EFFECTOR MODELING OF ACTION OF LIGANDS OF THE GABA RECEPTOR COMPLEX: MODIFICATION OF CONFORMATIONAL STATES OF THE COMPLEX BY COMBINED ADMINISTRATION OF BENZODIAZEPINES AND BARBITURATES

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KEY WORDS: GABA-receptor complex; barbiturates; benzodiazepines; anticonvulsant action.

The mutual effects of GABA, benzodiazepines (BD), and barbiturates (BB) on parameters of their binding by subunits of the supramolecular GABA-receptor complex (GABA-rc), found in experiments in vitro, determine the particular features of manifestation of cooperativeness of the pharmacological action of these drugs at the whole body level [12]. On the other hand, convulsive agents as a rule reduce affinity or the number of binding sites of GABA, BD, and BB on synaptosomal membranes [6, 9, 11]. Molecular biological investigations have demonstrated concrete types of interaction between ligands and anticonvulsants and their antagonists GABA and bicuculline (BC); BB and picrotoxin (PT). The mechanisms of action of BD and BB are classed in the metaffinoid and noncompetitive types of modulation (allosteric activation) of GABA-rc [5].

The aim of this investigation was to study the characteristics of function of GABA-rc on the basis of analysis of the results of the combined action of BD and BB, administered against the background of metrazol (M), PT, and BC.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 18-22 g. The animals were given an injection of phenazepam (0.08-5.6 mg/kg), 1,2,4,5-tetrahydrophenazepam (1.4-22.5 mg/kg), or barbital sodium (20-160 mg/kg) and, 1 h later, an intravenous injection of a 0.01, 0.3, or 1.0% solution of BC, PT, or M. The minimal effective doses of the convulsants, inducing clonicotonic convulsions (DCC) and tonic extension (DTE) were determined [1, 2]. The following scheme was used to determine the dynamics of the combined action of BD and BB: phenazepam in a dose of 0.7 mg/kg was injected 1 h before BB (20-160 mg/kg); phenazepam (0.35-2.8 mg/kg) was injected 1 h before injection of 40 mg/kg BB. DCC and DTE of BC, PT, and M were determined 1 h after injection of BB. The results were analyzed in accordance with algorithms given in [7, 8].

EXPERIMENTAL RESULTS

A linear increase of DCC and DTE ($ED_{50,j}$) of the chloride channel blockers PT and M, and also of BC against the background of increasing doses (D_j) of BB, was observed. The type of antagonism of BB and of the convulsant discovered cannot be explained by the direct interaction of the GABA-rc modulators tested on its structures [11]. Modification of GABA-rc by BB (noncompetitive activation) [5] consists of reducing the rate of conversion of the active (with high conductance of the chloride ionophore) [A]-conformation of the complex into the desensitized [C]-form [10, 13]. Since BB and PT, M, and BC (L_c) interact with different subunits of GABA-rc [3, 4], the results of experiments in vitro [10, 13] and in vivo [2] (see Fig. 1) can be adequately represented by a scheme of their heterotropic allosteric interaction [6, 10, 13] — a hyperbolic modification of parameters of conformational transitions of the complex:

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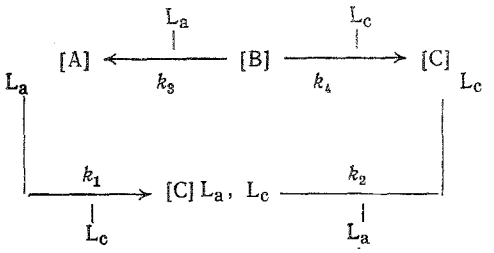


where L_a indicates an anticonvulsant agent (in the example to be examined; [B] is the energetically stable [4, 15] conformation of GABA-rc (the predominant form, in the absence of GABA and of other anticonvulsant compounds), k_1-k_4 are equilibrium constants; the arrows indicate equilibrium arches of the processes. The desensitized state of the complex [C] determines the abundance of the convulsant parameters. The conditions of hyperbolic (competitive) antagonism of BB to the effects of the convulsant (Fig. 1) are satisfied formally if $k_1 \ll k_3$ and k_4, which follows from the mechanism of modulation of GABA-rc by barbiturates [5, 10]. It follows from the relationships found that convulsants, independently of their chemical nature, are allosteric modifiers, destabilizing the [A]-conformation of GABA-rc.

