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EFFECTS OF MELANIN ON TYROSINE HYDROXYLASE AND PHENYLALANINE HYDROXYLASE

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Summary

Melanin inhibited rat liver phenylalanine hydroxylase, but activated tyrosine hydroxylase from rat brain (caudate nucleus), rat adrenal glands, and bovine adrenal medulla. Activation of tyrosine hydroxylase by melanin was demonstrated with the extensively dialyzed enzyme and in suboptimal concentrations of the substrate (tyrosine) and the cofactor (6-methyltetrahydropterin). Tyrosine hydroxylase from rat brain was activated by melanin more markedly than that from rat adrenal glands. Purified and extensively dialyzed bovine adrenal tyrosine hydroxylase had two K_m values with 6-methyltetrahydropterin, depending upon its concentrations, but the melanin-activated tyrosine hydroxylase had a single K_m value and showed the classical Michaelis-Menten kinetics.

Introduction

It is well known that the essential lesion of Parkinson's disease is an idiopathic degeneration of the nigro-striatal dopaminergic neurones which contain melanin. The concentration of dopamine in the pigmented neurones decreases simultaneously with the disappearance of melanin [1]. It has been also found that tyrosine hydroxylase activity is greatly decreased in nigro-striatum of Parkinsonian patients [2—4]. On the other hand, Katz et al. [5] reported that various polyanions such as heparin, chondroitin sulfate and phosphatidylserine activate tyrosine hydroxylase from the bovine caudate nucleus.

Since melanin is located together with tyrosine hydroxylase in the same nigral pigmented neurones and it has polyanionic properties [6], we have examined the effect of melanin on tyrosine hydroxylase from brain and adrenal

glands. The effect of melanin on phenylalanine hydroxylase, which is a similar tetrahydropterin-dependent monooxygenase but located in the liver cell, has been also examined for comparison.

Materials and Methods

6-Methyltetrahydropterin was purchased from Calbiochem. L-*erythro*-biopterin was isolated from the skin of the bullfrog [7], and the corresponding 5,6,7,8-tetrahydro derivative was prepared by catalytic hydrogenation in 0.1 M HCl using platinum oxide as a catalyst [8]. Tyrosine hydroxylase from rat brain (caudate nucleus) or adrenal glands was prepared by ammonium sulfate fractionation. Rat caudate nucleus or adrenal glands was homogenized in 9 vols. of 0.32 M sucrose with a glass Potter homogenizer, and the homogenate was centrifuged at 30 000 × *g* for 30 min. To the supernatant solid $(NH_4)_2SO_4$ was added to give 45% saturation, and the mixture was centrifuged at 10 000 × *g* for 20 min. The precipitate was dissolved in 20 mM potassium phosphate buffer (pH 6.2) and dialyzed against 200 vols. of 5 mM 2-(*N*-morpholino)-ethanesulfonic acid buffer (pH 6.2), changed 3 times. Bovine adrenal medulla tyrosine hydroxylase was purified from the soluble fraction by ammonium sulfate fractionation and column chromatography on Sephadex G-200 as described previously [9]. The enzyme solution was dialyzed against 5 mM buffer (pH 6.2) as in the case of rat brain or adrenal enzyme. Phenylalanine hydroxylase was prepared from rat liver by the method of Sugiyama (unpublished). Dihydropteridine reductase was purified from the extract of bovine liver by the method of Hasegawa [10]. Melanin was prepared from L-dopa [6], and the colloidal aqueous suspension was used. The concentration of melanin was tentatively expressed as dry weight per ml suspension.

Tyrosine hydroxylase activity was measured by estimating the formation of [^{14}C]dopa from L-[U-^{14}C]tyrosine [11,12]. The incubation mixture contained 0.1 M buffer (pH 6.2), 40 μg (2000 U) catalase, the enzyme, 200 μM (or at various concentrations for kinetic studies) 6-methyltetrahydropterin, 20 mM mercaptoethanol, 50 μM (or at various concentrations for kinetic studies) L-tyrosine containing 0.07 μCi L-[U-^{14}C]tyrosine (483 mCi/mmol) and water to make up a total volume of 0.5 ml. Incubation was carried out in air at 30°C for 10 min. For controls water was added instead of enzyme. Boiled-enzyme controls were also carried out, and both control values were similar (about 350 cpm).

