EFFECT OF NEUROLEPTICS ON SYNAPTOSOMAL TYROSINE HYDROXYLASE OF THE RAT HYPOTHALAMUS

M. F. Mineeva-Vyalykh and K. S. Raevskii

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The effect of various neuroleptics with different chemical structure on tyrosine hydroxylase isolated from synaptosomes of the rat hypothalamus was studied. A direct spectrophotometric method was used to determine the activity of the enzyme, based on measurement of absorption at 335 nm, at the isobestic point of oxidized forms of 6,7-dimethyl-5,6,7,8-tetrahydropterine. In the presence of 0.15 mM tyrosine, the initial velocity of the tyrosine-hydroxylase reaction was increased by haloperidol, haloanisone, and fluphenazine, but reduced by triperidol, droperidol, and carbidine. All the neuroleptics studied abolished the phenomenon of substrate inhibition of the enzyme normally arising when the tyrosine concentration is increased to 0.3 mM. The value of K_m for tyrosine (0.04 mmole) was unchanged by the action of the neuroleptics. The effect of the neuroleptics is considered to be allosteric in nature.

KEY WORDS: brain tyrosine hydroxylase; neuroleptics; allosteric regulation.

Neuroleptics accelerate the circulation of dopamine in the brain, and one way in which they do this may be by blocking central dopaminergic pathways, with consequent activation of dopamine synthesis by a feedback mechanism [2, 3]. In the light of these observations, it is interesting to study the effect of neuroleptics on tyrosine hydroxylase (EC 1.14.16.3) from the brain, a key enzyme in catecholamine synthesis [4].

The object of this investigation was to study the direct effect of neuroleptics of different types on tyrosine hydroxylase isolated from synaptosomes on the rat hypothalamus.

EXPERIMENTAL METHOD

Male albino rats weighing 180-200 g were used. After decapitation the brain was quickly removed and the hypothalamus isolated on ice. For one isolation of the enzyme, the brains of 20 animals were usually used. The enzyme was isolated by the method of Kuczenski and Mandell [5]. A weighed sample of tissue was homogenized in 10 volumes of 0.32 M sucrose, centrifuged at 1000 g for 10 min, and then washed with half a volume of the original sucrose solution; the fraction was then obtained from the pooled supernatants by centrifugation at 100,000 g for 1 h. Synaptosomes of this fraction were lysed by a hypotonic solution of K-phosphate buffer, pH 6.9-7.0, and centrifuged for 1 h at 100,000 g. The residue was layered on a 0.6 M–0.8 M–1 M sucrose gradient in the ratio of 1:1:1 and centrifuged at 80,000 g for 2 h. The membrane fraction was taken at the 0.8 M–1 M sucrose boundary. This fraction possesses the highest specific activity of the enzyme [5]. The residue was suspended in 2 M K-phosphate buffer, pH 6.9, and kept for 7 days at 0-4°C.

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TABLE 1. Effect of Neuroleptics on Activity of Membrane-Bound Tyrosine Hydroxylase with Substrate Present in Optimal and Inhibiting Concentrations (M ± m)

Substance (10^{-5} mM)	Activity of enzyme (in nmoles $DMPH_2$/mg protein/min)	
	tyrosine 0.15 mM	tyrosine 0.3 mM
Control	10±0,5	4±0,2
Haloperidol	34±2,0	10±0,4
Haloanisone	19±1,0	11±0,7
Triperidol	5±0,3	10±0,7
Droperidol	6±0,5	8±0,5
Fluphenazine	13±0,8	10±0,5
Carbidine	0	8±0,5

Legend. 1) Concentration of $DMPH_4$ 0.18 mM. 2) Data given from nine measurements, with triple isolation of enzyme.

