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INFLUENCE OF ROLIPRAM ON THE CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE RESPONSE TO HISTAMINE AND ADENOSINE IN SLICES OF GUINEA-PIG CEREBRAL CORTEX

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Abstract—The effect of the phosphodiesterase (PDE) inhibitor rolipram on the cyclic AMP responses to adenosine, histamine and combinations of these two agonists, was examined in [^{3}H]adenine-labelled slices of guinea-pig cerebral cortex. Constant levels of [^{3}H]-cyclic AMP were achieved within 10 min of agonist addition, both in the presence and absence of rolipram (0.1 mM). Histamine (1 mM) produced an 8-fold increase in [^{3}H]-cyclic AMP (compared with basal) which was increased 7-fold by rolipram. The responses to adenosine (0.1 mM) and adenosine and histamine in combination were larger than that to histamine alone (46-fold or more compared with basal) but the potentiation by rolipram was much smaller (2.5-fold or less). With both agonists the effect of rolipram was dose-dependent, the steady state [^{3}H]-cyclic AMP levels increasing 1–2-fold for a 10-fold increase in rolipram concentration. Removal of the histamine or adenosine stimulus once steady state had been reached resulted in a rapid fall in [^{3}H]-cyclic AMP levels with a half time of less than 5 min. Rolipram (0.1 mM) did not significantly alter the initial rates of fall in [^{3}H]-cyclic AMP levels but increased the time taken for them to return to basal levels.

The findings of higher steady state levels of cyclic AMP in the presence of rolipram, together with an almost unaltered rate of cyclic AMP turnover, are consistent with an interaction of rolipram with PDE which is overcome by an increase in cyclic AMP concentration. However, the relatively smaller effects of rolipram on the higher steady levels of cyclic AMP produced by adenosine and the rather shallow dose-dependence of the PDE inhibitor on the responses to both agonists are inconsistent with a simple competitive inhibition of total PDE activity in responding cells. The results can be explained, however, by the involvement of different forms of PDE, with the rolipram-sensitive, calcium-independent form dominating at low cyclic AMP levels and the rolipram-insensitive, calcium-dependent form becoming more important when cyclic AMP levels are higher.

It is now well established that a number of cellular responses (e.g. alterations in membrane potential, cell growth and secretion) elicited by neurotransmitters and hormones are mediated by changes in intracellular cyclic AMP levels [1–3]. There are several ways in which these agents can act. Some alter the rate of cyclic AMP generation directly by interacting with receptors coupled to guanine nucleotide regulatory components of adenylate cyclase [4]. Receptors coupled to the Ns subunit (e.g. histamine H_2, adenosine A_2) stimulate the cyclase, while those coupled to the Ni subunit (e.g. opiate, muscarinic) inhibit it [4–7]. In addition, some neurotransmitters can modify cyclic AMP levels in brain tissues via receptors whose link to the enzymes involved in cyclic AMP turnover seems to be indirect [8–11]. For example, in guinea-pig cerebral cortical slices, the accumulation of cyclic AMP produced by stimulation of adenosine A_2-receptors can be augmented by histamine H_1-receptor stimulation [12–15]. It remains to be established whether the potentiating effect on cyclic AMP levels is exerted by stimulation of the cyclase or by inhibition of the enzyme which breaks down cyclic AMP, phosphodiesterase (PDE).*

Because of the important role of cyclic AMP in cell function, there has been interest recently in modulating intracellular cyclic AMP concentration at the post-receptor level, with the use of inhibitors of PDE. We were interested in the potential use of these agents to study the mechanism by which histamine H_1-receptor stimulation potentiates adenosine-induced cyclic AMP accumulation in guinea-pig brain slices. However, the choice of PDE inhibitor for this purpose was made difficult by the fact that many PDE inhibitors such as the alkyl xanthines (e.g. 3-isobutyl-1-methylxanthine) are also potent adenosine receptor antagonists [16–18].

One PDE inhibitor which does not appear to antagonise adenosine receptors in brain slice preparations is the antidepressant drug rolipram, which potentiates the cyclic AMP responses to both histamine and adenosine [19]. This drug is a rather potent and competitive inhibitor of the cyclic AMP specific, calcium-independent isoform of PDE [20, 21]. This selective action on one particular form of PDE raised the possibility that its effect on the changes in cyclic AMP levels might throw some light on the form of PDE involved in cyclic AMP turnover

* Abbreviations used: PDE, phosphodiesterase.

in cells responding to histamine and adenosine. We report here the effect of rolipram on the accumulation of [^{3}H]-cyclic AMP in slices of guinea-pig cerebral cortex in response to histamine, adenosine and combinations of these two agonists.

MATERIALS AND METHODS

Measurement of [^{3}H]*-cyclic AMP accumulation*

Cyclic AMP production was determined using a modification of the [^{3}H]adenine prelabelling technique [22]. Hartley strain guinea pigs of either sex (200–400 g) were killed by cervical dislocation and decapitation. Slices (300 × 300 μm) of cerebral cortex were prepared with a McIlwain chopper. Slices from one brain were washed with Krebs–Henseleit solution (mM): 118 NaCl; 4.7 KCl; 1.2 $MgSO_4$; 2.5 $CaCl_2$; 1.2 KH_2PO_4; 25 $NaHCO_3$; 5.5 glucose, pH 7.4, gassed with O_2/CO_2 (95:5) and incubated in 40 ml Krebs solution at 37° for 1 hr in a shaking water bath. The Krebs medium was changed every 15 min during this period. The Krebs medium was then decanted and the slices resuspended in 20 ml Krebs solution containing 0.08 μM (40 μCi) [^{3}H]adenine, and the incubation continued for a further 40 min. The prelabelled slices were then washed three times with 50 ml Krebs solution at 37° and finally allowed to settle under gravity.

