ACTIVATION OF ADRENAL TYROSINE HYDROXYLASE BY ATP AND OTHER NUCLEOTIDES

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Received 24 September 1977

1. Introduction

Tyrosine hydroxylase (EC 1.14.16.2), which catalyzes the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), is known to be a rate-limiting enzyme in the biosynthesis of catecholamine. Therefore, the regulation of tyrosine hydroxylase activity is an important mechanism for modulating the tissue level of catecholamine. It has long been thought that end-product inhibition is the most important mechanism for regulation of this enzyme. However, recent evidence has shown that cyclic AMP participates in regulation of tyrosine hydroxylase [1–10]. Previously, we also reported that cyclic AMP activates tyrosine hydroxylase of bovine adrenal medulla, and that its effect depends on the presence of ATP [11,12]. During our experiments, we found that ATP itself activated tyrosine hydroxylase. This paper reports further studies on the effect of ATP and also on the effects of other nucleotides on tyrosine hydroxylase from bovine adrenal medulla.

2. Methods

Bovine adrenal glands were obtained at a slaughter house. 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 supernatant was recentrifuged at 105 000 × *g* for 60 min. The resulting supernatant was dialyzed overnight against at least 50 vol. 20 mM acetate buffer (pH 6.0), with three changes of the buffer. This dialyzed supernatant served as the source of tyrosine hydroxylase. For some experiments, the dialyzed supernatant was fractionated with ammonium sulfate as described [13]. Most of the tyrosine hydroxylase was precipitated with 25–35% saturation of ammonium sulfate. This fraction was dialyzed and used as the partially purified enzyme preparation. All steps in preparing the enzyme were done at 0–5°C.

Tyrosine hydroxylase activity was assayed by the method [13,14]. The standard incubation mixture (total vol. 1.0 ml) contained: 200 μmol acetate buffer (pH 6.0), 1.0 μmol $FeSO_4$, 10 μmol 2-mercaptoethanol, 0.1 μmol $DMPH_4$ (2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine, Pfaltz and Bauer Inc., USA) and enzyme solution. This mixture was preincubated for 5 min at 37°C in the presence or absence of nucleotides, and then the reaction was started by adding 0.1 μmol (0.05 μCi) of L-[^{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. [^{14}C]DOPA formed during the reaction was isolated on aluminum hydroxide and its radioactivity was assayed in a liquid scintillation counter (Packard Tri-Carb, Model 3385).

Protein was assayed by the method [15] with bovine serum albumin as the standard.

3. Results and discussion

The effects of various nucleotides at concentrations of 4 mM on tyrosine hydroxylase in the supernatant are shown in table 1; ATP, GTP, UTP and CTP increased the activity 15–20-fold, and ADP and AMP increased the activity about 6-, and 3-fold, respectively, but adenosine had no effect. These nucleotides also activated the partially purified enzyme preparation. Table 2 shows the effect of ATP concentration on the activity of partially purified enzyme. Activation was observed with concentrations of 0.5–1 mM ATP, and levelled off at about 8 mM ATP. The activations by GTP, UTP and CTP showed similar dose-responses to that by ATP.

Next, the nature of this activation was studied. In preliminary experiments we found that the enzyme activity increased with increase in the concentration of the cofactor, $DMPH_4$, but concomitantly activation by ATP and other nucleotides decreased and with 1 mM $DMPH_4$ (the saturation concentration) the latter had only slight effects. Therefore, we examined the effect of ATP on the K_m for cofactor. Lineweaver-Burke analysis showed that ATP reduced the apparent K_m of the enzyme for $DMPH_4$, but did not affect the V_{max} (fig.1). ATP caused about 5-fold decrease in the K_m for $DMPH_4$. Similar experiments were done with tyrosine concentrations of 10^{-5}–10^{-4} M at a saturating concentration of the cofactor, $DMPH_4$ (1 mM). ATP had no significant effect on the K_m of the enzyme for the substrate, tyrosine, or on the V_{max} (data not shown). Thus activation of tyrosine hydroxylase by ATP (and probably other nucleotides) appears to be due mainly to increase in the affinity of the enzyme for the pteridine cofactor.

