EFFECT OF COCAINE ON TYROSINE HYDROXYLASE OF RAT HYPOTHALAMUS

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The effect of cocaine (an adrenergic agent) on tyrosine hydroxylase of the rat hypothalamus was investigated in vivo (0.5 mg/kg) and in vitro (in concentrations of 10^{-6}-10^{-5} M). Cocaine reduces K_m for the coenzyme 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$) without altering the maximal reaction velocity. Internal administration of cocaine leads to a decrease in tyrosine hydroxylase activity after 10 min, especially of the membrane-bound form of that enzyme. After injection of cocaine a decrease in K_m was found for $DMPH_4$ and a decrease in the maximal reaction velocity. The decrease in the maximal reaction velocity is regarded as the result of the indirect action of cocaine.

KEY WORDS: cocaine; tyrosine hydroxylase.

Catecholamine synthesis occupies an important place in brain metabolism. Of special interest among the enzymes of catecholamine synthesis is tyrosine hydroxylase, which limits the overall velocity of the process [6]. Activation of the CNS is usually accompanied by an increase in catecholamine synthesis, produced as a rule by an increase in the tyrosine hydroxylase activity of brain tissue [8]. During inhibition of tyrosine hydroxylase, by α-methyl-p-tyrosine, for example, some behavioral responses are inhibited [9]. Many psychotropic drugs also modify tyrosine hydroxylase activity in various brain structures. The direction of these changes is connected with the character of action of the drug [4].

Cocaine has a well-marked central psychostimulant action, and catecholamines participate in its mechanism. It was shown previously [1] that cocaine potentiates the stimulant action of noradrenalin on the CNS and inhibits the reassimilation of noradrenalin by nerve endings [7].

In this investigation the effect of cocaine was studied, as a representative of adrenergic agents of psychostimulant type, on tyrosine hydroxylase activity in the rat hypothalamus, in which it is localized in the presynaptic membranes of nerve endings.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. After decapitation, the brain was removed quickly from 10 animals and tyrosine hydroxylase obtained [5] as the soluble and membrane-bound enzyme. The source of the membrane-bound enzyme was the membrane fraction sedimented at the boundary between 0.8 and 1 M sucrose, which has the greatest specific activity. The initial reaction velocity was determined by a direct spectrophotometric method, by measuring the velocity of oxidation of the pterine coenzyme of tyrosine hydroxylase, namely synthetic 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$). The increase in light absorption on account of the formation of the oxidized form of DMPH was recorded at 335 nm, at the isobestic point of the oxidized forms of the coenzyme [2]. The standard composition of the sample was: Tris–maleate buffer 0.1 M, pH 6.15, enzyme solution 80-100 μg protein, tyrosine 0.1 mM, $DMPH_4$ 0.03 mM. The volume of the sample was 3 ml. The comparison cell contained all components except tyrosine. Temperature 26°C. The enzyme was added to the cells after equalization of the temperature of the buffer solution for 3 min.

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TABLE 1. Effect of Intraperitoneal Injection of Cocaine (0.5 mg/kg) on Rat Brain Tyrosine Hydroxylase Activity (in μmoles/mg protein/min; M ± m)

Form of enzyme	Control	After injection of cocaine		
		10 min	60 min	120 min
Membrane-bound	17,1±1,7	2,8±0,3	4,1±0,5	8,5±1,5
Soluble	12,5±1,0	6,2±0,6	8,1±0,9	11,0±1,5

TABLE 2. Effect of Cocaine on Activity of Isolated Membrane-Bound Tyrosine Hydroxylase (M ± m)

Experimental conditions	Enzyme activity, μmoles/mg protein/min
Control	13,0±1,2
Cocaine:	
10^{-6}M	14,5±1,3
10^{-5}M	16,0±1,4

