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Cyclic AMP stimulation of amino acid uptake in bone and kidney

Many hormones increase the intracellular concentration of adenosine 3′,5′-cyclic monophosphate (cyclic AMP)[1]. Indeed, numerous investigators have reproduced specific hormone effects on tissue with cyclic AMP or its dibutyryl derivative, N^6-2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate (dibutyryl cyclic AMP)[1]. In certain instances, dibutyryl cyclic AMP has been more effective than cyclic AMP[2–4]. Presumably, dibutyryl cyclic AMP has greater cellular entry and/or slower degradation than cyclic AMP[1].

A mechanism of action of cyclic AMP may be its regulation of protein synthesis[5,6]. We considered whether cyclic AMP may stimulate amino acid uptake, which can in part control protein synthesis[7]. We now report that both cyclic AMP and dibutyryl cyclic AMP increase the uptake of amino acids by fetal rat calvaria and rat kidney cortex slices *in vitro*.

We prepared Sprague–Dawley fetal rat calvaria by the method of FINERMAN AND ROSENBERG[8] with minor modifications. Calvaria bisected along the sagittal suture provided matched pairs for controls. Using the method of ROSENBERG *et al.*[9] we prepared kidney cortex slices from 80–100 g male Sprague–Dawley rats. The same kidney furnished tissues for a treated preparation and its paired control. Each preparation incubated in 2 ml Krebs–Ringer bicarbonate buffer (pH 7.4) at 37° with a gas phase of O_2–CO_2 (95:5, v/v). The buffer contained glucose at a concentration of 10 mM for calvaria and 5 mM for kidney preparations.

Tissues were first subjected to a preincubation period during which amino acid was absent from the medium; nucleotide or parathyroid hormone was present only in the treated preparations. After preincubation we added the [^{14}C]amino acid mixed with its corresponding unlabeled amino acid to a desired concentration in the medium, and incubation followed for a specified time. Tissues were then rinsed in saline, blotted, weighed, and boiled in 2 ml of water for 10 min to release all free amino acids.

Using the difference in tissue weight before and after 24 h desiccation at 110°, as well as the tissue uptake of [*carboxyl*-^{14}C]inulin, we calculated the fraction of tissue weight represented by extracellular and intracellular water. Radioactivity from [^{14}C]-amino acids in the medium and [^{14}C]amino acids released from tissues was counted by liquid-scintillation spectrometry. We then calculated the [^{14}C]amino acid distribution ratio (ratio of intracellular to extracellular amino acid concentration) for each preparation. To verify that the calculated distribution ratios did not increase merely by changes in tissue-water spaces, we demonstrated that neither dibutyryl cyclic AMP nor parathyroid hormone changed these spaces in calvaria and kidney tissue.

We purchased unlabeled amino acids from Nutritional Biochemical Co., [^{14}C]-amino acids and [*carboxyl*-^{14}C]inulin from New England Nuclear Corp., and nucleotides from Calbiochem. Dr. Gerald Aurbach, National Institutes of Health, generously supplied us with purified bovine parathyroid hormone (1700 units/mg).

In fetal rat calvaria, dibutyryl cyclic AMP stimulated the uptake of α-aminoisobutyric acid, a nonmetabolized amino acid that shares transport mechanisms with certain neutral amino acids[8]. Furthermore, dibutyryl cyclic AMP increased distribution ratios of the neutral amino acid, glycine, as well as L-proline, a major constituent of calvarial protein. On the other hand, dibutyryl cyclic AMP failed to change

the distribution ratio of L-leucine. Cyclic AMP also stimulated α-aminoisobutyric acid uptake in calvaria, but the effect was less than with dibutyryl cyclic AMP (Table I) The specificity of the dibutyryl cyclic AMP effect was supported by the finding that dibutyryl cyclic AMP stimulates the uptake of some but not all amino acids as well as by the failure of noncyclic nucleotides to change α-aminoisobutyric acid uptake.

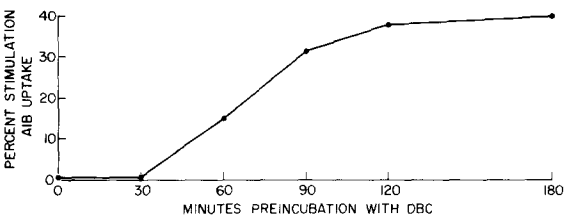

Fig. 1. Dibutyryl cyclic AMP stimulation of α-aminoisobutyric acid (AIB) uptake *versus* duration of dibutyryl cyclic AMP (DBC) exposure in fetal rat calvaria. Both treated and control preparations preincubated for 3 h. Dibutyryl cyclic AMP was added to the treated preparations at specified times prior to the end of the preincubation period. Following preincubation, α-amino-[^{14}C]isobutyric acid (0.07 mM) was added and incubation continued for 30 min. Data are expressed as percent increase in distribution ratios of treated preparations compared to paired controls. Each point represents the mean of at least 4 determinations.