Portions (50 μl) of the slices were added to 240 μl Krebs containing antagonist, rolipram or adenosine deaminase where appropriate, in flat bottomed insert vials. Tubes were gassed with O_2/CO_2 (95:5), capped and incubated for 20 min at 37°. Agonist was added after this step in 10 μl medium, the tubes gassed again and incubated for a further 10 min unless otherwise indicated. In experiments in which cyclic AMP breakdown was investigated, the adenosine or histamine stimulus was removed at this stage by the addition of adenosine deaminase (to yield a final activity of 1.2 U/ml) or tiotidine (final concentration 30 μM) respectively, in 10 μl of Krebs solution and incubations continued for the appropriate times.

Incubations were terminated by the addition of 200 μl 1 M HCl and left on ice for at least 15 min before being diluted with 0.75 ml distilled water. Slices were precipitated by centrifugation at 1000 *g* for 10 min and a 1 ml sample of the aqueous supernatant was taken for analysis of [^{3}H]-cyclic AMP by column chromatography. Extraction of tritium from the tissue was approximately 97%.

[^{3}H]-cyclic AMP was isolated by sequential Dowex-alumina chromatography essentially as described by Minneman *et al.* [23]. One-millilitre samples were applied to Dowex 50 ion exchange resin (0.6 ml) in plastic Econo columns (Bio-Rad), previously treated with 5 ml 1-M HCl and 10 ml distilled water. Columns were washed with 2 ml distilled water and were then placed directly above similar plastic columns containing 0.6 g neutral alumina (washed with 10 ml of 0.1 M imidazole before use). [^{3}H]-cyclic AMP was eluted with water (4 ml) from the Dowex onto the alumina columns. The alumina columns were then placed directly over scintillation vials and the [^{3}H]-cyclic AMP was eluted in 5 ml of 0.1 M imidazole. Ten millilitres of Packard emulsifier scintillator was added and radioactivity determined by liquid scintillation counting. Recovery of cyclic AMP from the columns was routinely 80%.

Theoretical analysis of the effect of a competitive inhibitor of PDE on the steady state level of cyclic AMP. For a simple competitive inhibition, the activity of PDE, v_{PDE}, is given by

$$v_{PDE} = v_{max} \cdot [A]/([A] + K_m\,(1 + [R]/K_i)) \quad (1)$$

where $[A]$ is the concentration of cyclic AMP, $[R]$ is the concentration of the inhibitor, v_{max} is the maximal PDE activity and K_m and K_i are the Michaelis constants for cyclic AMP and the inhibitor respectively.

Writing $K_m' = K_m\,(1 + [R]/K_i)$, it follows that at steady state, i.e. when the rate of breakdown and production of cyclic AMP are equal, the rate of cyclic AMP production (v_{prod}) is defined

$$v_{prod} = v_{max} \cdot [A]_s/([A]_s + K_m') \quad (2)$$

where $[A]_s$ is the cyclic AMP concentration in the steady state. Rearranging,

$$[A]_s = v_{prod} \cdot K_m'/(v_{max} - v_{prod})$$

The steady state concentration of cyclic AMP in the absence of inhibitor $[A]_s'$, is given by

$$[A]_s' = v_{prod} \cdot K_m/(V_{max} - v_{prod})$$

so that

$$[A]_s/[A]_s' = (1 + [R]/K_i) \quad (3)$$

This means that for a given concentration of inhibitor, the *relative* increase ($[A]_s/[A]_s'$) in cyclic AMP concentration will be the same for all values of v_{prod} (i.e. regardless of histamine and adenosine concentration). This result reflects the increase in cyclic AMP concentration required to overcome the competition from the inhibitor sufficiently for the rate of cyclic AMP breakdown to match the (unaltered) rate of production. Because of the hyperbolic dependence of v_{PDE} on $[A]$ (equation 1), the absolute increase in cyclic AMP needed is larger when v_{prod}, and hence also v_{PDE}, is larger, i.e. at high agonist concentration.

Equation (3) also shows that the ratio $[A]_s/[A]_s'$ is linearly dependent on the inhibitor concentration. This means that for inhibitor concentrations which are large compared with K_i an increase in $[R]$ will produce an almost proportional increase in the steady state level of cyclic AMP, e.g. if $[R]$ is increased from 10 K_i to 100 K_i, the ratio $[A]_s/[A]_s'$ will increase from 11 to 101.

Chemicals. Dowex 50W, H^+ form (200–400 mesh), 2-chloroadenosine, adenosine, adenosine deaminase (type VI), neutral alumina (type WN-3), imidazole and mepyramine maleate were obtained from Sigma. Histamine dihydrochloride was purchased from BDH and [8-^{3}H]adenine from Amersham. Gifts of rolipram (Schering) and tiotidine (ICI) are gratefully acknowledged.

