STEREOSPECIFICITY OF THE EFFECT OF FLUPENTHIXOL ISOMERS ON SUBSTRATE INHIBITION OF BRAIN TYROSINE HYDROXYLASE

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It was shown previously [4] that neuroleptics with different chemical structure can abolish substrate inhibition of rat brain tyrosine hydroxylase [TH (EC 1.14.16.2)], an enzyme limiting the rate of catecholamine biosynthesis [9]. The specific character of this effect was confirmed by experiments with tricyclic antidepressants, which do not possess these properties [12]. These facts suggested that the effect of neuroleptics on the kinetics of the tyrosine hydroxylase reaction, manifested as maintenance of high activity of the enzyme in the presence of inhibiting concentrations of substrate, may be related to the molecular mechanism of the specific psychotropic effect of neuroleptics.

The object of this investigation was to study the stereospecificity of this phenomenon, which can be regarded as the result of direct interaction of neuroleptics with TH. To assess the stereospecificity of the effect, stereoisomers of flupenthixol were used: the cis-(α)-isomer, with high neuroleptic activity, and the trans-(β)-isomer, with no such activity [6].

EXPERIMENTAL METHOD

Experiments were carried out on TH isolated from the bovine hypothalamus by biospecific chromatography, by the method described previously [3], using di-iodothyronine-sepharose 4B. An electrophoretically homogeneous fraction of the enzyme was used. The initial velocity of the tyrosine hydroxylase reaction was measured spectrophotometrically by recording the increase in absorption at 335 nm, due to oxidation of the pterine coenzyme, coupled with conversion of the reaction substrate L-tyrosine into L-dihydroxyphenylalanine [1]. As pterine coenzyme, 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$) was used. The composition of the sample was: 0.1 M Tris-maleate buffer, enzyme (2 μg in 1 ml), tyrosine (40-360 μM), $DMPH_4$ (140 μM). Flupenthixol was added to the sample at different stages of formation of the incubation medium: to enzyme unprotected by substrate (before tyrosine), to the enzyme–substrate complex (after addition of tyrosine), and also after the addition of $DMPH_4$, i.e., after the beginning of the reaction. The comparison cuvette contained the same components of the mixture, except tyrosine. Protein was determined by Lowry's method [10]. The following reagents were used: Tris and

TABLE 1. Effect of Flupenthixol Isomers and Fluphenazine on Velocity of Tyrosine Hydroxylase Reaction in the Presence of α-MT

Experimental conditions	Reaction velocity nmoles $DMPH_4$/min/mg protein
Control (tyrosine concentration 110 μM)	280±14
Control (tyrosine concentration 220 μM)	90±3
α-MT, $5 \cdot 10^{-4}$ M	85±3
Fluphenazine $5 \cdot 10^{-5}$ M, tyrosine 220 mM	285±10
Cis-flupenthixol $5 \cdot 10^{-5}$ M, tyrosine 220 mM	282±10
Fluphenazine $5 \cdot 10^{-5}$ M, α-MT $5 \cdot 10^{-4}$ M	87±4
Cis-flupenthixol $5 \cdot 10^{-5}$ M, α-MT $5 \cdot 10^{-4}$ M	86±3

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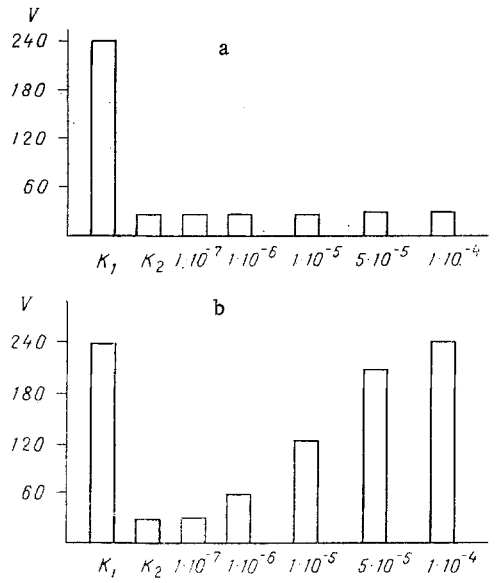

Fig. 1. Effect of geometric isomers of flupenthixol on substrate inhibition of TH as a function of their concentration. Incubation medium: 0.1 M Tris-maleate buffer, pH 6.0, $DMPH_4$ (140 μM), tyrosine (200 μM). Abscissa, K_1) control with tyrosine in concentration of 120 μM, K_2) control with tyrosine in concentration of 200 μM, 1×10^{-7} M to 1×10^{-4} M) samples with flupenthixol in corresponding concentrations; ordinate, velocity of enzyme reaction (in nmoles $DMPH_4$/min/mg protein). a) Trans-, b) cis-isomer.

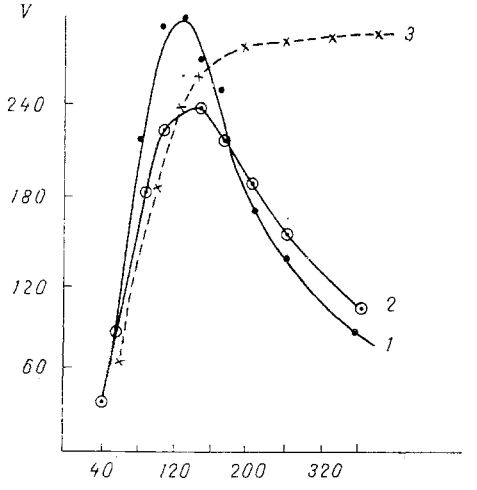

Fig. 2. Effect of cis- and trans-isomers of flupenthixol on dependence of velocity of tyrosine hydroxylase reaction on tyrosine concentration. Incubation medium: 0.1 M Tris-maleate buffer, pH 6.0, $DMPH_4$ (140 μM), tyrosine (40-360 μM). 1) Control, 2) trans-flupenthixol (5×10^{-5} M), 3) cis-flupenthixol (5×10^{-5} M). Abscissa, tyrosine concentration (in μM); ordinate, reaction velocity (in nmoles $DMPH_4$/min/mg protein).

