# ACTIVATION BY CYCLIC AMP OF SOLUBLE TYROSINE HYDROXYLASE IN BOVINE ADRENAL MEDULLA

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## 1. Introduction

Recent studies have shown that cyclic AMP (cAMP) may play a role in activation of tyrosine hydroxylase (EC 1.14.16.2), the rate-limiting enzyme in the biosynthesis of catecholamine. Goldstein et al. [1–3] and Harris et al. [4] reported that dibutyryl cAMP stimulated tyrosine hydroxylation in brain slices and synaptosomal preparations. Harris et al. [4] also reported that tyrosine hydroxylase in the supernatant fraction of brain was activated by addition of cAMP. This activation appeared to be related to changes in the affinities of the enzyme for substrate (tyrosine), pteridine cofactor and end-product inhibitor (catecholamine). Furthermore, it was suggested by Morgenroth et al. [5,6], Lovenberg et al. [7,8] and Lloyd and Kaufman [9] that the effect of cAMP might be mediated by a cAMP-dependent protein kinase, although actual phosphorylation of tyrosine hydroxylase might not occur.

Independently, we also reported [10] that cAMP and dibutyryl cAMP markedly increased [$^{14}$C] catecholamine synthesis from [$^{14}$C] tyrosine in slices of bovine adrenal medulla, mainly by increasing hydroxylation of tyrosine. In the present work, we examined the effects of cAMP on tyrosine hydroxylase in the supernatant fraction of bovine adrenal medulla. We found that the enzyme was activated by cAMP plus ATP, but not by cAMP alone. This activation was due to increased affinity of the enzyme for pteridine cofactor, without any change in its affinity for substrate. Furthermore, cAMP did not activate tyrosine hydroxylase in dialyzed supernatant, suggesting that some factor(*s*) that was removed or denatured during dialysis was required for the activation.

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## 2. Methods

Bovine adrenal glands were obtained at a slaughter house, and transported to the laboratory on ice. The medulla was separated from the cortex and homogenized with 0.25 M sucrose containing 20 mM sodium acetate buffer (pH 6.0). The homogenate was centrifuged at 17 000 × *g* for 30 min and the supernatant was recentrifuged at 105 000 × *g* for 60 min. The resulting supernatant was used as the enzyme preparation. All steps in preparing the enzyme were done at 0–5°C. For some experiments, the supernatant was dialyzed overnight against at least 50 vol. 20 mM acetate buffer (pH 6.0), with three changes of the buffer, before use.

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Tyrosine hydroxylase activity was measured by the method of Nagatsu et al. [11,12]. The incubation mixture (final vol. 1.0 ml) contained 200 μmol acetate buffer (pH 6.0), 1.0 μmol $FeSO_4$, 10 μmol 2-mercaptoethanol, 0.1 μmol 2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetra-hydropteridine ($DMPH_4$, Pfaltz and Bauer Inc. USA) and enzyme solution. This mixture was preincubated for 5 min at 37°C in the presence or absence of cAMP and other nucleotides and then the reaction was started by adding 0.1 μmol (0.05 μCi) of L-[U-$^{14}$C] tyrosine (Radiochemical Centre, England). The reaction was carried out for 20 min at 37°C and stopped by adding 4.0 ml 0.5 N perchloric acid. The $^{14}$C-labelled L-3, 4,-dihydroxyphenylalanine (Dopa) formed enzymatically was absorbed on aluminum hydroxide and its radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer, Model 3385. Protein was assayed by the method of Lowry et al. [13].

## 3. Results and discussion

The effects of cAMP and other nucleotides on tyrosine hydroxylase in the supernatant from adrenal medulla are shown in table 1. Incubations were done using a suboptimal concentration of cofactor, $DMPH_4$ (0.1 mM): ATP-activated tyrosine hydroxylase markedly and although cAMP alone had no significant effect on tyrosine hydroxylase activity, in the presence of ATP it activated the enzyme at a concentration as little as $10^{-7}$–$10^{-5}$ M. Therefore, it appears that the activation of tyrosine hydroxylase by cAMP is dependent upon ATP. GTP, like ATP, activated tyrosine hydroxylase, but cyclic GMP had no effect, either in the absence or presence of GTP.

Table 1
Effects of cAMP and other nucleotides on tyrosine hydroxylase in the supernatant fraction of bovine adrenal medulla

| Nucleotide | Tyrosine hydroxylase activity (nmol Dopa/mg protein/20 min) |
|---|---|
| None | 0.08 ± 0.02 |
| cAMP ($10^{-6}$ M) | 0.11 ± 0.05 |
| ATP (4 mM) | 1.15 ± 0.02 |
| ATP (4 mM) + cAMP ($10^{-7}$ M) | 1.82 ± 0.20 |
| ATP (4 mM) + cAMP ($10^{-6}$ M) | 2.22 ± 0.16 |
| ATP (4 mM) + cAMP ($10^{-5}$ M) | 2.10 ± 0.11 |
| GTP (4 mM) | 0.92 ± 0.10 |
| GTP (4 mM) + cGMP ($10^{-6}$ M) | 1.06 ± 0.09 |

Tyrosine hydroxylase activity was assayed as described under Methods with 0.1 mM $DMPH_4$. The values are means ± SD of results in at least four experiments.