RESULTS

Agonist-stimulated [^{3}H]*-cyclic AMP accumulation*

Incubation of cerebral cortical slices with histamine (1 mM) or adenosine (0.1 mM) for 10 min

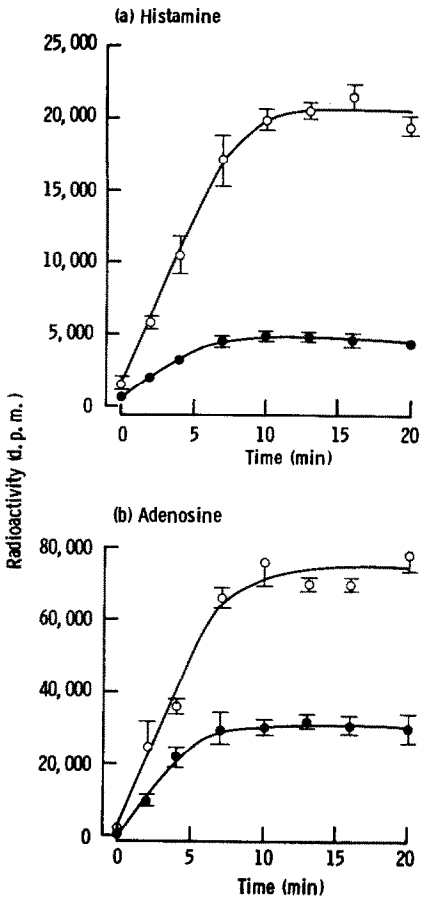

Fig. 1. Time course of the accumulation of [^{3}H]-cyclic AMP in guinea-pig cerebral cortical slices, in response to (a) 1 mM histamine and (b) 0.1 mM adenosine. Time courses were performed in the absence (●) and presence (○) of 0.1 mM rolipram (20 min preincubation). Data represent the mean ± SE of triplicate incubations in a single experiment. Similar results were obtained in two other experiments.

resulted in a stimulation of [^{3}H]-cyclic AMP accumulation of 7.8 ± 0.7-fold (N = 25) and 46.5 ± 4.5-fold (N = 15) respectively, over basal levels. Studies of the time course of these responses revealed that cyclic AMP levels were maximal within 10 min following exposure to either agonist, and remained constant for at least a further 10 min (Fig. 1).

Previous studies have indicated differences in the pharmacology of the histamine response in guinea-pig cerebral cortical slices. Some workers have observed an H_2-receptor mediated component in the response [24–26], whilst others have found the response to be mediated almost totally by H_1-receptors [13, 14, 27]. In view of these differences, we have examined the pharmacology of histamine-induced cyclic AMP accumulation in our system. The results of this study are shown in Fig. 2a. In these experiments, adenosine deaminase was routinely included in the incubations since preliminary studies showed that adenosine deaminase (1.2 U/ml) reduced the response to 1 mM histamine by 16.2 ± 5.9% (N = 6) (data not shown). This component of the response was presumably due to H_1-receptor-mediated potentiation of a cyclic AMP response caused by release of endogenous adenosine. The H_1-antagonist mepyramine (1 μM) produced a 40.4 ± 4.8% (N = 3) reduction in the cyclic AMP accumulating in response to 1 mM histamine, whilst the H_2-receptor antagonist tiotidine (30 μM) almost totally abolished the histamine response (95.7 ± 0.7% reduction, N = 3) (Fig. 2a). This suggests that, in the presence of adenosine deaminase, there is a small H_2-receptor effect which is potentiated by an indirect H_1-receptor mediated response.

Effect of rolipram on cyclic AMP accumulation

The PDE inhibitor rolipram produced a dose-dependent increase in the [^{3}H]-cyclic AMP accumulating in response to 1 mM histamine (Fig. 3a). The highest concentration of rolipram examined (1 mM) produced a 7.4 ± 0.7 (N = 3) fold augmentation of

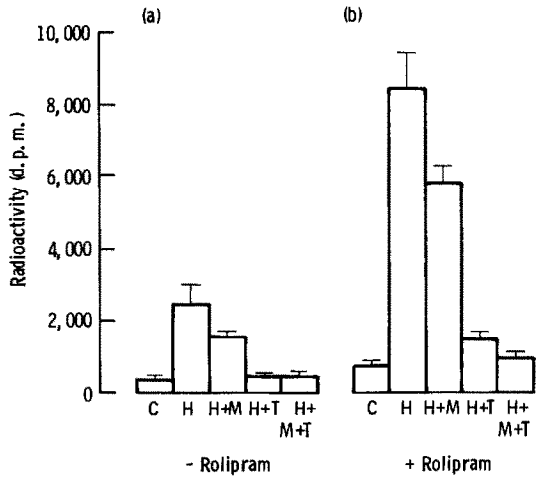

Fig. 2. Pharmacological profile of the cyclic AMP response to histamine (1 mM) in slices of guinea-pig cerebral cortex, determined in the absence (a) and presence (b) of 0.1 mM rolipram (20 min preincubation). Adenosine deaminase (1.2 U/ml) was included in each incubation. (C) Control; (H) histamine; (M) 1 μM mepyramine; (T) 30 μM tiotidine. Data represent the mean ± SE of triplicate determinations made in a single experiment. Similar results were obtained in two other experiments.