The assay mixture (1.0 ml) for phenylalanine hydroxylase contained 0.1 M potassium phosphate buffer (pH 6.8) 1 mM phenylalanine 0.2 mM NADPH 77 μg (300 mU) dihydropteridine reductase phenylalanine hydroxylase and 20 μM L-*erythro*-tetrahydrobiopterin. Incubation was carried out in air at 25°C for 30 min. Tyrosine formed was determined by the nitroso-naphthol procedure [13]. Melanin did not have any effect on nitroso-naphthol assay of tyrosine. Protein was measured by the method of Lowry et al. [15].

The K_m values with purified bovine adrenal tyrosine hydroxylase were determined from Lineweaver-Burk plots [15] on a Facom-230 computer using Wilkinson's program [16], and expressed as mean ± standard error of mean.

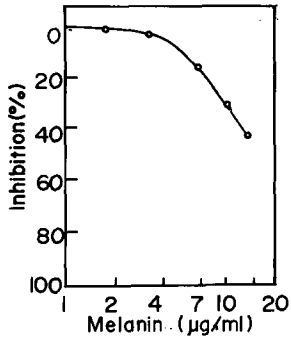
Results

Effect of melanin on rat liver phenylalanine hydroxylase

Melanin inhibited rat liver phenylalanine hydroxylase (Fig. 1). Preincubation of the enzyme with melanin in the presence or absence of phenylalanine gave similar inhibition rates.

Effect of melanin on tyrosine hydroxylase from rat brain and rat adrenal glands

The dialyzed crude tyrosine hydroxylase from brain (caudate nucleus) or adrenal glands (Fig. 2) was activated markedly by melanin; the activation of tyrosine hydroxylase was more pronounced with the brain enzyme (about 10-fold at 100 μg/ml of melanin) than that with the adrenal enzyme (about 3-fold at 100 μg of melanin). However, the activation curves with the increasing concentration of melanin were similar between brain and adrenal enzymes. The activation was observed only in the suboptimal concentrations of tyrosine (50 μM), and 6-methyltetrahydropterin (200 μM), and not in the saturated concentration of tyrosine (200 μM) and 6-methyltetrahydropterin (1.5 mM). As shown in Fig. 3, rat brain enzyme showed a deviation from classical Michaelis-Menten kinetics against 6-methyltetrahydropterin in the absence of melanin and two different K_m values were obtained depending on whether the concentrations were lower or higher than 200 μM. At low concentrations (less than 200 μM) a relatively low K_m value of about 120 μM was observed, whereas at higher concentrations (higher than 200 μM) the K_m values was higher and registered about 2.8 mM. The melanin-activated enzyme gave a normal Michaelis-Menten kinetics and a single K_m value of about 220 μM.



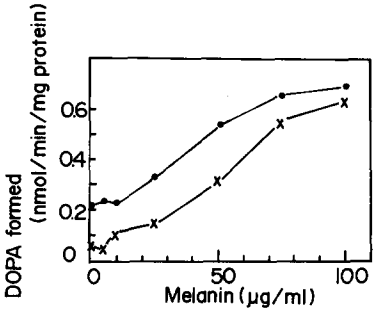


Fig. 1. Effect of melanin on rat liver phenylalanine hydroxylase. The reaction mixture (total volume, 1.0 ml) containing (in final concentrations) 0.1 M potassium phosphate buffer (pH 6.8), 0.2 mM NADPH, 300 mU (77 μg) dihydropteridine reductase, 1 mM phenylalanine, 92 μg phenylalanine hydroxylase, and various amounts of melanin was preincubated at 25°C for 10 min, and the reaction was started by the addition of 20 μM tetrahydrobiopterin. The incubation was carried out at 25°C for 30 min.

Fig. 2. Effect of melanin on rat brain (caudate nucleus) or rat adrenal tyrosine hydroxylase. The enzyme in the soluble fraction was fractionated with ammonium sulfate and extensively dialyzed, as described in the text. 480 μg of the brain enzyme and 450 μg of the adrenal enzyme were used for each assay in the standard incubation mixture including 50 μM tyrosine and 200 μM 6-methyltetrahydropterin as described in the text. The values are the mean of duplicate experiments. •———•, rat brain enzyme; X———X, rat adrenal enzyme.