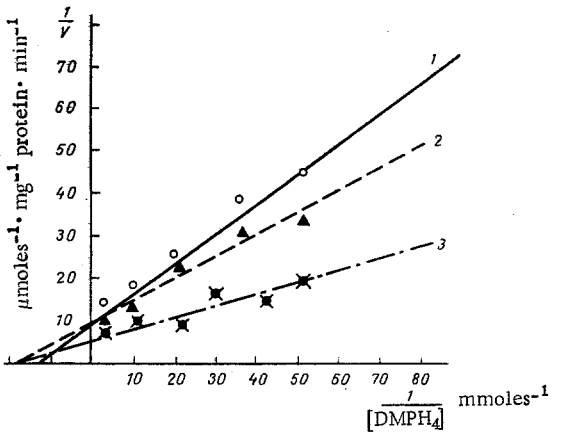

Fig. 1. Velocity of tyrosine hydroxylase reaction as a function of concentration of coenzyme $DMPH_4$: 1) control; 2) addition of cocaine in vitro; 3) injection of cocaine in vivo. Ordinate, 1/v (in $\mu moles^{-1} \cdot mg^{-1}$ protein $\cdot min^{-1}$); abscissa, $1/[DMPH_4]$ (in $mmoles^{-1}$).

Recording the reaction velocity began 10 sec after addition of the coenzyme. The initial reaction velocity was calculated in μmoles $DMPH_2$/min/mg protein of the sample. Oxidation of $DMPH_4$ into $DMPH_2$ was equimolar to dopa formation. A molar extinction coefficient of 4100 was used for the calculations. The final cocaine concentration in the experiments in vitro was 10^{-6}-10^{-5} M. In the experiments in vivo cocaine was injected intraperitoneally in a dose of 0.5 mg/kg. The animals were decapitated 10, 60, and 120 min after the injection.

EXPERIMENTAL RESULTS AND DISCUSSION

After 10 min cocaine caused a significant ($P < 0.05$) decrease in the initial velocity of the tyrosine hydroxylase reaction for the membrane-bound enzyme. The enzyme activity still remained low 1-2 h after injection of the cocaine. Activity of the soluble enzyme underwent a much smaller change after administration of cocaine, although the dynamics of the changes was similar (Table 1).

The direct action of cocaine on tyrosine hydroxylase was studied in experiments in vitro by adding it to the enzyme isolated from the brain of intact animals. In these experiments cocaine, in a concentration of 10^{-6}-10^{-5} M and in the presence of substrate, increased the initial reaction velocity a little (Table 2). This effect also was observed after the addition of cocaine to the complete system during the course of the reaction. However, enzyme activity was not proportional to the concentration of cocaine.

Cocaine thus can directly affect the velocity of the tyrosine hydroxylase reaction and can accelerate the hydroxylation of tyrosine. However, preliminary contact between cocaine and the enzyme inhibited the reaction in the complete system by 20-30%. Probably cocaine can interact with the enzyme protein, to occupy some of the binding sites intended for the substrate, thereby preventing the normal course of the reaction. In that case, the activating effect of cocaine must be due to its interaction with other sites on the enzyme molecule.

The results of determination of the velocity of the tyrosine hydroxylase reaction as a function of $DMPH_4$ concentration are given in Fig. 1. Clearly both after injection of cocaine in vivo and after its addition to the isolated enzyme K_m for $DMPH_4$ was reduced: In the control experiments it was 0.141, after injection of cocaine in vivo it was 0.107, and after addition of cocaine in a concentration of 10^{-5} M in vitro it was 0.111. This suggests that the decrease in K_m for $DMPH_4$ was the result of the direct action of cocaine. The maximal velocity of the tyrosine hydroxylase reaction in the experiments in vitro was unchanged, for it was 0.1 μmole $DMPH_4$/mg protein/min just as in the control, but after injection of cocaine in vivo it fell from 0.1 to 0.041. The decrease in the maximal velocity may perhaps be connected with the regulatory action of dopa, dopamine, or noradrenalin, the assimilation of which is inhibited by cocaine. Calculation of the ratio between the velocities of dissociation of the product [3] shows that the dissociation constant of the products after injection of cocaine was 2.4 times less than under normal conditions.

The observed decrease in tyrosine hydroxylase activity under the influence of cocaine was thus the combined result of its direct and indirect action on the enzyme. Changes in the intracellular medium following injection of cocaine, arising through inhibition of reassimilation and its action on the receptor, lead to changes in the kinetic characteristics of the enzymes, expressed as an increase in affinity for the coenzyme and, simultaneously, as a decrease in the velocity of dissociation of the product.

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