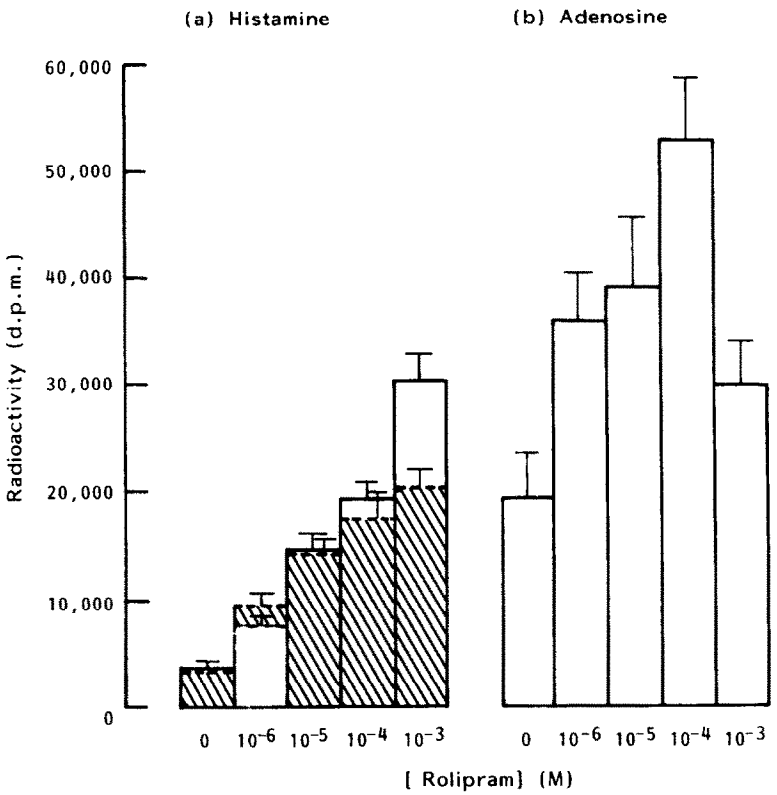

Fig. 3. Effect of rolipram concentration on the cyclic AMP response to (a) 1 mM histamine and (b) 0.1 mM adenosine in slices of guinea-pig cerebral cortex. Data represent the mean ± SE of three replicate determinations in a single experiment. Similar results were obtained on two other occasions. Shaded blocks represent results obtained in the presence of adenosine deaminase (1.2 U/ml).

the response to histamine. Data obtained in the absence and presence of adenosine deaminase was similar, with the notable exception of the results obtained with 1 mM rolipram. Adenosine deaminase reduced the histamine response in the presence of this high concentration of rolipram, suggesting that the PDE inhibitor was causing the release of endogenous adenosine. Rolipram also augmented the response to 0.1 mM adenosine. The stimulation by rolipram increased with dose up to a concentration of 0.1 mM (Fig. 3b), which produced a 2.5 ± 0.1 (N = 3) fold increase in the adenosine response. The augmentation produced by 1 mM rolipram, however, (1.7 ± 0.2 fold, N = 3) was significantly less ($P < 0.05$, Student t-test) (Fig. 3b). This suggests that at high doses, rolipram antagonises the cyclic AMP response to adenosine. A concentration of 0.1 mM rolipram was selected for further studies.

A number of experiments were carried out to determine whether the pharmacology of the histamine response was altered in the presence of rolipram. In 3 experiments performed in the presence of adenosine deaminase (1.2 U/ml), the absolute size of the histamine response was increased by rolipram but the relative sizes of the H_1- and H_2-receptor mediated components of the response were essentially unaffected (Fig. 2b). However, the reduction of the histamine-induced accumulation of [^{3}H]-cyclic AMP produced by tiotidine (30 μM) was slightly less in the presence of rolipram (87.0 ± 3.3%) than in its absence (95.7 ± 0.7%) (N = 3 in each case).

The increase in agonist-induced [^{3}H]-cyclic AMP accumulation produced by 0.1 mM rolipram appeared to depend on the rate of cyclic AMP production. Rolipram was particularly effective at low levels of stimulation such as that produced by 1 mM histamine. Thus, incubation of tissue slices with rolipram resulted in a 4.9 ± 0.4 (N = 22) fold augmentation of the response to histamine. Rolipram had a much smaller effect on the larger responses produced by adenosine (0.1 mM) alone and by a combination of adenosine (0.1 mM) and histamine (1 mM), producing a 2.0 ± 0.2 (N = 12) fold and a 1.2 ± 0.1 (N = 7) fold augmentation with adenosine alone and adenosine plus histamine respectively (Fig. 4). These studies also revealed that the extent of the H_1-receptor mediated augmentation of the adenosine response (3.5 ± 0.5, N = 11) (Fig. 4) was greater than the H_1-receptor mediated augmentation of the H_2-receptor response (1.7 ± 0.1, N = 3) (Fig. 2a).