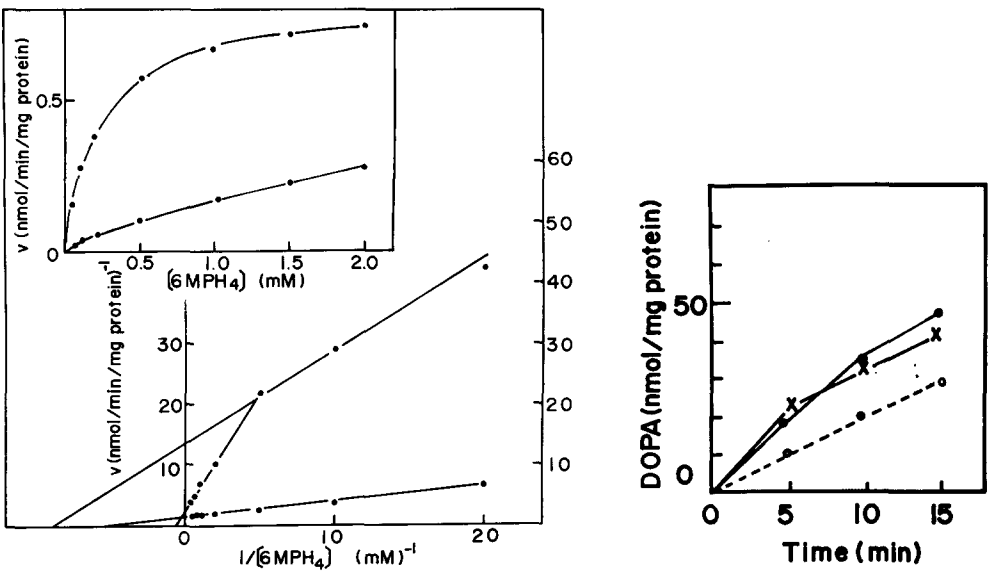


Fig. 3. Lineweaver-Burk and Michaelis-Menten plots illustrating the effect of 6-methyltetrahydropterin on the rate of dopa formation by rat brain tyrosine hydroxylase. The values are the mean of duplicate experiments. The standard incubation mixture including 480 μg of the enzyme, 50 μM tyrosine and 50 μg/ml melanin was used. ●———●, control (no melanin); ○———○, plus melanin 50 μg/ml.

Fig. 4. Effect of melanin on purified bovine adrenal tyrosine hydroxylase. Tyrosine hydroxylase was purified from the soluble fraction of bovine adrenal medulla and dialyzed extensively, as described in the text. The standard incubation mixture including 27 μg of the enzyme, 50 μM tyrosine and 5 μg/ml or 50 μg/ml of melanin was used. ○———○, control (no melanin); ●———●, plus melanin 5 μg/ml; X———X, plus melanin 50 μg/ml.

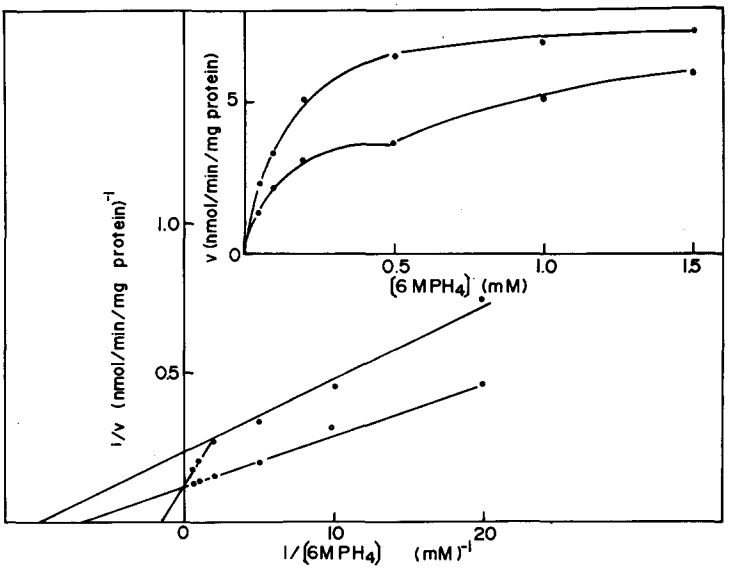


Fig. 5. Lineweaver-Burk and Michaelis-Menten plots illustrating the effect of 6-methyltetrahydropterin on the rate of dopa formation by purified bovine adrenal tyrosine hydroxylase. The standard incubation mixture including 50 μM tyrosine and 5 μg/ml of melanin was used. ●———●, control (no melanin); ○———○, plus melanin 5 μg/ml. The values are the mean of duplicate experiments.

