EFFECTOR MODELING OF THE ACTION OF GABA-RECEPTOR COMPLEX LIGANDS, COOPERATIVENESS OF THE PROCESSES, AND TYPE OF MODIFICATION OF THE COMPLEX BY CONVULSANTS AND THEIR REVERSE AGONISTS

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UDC 615.214.22.015.11

KEY WORDS: GABA-receptor complex; exogenous ligands; cooperativeness of effects.

Extensive molecular-biological investigations [3, 4, 7] have confirmed the structural model of the "quartet" (tetrameric) structure of the $GABA_{A_2}$-postsynaptic supramolecular receptor-channel (GABA-rc), consisting of four subunits, each incorporating two GABA and one benzodiazepine (BD) binding sites [3, 4, 8]. The tetrameric structure of GABA-rc suggests the manifestation of "kinetic cooperativeness" [3, 5] of pharmacologic effects – characteristics quantitatively describing their deviations from simple kinetic schemes.

The aim of the investigation was to determine coefficients of cooperativeness of pharmacological effects of convulsants, namely exogenous ligand of GABA-rc: picrotoxin (PC), metrazol (MT), bicuculline (BC), and bemegride (BM), and to establish the types of interaction of BM and anticonvulsants, namely exogenous ligands of the BD (phenazepam) and BB (barbital sodium) complex.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 18-22 g. Intact animals received an injection (0.01 ml/sec) of a 0.1, 0.5, 0.5, and 1.0% solution of BC, PC, BM, and MT respectively, into the caudal vein. One hour after intraperitoneal injection of BD (0.175-5.6 mg/kg) and BB (40-320 mg/kg) intraperitoneally, the animals were given an intravenous injection of 0.5% BM solution. The minimal effective doses of the convulsants, inducing clonicotonic convulsions (DCC) and tonic extension (DTE) were determined [1, 2]. The experimental data were analyzed by means of algorithms presented in [1, 2, 5, 6].

EXPERIMENTAL RESULTS

On the basis of the suggestion that dependence of the values of input activities (doses of exogenous ligands D_i) on changes in the output parameters (effects) of the biosystem (E_i) is hyperbolic (a hyperbola of the n-th order), the experimental data were formalized (as in [3]) in accordance with equation (1):

$$E_i = E_{max}(K_d^n D_i^{-n} + 1)^{-1} \quad (1)$$

where E_{max} denotes the maximal effect (at $D_i \to \infty$), K_d is a constant, and n the coefficient of cooperativeness of the process.

By using equation (1) it is possible to linearize the dose-effect relationships between coordinates (E_i; D^{-1}/n). As the experimental data (Fig. 1) show, for the convulsant action of MT and PC, cooperativeness of the effects was not observed with respect to either parameter (n-1).

It was shown previously [1, 2] that against the background of administration of increasing doses (d_i) of BD, values of $D_{50,i}$, MT, and PC rose on a hyperbolic course (n-1, m-1) relative to control values ($D_{50,c}$).

Department of Physicochemical Pharmacology, A. V. Bogatskii Physicochemical Institute, Academy of Sciences of the Ukrainian SSR, Odessa. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 453-456, April, 1986. Original article submitted May 20, 1988.

0007-4888/89/0004-0513$12.50

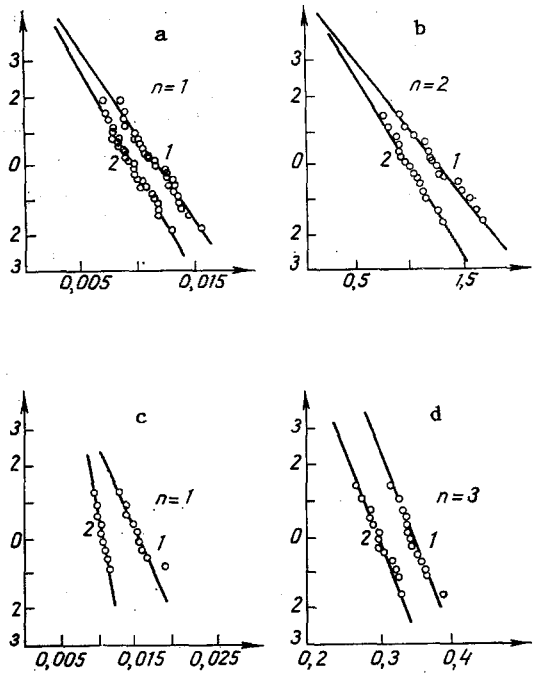


Fig. 1. Distribution of probability of values of minimal effective doses (D_i, mg/kg) of MT (a), BC (b), PC (c), and BM (d) inducing clonic convulsions (1) and tonic extension (2) in mice. Abscissa, reciprocals of recorded minimal effective doses $D_i^{-1/n}$; n denotes the coefficient the coefficient of cooperativeness of convulsive effects); ordinate, reciprocals of integral of probability of the normal distribution.