Kinetic studies

An examination of the time course of [^{3}H]-cyclic AMP accumulating in response to histamine or adenosine revealed that at all time points, [^{3}H]-cyclic AMP accumulation in the presence of 0.1 mM rolipram was greater than in its absence (Fig. 1). In both cases the cyclic AMP level achieved steady state

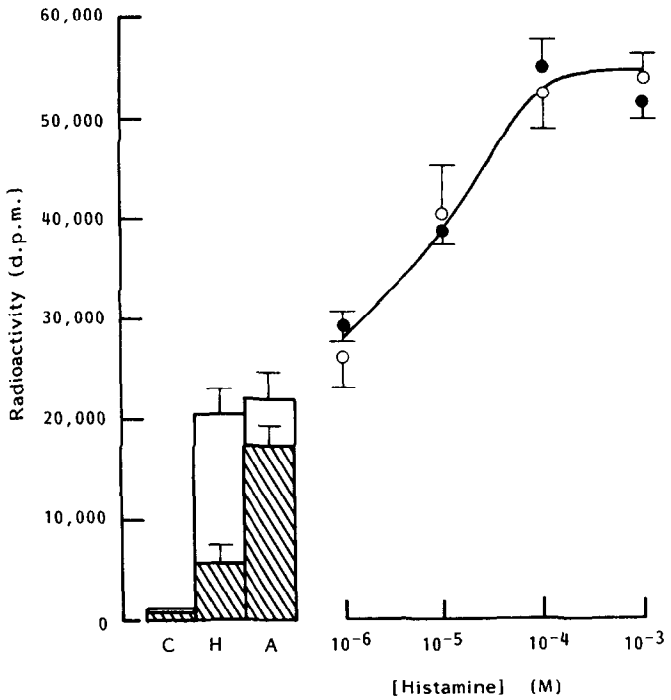

Fig. 4. The effect of rolipram (0.1 mM) on the cyclic AMP response to histamine and adenosine in slices of guinea-pig cerebral cortex. (C) Control; (H) 1 mM histamine; (A) 0.1 mM adenosine. Blocks represent results obtained in the absence (shaded) and presence (open) of rolipram. Dose–response curve to histamine was determined in the presence of 0.1 mM adenosine. Histamine and adenosine were added simultaneously. (●) Minus rolipram, (○) plus rolipram. Data represent the mean ± SE of triplicate determinations made in a single experiment. Similar results were obtained in four other experiments.

within the first 10 min and remained constant for at least a further 20 min (Figs 1 and 5). This is consistent with an inhibition of cyclic AMP breakdown by rolipram which is overcome by an increase in cyclic AMP concentration. If this is the case, the rate of cyclic AMP breakdown at steady state should be the same in the presence or absence of rolipram. The data in Fig. 5 support this view. In these experiments cerebral cortical slices were incubated with agonist for 10 min, after which the agonist stimulus was removed by addition of adenosine deaminase (final activity 1.2 U/ml) for adenosine or tiotidine (final concentration 30 μM) for histamine and the levels of [^{3}H]-cyclic AMP followed for a subsequent 20 min. The use of these agents to remove the stimuli was justified, since tiotidine totally abolished the histamine response (Fig. 2) and incubation of tissue slices with adenosine deaminase (1.2 U/ml) resulted in a 97% and a 99% reduction in the cyclic AMP accumulating in response to adenosine (0.1 mM) in two experiments (data not shown). The results of these studies are presented in Fig. 5.

These studies indicated that rolipram had little effect on the initial rate of cyclic AMP breakdown. The half time of cyclic AMP breakdown was, however, increased by the PDE inhibitor. In the absence of rolipram, cyclic AMP levels fell by 50% within 2.5 min following removal of the histamine or adenosine stimulus (Figs 5a and b). In the presence of rolipram the half time was increased, such that a 50% reduction in [^{3}H]-cyclic AMP levels was not achieved until 4–6 min after removal of the agonist stimulus (Figs 5c and d). However, within 10 min cyclic AMP levels had returned essentially to basal levels, both in the absence and presence of rolipram.

Effect of rolipram on the cyclic AMP response to 2-chloroadenosine

In order to investigate the possibility that the reduced effect of rolipram on adenosine and adenosine/histamine combinations was due to competition between adenosine and rolipram at the intracellular P-site [29], the effect of rolipram on the cyclic AMP response to 2-chloroadenosine was examined. 2-Chloroadenosine is a stable adenosine analogue which does not appear to be incorporated into cells [28–30] and is a poor substrate for the intracellular P-site [29]. Experiments were performed in the presence of adenosine deaminase (1.2U/ml) to eliminate endogenous adenosine which is continually released by cells. A fixed dose of histamine (0.1 mM) was included in every incubation, in order to achieve a greater range of cyclic AMP levels. The H_2-receptor mediated component of the histamine response was eliminated by the inclusion of 30 μM tiotidine in the incubations. Thus the only effect of histamine would be an H_1-receptor mediated potentiation of the 2-chloroadenosine response.

