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EFFECT OF BUTACLAMOL ENANTIOMERS ON TYROSINE HYDROXYLASE IN THE RAT HYPOTHALAMUS

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In view of its important regulatory role in catecholaminergic processes, the tyrosine hydroxylase reaction is interesting not only as an indicator of the velocity of these processes but also as a possible object for pharmacologic intervention [1]. Experiments *in vitro* showed previously that neuroleptics can exert a direct influence on tyrosine hydroxylase (TH), which is expressed as abolition of substrate inhibition of the enzyme [4]. The specificity [11] and stereospecificity of this effect have been established in relation to geometric isomers of flupenthixol [3]. At the present time butaclamol enantiomers, which differ in their clinical efficacy [10], their effect on animal behavior [11], and also in the specific competitive with spiroperidol binding test with membrane preparations from brain structures [13], are used as reference substances for the study of the mechanism of action of neuroleptics. Being equally soluble in lipids, the (+)- and (−)-enantiomers of butaclamol are virtually indistinguishable in their nonspecific membranotropic effects, and for that reason differences in their biological activity can be ascribed to their specific and stereoselective action [13].

The aim of this investigation was to study effects of butaclamol enantiomers, both direct and mediated through the synaptosomal membrane, on TH in the rat hypothalamus.

EXPERIMENTAL METHOD

Rats were decapitated and the hypothalamus removed in the cold. To obtain fractions, tissue from 10 rats was pooled, and the fractions for these experiments were isolated three times. A 10% homogenate was obtained in 0.32 M sucrose, large cell fragments were sedimented at 1000 g for 10 min, and the residue was washed with half the original volume of 0.32 M sucrose, after which unpurified synaptosomes and microsomes were obtained (100,000g, 60 min). The residue of unpurified synaptosomes was homogenized in 0.001 M K-phosphate buffer, pH 7.0, containing 0.002 M $CaCl_2$ to disintegrate the synaptosomes and interacellular organelles. The homogenate was centrifuged (100,000g, 60 min), the residue was suspended in 15 ml of 0.32 M sucrose, layered on a 0.6-0.8-1.2 M sucrose gradient, and centrifuged for 120 min at 80,000g in a bucket rotor. The fraction at the boundary between 0.8 and 1.2 M sucrose was collected. This membrane fraction is rich in TH [8] and does not contain myelin or mitochondrial material. The fraction was diluted with cold distilled water to a sucrose concentration 0.32 M, centrifuged at 100,000g for 60 min, and homogenized in 0.001 M K-phosphate buffer, pH 7.0, at the rate of 0.8 ml buffer per gram of obtained tissue. This homogenate was used as membrane-bound TH. Experiments on synaptosomes were carried out by the method described previously [5]. Synaptosomes isolated by the method in [7] were incubated in Krebs' phosphate buffer, pH 7.4, at 37°C for 15 min. After incubation the synaptosomes were separated from incubation medium by centrifugation at 25,000g (10 min). Membrane-bound TH was isolated from the residue thus obtained by the method in [8]. To obtain synaptosomes in these experiments, hypothalamic tissue from 40 rats was pooled each time (each sample during incubation contained material from six or seven rats). Altogether three experiments were carried out with incubation of synaptosomes. When the reaction velocity was measured, three

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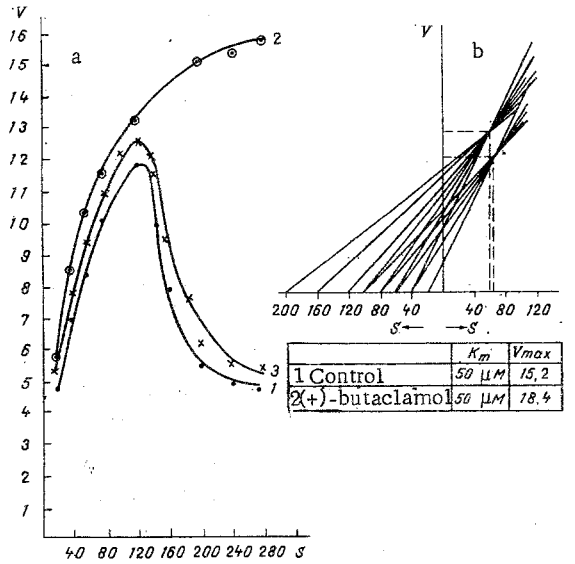

