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ACTION OF ETHMOZINE AND ETHACIZINE ON DOPAMINERGIC ADENYLATE CYCLASE OF THE BRAIN STRIATAL SYSTEM

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KEY WORDS: adenylate cyclase; dopamine receptors; corpus striatum; rabbit brain; neuroleptics; ethmozine; ethacizine.

Ethmozine and ethacizine (the diethylamino analog of ethmozine) are the first phenothiazine derivatives to be introduced into medical practice for the treatment of arrhythmias [2-4, 6]. These substances have a marked cardiotropic action — their antiarrhythmic effects develop as a result of their direct effect on the heart [3, 4].

Many compounds of the phenothiazine group are known to be neuroleptics [5]. During clinical trials of ethmozine and ethacizine no marked neuroleptic or other psychotropic effect was found. This fact can be explained on the grounds that either these compounds do not pass through the blood—brain barrier or, unlike chlorpromizine, trifluoroperazine, and other phenothiazines, they have low affinity for receptors of the mediator systems of the brain.

One target for the action of neuroleptics of the phenothiazine series is the dopamine-sensitive adenylate cyclase system of the brain, coupled with the dopamine D-1 receptor [7, 9, 10-12]. Although the role of the dopaminergic adenylate cyclase system and of the dopamine D-1 receptor in the brain is not sufficiently clear, there is much evidence to indicate that the neuroleptic effect of the phenothiazines (and of certain other groups of preparations with similar structure) are interlinked with their ability to inhibit stimulation of adenylate cyclase by agonists, to bind with the D-1 receptor, and to displace agonists from receptors [7, 9, 10-12].

In this investigation the action of ethmozine and ethacizine and also of typical neuroleptics of the phenothiazine and butyrophenone series on adenylate cyclase in the corpus striatium of the rabbit brain was compared.

EXPERIMENTAL METHOD

A membrane preparation of adenylate cyclase was obtained from the rabbit corpus striatum by a modified method [8]. The corpus striatum and caudate nucleus [1], separated from the remainder of the brain, were homogenized in a Potter—Elvehjem homogenizer in 25 volumes of homogenization medium containing 0.32 M sucrose, 10 mM Tris-HCl, 0.1 mM EDTA, and 1 mM DTTE,

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TABLE 1. Effect of Catecholamines (0.1 mM) and Their Antagonists (0.1 mM) on Adenylate Cyclase (in picomoles cAMP/mg protein/min) in Striatal System of Rabbit Brain (M ± m)

Addition	Nothing added	Phentol-amine	Al-prenolol	Trifluoro-perazine
Nothing added	40,0±2,2	39,8±2,4	40,8±2,0	37,1±2,0
Dopamine	84,2±6,6	50,7±2,0	88,0±7,1	48,4±1,2
Noradrenalin	66,0±5,0	46,1±3,0	69,3±1,8	46,0±3,1
Adrenalin	52,1±4,1	45,0±1,2	54,1±3,0	44,1±2,2
Isoproterenol	44,2±3,7	42,7±2,0	42,0±0,8	42,0±0,7

TABLE 2. Effect of Neuroleptics and Anti-arrhythmic Drugs on Adenylate Cyclase (in picomoles cAMP/mg protein/min) of Brain Striatal System (M ± m)

Substance added, 10^{-5} M	Nothing added	Dopamine 10^{-4} M
Nothing added	42,1±3,7	90,0±6,2
Haloperidol	41,1±1,1	46,1±2,6
Trifluoperidol	40,7±2,0	48,8±3,3
Droperidol	42,0±0,2	46,0±2,2
Trifluoroperazine	40,9±0,9	44,0±1,8
Metofenazate	42,5±1,4	47,9±3,9
Chlorpromazine	39,0±3,0	47,4±4,1
Levomepromazine	41,8±2,1	46,6±3,8
Ethmozine	41,5±2,0	82,4±5,0
DAA-ethmozine (ethacizine)	41,0±2,7	77,3±4,2

pH 7.4, at 2-4°C. The resulting homogenate was centrifuged for 10 min at 1000g in a Beckman T-21 centrifuge. The supernatant was collected and the residue rehomogenized in 10 volumes of the same medium and recentrifuged under the same conditions. The supernatants were pooled and centrifuged for 20 min at 40,000g. The residue was suspended in 10 volumes of medium contaiing 5 mM Tris-HCl, pH 7.4, at 2-4°C and centrifuged for 20 min at 40,000g. This last procedure was repeated twice. All operations were carried out at 0-4°C. The final residue of membranes was suspended in 5 mM Tris-HCl, pH 7.4, at 4°C, frozen, and kept in liquid nitrogen.

