiation is the report [13] that 2-chloroadenosine-elicited accumulation of cyclic AMP in particulate preparations from guinea-pig cerebral cortex is enhanced by PMA. However, very much higher concentrations of PMA (>2 μM) were required than those normally necessary for protein kinase C activation (1–10 nM) [11]. We have set out to examine the effect of PMA on the response to adenosine in slices of guinea-pig cerebral cortex and to determine whether a stimulation of cyclic AMP accumulation can be obtained at lower concentrations of PMA if activation of the other arm of the inositol phospholipid pathway, Ca^{2+} release induced by inositol 1,4,5-trisphosphate, is mimicked by the presence of small concentrations of the calcium ionophore A23187.

Materials and methods

Cyclic AMP assay. The accumulation of cyclic AMP in cross-chopped slices (300 × 300 μm, McIlwain tissue chopper) from guinea-pig (Dunkin–Hartley strain, males, 300–400 g) cerebral cortex, preincubated with 4 μM adenine, was measured as described previously [5] using the protein binding assay of Brown *et al.* [14]. Incubation with agonists was for 10 min. Each incubation (total volume 280–300 μl) contained 30–50 μl of the slice suspension. The protein content of successive aliquots varied only modestly (mean ± SE of 22 50-μl samples in one experiment, 2.3 ± 0.1 mg protein) and cyclic AMP content was normally expressed as pmol cyclic AMP/incubation. Quadruplicate measurements were made at each incubation condition and the order of groups (basal, +100 μM adenosine, etc.) was randomised in experiments in a series. The accumulation of cyclic AMP induced by 100 μM adenosine (30–70 pmol per incubation) and 100 μM adenosine + 100 μM histamine (80–160 pmol per incubation) varied between experiments and results from different slice preparations were normalised by setting the response to 100 μM adenosine = 100%.

PMA was dissolved in dimethylsulphoxide and A23187 in ethanol. Equivalent volumes of dimethylsulphoxide or ethanol added alone had no significant effect on basal or adenosine-stimulated cyclic AMP accumulation.

Materials. Adenine, adenosine, adenosine 3′,5′-cyclic monophosphate, adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), forskolin, histamine dihydrochloride, PMA (phorbol 12-myristate-13-acetate) and theophylline were obtained from Sigma, A23187 from Calbiotech, La Jolla, CA, and [8-^{3}H]adenosine 3′,5′-cyclic monophosphate, ammonium salt, from Amersham International.

Results and discussion

Histamine (100 μM) routinely produced a 2–3-fold potentiation of cyclic AMP accumulation induced by 100 μM adenosine. The response to histamine in the absence of added adenosine appeared to be dependent on adenosine released from the tissue, since the addition of adenosine deaminase (2.5 U/ml) reduced the cyclic AMP accumulated to a level below the detection limit of the assay (approx. 3 pmol/incubation), consistent with earlier reports [4, 7]. The EC_{50} for the potentiation by histamine of the response to 100 μM adenosine was 8.4 ± 1.9 μM, with a Hill coefficient (the exponent parameter in the logistic equation fitted, see ref [5]) for the concentration response curve 0.99 ± 0.11 (best-fit values for the combined data from three independent experiments). These values are in accord with those reported previously, 5.1 ± 1.0 μM and 0.97 ± 0.12, respectively [5]. The stimulation of the adenosine response by 100 μM histamine was practically abolished by 1 μM mepyramine, consistent with H_1-receptor involvement.

PMA, in the range 1–100 nM, had no stimulatory effect on the response to 100 μM adenosine (Table 1). On the contrary there appeared to be a progressive inhibition of the response which was statistically significant at 100 nM PMA. A similar percentage inhibition was observed of the response to 100 μM adenosine in the presence of 100 μM histamine (Table 1). The inhibitory effect is not due to enhanced cyclic AMP breakdown. In an experiment in which the cyclic AMP level in the slices was elevated by 20 min exposure to 10 μM forskolin, before addition of PMA (10 nM, 100 nM and 1 μM) and incubation for a further 10 min, there was no significant reduction in the level of cyclic AMP (basal 12 ± 2 pmol/incubation, after forskolin 83.5 pmol/incubation).

