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EFFECTOR MODELLING OF THE ACTION OF GABA-RECEPTOR-COMPLEX LIGANDS. FUNCTIONAL INTERACTION BETWEEN SUBUNITS OF THE COMPLEX

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KEY WORDS: GABA-receptor complex; exogenous ligands; pharmacological effect; cooperativeness of interaction

The study of the structure and function of the GABA-receptor complex (GABA-RC), the postsynaptic supramolecular receptor-channel assembly, including receptors of GABA, bicuculline (BC), benzodiazepines (BD), their reciprocal agonists and antagonists, barbiturates (BB), and picrotoxin (PT), has mainly been undertaken by radioligand methods in vitro [6, 12-14]. The principles of function of GABA-RC in vivo can be determined by studying the time course of the rapidly reversible pharmacological effects of exogenous ligands by the use of techniques ensuring mutually equal agreement between state of the biological systems and the influences to which it is exposed [8, 10]. These demands are satisfied by investigations of changes in minimal effective doses and the shape of the distribution of probability of the recorded effects during infusion of convulsive agents which are GABA-RC ligands against the background of modulating influences of BB and BD.

Radioligand studies [7, 12-14] and the use of photoaffinity labeling of BD-receptors [12, 15] have shown that GABA-RC incorporates four BD binding sites. The ratio between the number of binding sites of BD and GABA is 1:2 [12, 13]. As a result, the hypothesis of the "quartet" model of GABA-RC, represented by four subunits, incorporating one BD binding site and two GABA binding sites, was confirmed [7, 13]. It is suggested that reception of the ligand by one of the four subunits leads to modification of the state of the whole supramolecular complex [13].

To study modifications of the state of GABA-RC under physiological conditions and to establish the parameters of these processes determined at the whole organism level, in the investigation described below the character of the changes in parameters of the convulsant effect of BC, PT, and metrazol (MT), namely the distribution of minimal effective doses, and of thiosemicarbazide (TS), namely the distribution of probability of a recordable effect following administration of BB (barbital sodium) and BD (phenazepan,* 1,2,4,5-tetrahydrophenazepam), were investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 18-22 g. Animals in the control group and animals 1 h after intraperitoneal injection of phenazepam (0.08-5.6 mg/kg), 1,2,4,5-tetrahydrophenazepam (1.4-22.5 mg/kg), and barbital sodium (20-160 mg/kg), received BC, PT, and MT in the form of 0.1, 0.3, and 1% solutions respectively, by intravenous infusion (into the caudal vein) at the rate of 0.01 ml/sec. Minimal effective doses inducing clonicotonic convulsions (CTD) and tonic extension (TED) were determined [2-4]. The probability of development of clonic convulsions (CC) and tonic extension (TE) was determined after injection of 5-400 mg/kg TS into control mice after preliminary (30 min earlier) injection of phenazepam (0.35-2.8 mg/kg) and barbital sodium (25-200 mg/kg). The results were analyzed by algorithms described in [5, 9].

*7-bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

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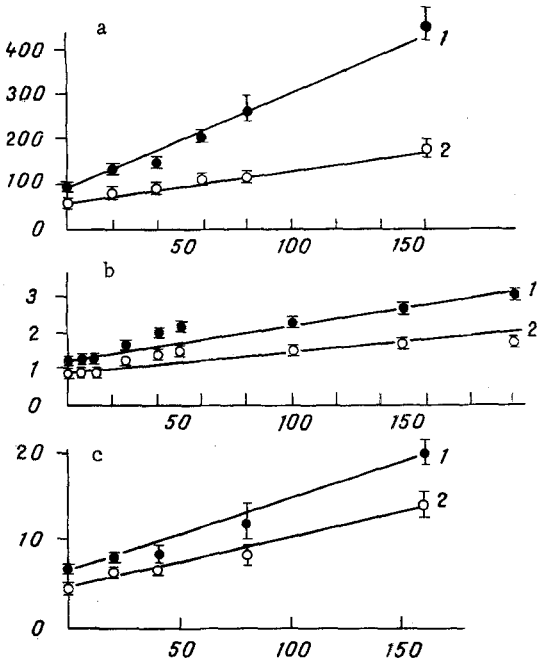

Fig. 1. Changes in minimal effective doses of metrazol (a), bicuculline (b), and picrotoxin (c) inducing clonicotonic convulsions (CTD) and tonic extension (TED), against the background of administration of increasing doses of barbital sodium to mice. Abscissa, dose of barbital sodium (in mg/kg); ordinate, minimal effective dose (in mg/kg). 1) TED; 2) CTD.