Preincubation with dibutyryl cyclic AMP for at least 60 min was a prerequisite for significant stimulation of α-aminoisobutyric acid uptake in calvaria. The effect increased with longer dibutyryl cyclic AMP exposure but reached a plateau after 120 min (Fig. 1). Dibutyryl cyclic AMP produced greater stimulation with increasing dibutyryl cyclic AMP concentration in the range of 0.05–0.2 mM. Higher concentrations of dibutyryl cyclic AMP, however, were without additional effect. In dibutyryl cyclic AMP-treated tissues, distribution ratios were found to be increased as early as 15 min after α-aminoisobutyric acid addition. The effect continued throughout 3 h of incubation with α-aminoisobutyric acid. Therefore, dibutyryl cyclic AMP stimulated α-aminoisobutyric acid entry as well as the intracellular accumulation of α-aminoisobutyric acid.

In bone, parathyroid hormone activates adenyl cyclase and thereby increases the intracellular concentration of cyclic AMP[10]. Hence, we expected that parathyroid hormone would also increase the uptake of amino acids in bone. As expected, our studies showed a significant increase in the distribution ratio of α-aminoisobutyric acid with purified bovine parathyroid hormone (Table I). Parathyroid hormone also stimulated the uptake of L-proline but not of L-leucine. When heat-inactivated at 95° for 15 min, parathyroid hormone did not change the α-aminoisobutyric acid distribution ratio.

Studies of proline incorporation and hydroxyproline formation in bone have shown that parathyroid hormone may inhibit collagen synthesis[11,12]. On the other hand, parathyroid hormone under certain conditions may stimulate collagen synthesis[13]. In all of these studies, changes in intracellular amino acid concentration may effect the rate of collagen formation[11]. Our study, then, suggests that amino acid transport may be worthy of consideration in the study of parathyroid hormone effects on bone.

Although cyclic AMP and parathyroid hormone stimulate the uptake of certain

TABLE I

AMINO ACID UPTAKE IN TREATED AND CONTROL PREPARATIONS

All tissues were preincubated for 120 min. In treated preparations, the medium contained nucleotide or parathyroid hormone. Following preincubation, [^{14}C]amino acid and the corresponding unlabeled amino acid were added to a desired initial medium concentration. Incubation then continued for 30 min. Distribution ratios are expressed as mean ± S.E.

Number of pairs	*Treatment*	*Amino acid*	*Distribution ratio*	
			Control	*Treated*
Fetal rat calvaria				
13	Dibutyryl cyclic AMP (0.2 mM)	α-Aminoisobutyric acid (0.07 mM)	11.8 ± 0.3	16.0 ± 0.4 *
6	Dibutyryl cyclic AMP (0.2 mM)	Glycine (0.05 mM)	14.7 ± 0.4	21.4 ± 1.0 *
6	Dibutyryl cyclic AMP (0.2 mM)	L-Proline (0.05 mM)	14.4 ± 0.7	19.6 ± 1.1 *
6	Dibutyryl cyclic AMP (0.2 mM)	L-Leucine (0.05 mM)	4.7 ± 0.5	4.4 ± 0.5 **
20	Cyclic AMP (1.0 mM)	α-Aminoisobutyric acid (0.07 mM)	11.6 ± 0.4	14.1 ± 0.6 *
8	AMP (0.2 mM)	α-Aminoisobutyric acid (0.07 mM)	11.9 ± 0.5	12.3 ± 0.5 **
8	ATP (0.2 mM)	α-Aminoisobutyric acid (0.07 mM)	11.8 ± 0.6	12.0 ± 0.7 **
8	GMP (0.2 mM)	α-Aminoisobutyric acid (0.07 mM)	11.8 ± 0.2	11.2 ± 0.7 **
9	Parathyroid hormone (0.6 μg/ml)	α-Aminoisobutyric acid (0.07 mM)	13.9 ± 0.5	17.1 ± 0.7 *
8	Parathyroid hormone (0.6 μg/ml)	L-Proline (0.07 mM)	13.2 ± 0.7	16.3 ± 0.4 *
8	Parathyroid hormone (0.6 μg/ml)	L-Leucine (0.07 mM)	5.0 ± 0.5	4.7 ± 0.4 **
Kidney cortex slices				
22	Dibutyryl cyclic AMP (1.0 mM)	α-Aminoisobutyric acid (0.01 mM)	4.58 ± 0.30	5.90 ± 0.36 *
32	Cyclic AMP (1.0 mM)	α-Aminoisobutyric acid (0.01 mM)	3.94 ± 0.13	4.48 ± 0.19 ***
11	ATP (1.0 mM)	α-Aminoisobutyric acid (0.01 mM)	5.01 ± 0.30	4.87 ± 0.27 **
11	AMP (1.0 mM)	α-Aminoisobutyric acid (0.01 mM)	4.68 ± 0.38	4.73 ± 0.30 **

