ACTIVATION OF BOVINE ADRENAL DOPAMINE β-HYDROXYLASE BY ADP AND OTHER NUCLEOTIDES

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Received 5 February 1979

1. Introduction

Dopamine β-hydroxylase (DBH; EC 1.14.2.1: 3,4-dihydroxylphenylethylamine, ascorbate; oxygen oxidoreductase) is known to be localized in chromaffin granules in the adrenal medulla [1–3] and is regarded as an important enzyme for modulating the tissue level of noradrenaline [4,5].

During work on regulation of catecholamine synthesis [6], we found that adrenal tyrosine hydroxylase, a rate limiting enzyme in catecholamine synthesis, was markedly activated by ATP, GTP, UTP and CTP, and also by ADP and AMP.

In this work we have studied the effects of these nucleotides on DBH from bovine adrenal medulla. Results showed that these nucleotides, and especially ADP, also activated DBH. ADP increased the V_{max} of the reaction without affecting the K_m for the substrate, tyramine.

2. Materials and methods

For isolation of chromaffin granules, bovine adrenal medulla was homogenized with 0.25 M sucrose containing 20 mM Tris–HCl (pH 7.4). The homogenate was centrifuged at 600 × *g* for 10 min and the supernatant was recentrifuged at 11 000 × *g* for 30 min. The resulting pellet, containing intact chromaffin granules, was suspended in 0.25 M sucrose containing 20 mM Tris–HCl buffer (pH 7.4). This suspension (1 vol.) was layered over (3 vol.) of 1.6 M sucrose containing 20 mM Tris–HCl buffer (pH 7.4) and the chromaffin granules were precipitated by centrifugation at 105 000 × *g* for 1 h [7]. DBH is known to be present in the granules in two forms: one form is readily solubilized by osmotic lysis, while the other appears to be firmly bound to the membranes [8]. Therefore, the chromaffin granules were lysed by suspending them in 10 mM Tris–HCl buffer (pH 7.0), freeze-thawing the mixture 3 times and centrifuging it at 25 000 × *g* for 30 min. The precipitate was washed with 10 mM Tris–HCl buffer (pH 7.0) by centrifugation and used as membrane-bound DBH; the supernatant was dialyzed against 100 vol. of the same buffer for 48 h with 6 changes of the buffer and used as soluble DBH.

DBH, extracted from the chromaffin granules with 0.1% Triton X-100, was purified by fractionation with ammonium sulfate, chromatography on DEAE-cellulose and gel filtration on Sephadex G-200 [9–11]. The purified enzyme appeared essentially homogeneous, giving a single band on polyacrylamide gel electrophoresis.

DBH activity was routinely assayed with tyramine as substrate [12]. The standard incubation mixture (total vol. 1.0 ml) contained 20 mM tyramine, 10 mM ascorbic acid, 20 mM Tris–HCl buffer (pH 7.0) and enzyme solution. After incubation for 10 min at 37°C, the reaction was stopped by adding 4 ml of 0.5 N PCA and formation of the β-hydroxylated product octopamine was determined spectrophotometrically by the periodate method [12,13].

The nucleotides used were obtained from Sigma.

Table 1
Effects of various nucleotides on membrane bound and soluble DBH from bovine adrenal medullary chromaffin granules

Nucleotide	Membrane-bound DBH	Soluble DBH
None	0.26 ± 0.02	0.19 ± 0.03
Adenosine	0.32 ± 0.03	0.20 ± 0.02
AMP	0.28 ± 0.05	0.25 ± 0.01
ADP	1.15 ± 0.11	0.84 ± 0.02
ATP	0.43 ± 0.05	0.50 ± 0.07
GDP	0.50 ± 0.04	0.38 ± 0.07
GTP	0.45 ± 0.01	0.35 ± 0.02
UDP	0.81 ± 0.05	0.51 ± 0.01

DBH activity was assayed as in section 2 with tyramine as substrate. DBH activity is expressed as μmol octopamine formed/mg protein/10 min. Values are means ± SD of results in at least 4 experiments. Nucleotides were added at 2 mM

3. Results and discussion

The effects of various nucleotides at 2 mM on membrane-bound and soluble DBH activities from chromaffin granules are shown in table 1. Both forms of DBH were activated by ADP, ATP, GDP, GTP and UDP, but not AMP or adenosine. ADP caused the highest activation; 2 mM ADP increased the activities of both membrane-bound and soluble DBH 4–5-fold.