EXPERIMENTAL RESULTS

Injection of increasing doses of BB caused a linear increase in the effective doses (CTD and TED) of PT, MT, and BC, in conformity with the competitive type of interaction between pharmacologic agents [6] (Fig. 1). The mechanism of modification of GABA-RC, modulated by BB, consists of stabilization of the activated (with high conductance of the chlorine ionophore) conformation (A) of the complex [5] and slowing of transition of the A conformer into the desensitized state [11]. The types of dependences (Fig. 1) discovered suggest destabilization of the A-conformation of GABA-RC by its exogenous ligands (PT and MT) or reversible modification of the complex by the competitive GABA antagonist BC, under physiological conditions. For the given types of dependences, values of the regression coefficients coincide formally with values of inhibition indices, determined for all the data subjected to analysis. Indices normalized relative to values of CTD and TED in the control groups of animals allow a comparative estimate to be made of effects of GABA-RC modulators of different nature at the whole body level. For the convulsant action of chloride channel blockers (PT and MT) the normalized inhibition indices with respect to CTD are 0.0127 ± 0.0014 and 0.017 ± 0.0010, respectively, for TED they are 0.0126 ± 0.0014 and 0.025 ± 0.0008, and for the convulsant action of BC, lower than values given for MT ($p \leq 0.001$), they are 0.0067 ± 0.0003 and 0.0045 ± 0.0002, respectively.

The values of effective doses (ED_{50}) of BC with respect to CTD and TED, against the background of administration of the (d_i)-dose of BD ($ED_{50,i}$) exceed (conditionally) the corresponding parameters in animals of the control group ($ED_{50,k}$) by certain values which tend at the limit toward the value $ED_{50,max}$, where $ED_{50,max}$ denotes minimal effective doses of the convulsant provided that the d_i $-\infty$-dose of BD is injected. The shape of the rise of the values of ED_{50} is determined by the order (m) and nature (K_{di}) of the interaction process between the anticonvulsant (d_i^m) and the complex; and by the order (n) of the process of formation of the convulsant effect against the background of injection of d_i;

$$ED_{50,i} - ED_{50,k} = (ED_{50,max} - ED_{50,k}) \times \left[d_i^m \left(K_d^m + d_i^m\right)^{-1}\right]^n. \quad (1)$$

Interaction between BC and BD takes place at n = 1.75-1.8 (Fig. 2), unlike data for the previous investigation [2, 3] of changes in values of CTD and TED for infusion of K_d against the background of injection of diazepam and 1,2,4,5-tetrahydrophenazepam (dose–effect) and of ^{14}C-phenazepam (dose–concentration–effect), where n = 1. The fundamental processes of modulation of GABA-RC by BD in vitro consist of destabilization of the energetically stable (b) conformation of the complex [13], which, in particular, causes an increase in the affinity of GABA-RC for GABA [12, 13]. It follows from the data described that BC, unlike the chloride channel blocker – MT – interacts with two GABA binding sites in the structure of GABA-RC during the formation of the convulsant effect against the background of modification of the complex by BD. Calculated values ($ED_{50,max}/ED_{50,k} \approx 2$ and of K_d for BD were only half of

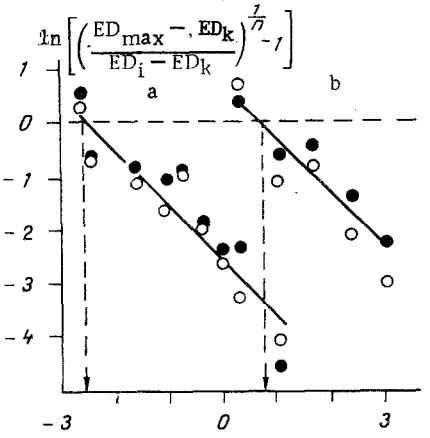

Fig. 2

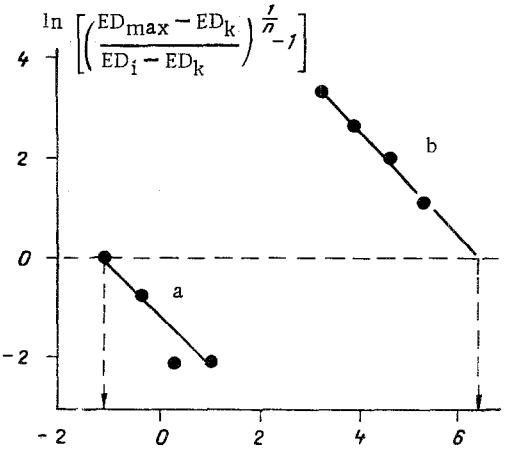

Fig. 3

Fig. 2. Correlation between values of logarithms of minimal effective doses with respect to CTD (empty circles) and TED (filled circles) of bicuculline and control and calculated maximal values for n = 2 (ordinate) and logarithms of injected doses of phenazepam (a) and of 1,2,4,5-tetrahydrophenazepam (b). Here and in Fig. 3, arrows indicate values of ln K_d for a and b.