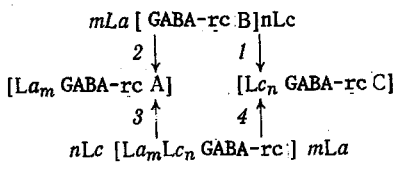
$$(D^n_{50,i}-D^n_{50,c})(D^n_{50,\max}-D^n_{50,c})^{-1}=\left[(d_{50}d_i^{-1})^m+1\right]^{-1}, \quad (2)$$

where $D^n_{50,max}$ denotes the calculated value of $D^n_{50,i}$ at $d_i \to \infty$; d_{50} the value of d_i, inducing an increase in the value of $D^n_{50,i}$ to 0.5 ($D^n_{50,max} + D^n_{50,c}$); m the coefficient of cooperativeness of the anticonvulsant effect.

The form of the dependences of the pharmacological effects of BD (n-1) in vivo [1, 2] coincides with that established previously under conditions of concentration equilibrium in vitro [3].

For the convulsant action of BC (Fig. 1) coefficients of cooperativeness (n-2) corresponding to those found previously in experiments in vitro [3] were found for both parameters of the pharmacological effect. This dependence (n-2, m-1) was preserved after injection of increasing doses of BD [1, 2].

During interaction with convulsive agents (Lc) and anticonvulsant compounds (La), GABA-rc in the native form (GABA-rc B) modifies the conformation state [8] with predominance in the first case of the form with low conductance of the chlorine ionophore (GABA-rc C) and in the second case, with high (GABA-rc A) conductance – see the Scheme below:



The type of interaction of La and Lc is determined by the ratio between the velocities of the processes 1:3 and 2:4, the preferred conformation (A- or C-form) of the [La_mLc_n GABA-rc] complex. The formation of the GABA-rc C-form of the complex is accompanied by the development of a recordable (convulsive) effect.

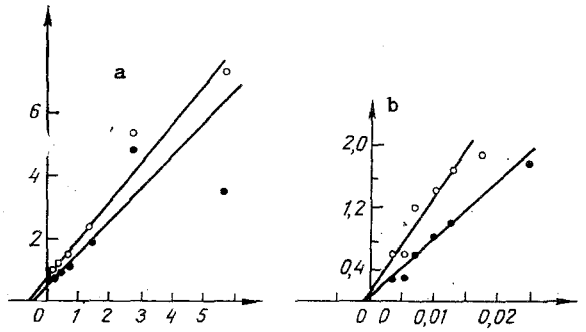


Fig. 2. Changes relative to control values ($D_{50,c}$) in mean effective doses ($D_{50,i}$) of bemegride (in mg/kg) inducing clonicotonic convulsions (empty circles) and tonic extension (filled circles), after administration of increasing doses (in mg/kg) of anticonvulsants. Abscissa, reciprocals of injected doses of phenazepam (a) and barbital sodium (b); ordinate, reciprocals of anticonvulsant effect $(D^n_{50,i} - D^n_{50,c})^{-1}$ (n is the coefficient of cooperativeness of the convulsive action of bemegride).

The consequences of interaction of La and Lc on the structures of GABA-rc during allosteric modulation of its functions can be reduced to the following types.

I. The complex of GABA-rc, La, and Lc formed as a result of processes 3 and 4 (see Scheme) has a predominant conformation with low conductance of the chlorine ionophore [La_m· Lc_n GABA-rc C], and this is manifested as a limited increase in the anticonvulsant effect (values of $D_{50,i} - D_{50,c}$ after injection of increasing doses of La. This dependence (equation 2) is characteristic of the anticonvulsant action of BD after administration of MT, PC, and BC [1, 2].

II. The complex of GABA-rc, La, and Lc has high conductance of the chlorine ionophore. This type of interaction was not found experimentally.