Adenylate cyclase activity was determined in 50 µl of medium containing 50 mM Tris-HCl pH 7.5 (at 30°C), 5 mM $MgCl_2$, 1 mM cAMP, 1 mM isobutylmethylxanthine, 0.1-0.5 mM [α-^{32}P]-ATP (500,000-1,000,000 cpm per sample), 20 mM creatine phosphate, 0.5 mg/ml of creatine kinase with an activity of not less than 100 units, and 0.1 mM GTP. To exclude any effect of calmodulin the incubation medium contained 2 mM EGTA. The incubation time was 10 min at 37°C and the protein content from 10 to 50 µg per sample. The [^{32}P]-cAMP was assayed as in [14].

EXPERIMENTAL RESULTS

Adenylate cyclase activity of the rabbit corpus striatum is regulated by catecholamines. The enzyme is activated twofold by dopamine, by 50-60% by noradrenalin, and by 10-20% by adrenalin, but is insensitive to isoproterenol (Table 1). Alprenolol, an antagonist of β-adrenergic receptors, did not inhibit the effects of catecholamines. Weak stimulation of adenylate cyclase by adrenalin and the absence of any effect of isoproterenol, and also insensitivity to alprenolol thus leads to the conclusion that β-adrenoreceptors do not participate in regulation of adenylate cyclase in the membrane preparation from the striatal system. As Table 1 shows, phentolamine, an α-adreno-receptor antagonist, inhibits activation of adenylate cyclase by catecholamines sufficiently effectively. The virtually complete suppression of the effects of catecholamines is produced by trifluoroperazine, a blocker of dopaminergic receptors and a typical neuroleptic of the phenothiazine series (Table 1).

The activation constant of the adenylate cyclase preparation obtained from the rabbit corpus striatum of noradrenalin was 5.2×10^{-5} M, and for dopamine 5.6×10^{-6} M. Such a significant difference in the affinity of these two agonists for the enzyme, together with data in the literature on the ability of phentolamine to block not only α-adrenoreceptors, but also dopaminergic receptors [7, 9, 10-12], leads to the conclusion that catecholamines activate adenylate cyclase of the rabbit corpus striatum through dopamine receptors of D-1 type [7, 10,

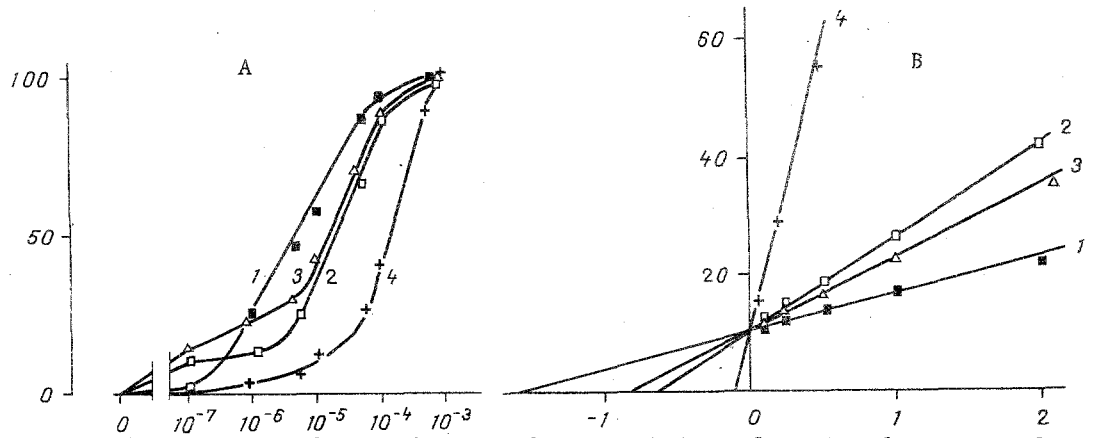

Fig. 1. Dependence of adenylate cyclase activity of striatal system of rabbit brain on dopamine concentration, expressed as direct values (A) and double reciprocals (B). 1) In absence of phenothiazines, 2) in presence of 5×10^{-6} M ethacizine, 3) 5×10^{-6} M ethmozine, 4) 10^{-6} M trifluoroperazine. Abscissa: A) dopamine concentration (in M). B) Reciprocals of dopamine concentration (in $M^{-1} \cdot 10^{-5}$); ordinate: A) enzyme activity (in %), B) reciprocals of enzyme activity (in $\%^{-1} \cdot 10^{3}$).