Fig. 3. Correlation between values of logarithms of effective doses of thiosemicarbazide (relative to TE) and control and calculated maximal values at n = 2 (ordinate) and logirithms of injected doses of phenazepam (a) and barbital sodium (b) (abscissa).

the corresponding values determined for MT against the background of injection of both BD (a shift toward a noncompetitive type of interaction of BC and BD). Dependences of similar shape were observed when TS was injected into mice (Fig. 3). In the case of animals receiving BB and BD (phenazepam), values of $ED_{50,i}$ of TS increased in accordance with the following expression:

$$\ln\left\{\left[(ED_{50,\max}-ED_{50,к})(ED_{50,i}-ED_{50,к})^{-1}\right]^{\frac{1}{n}}-1\right\}=m\ln K_d-m\ln d_i \qquad (2)$$

(anamorphosis of (1) for n/1); this suggests cooperativeness of the processes of modification, through the intermediary of GABA, of chloride channel conductance under the influence of BD and BB. Coincidence of the power indices (n), which were 1.8 and 2 for the two experiments in vitro during the study of the shape of dependence of concentration–effect of the action of GABA and the parameters of ionic (chloride) conductance [1], will be noted. The same investigation showed that the concentration–effect curve of chlordiazepoxide is described by a hyperbola of the first order.

It can be concluded from the results obtained in experiments on the whole organism that quantitative parameters of pharmacologic effects of GABA-RC ligands reflect the character of their interaction with the complex (in particular, modification of its conformations). The forms of dose–effect dependences discovered (coefficient of cooperativeness of the effect ≈2 for TS and BC and ≈1 for PT, MT, BD, and BB) suggest that GABA-RC functions under physiological conditions in accordance with the hypothesis of the "quartet" structural-functional model. It can be postulated that modification of one of the four subunits of GABA-RC (containing 2 GABA-BC and one GABA-BD binding sites) by exogenous ligands modifies the functional state of chloride ionophores, which are effectors of receptor-channel assemblies, which modify the output parameters (physiological state) of the biological system in a manner determined relative to the input (exogenous ligand) activity.

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EFFECT OF SOMATOTROPHIC HORMONE IN SYNAPTOSOMAL MEMBRANE Na,K-ATPase IN THE YOUNG RAT BRAIN

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Somatotrophic hormone (STH) exhibits epileptogenic properties and plays an important role in processes of regeneration and formation of epileptic activity (EA), especially after trauma. The excitatory effect of STH, compensating for the synaptic deficit during recovery after local brain damage brings about hyperexcitability of this region and the development of EA [6, 9]. Hyperactivity of neurons, incidentally, is most characteristic of postnatal development, and the predisposition of the brain to seizures and the possibility of development of epilepsy are particularly great in childhood. STH has a decisive influence on the formation and differentiation of cells of the growing organism and, in particular of neurons [10].

According to the membrane hypothesis of development of EA [3, 4], an important role in the epileptization of neurons is played by structural changes in neuron membranes, leading to reversible inactivation of Na,K-ATPase which, in turn, is a trigger factor in the development of prolonged and persistent depolarization of neuron membranes and the formation of generators of pathologically enhanced excitation [5]. Inactivation of the Na,K-pump of neuron membranes is also a trigger factor coupling depolarization and secretion of mediators by nerve endings, i.e., it is a factor in not only pathological, but also physiological hyperactivity of neurons [2].

The action of STH inducing hyperactivity of neurons may be effected through receptors on the electrogenic Na,K-ATPase of neuron membranes.

The aim of the present investigation was to study the action of STH in experiments in vivo on the state of activity of transport Na,K-ATPase of synaptosomal membranes of the brain of young and adult rats.

EXPERIMENTAL METHOD

Unpurified, osmotically destroyed synaptosomes were obtained from formation of the brain stem and cerebral hemispheres of young male rats aged 3 weeks, weighing 35 g, and of

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