III. Processes 3 and 4 virtually do not take place. The principal role in the formation of the pharmacological effect is played by interactions 1 and 2. Formally the process is analogous to competitive – the dose (d_i) – effect ($D_{50,i} - D_{50,c}$) curves rise indefinitely (linearly). This type of interaction is characteristic of the anticonvulsant effects of BB during the convulsant action of MT, PC, and BC [1, 2].

The pharmacological action of the convulsant BM is produced with a marked cooperative (n-3) effect (Fig. 1). Linearization of the experimental data in accordance with equation (2) assumes cooperativeness (n-3) of the pharmacological effect of BM, after previous administration of BD, and the first type (Fig. 2a) of modulation of the functions of GABA-rc. A similar dependence (cooperativeness of effect and type of modulation of GABA-rc) was observed during interaction of the pharmacological effects of BM and BB (Fig. 2b).

Data in the literature [1-3] and the results described above are evidence of the absence of negative cooperativeness of the pharmacological effects of exogenous GABA-rc ligands (n, $m \geq 1$). Positive cooperativeness of the action of blockers of the chlorine ionophore (MT and PC) and of the exogenous ligand of this GABA-rc subunit, namely BB, was not found. A similar dependence (n-1) is characteristic of the pharmacological effects of BD. Positive cooperativeness (n-2) was established for the effects of modifiers of GABA receptors in the structure of GABA-rc (GABA and its competitive antagonist BC). In experiments in vitro, interaction of GABA with GABA-rc was determined as cooperative (n-2 and 3) [3]. The pharmacological effects of BM took place with the highest values n-3. Interaction of pharmacological effects of BD and of all the convulsive agents tested that are exogenous ligands of the various subunits of GABA-rc, and also of BB and BM is described by the same kinetic scheme (2). This agreement between the coefficients of cooperativeness of pharmacological effects of ligands of the complex, on the one hand, and the number of their binding sites on one of the four elements of the structural model of GABA-rc, on the other hand [3, 4, 7], and the stability of these values during the modulating action of anticonvulsant compounds (BD and BB) suggest the singling out, as functional element of GABA-rc (the functional model of GABA-rc) of a structure containing one binding site for BD (BD-receptor), two binding sites for GABA and (competitively) BC (GABA-receptors), and one binding site for BM, none of which coincide with the binding site for BB.

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DOES ETHANOL INDUCE STRESS IN RATS WITH ESTABLISHED ALCOHOL MOTIVATION?

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UDC 616.89-008.441.13-092.9-07:[616.154:577.175.325]-02:[615.917:549.262]

KEY WORDS: stress; ethanol; ACTH.

A single dose of ethanol of more than 1 g/kg induces considerable release of stressor hormones of pituitary and adrenal origin [3, 5]. In low doses (0.5-1 g/kg), however, ethanol may behave as an antistressor factor, and this can be recorded both in behavioral experiments and as lowering of the plasma corticosterone level in animals previously exposed to stress [1, 4]. Prolonged alcoholization is accompanied by activation of the hypothalamo-hypophyseoadrenal system with disturbance of internal feedback mechanisms, causing changes in the response of this system to classical stress-inducing stimuli [2, 7, 9].

The problem of possible reversal of the antistressor and stress-inducing properties of small and large doses of ethanol in animals with chronic contact with ethanol accordingly arises. The aim of this investigation was to compared the time course of the plasma ACTH level in intact rats and rats with established alcohol motivation.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-250 g were used. To study the effect of ethanol on the plasma ACTH level of intact rats, animals kept in communal cages were divided into three groups. Animals of group 1 received an intraperitoneal injection of physiological saline, those of groups 2 and 3 received a 25% solution of ethanol in doses of 1 and 4 g/kg respectively; the animals were decapitated (5-6 rats in each subgroup) 15, 30, and 60 min later.

Other rats were kept in individual cages with free access to water and 15% ethanol solution, and their liquid consumption was recorded daily for 14 days. The rats were then divided into four groups: animals of group 1, with no access to alcohol, received an intraperitoneal injection of physiological saline (water control); the remaining animals, consuming ethanol by preference, in approximately equal amounts, were given an intraperitoneal injection of physiological saline (group 2 – alcohol control) and a 25% solution of ethanol in doses of 1 and 4 g/kg (groups 3 and 4 respectively), and the animals (5-6 rats in each subgroup) were decapitated 15, 30, and 60 min later.

Department of Neuropharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 456-458, April, 1989. Original article submitted October 19, 1987.

 0007-4888/89/0004-0516$12.50