Using a saturating concentration of cofactor, $DMPH_4$ (1 mM) we found that neither ATP nor ATP plus cAMP caused activation. Therefore, we examined the effects of ATP and cAMP on the $K_m$ for cofactor. Analysis of Lineweaver-Burke plots showed that ATP decreased the apparent $K_m$ for $DMPH_4$ without changing the $V_{max}$ and that cAMP increased the effect of ATP (fig.1). Similar experiments were done with tyrosine concentrations of $1.2 \times 10^{-5}$–$10^{-4}$ M at a saturating concentration of cofactor, $DMPH_4$ (1 mM). The results all showed that ATP and ATP plus cAMP did not change the $K_m$ for tyrosine without changing the $V_{max}$ (data not shown). Thus the activation by ATP or ATP plus cAMP seems to be due to increase in affinity of the enzyme for cofactor, $DMPH_4$.

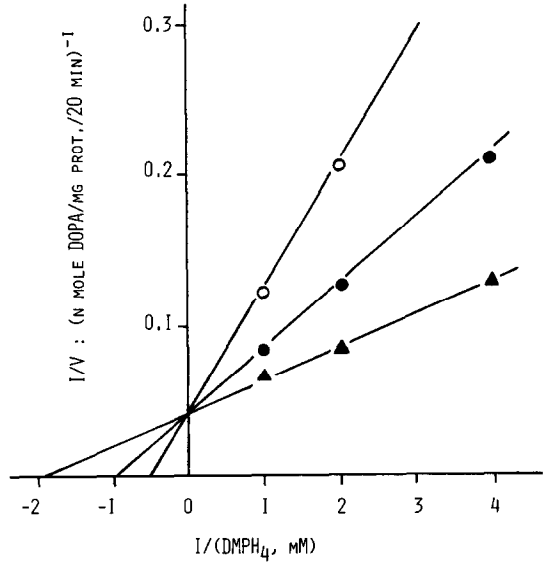


Fig.1. Effects of ATP and ATP plus cAMP on the apparent $K_m$ for $DMPH_4$ of tyrosine hydroxylase in the supernatant of bovine adrenal medulla. Tyrosine hydroxylase activity was measured as described under Methods with various concentrations of $DMPH_4$ and $10^{-4}$ M tyrosine. (○) Control, (●) 4 mM ATP, (▲) 4 mM ATP + $10^{-6}$ M cyclic AMP.

Table 2
Effects of cAMP and ATP on tyrosine hydroxylase in the dialyzed supernatant fraction of bovine adrenal medulla

| Nucleotide | Tyrosine hydroxylase activity (nmol Dopa/mg protein/20 min) |
|---|---|
| None | 0.28 ± 0.04 |
| cAMP ($10^{-6}$ M) | 0.29 ± 0.02 |
| ATP (4 mM) | 5.66 ± 0.28 |
| ATP (4 mM) + cAMP ($10^{-6}$ M) | 5.93 ± 0.12 |

Tyrosine hydroxylase activity was assayed in the dialyzed supernatant as described under Methods with 0.1 mM $DMPH_4$. The values are means ± SD of results in at least four experiments.

Similar results were obtained by Lovenberg et al. [7,8] for brain tyrosine hydroxylase but Harris et al. [4] found that cAMP alone activated tyrosine hydroxylase in the supernatant from brain by lowering the $K_m$-values for both the cofactor and the substrate. The reason for this difference is unknown, but it could be due to differences in the enzyme source and enzyme assay system used: Harris et al. [4] used a sheep liver pteridine reductase system to reduce the pteridine cofactor oxidized during the reaction, whereas Lovenberg et al. [7,8] and we used 2-mercaptoethanol to reduce the 'oxidized pteridine'.

When dialyzed supernatant was used as enzyme instead of nondialyzed supernatant, the basal activity of tyrosine hydroxylase was higher and ATP caused more activation (table 2). However, cAMP did not cause activation, even in the presence of ATP.

These findings suggest that cAMP and ATP activate tyrosine hydroxylase by different mechanisms and that some factor(s) that is removed or denatured during dialysis may be required for the activation by cAMP of tyrosine hydroxylase. Further experiments are in progress on the factor required for activation by cAMP and on the mechanism of this activation.

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