L-tyrosine from Sigma, USA; twice-recrystallized maleic acid was from the Erevan Chemical Factory; $DMPH_4$ was synthesized by ourselves by the method in [11]; the flupenthixol isomers, generously provided for this investigation by Dr. Moller-Nielssen, were from Lundbeck and Co., Copenhagen, Denmark.

EXPERIMENTAL RESULTS

The effect of geometric isomers of flupenthixol in concentrations of 1×10^{-7} to 1×10^{-4} M on the velocity of the tyrosine hydroxylase reaction under substrate inhibition conditions and also the effect of flupenthixol isomers in a concentration of 1×10^{-5} M on the reaction velocity in the presence of different concentrations of tyrosine were studied. As Fig. 1a shows, the trans-isomer of flupenthixol, when added to the sample in concentrations of 1×10^{-7} to 1×10^{-4} M at the beginning of the reaction, did not abolish substrate inhibition of TH. Similar results were obtained when the trans-isomer was added to the sample before tyrosine. The trans-isomer thus does not affect the velocity of the tyrosine hydroxylase reaction in the presence of a high concentration of tyrosine (2×10^{-4} M).

By contrast to this, the cis-isomer of flupenthixol abolished substrate inhibition of TH (Fig. 1b). This effect was clearly dependent on the concentration of the neuroleptic: it first appeared in the presence of the cis-isomer in a concentration of 1×10^{-6} M, and gradually increased to reach a maximum at 1×10^{-4} M. The cis-isomer increased the velocity of the tyrosine hydroxylase reaction under substrate inhibition conditions irrespective of the order of its addition to the sample. As Fig. 1b shows, if the cis-isomer was added to the sample after $DMPH_4$ in the course of the enzyme reaction the degree of abolition of substrate inhibition of TH depended on the concentration of the cis-isomer. Similar results were obtained when the cis-isomer was added to the sample before tyrosine. The concentration of the cis-isomer of 1×10^{-4} M, which was essential for complete abolition of substrate inhibition of the enzyme, did not exceed the tyrosine concentration (2×10^{-4} M) at which substrate inhibition of TH was observed, evidence of the high affinity of flupenthixol and, possibly also, of other neuroleptics, for the enzyme. The results show that abolition of substrate inhibition of TH by neuroleptics is a stereospecific effect.

To shed light on the possible mechanism of this effect, the effect of flupenthixol isomers on the velocity of the tyrosine hydroxylase reaction was studied in the presence of different concentrations of tyrosine. As Fig. 2 shows, dependence of reaction velocity on tyrosine concentration for the enzyme isolated from bovine brain (curve 1) is described, like that for TH from other sources [13], by a curve with a maximum. The region of saturating concentrations of tyrosine (100-140 μM) passes into a region of inhibiting concentrations of substrate (over 140 μM). Trans-flupenthixol in a concentration of 5×10^{-5} M (curve 2) had no appreciable effect on the position of the maximum of the curve, it slowed the reaction velocity by 20% in the presence of tyrosine in a concentration of 40-60 μM, and it considerably increased the reaction velocity in the presence of inhibitory concentrations of tyrosine (160-360 mM). On the addition of 5×10^{-5} M cis-flupenthixol (curve 3) not only was an increase in the saturating concentration of tyrosine observed, but the region of saturation also was widened, so that it formed a plateau between tyrosine concentrations of 160 and 360 μM. In the presence of cis-flupenthixol, the kinetics of the tyrosine-hydroxylase reaction relative to tyrosine completely obeys the Michaelis–Menten law, unlike the kinetics of this reaction under ordinary conditions.

If curves 2 and 3 are compared, a clear difference can be seen in the right hand side of these curves corresponding to the region of tyrosine in concentrations of 160-360 mM, whereas their left hand sides, corresponding to tyrosine in concentrations not exceeding saturating values, coincide almost completely. The stereospecificity of the effect of flupenthixol isomers on the tyrosine-hydroxylase reaction is thus manifested in the region of substrate inhibition of the enzyme but is not found to an appreciable degree in the presence of lower tyrosine concentrations. This fact supports the writers' hypothesis [2] on the presence of two tyrosine binding sites in the TH molecule, one in the catalytic center, the other in the noncatalytic region. This hypothesis is also supported by the results of experiments with the TH inhibitor α-methyl-p-tyrosine (α-MT). As Table 1 shows, the cis-isomer of flupenthixol, and fluphenazine, a neuroleptic of the phenothiazine series, do not abolish the inhibitory effect of α-MT, a competitive inhibitor of TH, whose action is directed toward the catalytic center of the enzyme. In the writers' view, neuroleptics which interact with TH in the regulatory region prevent the binding of tyrosine in the noncatalytic center responsible for substrate inhibition of the enzyme. It has been shown that substrate inhibition of TH may participate in regulation of the activity of this enzyme by presynaptic receptors [5], and the result of its removal, as we know from the literature, is an increase in the velocity of hydroxylation of tyrosine, which is observed on systemic administration of neuroleptics [15]. The mechanism of action of neuroleptics is traditionally associated with their inhibitory effect on dopamine receptors [7, 8]. This property correlates on the whole with the clinical