Inhibition of dihydropteridine reductase by dopamine

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Dihydropteridine reductase has been purified 900-fold from rat liver. Dopamine inhibited the enzyme up to 50% at a concentration of 0.11 mM. In the presence of dopamine the enzyme gave non-hyperbolic *v*-against-[S] plots. This enzyme may have a role in control of dopamine biosynthesis.

The rate-limiting step for dopamine biosynthesis is tyrosine hydroxylase, but the rate-limiting factor is not the concentration of the enzyme, but the concentration of its cofactor, tetrahydrobiopterin (Kettler *et al.*, 1974). Alteration of tetrahydrobiopterin concentrations would, therefore, alter tyrosine hydroxylase activity within the cell. During the enzymic hydroxylation of tyrosine to dopa, tetrahydrobiopterin is oxidized to quinonoid dihydrobiopterin (Kaufman, 1964). Quinonoid dihydrobiopterin is reduced to tetrahydrobiopterin by dihydropteridine reductase (EC 1.6.99.10), which requires NADH for the reduction. Quinonoid dihydrobiopterin is unstable, and rapidly rearranges to 7,8-dihydrobiopterin, which is not a substrate for dihydropteridine reductase.

The essential role of dihydropteridine reductase is clearly demonstrated in children congenitally deficient in this enzyme. These children are usually diagnosed as having phenylketonuria, but fail to respond to a low phenylalanine diet. They are unable to synthesize the neurotransmitters dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine (Kaufman *et al.*, 1975), and have high serum concentrations of 7,8-dihydrobiopterin. It is therefore possible that the rate-limiting factor for dopamine biosynthesis is dihydropteridine reductase, and that this enzyme could be a site for control of dopamine biosynthesis. Tyrosine hydroxylase itself is known to be a regulatory enzyme for dopamine biosynthesis, but its behaviour is not wholly explained by its allosteric inhibition by dopamine (Waggoner *et al.*, 1980). In the present paper we report the effect of dopamine on dihydropteridine reductase.

Materials and methods

Purification of rat liver dihydropteridine reductase

Rat liver dihydropteridine reductase was purified by the affinity chromatography method of Cotton &

Jennings (1978). Male Wistar rats (approx. 200 g body wt.) were killed and the livers were quickly removed into ice-cold beakers. After preparation of the rat liver supernatant fraction, the enzyme was partially purified by chromatography on Sephadex G-150 before affinity chromatography on AH Sepharose 4B with 1,2-naphthoquinone 4-sulphonate as the affinity ligand. Protein concentrations were determined by the method of Lowry *et al.* (1951).

Assay of enzyme activity

Dihydropteridine reductase activity was measured by two methods. Method 1 was that of Craine *et al.* (1972), which uses H₂O₂ and horseradish peroxidase to generate the quinonoid dihydropterin or method 2 (Cheema *et al.*, 1973), in which 2,6-dichlorophenol-indophenol is used to generate the quinonoid dihydropterin. Both methods use an artificial substrate, 6,7-dimethyl-5,6,7,8-tetrahydropterin instead of tetrahydrobiopterin.

The reaction was started by addition of NADH. Enzyme activity was monitored by measuring the decrease in absorbance at 340 nm using a Pye-Unicam 1750 recording spectrophotometer.

Inhibitor studies

Dihydropteridine reductase activity was assayed by methods 1 and 2 in the presence of dopamine (1.1 mM, 0.53 mM, 0.11 mM). Dopamine inhibition was determined by using either 6,7-dimethyl-5,6,7,8-tetrahydropterin or NADH as the variable substrate.

Chemicals

6,7-Dimethyl-5,6,7,8-tetrahydropterin, dopamine, NADH, horseradish peroxidase and 2,6-dichlorophenol-indophenol were purchased from Sigma London Chemical Co., Poole, Dorset, U.K. Sodium naphthoquinone 4-sulphonate was purchased from Cambrian Chemicals, Croydon, CRO 4XB, U.K. AH Sepharose 4B and Sephadex G-150

Table 1. *Purification of dihydropteridine reductase*
For details see the text.

	Enzyme activity ($\mu\text{mol/ml per min}$)	Protein (mg/ml)	Specific activity ($\mu\text{mol/min per mg of protein}$)	Purification factor	Recovery (%)
Rat liver supernatant	2.72	49.0	0.06	1	100
Enzyme after Sephadex G-150 chromatography	9.67	1.82	5.32	89	68
Enzyme after affinity chromatography	5.51	0.10	55.1	918	25

were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were of Analar grade or its equivalent.

Results and discussion

Purification of dihydropteridine reductase

Purification data for dihydropteridine reductase are given in Table 1. The enzyme was purified 900-fold compared with rat liver supernatant and this purified enzyme was used for all further studies. The purified enzyme gave a K_m value of $32\mu\text{M}$ for NADH and $15\mu\text{M}$ for 6,7-dimethyl-5,6,7,8-tetrahydropterin. These values are in agreement with published values (Craine *et al.*, 1972; Webber, *et al.*, 1978; Webber & Whiteley, 1978).

Inhibition of dihydropteridine reductase

Dihydropteridine reductase activity was measured in the presence of dopamine (1.1 mM, 0.53 mM and 0.11 mM). Dopamine inhibited dihydropteridine reductase whether assayed by method 1 or method 2, indicating that inhibition by dopamine was by an action on dihydropteridine reductase, and not on the system used to generate the quinonoid dihydropterin. Fig. 1 shows v -against-[S] plots for dihydropteridine reductase at a constant concentration of NADH, varying the concentration of 6,7-dimethyl-5,6,7,8-tetrahydropterin. Similar results were obtained when NADH was the variable substrate and the concentration of 6,7-dimethyl-5,6,7,8-tetrahydropterin was constant.

Plots of v against [S] in the absence of dopamine for both NADH and the tetrahydropterin were rectangular hyperbolas, and the data gave good straight-line plots using the methods of Lineweaver and Burke and Eadie and Hofstee. In the presence of dopamine the v -against-[S] plots are no longer rectangular hyperbolas, and it was not possible to draw any straight-line plots with these data. In the presence of dopamine, v -against-[S] plots show positive co-operativity with respect to both 6,7-dimethyl-5,6,7,8-tetrahydropterin and NADH. We therefore suggest that dihydropteridine reductase

Fig. 1. *Effect of dopamine on dihydropteridine reductase*
Enzyme activity was assayed by method 2. O, No dopamine; Δ , 0.11 mM-dopamine; $\square$, 0.53 mM-dopamine.

may be a regulatory enzyme involved in the control of dopamine biosynthesis. Waggoner *et al.* (1980) have shown that dopamine inhibits tyrosine hydroxylase activity both when the enzyme is in synaptosomes and when it has been solubilized. Their results showed that there was greater inhibition of tyrosine hydroxylase by dopamine in synaptosomal preparations than when the enzyme was solubilized. They were unable to explain their results, but suggested that dopamine could function by lowering reduced cofactor concentrations (tetrahydrobiopterin).

Our results show a marked inhibition of dihydropteridine reductase by dopamine, suggesting a possible role for this enzyme in the control of dopamine biosynthesis.

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