LACK OF EFFECT OF EXCITATORY AMINO ACIDS ON ENDOGENOUS CYCLIC AMP LEVELS IN RAT CEREBROCORTICAL TISSUE IN VITRO

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SUMMARY

In contrast to results reported for guinea pig brain, neuro-excitatory amino acids did not raise endogenous cyclic AMP levels in rat cerebrocortical tissue in vitro.

INTRODUCTION

Glutamate and structurally related amino acids, which are known to depolarise neurones in vivo (1) and in vitro (2), have been reported to produce large increases in endogenous cyclic AMP levels and in rates of synthesis of [^{14}C]-cyclic AMP from preloaded [^{14}C]-adenine in guinea pig cerebrocortical tissue slices (3,4). However, while the glutamate analogue kainate, which is a potent neuroexcitant (5), caused a large increase in formation of cyclic AMP from preloaded adenine in guinea pig cerebrocortical tissue (6), no effect was observed in another investigation on endogeous cyclic AMP levels in the rat (7).

In the present study, the effect of glutamate on endogenous cyclic AMP levels in the rat cerebral cortex has been investigated, and the project extended to include a range of related excitatory amino acids. In addition, 2,3-diaminopropionic acid, which has been reported to antagonise the effects of excitatory amino acids on guinea pig brain cyclic AMP (6), was also included in the range of compounds studied.

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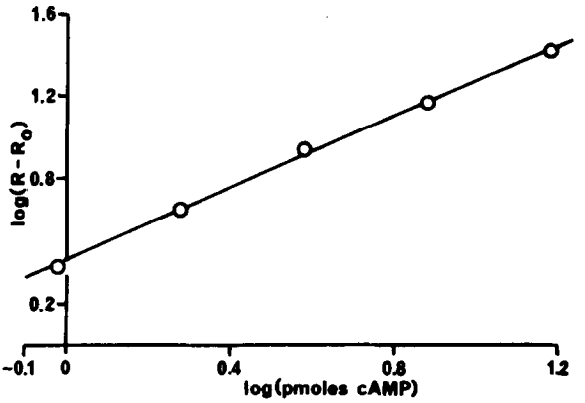

FIG. 1: FUNCTION USED IN ESTIMATING ENDOGENOUS CYCLIC AMP IN TISSUE

METHODS

Materials were obtained as follows: D-homocystine, DL-2,3-diamino-propionic acid hydrochloride, bovine serum albumin: Sigma Chemical Co., London; sodium-L-glutamate, theophylline: B.D.H. Chemicals Ltd., Poole; cyclic AMP: Boehringer Corporation, Lewes; D-glutamic acid: Koch-Light Labs, Ltd., Colnbrook; L-homocystine: Fluorochem Ltd., Glossop; GSX charcoal; Norit-Clydesdale Co.Ltd., Glasgow; [8-^{3}H]-cyclic AMP: Radiochemical Centre, Amersham. Bovine adrenal cyclic AMP-binding protein was a kind gift from Dr. Janet Albano. D- and L-homocysteic acid were prepared from D- and L-homocystine according to the method of Watkins (8).

Cerebrocortical slices, weighing about 50 mg, were prepared from male adult Porton rats as described by McIlwain (9). The slices were preincubated for 37-43 min at 37° in 4.0 ml Krebs-bicarbonate medium (mM: NaCl, 124; KCl, 5; KH_2PO_4, 1.2; $MgSO_4$, 1.3; $CaCl_2$, 1.4; $NaHCO_3$, 26; D-glucose, 10; gassed continuously with 95% O_2: 5% CO_2; pH 7.4) and then transferred to fresh medium with or without 2 mM DL-2,3-diamino-propionate. After 2 min various excitatory amino acids were added to this medium (at pH 7.4) to give a final concentration of 2 mM. Following a further 8 min incubation, slices were placed unthawed into 0.5 ml boiling 6 mM theophylline for 10 min, before homogenisation and extraction of cyclic AMP as described by Albano and colleagues (10). Extracted cyclic AMP was assayed by the saturation binding assay of Brown and colleagues (11).