The effect of rolipram on the cyclic AMP response to a combination of histamine and 2-chloroadenosine (Figs 6a and b) was similar to that obtained with histamine and adenosine (Fig. 4). Thus at low levels of stimulation, rolipram produced a larger aug-

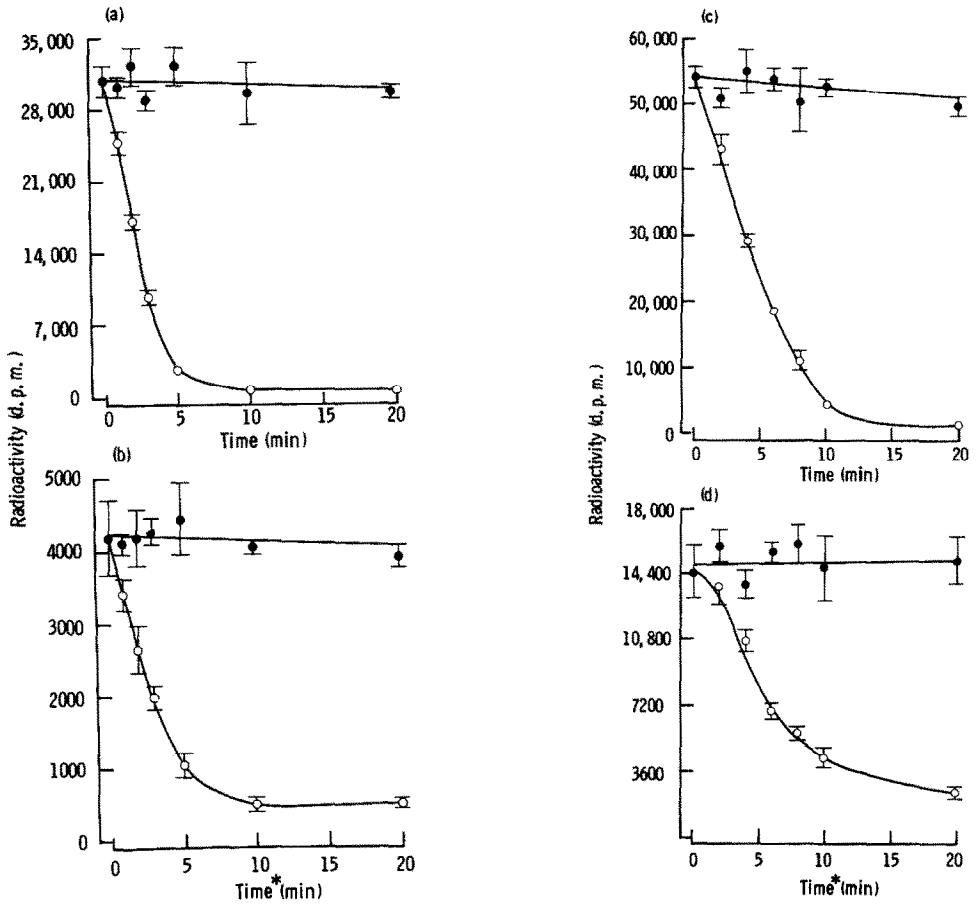

Fig. 5. Effect of rolipram (0.1 mM) on the breakdown of [^{3}H]-cyclic AMP accumulated in response to 1 mM histamine or 0.1 mM adenosine. (a) Adenosine; (b) histamine; (c) adenosine + rolipram; (d) histamine + rolipram. Histamine experiments were performed in the presence of adenosine deaminase (1.2 U/ml). Slices were incubated with agonist for 10 min before the agonist stimulus was removed (○) with tiotidine (final concentration 30 μM, for histamine) or adenosine deaminase (final activity 1.2 U/ml for adenosine) and [^{3}H]-cyclic AMP breakdown followed. (●) Control, i.e. agonist stimulus present throughout the duration of the experiment. * Zero time represents the time of addition of adenosine deaminase, tiotidine or Krebs (controls). Data represent mean ± SE of three replicate determinations in a single experiment. Similar results were obtained on two other occasions.

mentation of the agonist-induced cyclic AMP response than at high levels of stimulation (e.g. $P < 0.05$, Student t-test, augmentation of the response to 1 μM 2-chloroadenosine compared to augmentation of response to 100 μM 2-chloroadenosine) (Fig. 6a).

DISCUSSION

Histamine produced an approximately 8-fold stimulation of the accumulation of [^{3}H]-cyclic AMP in slices of guinea-pig cerebral cortex. This finding is in agreement with results obtained by other workers [13, 14, 24–27]. However, in these previous studies there is disagreement as to the pharmacological characteristics of the histamine response. Some workers have found no evidence for an H_2-receptor mediated component [13, 14, 27], the histamine response being unaffected by H_2-receptor antagonists. In these circumstances the response to histamine alone appeared to be due to the potentiation of the response to endogenous adenosine, since it was abolished by adenosine deaminase treatment [13]. Other workers have reported that about 50% of the histamine-induced cyclic AMP response in cerebral cortical slices is mediated by H_2-receptors [24, 25, 31]. The reason for this discrepancy is not clear. However, it is notable that in studies in which an H_2-receptor component was detected, cyclic AMP accumulation was generally measured by the [^{3}H]-adenine prelabelling technique, whilst in those in which an H_2-receptor component was not observed, total cyclic AMP levels were measured by a sensitive protein binding assay.