Fig. 1. Effect of butaclamol enantiomers on velocity of tyrosine hydroxylase reaction depending on tyrosine concentration. Abscissa, concentration (S) of tyrosine (in μM); ordinate, initial reaction velocity (V; in nanomoles $DMPH_2$/min/mg protein); a) in direct coordinates, b) in Cornish-Bowden coordinates. Reaction conditions: 0.1 M Tris-maleate, pH 6.15, $DMPH_4$ (140 μM), tyrosine (40-320 μM). 1) Control, 2) (+)-butaclamol, 10^{-5} M, 3) (−)-butaclamol, 10^{-5} M.

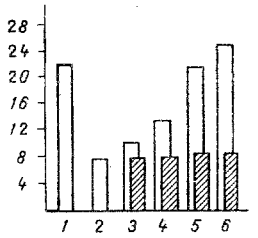

Fig. 2. Effect of butaclamol enantiomers on substrate inhibition of hypothalamic TH depending on their concentration. Abscissa, designation of samples; ordinate, initial reaction velocity (in nanomoles/min/mg protein). 1) Control 1, tyrosine concentration 120 μM, 2) control 2, tyrosine concentration 320 μM (substrate inhibition). 3-6) Addition of butaclamol in concentrations of 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M respectively. Unshaded columns (+)-butaclamol, shaded columns (−)-butaclamol.

parallel determinations were made for each experimental point in each of three experiments. The initial reaction velocity was measured spectrophotometrically [2]. Protein was determined by Lowry's method [9].

EXPERIMENTAL RESULTS

The results of experiments to study the effect of butaclamol enantiomers on hypothalamic TH are given in Fig. 1. They show that the (+)- and (−)-enantiomers of butaclamol differ essentially in their action on hypothalamic TH. The kinetics of the TH reaction, measured as tyrosine, is characterized by substrate inhibition. In the region of activating tyrosine concentrations (the left branch of the curve) both enantiomers have an activating effect, and the difference in magnitude of the effect is negligible. In the region of inhibitory concentrations of tyrosine the (+)- and (−)-enantiomers of butaclamol differ in principle. In the presence of (+)-butaclamol (Fig. 1: 2) substrate inhibition of the enzyme was not found and the reaction kinetics obeyed the Michaelis–Menten law. In the presence of (−)-butaclamol substrate inhibition of TH was preserved (Fig. 1: 3). Thus the (+)- and (−)-enantiomers of butaclamol exhibit stereospecificity relative to substrate inhibition of TH. The effect of (+)-butaclamol depends on its concentration and is clearly apparent in a concen-

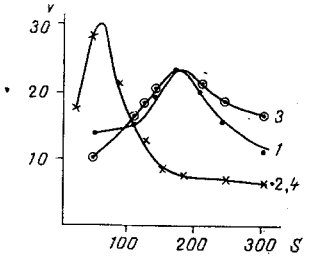

Fig. 3. Kinetic properties of TH from hypothalamic synaptosomes incubated in presence of dopamine and butaclamol enantiomers, relative to tyrosine. Abscissa, tyrosine concentration (in μM); ordinate, initial reaction velocity (in nanomoles $DMPH_2$/min/mg protein). 1) Control, 2) enzyme from sample with dopamine, 3) enzyme in sample with dopamine and (+)-butaclamol, 4) enzyme in sample with dopamine and (−)-butaclamol.

tration of 10^{-7} M and reaches a maximum in a concentration of 10^{-6} M; (−)-butaclamol has no effect on substrate inhibition of TH even in a concentration of 10^{-4} M (Fig. 2). Abolition of substrate inhibition of TH is unconnected with any change in K_m for tyrosine. The abolition of inhibition is noncompetitive in character (Fig. 1b).