The velocity of the tyrosine hydroxylase reaction was determined spectrophotometrically at 26°C by the writers' own method, using the Aminco-Chance DW-2 spectrophotometer, based on oxidation of the pteridine coenzyme of tyrosine hydroxylase, for which a synthetic 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_2$), dissolved in 5 mM HCl, was used. The increase in absorption at 335 nm was recorded. The comparison cell contained all the components but tyrosine. The composition of the sample was: 2.9 ml tris-maleate buffer (0.1 M) pH 6.15, 0.02 ml of the enzyme preparation (50-90 μg), 0.1 ml tyrosine solution, and 0.005 ml of $DMPH_4$ solution (10 mM). The reaction was started by addition of the coenxyme. The neuroleptics in a final concentration of 10^{-5} mM were added (0.005 ml) after the substrate. The final concentration of tyrosine was 0.15 mM (optimal concentration) or 0.3 mM, at which inhibition of the enzyme was observed. Before addition of the enzyme, the buffer was kept for 3 min in the cell to equalize the temperature. Recording of the reaction velocity began 10 sec after addition of the coenzyme and continued without interruption for 5-10 min. The reaction velocity was calculated from the formation of $DMPH_2$ (in nanomoles) per minute per milligram protein of the sample. Oxidation of $DMPH_4$ to $DMPH_2$ is equimolar to the formation of 3,4-dihydroxyphenylalanine (dopa). For the calculations the extinction coefficient at 335 nm was taken to be 4100.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the experiments in vitro with neuroleptics of different chemical structure are given in Table 1, from which it can be seen that with the substrate in an optimal concentration of 0.15 mM the neuroleptics affected the velocity of the tyrosine hydroxylase reaction differently. Haloperidol and haloanisone, in a concentration of 10^{-5} mM, caused a marked increase in enzyme activity, and fluphenazine increased the velocity of the enzyme reaction by 30%. The other neuroleptics in the same concentration had the opposite action. Triperidol and droperidol inhibited the activity of the enzyme by 50% and 40%, respectively. A special place among the neuroleptics was occupied by carbidine* [1], which in these experiments completely suppressed tyrosine hydroxylase activity.

As Table 1 shows, in the presence of 0.3 mM tyrosine the velocity of the tyrosine hydroxylase reaction was reduced to 40% of its initial level. Substrate inhibition for tyrosine hydroxylase is described in the literature [5]. Under these experimental conditions the neuroleptics shared the common property of abolishing substrate inhibition of the enzyme. As Table 1 shows, haloperidol, haloanisone, triperidol, and fluphenazine completely abolished substrate inhibition of the enzyme. In the presence of droperidol and carbidine, activity of the enzyme was restored to 80% of its initial level. Substrate inhibition also was abol-abolished when the neuroleptics were added to the complete system during the reaction, usually 3 min after its beginning. Abolition of inhibition by haloperidol developed in the course of time under these conditions and the curve was sigmoid in shape (Fig. 1).

*3,6-Dimethyl-1,2,3,4,4a,9a-hexahydro-γ-carboline di-hydrochloride – Translator.

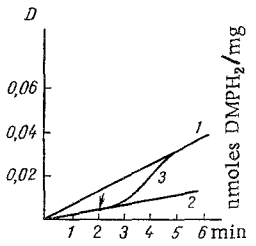

Fig. 1

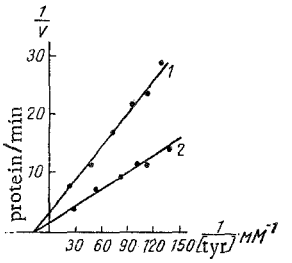

Fig. 2

Fig. 1. Abolition of substrate inhibition of tyrosine hydroxylase by haloperidol. 1, 2) Velocity of tyrosine hydroxylase reaction in the presence of substrate in concentrations of 0.15 and 0.3 mM, respectively; 3) the same after addition (time indicated by arrow) of haloperidol (10^{-5} mM). Abscissa, time (in min); ordinate, absorption of $DMPH_2$ at 335 nm.

Fig. 2. Effect of haloperidol on velocity of tyrosine hydroxylase reaction. 1) Control; 2) haloperidol (10^{-5} mM). Abscissa, reciprocal of tyrosine concentration; ordinate, reciprocal of initial reaction velocity.

The Michaelis constant for tyrosine in these experiments was 0.04 mmole, in agreement with the value described in the literature [5]. In the presence of neuroleptics the value of K_m was unchanged, also in agreement with observations by other workers [6]. The Lineweaver–Burk plot of reaction velocity as a function of tyrosine concentration in the control and in the presence of 10^{-5} mM haloperidol, shown in Fig. 2, demonstrates activation of tyrosine hydroxylase under these conditions in the absence of any change in the value of K_m.

These experiments thus showed that neuroleptics can directly influence the velocity of the tyrosine hydroxylase reaction and abolish substrate inhibition. Considering the absence of any change in the value of K_m for tyrosine and also the abolition of substrate inhibition observed even if the neuroleptics are added to the complete system during the reaction, when the active site of the enzyme is already formed, it must be assumed that the abolition of substrate inhibition effect is due to the action of the neuroleptics on the allosteric region of the enzyme molecule. In the case activation of the enzyme of haloperidol and the other neuroleptics must also be allosteric in nature. Inhibition of activity of the enzyme by triperidol, droperidol, and carbine is evidently due to other causes, such as nonspecific interaction with the enzyme protein, which may mask the allosteric type of enzyme activation characteristic of the neuroleptics.

The property of neuroleptics to influence the allosteric region of the enzyme molecule and to abolish substrate inhibition of tyrosine hydroxylase revealed by these experiments may, it can be suggested, play an important role in the psychotropic effect of these drugs.

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