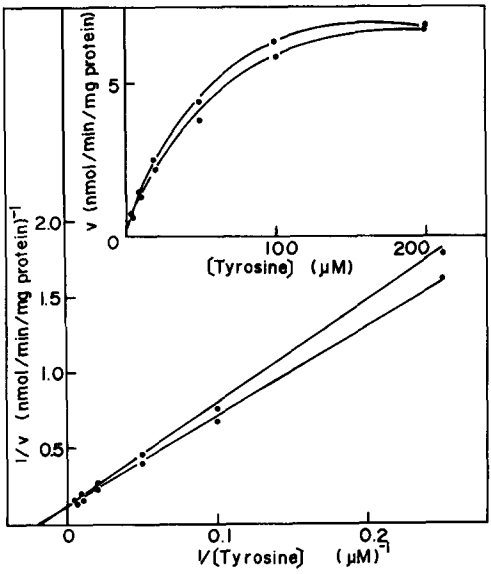


Fig. 6. Lineweaver-Burk and Michaelis-Menten plots illustrating the effect of tyrosine on the rate of dopa formation by purified bovine adrenal tyrosine hydroxylase. The standard incubation mixture including 1.5 mM 6-methyltetrahydropterin and 5 μg/ml of melanin was used. ●———●, control; ○———○, plus melanin 5 μg/ml. The values are the mean of duplicate experiments.

Effect of melanin on tyrosine hydroxylase purified from bovine adrenal medulla

To investigate precisely the kinetic properties of the activation of tyrosine hydroxylase by melanin, tyrosine hydroxylase was purified from bovine adrenal medulla. The purified enzyme was activated by melanin at much lower concentrations (5 μg/ml) than the crude rat adrenal or brain enzyme. Similar degrees of activation were observed either without or with preincubation of the enzyme with melanin. Melanin at higher concentrations (50 μg/ml) showed similar activation as at 5 μg/ml (Fig. 4). As shown in Fig. 5, the purified and dialyzed tyrosine hydroxylase, like crude rat brain enzyme (Fig. 3), showed a pronounced deviation from classical Michaelis-Menten kinetics against 6-methyltetrahydropterin in the absence of melanin; at low concentrations (less than 500 μM) of 6-methyltetrahydropterin a relatively low K_m value of 102 ± 15 μM was observed, whereas at higher concentrations (higher than 500 μM) the K_m value was much higher and registered 659 ± 20 μM. In contrast, the melanin-activated tyrosine hydroxylase gave a single K_m value of about 141 ± 15 μM. The activation was observed only in the suboptimal concentrations of tyrosine and 6-methyltetrahydropterin. The K_m value of tyrosine was measured with 6-methyltetrahydropterin cofactor at 1.5 mM. As shown in Fig. 6, a classical Michaelis-Menten curve was observed. K_m values of tyrosine in the absence and presence of melanin were 65 ± 7 μM and 47 ± 8 μM, respectively.

Discussion

The above data demonstrate that melanin which is present in the nigral pigmented neurones together with tyrosine hydroxylase activates tyrosine

hydroxylase from brain (caudate nucleus) and adrenal glands. The stimulation by melanin occurs only in the presence of subsaturating concentrations of substrate and pterin cofactor. The data presented indicate that melanin affects primarily the cofactor affinity. Interestingly, another similar pterin-dependent monooxygenase, rat liver phenylalanine hydroxylase, was not activated but inhibited by melanin. The mechanism of activation of tyrosine hydroxylase by melanin may be due to the interaction of melanin with the enzyme as polyanion, as reported by Katz et al. [5]. The polyanionic nature of melanin was described by Akino (ref. 6, see also ref. 17). The activation by melanin was observed only in the buffer with low ionic strength, and only with the extensively dialyzed enzyme. We reported that 6-methyltetrahydropterin showed a single K_m value with the purified undialyzed bovine adrenal tyrosine hydroxylase in the incubation mixture with a high (0.2 M) sodium acetate buffer concentration [9]. In the present study, it has been found that two different K_m values of 6-methyltetrahydropterin were obtained with the dialyzed enzyme and under low salt concentrations. This deviation from classical Michaelis-Menten curves was normalized by melanin as the enzyme was activated. Melanin may introduce a conformational change on tyrosine hydroxylase by interacting with the enzyme in its active site, as proposed by Katz et al. on various polyanions [5]. Although it is difficult to extrapolate present in vitro phenomenon of melanin activation of tyrosine hydroxylase to the enzyme regulation in vivo, it is interesting that the natural substance which presents with tyrosine hydroxylase in the pigmented brain neurones can activate tyrosine hydroxylase.

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