The results of this series of experiments confirm the conclusion on stereospecificity of the effect of abolition of substrate inhibition of TH by neuroleptics drawn previously from experiments with cis- and trans-isomers of flupenthixol — a neuroleptic with a different chemical structure [3].

The possible mechanism of the effect of butaclamol on regulation of TH by presynaptic receptors was studied in another series of experiments conducted on hypothalamic synaptosomes. Butaclamol enantiomers, if added to incubation medium without dopamine, had no appreciable effect on the kinetics of the tyrosine hydroxylase reaction in resting synpatosomes. As will be clear from Fig. 3, incubation of synaptosomes in the presence of dopamine led to a change in the kinetic properties of the enzyme relative to tyrosine (Fig. 3: 2), in agreement with previous results [5]. The butaclamol enantiomers had no effect when added in the absence of dopamine.

After combined addition of dopamine and (+)-butaclamol to the incubation medium, changes in the kinetics of the tyrosine hydroxylase reaction characteristic of the dopamine effect were not found (Fig. 3: 2, 3). In addition, the curve of reaction velocity as a function of tyrosine concentration for enzyme from the sample with (+)-butaclamol and dopamine (Fig. 3: 3) differed from the control (see Fig. 3: 1), and resembled curve 2 in Fig. 1a, characterizing the effect of (+)-butaclamol when interacting directly with TH. It can be tentatively suggested that treatment of the presynaptic membrane with dopamine creates conditions for penetration of (+)-butaclamol inside the nerve ending and for its direct contact with the enzyme, whereas (−)-butaclamol has no such action (Fig. 3). In this case the same changes were observed in the kinetics of the TH reaction as in the test with dopamine without addition of (−)-butaclamol (curves 2 and 4 coincide).

The stereospecificity of the effect of butaclamol enantiomers on regulation of the tyrosine hydroxylase reaction by presynaptic synaptosomal receptors was thus exhibited in a concentration of 10^{-5} M. In experiments to study specific (competitive with spiroperidol) binding of butaclamol enantiomers with brain membrane preparations [12], high stereospecificity of the effect was observed only when these substances were present in nanomole concentrations, when the (+)- and (−)-enantiomers of butaclamol differed in their binding constant by a factor of 1000. In concentrations of 10^{-6} M and higher, stereospecificity of butaclamol was virtually not observed in this test. In the present investigation stereospecificity of (+)- and (−)-enantiomers of butaclamol relative to TH was observed in a concentration of 10^{-5} M. According to the concentration criterion of specificity of action of neuroleptics, manifested on binding with membranes, the effect achieved with the drug in concentrations of the nanomole order is taken [13], for this concentration corresponds to blood levels of antipsychotic drugs in patients [12]. The total concentration of a neuroleptic (thioridazine) in the blood of patients with acute psychosis after a single dose was 1-4 μM. Considering the high density of the synaptosomal suspension (4 mg protein in 1 ml of incubation medium), dopamine, an agonist of presynaptic receptors, was added in a concentration of 10^{-5} M, in accordance with the results of an investigation [14] which showed that the concentration of

the agonist of catecholamine receptors in experiments on a cell suspension must be chosen depending on their total surface area in the suspension, and must be not less than 10^{-5} M for a concentration of 3-4 mg/ml. The concentration of (+)-butaclamol (10^{-6} M) in experiments with direct interaction with TH *in vitro* cannot rightly be compared with data on the binding of this substance with membranes (micromolar and nanomolar values respectively), but in both cases examined above distinct stereospecificity of action of butaclamol was found.

The results complement the data published by the writers previously [3] and confirm that abolition of substrate inhibition of TH is a specific, stereoselective effect of neuroleptics and may be a prognostically important criterion for evaluation of compounds with potential neuroleptic activity. The results also are evidence of the complexity of the antagonistic relation between neuroleptics and dopamine in indirect regulation of TH through the presynaptic membrane.

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