Injection of increasing doses (d_i) of phenazepam and of other BD causes a limited increase in the minimal effective doses ($ED_{50,i}$) in relation to values of DCC and DTE for M, BC, and PT. The relationships observed correspond to 1st-order hyperbolas for M and PT and a 2nd-order hyperbola for BC.

$$ED_{50,i} - ED_{50,k} = (ED_{50,m} - ED_{50,k})[d_1 (d_{50} + d_i)^{-1}]^n \quad (1)$$

where $ED_{50,k}$ denotes values of DCC and DTE in the control groups of animals, $ED_{50,m}$ the calculated value corresponding to $ED_{50,i}$ preceded by injection of $d_i \to \infty$ BD; d_{50} denotes the dose of BD on injection of which $ED_{50,i} = 0.5\ (ED_{50,m} + ED_{50,k})$; n is the order of the process. Processes of modification of the functions of GABA-rc by anticonvulsant compounds (BD) follow a course that corresponds to that given in scheme 1 under the stipulation that $k_3 > k_2$, which follows from the data shown in (Fig. 2) and from determination of the mechanism of action of BD, which consists of destabilization of the [B]-form of GABA-rc [4, 14]. The existence of GABA-rc in the [C] form, and L_c and L_a (scheme 1) is confirmed by the mixed type of antagonism found between L_c and the anticonvulsant effect of BD.

The study of the dynamics of the combined action of BB and BD in the case of injection of an assigned dose (D_j) of BB and of increasing doses of BD revealed a change in the form of the dependence (1), namely reduction of the value of D_{50} relative to both parameters:

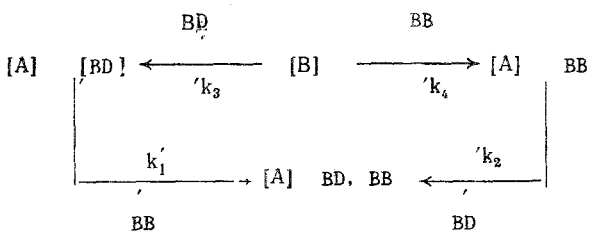
$$ED_{50,i,j} = ED_{50,j} + (ED_{50,m} - ED_{50,k})\{1 + d_{50}(1 + aD_j)[d_1 (1 + bD_j)]^{-1}\}^{-1} \quad (2)$$

where a and b are constants.

Potentiation of the anticonvulsant effect of an assigned dose (d_i = 0.7 mg/kg) of BD by increasing doses of BD (Fig. 1) consisted of a limited change in the value of $ED_{50,i,j}$ from $ED_{50,i}$ (at $D_j = 0$) to

$$\{ED_{50,j} + (ED_{50,m} - ED_{50,k})\, d_1 (d_{50}\, ab^{-1} + d_i)^{-1}\}$$

for high values ($D_j > 1$) of injected doses of BB. The form of potentiation by BB of the anticonvulsant effects of BD observed suggests the following scheme of their interaction on structures of GABA-rc:



where $'k_1$-$'k_4$ are values proportional to the equilibrium constant of processes of interaction of the modulators with GABA-rc. Hyperbolic activation of the anticonvulsant effects of BD by BB presupposes that $'k_1 > k_4$; $'k_1\ 'k_4^{-1} = ba^{-1}$. The suggested model (scheme 1, equation 1) explains the single mechanism of the change in ionic permeability of cell membranes under the influence of BD, its antagonists and counteragonists [11] as the result of hyperbolic modification

of interconversion of GABA-rc conformers, differing in the conductance of the chloride ionophore. It follows from the scheme that $ED_{50,m}\ ED_{50,k}^{-1} = k_3k_2^{-1} > 1$ and is stationary in the BD group investigated; ≈1 for their antagonists (stabilizers of the [B]-form [14]), and <1 for their counteragonists (stabilizers of the [C]-form of BD receptor [13]). Potentiation of the effects observed in the presence of the combined anticonvulsant action of different types of GABA-rc modulators (BD and BB) assumes the existence of more than two ([A], and [B] and [C]) conformational states of GABA-rc, which under physiological conditions definitely determine the recorded parameters of the biological system.

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EFFECTIVENESS OF ADRENERGIC TRANSMISSION AND MECHANISMS CONTROLLING IT IN RATS EXPOSED PRENATALLY TO ETHANOL

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KEY WORDS: noradrenalin secretion; ethanol; prenatal development; rat brain.

Prenatal exposure to ethanol leads to disturbances of conditioned-reflex activity [7, 10, 11]. It was shown previously that conditioned reflex formation and preservation correlate with specific changes in the effectiveness of adrenergic transmission: with changes in the intensity and stability of noradrenalin (NA) secretion [3]. The intensity of NA secretion is controlled by α- and β-autoadrenoreceptors, whose physiological role is to stabilize NA secretion in a series of consecutive depolarizations and to realize the process of frequency potentiation [4-6].

This paper describes the study of the effects of prenatal exposure to ethanol on the intensity and stability of NA secretion in a series of consecutive depolarizations and on activity of the autoreceptors regulating these processes.

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