In the present study, a small proportion of the histamine response appeared to be due to the potentiation of a cyclic AMP response to endogenous adenosine, since adenosine deaminase treatment produced a small (15%) reduction in the cyclic AMP response to histamine. In subsequent studies, performed in the presence of adenosine deaminase, the H_1-antagonist mepyramine (1 μM) produced a 40%

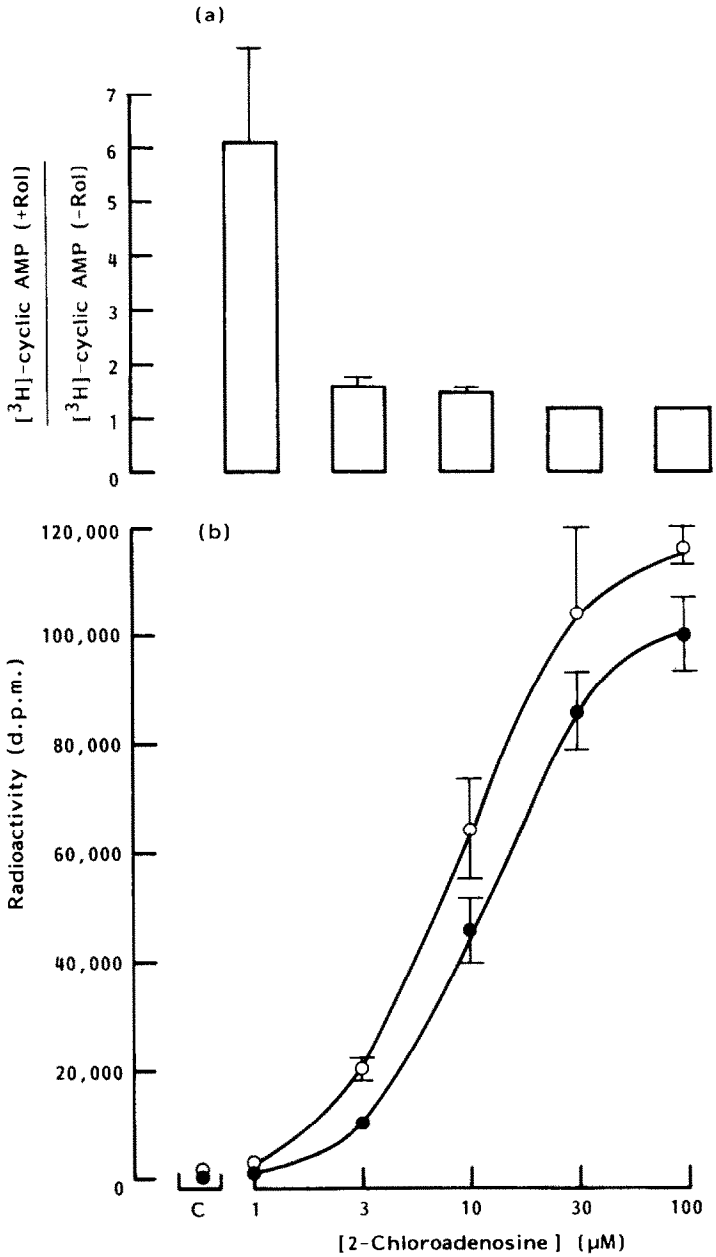

Fig. 6. Effect of rolipram (0.1 mM) (○) on the cyclic AMP response to 2-chloroadenosine (●). (C) Control. Determinations were made in the presence of histamine (0.1 mM), tiotidine (30 μM) and adenosine deaminase (1.2 U/ml). Histamine and 2-chloroadenosine were added simultaneously. Histamine was included in all incubations to amplify (via H_1-receptors) the response to 2-chloroadenosine and give a wide range of [^{3}H]-cyclic AMP levels. Tiotidine and adenosine deaminase were present to remove the direct actions of histamine (via H_2-receptors) and endogenous adenosine. Data in (b) represent the mean ± SE of three replicate determinations made in a single experiment. Similar results were obtained in two other experiments. The histogram (a) shows the extent of the augmentation produced by rolipram at different concentrations of 2-chloroadenosine and is expressed as a ratio of the cyclic AMP produced in the presence (+Rol) and absence (−Rol) of rolipram. Data represent the mean ± SE of results obtained in three experiments.

reduction in the histamine response whilst the H_2-antagonist tiotidine (30 μM) effectively abolished it. This suggests that about 60% of the response to histamine was produced by H_2-receptor stimulation, and that the remaining H_1-receptor component was due to an indirect potentiation of the H_2-response. It is notable that the size of the H_1-receptor mediated augmentation of the H_2-response was small (1.7-fold) compared to the H_1-receptor mediated augmentation of the response to adenosine (3.5-fold). Furthermore, the extent of the H_1-induced augmentation of the histamine response was not

increased when the size of the H_2-response was increased by rolipram. These results suggest that the H_2- and adenosine-receptor responses occur in different compartments within the preparation, e.g. in different cells.

Rolipram (0.1 mM) produced a large potentiation of the cyclic AMP response to histamine. As with experiments performed in the absence of rolipram, a steady state level of cyclic AMP was achieved within 10 min. The level of cyclic AMP at which steady state was achieved was, however, greater in the presence than in the absence of the PDE inhibitor. Studies with H_1- and H_2-receptor antagonists suggested that the pharmacological profile of the response to histamine was not significantly altered by rolipram. However, a residual 13% of the response was not abolished by tiotidine. The reason for this is unclear. Since rolipram has been reported to increase noradrenaline turnover in rat cerebral cortex [32], it is possible that this component of the response may be due to α- or β-receptor activation. However, preliminary experiments in our laboratory have indicated that the histamine-induced [^{3}H]-cyclic AMP response in the presence of rolipram is insensitive to inhibition by α- and β-antagonists.