The stimulatory effect of PMA in rat pinealocytes [8] or S49 lymphoma cells [9] is rapid in onset, but could be slower in tissue slices, although the inhibitory action of PMA argues against this. To test the possibility of a slow onset slices were incubated with PMA (1, 10 and 100 nM) for 20 min before addition of adenosine and incubation then continued for a further 10 min. No stimulation was observed in two independent experiments and the only difference from the experiments in which PMA was added together with adenosine was that 100 nM PMA did not now cause any inhibition of the adenosine response.

The calcium ionophore A23187 (0.1, 1 and 10 μM) similarly failed to stimulate the response to adenosine (Table 2). At the highest concentration (10 μM) there was a pronounced inhibition (47 ± 3%). In view of the inhibitory

Table 1. Effect of PMA on adenosine- and adenosine + histamine-stimulated accumulation of cyclic AMP

	Cyclic AMP accumulated (% of response to 100 μM adenosine alone) PMA added with	
	No histamine	100 μM histamine
Adenosine + histamine	197 ± 10	212 ± 8
Adenosine	100 ± 6	100 ± 4
PMA (1 nM)	98 ± 6	—
PMA (10 nM)	86 ± 4	191 ± 8
PMA (100 nM)	72 ± 6*	165 ± 6*
PMA (1 μM)	—	155 ± 5*

Values are the weighted means ± SE (calculated as described in ref [15]) from four independent experiments.

* Significantly different ($P < 0.05$) from the value for 100 μM adenosine alone (column 1) or 100 μM adenosine + 100 μM histamine (column 2).

Table 2. Effect of the calcium ionophore A23187 on adenosine-stimulated accumulation of cyclic AMP

Addition	Cyclic AMP accumulated (% of response to 100 μM adenosine)
Adenosine (100 μM) +	
Histamine (100 μM)	237 ± 6 (6)
A23187 (0.1 μM)	79 ± 4* (5)
A23187 (1 μM)	107 ± 4 (6)
A23187 (10 μM)	53 ± 2* (3)

Values are the weighted means ± SE from the number of independent experiments given in parentheses. * Significantly different ($P < 0.05$) from 100 μM adenosine alone (100 ± 3%).

Table 3. Effect of PMA and A23187 in combination on adenosine-stimulated cyclic AMP accumulation

Addition	Cyclic AMP accumulated (% of response to 100 μM adenosine)
Adenosine (100 μM) +	
Histamine (100 μM)	220 ± 8
PMA (10 nM)	80 ± 1*
A23187 (0.1 μM)	101 ± 5
PMA + A23187	85 ± 3

Values are the weighted means from three independent experiments.

* Significantly different ($P < 0.05$) from 100 μm adenosine alone 100 ± 6%.

actions of higher concentrations of both PMA and A23187, concentrations of 10 nM PMA and 100 nM A23187 were selected for experiments designed to look for synergistic effects of raised intracellular Ca^{2+} and activated protein kinase C. The need to avoid high concentrations, and hence non-specific effects, with both these agents has been stressed [10]. However, in 3 independent experiments PMA and A23187, alone or in combination, failed to produce any significant stimulation of adenosine-induced cyclic AMP accumulation (Table 3).