The mechanism by which ATP activates the

Table 1
Effects of various nucleotides on tyrosine hydroxylase in the supernatant fraction of bovine adrenal medulla

Nucleotide	Tyrosine hydroxylase activity (nmol DOPA/mg protein/20 min)
None	0.28 ± 0.04
Adenosine	0.38 ± 0.27
AMP	0.86 ± 0.22
ADP	1.73 ± 0.09
ATP	5.66 ± 0.28
GTP	4.88 ± 0.15
UTP	4.20 ± 0.32
CTP	4.88 ± 0.18

Tyrosine hydroxylase activity was assayed as described under Methods with 0.1 mM $DMPH_4$. Values are means ± SD of results in at least four experiments. Nucleotides were added at concentrations of 4 mM

Table 2
Effect of ATP concentration on partially purified tyrosine hydroxylase from bovine adrenal medulla

ATP concentration (mM)	Tyrosine hydroxylase activity (nmol DOPA/mg protein/20 min)
0	5.35 ± 0.61
0.5	7.26 ± 0.52
1	9.35 ± 0.41
2	35.12 ± 1.31
4	70.31 ± 4.04
8	95.85 ± 1.21

Tyrosine hydroxylase activity was assayed as described under Methods with 0.1 mM $DMPH_4$. Values are means ± SD of results in three experiments

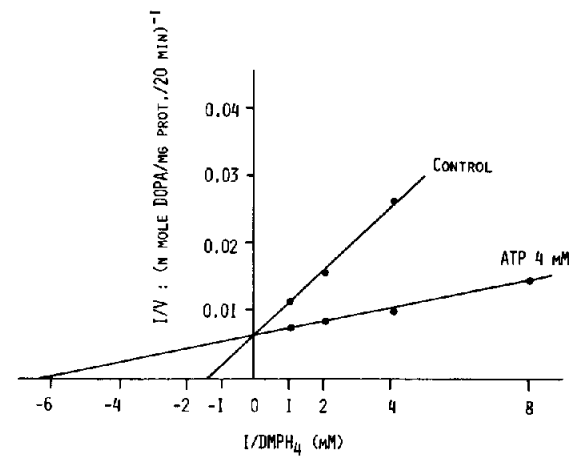

Fig.1. Effect of ATP on the reciprocal (Lineweaver-Burke) plot of the rate of the reaction versus the concentration of the cofactor $DMPH_4$. Tyrosine hydroxylase activity was measured as described under Methods with various concentrations of $DMPH_4$ and 10^{-4} M tyrosine.

Table 3
Effects of calcium, magnesium, and EGTA on tyrosine hydroxylase from bovine adrenal medulla

Additions	Tyrosine hydroxylase activity (nmol DOPA/mg protein/20 min)
Supernatant fraction	
None	0.28 ± 0.04
EGTA 1 mM	0.26 ± 0.04
ATP 4 mM	5.66 ± 0.27
ATP 4 mM + EGTA 1 mM	6.73 ± 0.41
Partially purified enzyme	
None	6.12 ± 0.61
$CaCl_2$ 1 mM	6.20 ± 0.77
$MgSO_4$ 1 mM	5.42 ± 0.52
ATP 4 mM	71.66 ± 1.26
ATP 4 mM + $CaCl_2$ 1 mM	65.56 ± 2.18
ATP 4 mM + $MgSO_4$ 1 mM	64.70 ± 3.63

Tyrosine hydroxylase activity was assayed as described under Methods with 0.1 mM $DMPH_4$. Values are means ± SD of results in three experiments

tyrosine hydroxylase is still unknown. However, under our experimental conditions in which ATP activated the tyrosine hydroxylase in the supernatant, liberation of inorganic phosphate from ATP was not observed. Moreover, neither calcium nor magnesium had any affect on the basal activity of tyrosine hydroxylase or on its activation by ATP. These properties were also not significantly affected by the addition of EGTA (calcium chelator, ethyleneglycol bis (β-aminoethylether)-tetraacetic acid), suggesting that the properties are not dependent upon trace concentrations of calcium (table 3). These results suggest that the activation by ATP is probably due to a physical interaction of the enzyme with ATP rather than to a specific enzymic reaction. It was reported [16–18] that the activity of brain tyrosine hydroxylase was increased by heparin, phosphatidylserine and polyanions which reduced the K_m of the enzyme for pteridine cofactor. Recently, it was reported that these polyanions could also activate the bovine adrenal enzyme [19]. Therefore, the activation by nucleotides may also be somehow related to the polyanionic character of nucleotides.

To summarize: the present findings indicate that ATP (and probably other nucleotides) play a basic role in activation of adrenal tyrosine hydroxylase, mainly by increasing the affinity of the enzyme for the pteridine cofactor. However, the physiological significance in vivo of this mechanism of regulation of tyrosine hydroxylase requires further investigation.

Acknowledgements

We thank Miss Takako Nagao and Mrs Keiko Tachibana for technical assistance. This study was supported by Grants No. 157062 and No. 137015 from the Japanese Ministry of Education, Science and Culture.

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