EFFECTS OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE AND ITS DIBUTYRYL DERIVATIVES ON CATECHOLAMINE SYNTHESIS IN BOVINE ADRENAL MEDULLARY SLICES

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1. Introduction

Recent results have suggested the possible role of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in control of catecholamine biosynthesis. Kvetnansky et al. found that administration of dibutyryl adenosine 3',5'-monophosphate (DB-cyclic AMP) restored the adrenal activities of tyrosine hydroxylase and dopamine-β-hydroxylase in hypophysectomized rats [1]. Waymire et al. observed that culture of mouse neuroblastoma tissue with DB-cyclic AMP for 1 and 3 days increased the tyrosine hydroxylase activity [2]. Keen and McLean also reported that incubation of isolated rat superior cervical ganglia with DB-cyclic AMP increased the levels of dopamine-β-hydroxylase and noradrenaline (NA) [3]. Furthermore, Guidotti and Costa found that following administration of reserpine, aminophylline or carbamylcholine, the concentration of cyclic AMP in the adrenal medulla of rats increased several-fold [4]. They suggested that this might cause increase in tyrosine hydroxylase activity in the medulla [4,5].

This paper reports studies on the effects of cyclic AMP and DA-cyclic AMP on the synthesis of [^{14}C]-catecholamine (CA) from [^{14}C]tyrosine in bovine adrenal medullary slices. Results showed that these cyclic nucleotides seem to increase [^{14}C]CA synthesis mainly through their effects on the rate-limiting step catalyzed by tyrosine hydroxylase.

2. Methods

Bovine adrenal glands were obtained at a slaughter house and immediately cooled on ice. The medulla was free from cortical tissue and sliced by hand with a Stadie-Rigg slicer. Usually three slices from different parts of the medulla, weighing a total of 150–180 mg wet weight, were incubated at 37°C in 2 ml of medium with [^{14}C] L-tyrosine (final concentration 2×10^{-5} M; 175×10^4 cpm) as described in the footnote of table 1. In some experiments, [^{14}C]L-DOPA (2×10^{-5} M; 340×10^4 cpm) was used as substrate instead of [^{14}C]tyrosine.

After incubation, the tubes were rapidly chilled in ice and the slices were removed from the medium and homogenized in 5 ml of 0.4 N perchloric acid (PCA). The incubation media were acidified with 0.5 ml of 2 N PCA. Precipitated protein was removed by centrifugation at 3000 rev/min for 10 min and the supernatants were analysed for ^{14}C-labelled substances (tyrosine, DOPA, CA and its deaminated metabolites) by ion exchange chromatography on Duolite C-25 columns (H^+ form, 0.4 × 7.0 cm) as reported previously from our laboratory [6]. All samples were counted in a Packard Tricarb Scintillation Counter.

3. Results and discussion

3.1. *Effects of cyclic AMP and DB-cyclic AMP on [^{14}C]CA synthesis from [^{14}C]tyrosine*

The [^{14}C]tyrosine added to the incubation medium was rapidly taken up into the slices and its concentration in the slices reached a maximum after 30 min and remained at this level for up to 90 min (approx. 140 cpm × 10^4/g wet wt.). The synthesis of [^{14}C]CA from [^{14}C]tyrosine was linear for at least 90 min and more than 80% of the newly synthesized [^{14}C]CA was

Table 1
Effects of cyclic nucleotides on [^{14}C]CA synthesis from [^{14}C]tyrosine

		[^{14}C]CA Synthesis	
			Ca^{++}-free medium
Control		20.5 ± 4.1 (12)	31.2 ± 4.3 (9)
Cyclic AMP	0.2 mM	24.6 ± 4.2 (6)	48.6 ± 8.6 (4)
Cyclic AMP	1 mM	39.6 ± 8.2 (6)	68.5 ± 10.5 (4)
Cyclic AMP	5 mM	46.1 ± 8.3 (6)	108.6 ± 15.3 (5)
DB-cyclic AMP	0.2 mM	47.5 ± 9.0 (9)	68.3 ± 11.8 (4)
DB-cyclic AMP	1 mM	83.6 ± 15.8 (9)	90.1 ± 15.4 (4)
DB-cyclic AMP	5 mM	122.2 ± 23.6 (9)	128.8 ± 24.2 (6)
Cyclic GMP	1 mM	19.3 ± 3.8 (4)	
Cyclic GMP	5 mM	20.8 ± 4.0 (4)	