ADP also activated the purified preparation of DBH. As shown in table 2, activation increased with the concentration of ADP at 0.1–0.5 mM ADP and leveled off at 8 mM ADP. The time course of activation of DBH by ADP is shown in fig.1; activation was already marked after 1 min of incubation, indicating that ADP caused rapid activation of DBH.

Next, the nature of this activation was studied. Figure 2 shows the effect of ADP on the K_m for the substrate, tyramine, and the V_{max} of the reaction.

Table 2
Effect of ADP on purified DBH from bovine adrenal medullary granules

ADP (mM)	DBH activity (μmol octopamine/mg protein/10 min)
0	7.74 ± 0.10
0.1	10.41 ± 0.09
0.5	18.84 ± 0.47
1.0	24.19 ± 1.79
2.0	36.91 ± 1.50
4.0	58.32 ± 1.00
8.0	67.07 ± 2.10
10.0	69.19 ± 2.79

DBH activity was assayed as in section 2 with tyramine as substrate. Values are means ± SD of results in 4 experiments

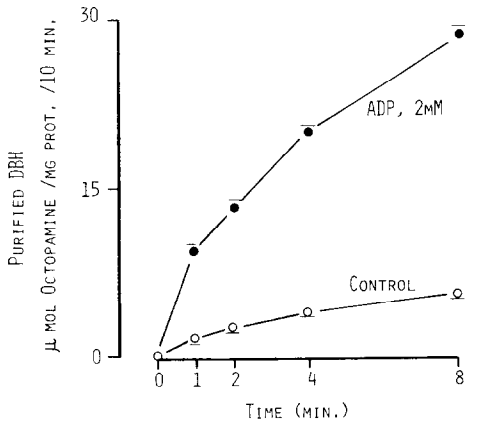

Fig.1. Time course of activation of purified DBH by ADP. DBH activity was assayed as in section 2 with tyramine as substrate. Values are means of 4 determinations with SD. The concentration of ADP was 2 mM.

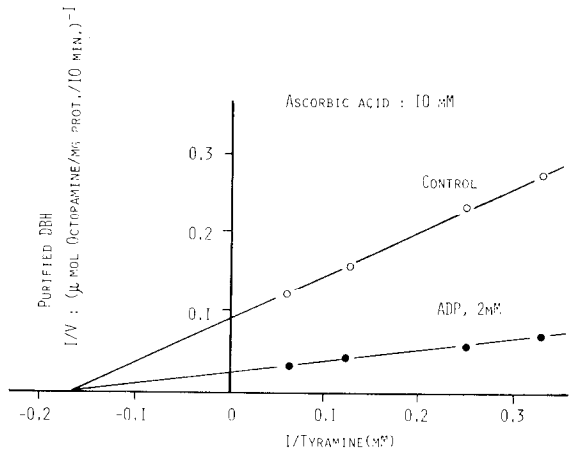

Fig.2. Effect of ADP on the reciprocal of the rate of the reaction versus the concentration of substrate, tyramine (Lineweaver-Burke plot). DBH activity was measured as in section 2 with various concentrations of tyramine and 10 mM ascorbic acid.

ADP increased the V_{max} of the reaction without affecting the K_m for tyramine. Thus it appears to increase, not the affinity of the enzyme for the substrate, but the catalytic properties of the enzyme itself.

DBH activity is known to increase on lowering the pH of the reaction, or adding fumarate, *N*-ethyl-maleimide or catalase [12]. Therefore, the effect of ADP on DBH activity was examined at various pH values (pH 4.5–7.5) and in the presence of fumarate (1–20 mM), *N*-ethyl-maleimide (1–20 mM) or catalase (650–1300 units). Activation of DBH by ADP was marked under all the conditions tested, suggesting that the mechanism of action of ADP is different from those of other activators. Recently, we found in preliminary studies that ADP induces a conformational change of the DBH protein, detectable by measuring circular dichroism.

It is still unknown whether ADP actually regulates DBH in vivo. However, it seems likely that when dopamine is taken up into the chromaffin granules by an ATP-Mg^{2+}-dependent process [14,15], the ADP formed from ATP stimulates the conversion of dopamine to noradrenaline, which is then stored in the chromaffin granules.

Acknowledgements

We thank Mrs Keiko Tachibana and Miss Youko Sugiura for technical assistance. This study was supported by Grants from the Japanese Ministry of Education, Science and Culture.

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