RESULTS

Data from triplicate assay standards, transformed to produce a linear function (11), are shown in Fig.1. Here, $\log_{10}(R - \bar{R}_o)$ is plotted against log pmole unlabelled cyclic AMP, where R is the ratio of free [^{3}H]-cyclic AMP: bound [^{3}H]-cyclic AMP in a particular standard tube and $\bar{R}_o$ is the mean ratio in

TABLE 1: EFFECTS OF EXCITANTS ON ENDOGENOUS LEVELS OF CYCLIC AMP

Addition	Endogenous Level nmole/g i.w.w.			
	n	-DAP	n	+DAP
None	7	0.51 ± 0.13	4	0.51 ± 0.14
L-Glutamate	8	0.81 ± 0.26	4	0.49 ± 0.12
D-Glutamate	4	0.55 ± 0.22	4	0.56 ± 0.19
L-Homocysteate	4	0.49 ± 0.17	4	0.35 ± 0.05
D-Homocysteate	4	0.41 ± 0.12	4	0.28 ± 0.10

Slices were preincubated for 37-43 min before transfer to fresh medium with or without 2 mM 2,3-diaminopropionic acid (+DAP or -DAP respectively). Two min later, the amino acids listed were added to give a final concentration of 2 mM. The incubation was then continued for a further 8 min. Results are expressed as the means and standard errors of the sample sizes shown in nmole/g initial wet weight of tissue.

blank tubes without any unlabelled nucleotide. The levels of endogenous cyclic AMP in tissue samples were determined from this plot.

Assay of slices to which exogenous cyclic AMP had been added (about 60 pmole/tube prior to homogenisation of theophylline-treated slices) suggested that 86% of the exogenous cyclic AMP, and therefore presumably of the endogenous cyclic AMP was recoverable after the extraction and assay procedure. This figure was then used to correct observed endogenous levels of cyclic AMP.

The results obtained are shown in Table 1. After 10 min incubation of preincubated slices, control values of 0.51 ± 0.13 nmole cyclic AMP per g initial wet weight of tissue were obtained. This value was not changed significantly by any of the excitants tested. DL-2,3-diamino-propionate had no significant effect on cyclic AMP levels either in control slices or in slices incubated with excitants.

DISCUSSION

Assuming a figure of 80 mg protein per g initial wet weight of tissue (12), the control levels of endogenous cyclic AMP observed here are equivalent to about 6pmole per mg protein, a figure which agrees fairly well with the values of 8-20 pmole per mg protein reported elsewhere for rat brain *in situ* (13,14) or for diced incubated cerebrocortical tissue (guinea pig: 3,4,6; rat: 7).

Although 12-fold increases in endogenous cyclic AMP levels have been reported on incubation of diced guinea pig tissue with 2 mM L-glutamate (6), and 20-30 fold stimulation of rates of [^{14}C]-cyclic AMP formation from preloaded [^{14}C]-adenine in the presence of D- and L-glutamate and L-homocysteate has been observed (3,4), no significant effects of these amino acids were observed here.

There are several possible explanations for the disparity between the present results and those reported for the guinea pig. Firstly, intact slices were used in this study rather than diced tissue. However, the greater morphological integrity of intact tissue (15,16) would be expected to enhance any effects mediated by a membrane-bound enzyme such as adenylate cyclase. It may nevertheless be significant that dicing appears to selectively damage glial cells (16). Secondly, although conditions of incubations were very similar to those used in the work with the guinea pig, the same was not true for the method of extraction and assay of cyclic nucleotide. Nevertheless, control extraction and assay experiments indicated good recoveries when known amounts of cyclic AMP were added prior to tissue homogenisation. Thirdly, the results may indeed reflect a genuine species difference between rat and guinea pig.

The mechanism of regulation of cyclic GMP levels has been shown to vary considerably between closely related species (17). Thus a dose of L-glutamate sufficient to increase endogenous levels of cyclic GMP by ten-fold in guinea pig brain will only elicit a 30% increase in rat brain. The same situation may apply to cyclic AMP since it has been shown that rates of formation of

[^{14}C]-cyclic AMP from preloaded [^{14}C]-adenine are about two-fold more susceptible in the guinea pig than in the rat to stimulation by the same doses of amino acids (4). However, the observation that amino acid-induced increases in endogenous cyclic AMP levels in the guinea pig are considerably more than two times the increases reported here for the rat is difficult to explain, unless, in the rat, endogenous cyclic AMP levels are less susceptible to modulation by amino acids than is formation of cyclic AMP from preloaded adenine. Such a situation would imply that amino acid-sensitive formation of cyclic AMP from preloaded adenine is limited to a smaller compartment of cyclic AMP metabolism in the rat than in the guinea pig. Compartmentation of cyclic AMP metabolism has been demonstrated in the mouse (18).

Further research is required to confirm and clarify the situation.

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