12]. This conclusion is supported by the data in Fig. 1, which shows that trifluoroperazine competitively inhibits stimulation of adenylate cyclase by dopamine and has high affinity for the receptor (inhibition constant 2×10^{-7} M). We know in this regard that α- and β-adrenolytic properties of trifluoroperzine are exhibited in concentrations 2 or 3 orders of magnitude higher [12, 13, 15] than its dopaminolytic properties.

The effects of catecholamines and also of antagonists of α- and β-adrenoreceptors and of dopaminergic recetors obtained (Table 1, Fig. 1) are in full agreement with data in the literature [7, 9, 10-12] and it can be concluded from them that regulation of adenylate cyclase in the rabbit corpus striatum by catecholamines and phenothiazine is effected through dopamine D-1 receptors.

It will be clear from Fig. 1 that affinity for these receptors in the case of ethmozine and ethacizine is significantly lower than affinity of the typical neuroleptic trifluoroperazine. Inhibition constants, calculated from the data in Fig. 1, are 3×10^{-6} M for ethmozine and 4×10^{-6} M for ethacizine, 15-20 times higher than the inhibition constant for trifluoroperazine (2×10^{-7} M).

The virtual absence of psychotropic effects of the antiarrhythmic preparations of the phenothiazine series under clinical conditions can thus be explained by the low affinity of these substances for brain dopamine receptors.

The action of ethmozine and ethacizine on dopaminergic adenylate cyclase was compared with the action of seven neuroleptics used in clinical practice (Table 2).

These neuroleptics belong to two different classes of chemical compounds: Trifluoroperazine, metofenazate, chloropromazine, and levomepromazine are derivatives of phenothiazine, whereas haloperidol and droperidol are deriviatives of butyrophenone. A common feature of all these compounds is that their pharmacologic effect is realized through blocking of dopaminergic transmission in the brain [7, 9, 10-12]. In the present experiments all the neuroleptics studied completely inhibited activation of adenylate cyclase in the striatal system of the brain by dopamine, yet had virtually no effect on basal adenylate cyclase activity. Under these same conditions ethmozine and ethacizine had a very weak inhibitory effect on dopamine-activated adenylate cyclase (Table 2).

Close correlation exists between the clinical effects of neuroleptics and their ability to bind with dopamine receptors and to displace agonists from receptors [7, 9, 10, 12]. It must be pointed out that such correlation between the pharmacologic effect of neuroleptics and inhibition of stimulation of the adenylate cyclase of the brain striatal system and, correspondingly, binding with dopamine D-1 receptors is observed only for neuroleptics of the phenothiazine series and of the related group of thioxanthines [9, 10, 12]. According to the data given in this paper ethmozine and ethacizine, which belong to the phenothiazine group, have significantly less affinity for dopamine-sensitive adenylate cyclase of the

corpus striatum, coupled with the dopamine D-1 receptor and also, evidently, for other dopaminergic receptors in the brain than typical neuroleptics. Consequently it may be expected that the use of thse antiarrhythmic phenothiazine preparations will not be complicated by any significant neurotropic action or by any influence on peripheral dopaminergic receptors.

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AMPHETAMINE STEREOTYPY AS A STABLE RHYTHMIC PROCESS

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KEY WORDS: amphetamine; stereotypy; mental disturbances.

Stereotyped behavior induced by large doses of amphetamine is a well known state at the present time that is widely used as a model of psychopathology and for screening psychotropic drugs [1, 2].

Stereotypy in different species of animals is characterized by an assortment of automatized actions (head turning, sniffing, licking, and so on). Their frequency characteristics and time course have not been adequately studied. Yet such an approach could be important for our understanding of the nature of mental disturbances. With this in mind, in the investigation described below some temporal parameters of stereotyped behavior were studied in detail for the first time in experiments on cats.

EXPERIMENTAL METHOD

Nineteen cats of both sexes weighing from 2 to 3.5 kg were used. Horizontal motor activity of the animals was recorded in a special chamber by means of an electromechanical rotameter of original design. To connect the cat's head securely to the mechanical part of the rotameter, the animal was anesthetized with ether and a socket was fixed to the vault of the skull. Head movements to right and left were recorded on a 4-channel N338-4P automatic writer and monitored visually.

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