Slices (each about 50–60 mg; total 150–180 mg) were incubated for 60 min at 37°C in 2 ml of medium containing [^{14}C]L-tyrosine (final concentration, 2×10^{-5} M; 175×10^4 cpm), with or without cyclic nucleotides. The medium contained 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 10 mM glucose and 10 mM buffer (pH 7.4). Values are means ± S.D., expressed as cpm $\times 10^4$/g wet wt, and numbers of experiments are given in parentheses.

accumulated in the slices. [^{14}C]DA and [^{14}C]NA plus [^{14}C]Ad constituted 66% and 34% of the total labelled CA, respectively. The [^{14}C]DOPA content of the slices was low, indicating that [^{14}C]DOPA formed from [^{14}C]tyrosine was rapidly converted to [^{14}C]CA by DOPA decarboxylase. Moreover, the amount of [^{14}C]deaminated metabolites was less than 30% of the newly synthesized CA and most of this was found in the medium.

The effects of different concentrations of cyclic AMP and DB-cyclic AMP on [^{14}C]CA synthesis from [^{14}C]tyrosine in adrenal medullary slices are shown in table 1. Cyclic AMP and DB-cyclic AMP did not affect the uptake of [^{14}C]tyrosine into the slices. The synthesis of [^{14}C]CA from [^{14}C]tyrosine increased on addition of 1 mM to 5 mM cyclic AMP but at a concentration of less than 0.2 mM, cyclic AMP had little effect on the synthesis of [^{14}C]CA. DB-cyclic AMP, which is known to permeate the cell membrane more rapidly than cyclic AMP and to be more resistant than cyclic AMP to phosphodiesterase, caused great increase in [^{14}C]CA synthesis from [^{14}C]tyrosine at concentrations of 0.2 mM to 5 mM. The percentages of newly synthesized [^{14}C]DA and [^{14}C]NA plus [^{14}C]Ad in the slices were similar on incubation with and without cyclic AMP or DB-cyclic AMP. The increases in [^{14}C]CA synthesis caused by cyclic AMP and DB-cyclic AMP were observed during incubation periods of 30 to 90 min. Cyclic GMP (1 mM – 5 mM) had no significant effect on the synthesis of [^{14}C]CA from [^{14}C]tyrosine.

Omission of Ca^{++} from the medium caused a slight increase in [^{14}C]tyrosine caused by cyclic AMP. In the absence of Ca^{++}, the stimulation of [^{14}C]CA synthesis by cyclic AMP was similar to that by DB-cyclic AMP. However, the effect of DB-cyclic AMP on [^{14}C]CA synthesis was not significantly affected by the absence of Ca^{++} in the medium. Therefore, the increased stimulation of [^{14}C]CA synthesis by cyclic AMP observed on removal of Ca^{++} may be due either to increased membrane permeability to cyclic AMP or to inhibition of phosphodiesterase which destroys cyclic AMP.

3.2. *Effects of cyclic AMP and DB-cyclic AMP on [^{14}C]CA synthesis in slices preincubated with [^{14}C]tyrosine*

To confirm that the increase in [^{14}C]CA synthesis caused by these nucleotides was not due to increased [^{14}C]tyrosine uptake by the slices, the following experiments were carried out. Slices were preincubated for 50 min in medium containing [^{14}C]tyrosine, and then washed with fresh medium and incubated for 40 min in medium without [^{14}C]tyrosine and with and without cyclic nucleotide. Stimulation of [^{14}C]CA synthesis by cyclic AMP or DB-cyclic AMP was still

Table 2
Effects of cyclic AMP and DB-cyclic AMP on [^{14}C]CA synthesis in slices preincubated with [^{14}C]tyrosine

		[^{14}C]CA Synthesis
Control	(4)	8.3 ± 2.1
Cyclic AMP	(4)	15.8 ± 3.0
DB-cyclic AMP	(4)	44.3 ± 8.6

Slices were preincubated for 50 min in medium containing [^{14}C]L-tyrosine, and then washed with fresh medium and incubated for 40 min in medium without [^{14}C]tyrosine and with and without cyclic nucleotide (5 mM). Values show means ± S.D. of [^{14}C]CA synthesized during the second incubation period, expressed as cpm × 10^4/g wet wt. Numbers of experiments are given in parentheses.

observed under these conditions where the uptake of [^{14}C]tyrosine into the slices was not affected by cyclic nucleotides (table 2).

Furthermore, the incorporation of [^{14}C]tyrosine into the tissue protein fraction (0.4 N PCA insoluble fraction) was not affected by these cyclic nucleotides. The release of newly synthesized [^{14}C]CA or endogenous CA into the medium and formation of [^{14}C]deaminated metabolites from labelled CA were also not affected significantly by cyclic AMP and DB-cyclic AMP. Thus the increase in [^{14}C]CA synthesis caused by cyclic AMP and DB-cyclic AMP seems be due to the direct effects of these compounds on [^{14}C]CA synthesis from [^{14}C]tyrosine.