* $P < 0.01$.
** Not significant.
*** $P < 0.02$.

amino acids in bone, this effect may be related to the well-known action of parathyroid hormone on bone resorption. We therefore considered whether cyclic AMP would also affect amino acid uptake in kidney cortex slices. Indeed, both cyclic AMP and dibutyryl cyclic AMP increased the distribution ratio of α-aminoisobutyric acid in kidney cortex slices (Table I). As in calvaria, dibutyryl cyclic AMP was more effective than cyclic AMP in stimulating α-aminoisobutyric acid uptake.

Our studies demonstrate that cyclic AMP stimulates amino acid uptake in two tissues known to contain hormone-sensitive adenyl cyclase[10,14]. This effect may also occur in other tissues. In adipose tissue, however, cyclic AMP inhibits amino acid transport and protein synthesis[15]. Therefore, there are tissue differences in the response of amino acid transport to cyclic AMP. Insulin[16] and growth hormone[17] both stimulate amino acid uptake, but do not appear to activate adenyl cyclase. In fact, insulin may inhibit adenyl cyclase activity under certain conditions[18]. Clearly, there are hormonal mechanisms which can regulate amino acid uptake independent of cyclic AMP. Nevertheless, cyclic AMP may have a role in regulating amino acid uptake in bone and kidney.

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1 E. W. SUTHERLAND, G. A. ROBINSON AND R. W. BUTCHER, *Circulation*, 3 (1968) 279.
2 R. W. BUTCHER, R. J. HO, H. C. MENG AND E. W. SUTHERLAND, *J. Biol. Chem.*, 240 (1965) 4515.
3 H. IMURA, S. MATSUKURA, H. MATSUYAMA, T. SETSUDA AND T. MIYAKE, *Endocrinology*, 76 (1965) 933.
4 G. VAES, *Nature*, 219 (1968) 939.
5 J. J. FERGUSON, *J. Biol. Chem.*, 238 (1963) 2754.
6 L. D. GARREN, R. L. NEY AND W. W. DAVIS, *Proc. Natl. Acad. Sci. U.S.*, 53 (1965) 1443.
7 G. A. M. FINERMAN, S. DOWNING AND L. E. ROSENBERG, *Biochim. Biophys. Acta*, 135 (1967) 1008.
8 G. A. M. FINERMAN AND L. E. ROSENBERG, *J. Biol. Chem.*, 241 (1966) 1487.
9 L. E. ROSENBERG, A. BLAIR AND S. SEGAL, *Biochim. Biophys. Acta*, 54 (1961) 479.
10 L. R. CHASE, S. A. FEDAK AND G. D. AURBACH, *Endocrinology*, 84 (1969) 761.
11 C. C. JOHNSTON, JR., W. P. DEISS, JR. AND E. B. MINER, *J. Biol. Chem.*, 237 (1962) 3560.
12 B. FLANAGAN AND G. NICHOLS, JR., *J. Clin. Invest.*, 48 (1969) 595.
13 B. FLANAGAN AND G. NICHOLS, JR., *J. Clin. Invest.*, 44 (1965) 1795.
14 L. R. CHASE AND G. D. AURBACH, *Science*, 159 (1968) 545.
15 F. BRUCHHAUSEN, *Z. Physiol. Chem.*, 349 (1968) 1437.
16 D. M. KIPNIS AND J. E. PARRISH, *Federation Proc.*, 24 (1965) 1051.
17 J. L. KOSTYO, *Endocrinology*, 75 (1964) 113.
18 R. L. JUNGAS, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 757.

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