Rolipram (0.1 mM) also augmented the response to adenosine (0.1 mM). As for histamine, steady state was achieved within 10 min both in the absence and presence of the PDE inhibitor. The achievement of steady state in the presence of rolipram was consistent with an inhibition of cyclic AMP breakdown by rolipram, which could be overcome by an increase in cyclic AMP concentration. At steady state the rate of production of cyclic AMP is equal to the rate of breakdown. Thus, the absolute rates of cyclic AMP breakdown will be the same in the absence and presence of a PDE inhibitor, provided the rate of cyclic AMP production is not altered by the inhibitor. The actual level of cyclic AMP at which steady state is achieved will, however, be greater. Studies in which cyclic AMP breakdown was measured after removal of the agonist stimulus, confirmed that 0.1 mM rolipram had little effect on cyclic AMP turnover in the steady state (Fig. 5).

The achievement of a steady state level in the presence of rolipram (0.1 mM) could be explained if rolipram were acting as a simple competitive inhibitor. Thus cyclic AMP levels would increase until the competition by the inhibitor was overcome sufficiently for the rate of cyclic AMP breakdown to match the unaltered rate of production. However, the results of experiments in which the effect of rolipram on the response to different levels of agonist stimulation were examined, were not consistent with simple competition. Theory predicts (see Materials and Methods) that in this situation, the augmentation by rolipram should be the same at all cyclic AMP levels. However, with 0.1 mM rolipram, the potentiation of the response to histamine (5-fold) was much greater than its effect on the larger cyclic AMP responses produced by adenosine (0.1 mM) and adenosine plus histamine (1 mM) (2.0- and 1.2-fold respectively). Similarly, rolipram produced a much greater augmentation of the response to low concentrations of 2-chloradenosine (6-fold, 1 μM) than of the response to high concentrations of the agonist (1.2-fold, 100 μM). The explanation for these effects seems unlikely to be due to an interference of rolipram with production, since 0.1 mM rolipram had little effect on the rate of cyclic AMP turnover in the steady state.

The results of the study in which the effects of different concentrations of rolipram were examined on the cyclic AMP response to adenosine and histamine, were also inconsistent with a simple competitive inhibition. The theoretical analysis outlined under Materials and Methods would predict that an increase in the concentration of rolipram would produce an almost proportional increase in the steady state level of cyclic AMP. Thus, if the inhibitor concentration is increased by 10-fold then the cyclic AMP concentration should increase by almost 10-fold. However, for a 10-fold increase in rolipram concentration, the increase in cyclic AMP was only of the order of 1.5-fold. It should be noted, however, that although the extracellular concentration of rolipram is increased by 10-fold, a 10-fold increase may not be achieved intracellularly. These experiments also revealed that at high concentrations (1 mM), rolipram caused a reduction in the steady state level of cyclic AMP, suggesting that it interfered with cyclic AMP production. Thus, at high concentrations, rolipram appears both to cause the release of endogenous adenosine and to act as an adenosine receptor antagonist.

The effects of rolipram on histamine- and adenosine-stimulated cyclic AMP accumulation cannot therefore be explained by simple competition. In general, most tissues contain at least three isoforms of PDE; (1) a calcium-stimulated activity, (2) a specific cyclic GMP activity and (3) a specific high affinity cyclic AMP PDE activity [20, 33, 34]. This latter form of the enzyme is quantitatively very minor in brain, but highly specific for cyclic AMP [34, 35]. The majority of the PDE activity in brain is calcium–calmodulin sensitive [34, 35]. However, nothing is known about the localisation of the various PDE isoenzymes in different cell types or cellular compartments within the central nervous system. Furthermore the physiological relationship and relative importance of calmodulin-dependent and independent forms of cyclic nucleotide metabolism is unclear at present. Rolipram is a selective and competitive inhibitor of the calcium-independent cyclic AMP specific form of PDE in broken cell preparations [20, 21, 36]. The reduced ability of rolipram to increase the cyclic AMP levels at steady state when the rate of cyclic AMP production is high, may therefore be explained if the rolipram-insensitive calcium-dependent form of the enzyme becomes more important as the levels of cyclic AMP increase.

In conclusion, rolipram is an effective PDE inhibitor at low rates of cyclic AMP production, such as those obtained in response to histamine or low concentrations of 2-chloroadenosine. This suggests that the calcium independent form of PDE is important for cyclic AMP metabolism at these low cyclic AMP levels. However, when the rate of cyclic AMP synthesis is increased rolipram becomes less effective and the calcium dependent form of the enzyme may be more important. These studies also indicate the need for caution in the use of PDE inhibitors in brain

slice preparations. Firstly, it should not be assumed that a particular PDE inhibitor will always increase cyclic AMP levels. Secondly, in the steady state a pure competitive inhibitor will not reduce the PDE activity. Instead, a new steady state will be achieved with a higher cyclic AMP level which will overcome the effect of the PDE inhibitor and result in a rate of cyclic AMP turnover similar to that without the inhibitor. Thus, for a total inhibition of cyclic AMP breakdown, irreversible inhibitors of all forms of PDE activity will be required.

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