These observations give no indication that the action of histamine in potentiating adenosine-stimulated cyclic AMP accumulation in slices of guinea-pig cerebral cortex is mediated by activation of protein kinase C. Interactions between what may well be the two major second messenger systems, cyclic AMP formation and phosphoinositide breakdown, are now well established. However, of 20 reports since 1984 of studies of phorbol ester effects on cyclic AMP production only seven observed an enhancement, while the remainder reported an inhibition. The effect observed clearly varies between tissues and between cell types and it is conceivable that in tissues containing multiple cell types, such as brain slices, phorbol esters might have effects in opposite directions in differing cell types. The likelihood of actions of PMA other than activation of protein kinase C alone has also been stressed [11], particularly where concentrations of PMA are used which are 100- or 1000-fold greater than that necessary for acti-

vation of the enzyme. The effective concentration of PMA at the cells involved in the adenosine/histamine interaction in our slice preparation is unknown. However, the inhibitory effect on adenosine-induced cyclic AMP accumulation consistently observed with concentrations of PMA ≥ 10 nM argues strongly that PMA does penetrate to the relevant cells. This argument holds even more strongly at higher concentrations of PMA at which the inhibition is greater and there seems no reason to doubt that protein kinase C would be activated, irrespective of whether it has any role in the inhibitory effect. In view of the marked inhibition we have not examined the effect of concentrations of PMA above 1 μM, but in summary, the lack of any potentiation of the adenosine response by lower, and probably physiologically more relevant, concentrations, alone or in combination with A23187, suggests that stimulation of protein kinase C, with or without raised intracellular calcium, is not the mechanism by which histamine and other H_1-agonists have their effect in potentiating cyclic AMP accumulation.

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Effect of gentamicin on the transition temperature and permeability to glycerol of phosphatidylinositol-containing liposomes

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Gentamicin, a cationic aminoglycoside antibiotic widely used in the treatment of gram negative infections [1], accumulates in renal proximal tubular cells [2] where it causes biochemical and structural changes which eventuate in cell necrosis [3, 4]. At physiologic pH, gentamicin carries a net charge of +3.5 and is thought to interact electrostatically with fixed anionic sites on the brush border membrane [2]. These fixed anionic sites may be acidic phospholipids, especially phosphoinositides [5–9], which play an important role in the biochemical and biophysical characteristics of membranes [9, 10]. Binding of gentamicin to these lipids may alter the properties of the membrane, and such alterations may be relevant to the pathogenesis of gentamicin nephrotoxicity. We investigated the effects of gentamicin on the differential scanning calorimetry (DSC*) properties and glycerol permeability of dipalmitoyl phosphatidylcholine (DPPC): phosphatidylinositol (PI) liposomes.

Materials and methods

DPPC and PI (bovine brain) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Gentamicin sulfate was a gift from the Schering Corp. (Bloomfield, NJ).

DSC studies. Multilamellar liposomes of DPPC and DPPC:PI (1:1) were prepared according to published methods [11]. Liposomes, 15 μmol lipid/ml, were incubated with and without gentamicin, 0 to 10^{-4} M, at 50° for 2 hr. Liposomes (20-μl samples) were transferred to aluminium pans and scanned 5° to 60° at a rate of 5°/min in a Perkin–Elmer differential scanning calorimeter in both the heating and cooling modes which gave similar results. The instrument was calibrated with benzoic acid and indium.

Glycerol permeability. Multilamellar liposomes of DPPC:PI (1:1) were prepared as above in 0.15 M KCl, 10 mM Tris, pH 7.0, and incubated with and without gentamicin, 10^{-4} M, at 50° for 1 hr. Liposomes (20 μl) were added to 1 ml of 0.3 M glycerol, and the change in absorbance at 450 nm due to liposome swelling was monitored. The relative permeability coefficient (P) was calculated as $\bar{P} = dA/A_o^2 t^{-1}$ where dA = change of absorbance over time and A_o = initial absorbance [11, 12]. Measurements were done at temperatures ranging between 25° and 32° which is below the T_m of DPPC:PI liposomes. Although most studies of permeability to non-electrolytes have been performed at temperatures above the T_m, studies of non-electrolyte permeation of lipid bilayers can be performed below the T_m [12, 13]. We chose the latter for convenience.

* Abbreviations: DSC, differential scanning calorimetry; E_a, activation energy; DPPC, dipalmitoyl phosphatidylcholine; PI, phosphatidylinositol; and T_m, transition temperature.