3.3. *Effect of 5′-AMP on [^{14}C]CA synthesis from [^{14}C]tyrosine and on stimulation of [^{14}C]CA synthesis caused by DB-cyclic AMP*

The effect of 5′-AMP on the synthesis of [^{14}C]CA from [^{14}C]tyrosine was compared with those of cyclic nucleotides. 5′-AMP was found to inhibit the synthesis of [^{14}C]CA from [^{14}C]tyrosine by approximately 50% and 80% at concentrations of 1 mM and 5 mM, respectively (table 3). It was also found that the stimulation of [^{14}C]CA synthesis caused by DB-cyclic AMP was inhibited by the presence of 5′-AMP (1 mM – 5 mM). Although the possibility that 5′-AMP interferes with the uptake of cyclic nucleotide into the slices cannot be excluded at present, it is interesting to speculate that 5′-AMP, a degradation product of cyclic AMP, may play a regulatory role not only in the basal rate of CA synthesis from tyrosine but also

Table 3
Effects of 5′-AMP on [^{14}C]CA synthesis from [^{14}C]tyrosine and stimulation of [^{14}C]CA synthesis by DB-cyclic AMP

		[^{14}C]CA Synthesis
Control	(6)	21.0 ± 4.4
5′-AMP	1 mM (4)	10.5 ± 2.2
5′-AMP	5 mM (4)	4.4 ± 0.9
DB-cyclic AMP	5 mM (4)	121.3 ± 21.8
DB-cyclic + 5′-AMP	1 mM (4)	86.1 ± 15.6
DB-cyclic + 5′-AMP	5 mM (4)	69.2 ± 13.4

Slices were incubated for 60 min in medium containing [^{14}C]L-tyrosine with and without 5′-AMP or DB-cyclic AMP. Values are means ± S.D., expressed as cpm × 10^4/g wet wt, and numbers of experiments are given in parentheses.

in the stimulation of CA synthesis caused by cyclic AMP.

3.4. *Effect of DB-cyclic AMP on [^{14}C]CA synthesis from [^{14}C]DOPA*

To ascertain which step in the synthesis of CA from tyrosine was stimulated by cyclic nucleotide, we used [^{14}C]DOPA as starting substrates instead of [^{14}C]tyrosine. Using [^{14}C]DOPA as substrate, [^{14}C]CA synthesis was slightly increased in the presence of DB-cyclic AMP (table 4). The increase in [^{14}C]CA synthesis induced by DB-cyclic AMP was much less using [^{14}C]DOPA than using [^{14}C]tyrosine as substrate. Therefore, it appears that the stimulation of [^{14}C]CA synthesis from [^{14}C]tyrosine caused by cyclic nucleotide must occur mainly before the DOPA decarboxylase step.

Table 4
Effect of DB-cyclic AMP on [^{14}C]CA synthesis from [^{14}C]DOPA

		[^{14}C]CA Synthesis
Control	(4)	107.2 ± 19.4
DB-cyclic AMP	(4)	162.9 ± 28.2

Slices were incubated for 60 min in medium containing [^{14}C]L-DOPA (final concentration, 2×10^{-5} M; 340×10^4 cpm) with or without DB-cyclic AMP (5 mM). Values are means ± S.D., expressed as cpm × 10^4/g wet wt and numbers of experiments are given in parentheses.

4. Summary of results

Cyclic AMP and dibutyryl-cyclic AMP (DB-cyclic AMP) caused marked increase in [^{14}C]catecholamine (CA) synthesis from [^{14}C]tyrosine in bovine adrenal medullary slices. Cyclic GMP had no significant effect on the synthesis of [^{14}C]CA. Removal of Ca^{++} from the medium potentiated the stimulatory effect of cyclic AMP on [^{14}C]CA synthesis, but did not significant affect the stimulatory effect of DB-cyclic AMP. On the other hand, 5′-AMP inhibited both the basal rate of [^{14}C]CA synthesis from [^{14}C]tyrosine and the stimulation of [^{14}C]CA synthesis caused by cyclic nucleotide. The stimulatory effect of cyclic nucleotide on [^{14}C]CA synthesis was less with [^{14}C]DOPA as substrate than with [^{14}C]tyrosine. Thus, cyclic nucleotide seems to stimulate [^{14}C]CA synthesis through its effect on the hydroxylation of tyrosine, the step which is thought to be rate-limiting in the synthesis of CA.

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