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EFFECTOR MODELING OF THE ACTION OF GABA—RECEPTOR-COMPLEX LIGANDS. FUNCTIONAL INTERACTION OF THE HYPOTHETICAL ALCOHOL RECEPTOR AND OTHER SUBUNITS OF THE COMPLEX

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KEY WORDS: aliphatic alcohols; GABA-receptor antagonists; barbiturates

Introduction of ethanol (E) into the body modifies the pharmacologic effects of exogenous ligands of the GABA-receptor—ionophore complex (GABA-RC) [7, 9], and in particular, it potentiates the action of 1,4-benzodiazepine (BD) derivatives [7]. Aliphatic alcohols (AA) have been shown to affect the binding parameters of barbiturate (BB) antagonists by synaptosomes [8] and conductance of the chloride ionophore of GABA-RC [5]. One suggested explanation is that the GABA-RC possesses in its structure a binding site or sites for E or AA, namely the hypothetical E receptor [2], with an influence on the conformational state of the complex.

The aim of the investigation was to determine the character of interaction of alcohols with the hypothetical AA-subunit of the complex and to determine its functions in a structural and functional model of interaction of GABA-RC and its ligands.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 18-22 g. Animals of the control groups and 30 min after internal administration of 0.01% solution of AA (methanol, ethanol, propranol, n-butanol, isoamyl alcohol, and n-heptanol, 0.75-8 g/kg) received an intravenous (into the caudal vein) injection of solutions of bicuculline (BC; 0.01%), bemegride (BM) and picrotoxin (PC; 0.5%), and metrazol (ME; 1%), and the minimal effective doses of the convulsant inducing clonicotonic convulsions (DCTC) and tonic extension (DTE) were recorded [1, 2]. Thiosemicarbazide (TS; 6-20 mg/kg) was injected intraperitoneally into the control animals and, 30 min after injection of E, the probability of onset of clonicotonic convulsions (CC) and tonic extension (TE) was recorded. The experimental results were analyzed by algorithms given in [4].

EXPERIMENTAL RESULTS

The writers showed previously that during intravenous infusion of convulsants (L_c) which are exogeneous ligands of GABA-RC, the minimal effective doses inducing rapidly reversible effects in mice (responses of integral biosystem) are determined by doses (concentrations in the biophase of action) of anticonvulsant compounds (L_a) such as BB and BD [1, 2]. To determine the type of interaction of L_a, L_c, and GABA-RC, in experiments in vivo, the fundamental issue [1] is determination of the final (under conditions of reception of L_a and L_c) state of the receptor-

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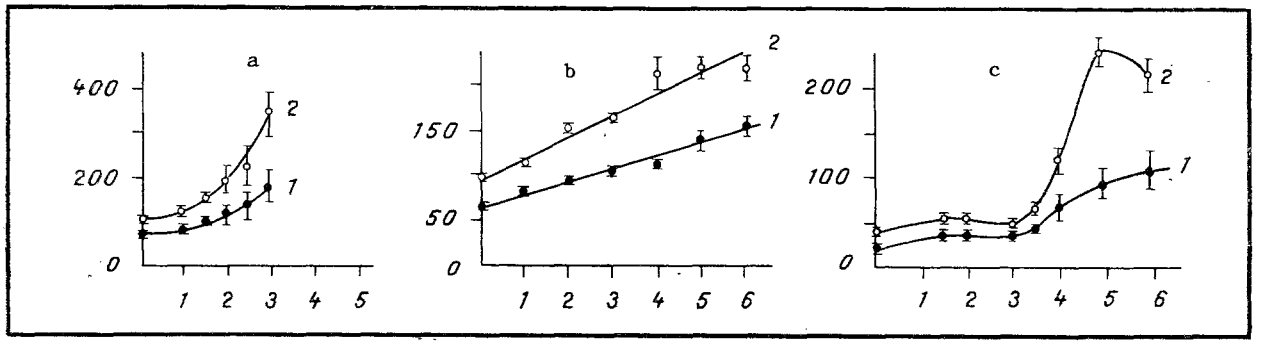
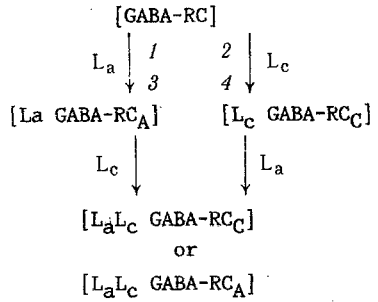


Fig. 1. Changes in minimal effective doses of antagonist of barbiturate subunit metrazol (a), picrotoxin (b), and bemegride (c), inducing DCTC (1) and DTE (2) in mice receiving ethanol by the peroral route. Ordinate, minimal effective doses of convulsants (ED_{50}, mg/kg); abscissa, dose of ethanol given (d, g/kg).

channel assembly with high (GABA-RC_A conformation) or with low (GABA-RC_C conformation) conductance of the ionophore (see the diagram below).



The experimental data in Fig. 1 show that administration of increasing doses of E to the animals changed the values of the minimal effective doses of BB antagonists: PC, ME, and BM. Determination of the type of antagonism of E to the convulsant effect of exogeneous ligands of the GABA-RC subunit including the chloride inophore and BB reception sites is particularly important because in a number of investigations [8] modification of the functions of the complex is linked with the action of E on this particular substructure of GABA-RC.

The consequences of interaction between E and the convulsant (L_c) belonging to the group of BB antagonists corresponded (Fig. 1) to all three types of modification of the complex (see Scheme):

1. The complex formed by GABA-RC with E and BM, formed as a result of processes 3 and 4, has a predominant conformation with low inophore conductance (L_aL_c GABA-RC_C), which is manifested as a limited enhancement of the anticonvulsant effect against the background of administration of increasing doses of L_a (E). A similar type of interaction was characteristic of the anticonvulsant effects of BB against PC, ME, and BC [1, 2].

2. During interaction of E and PC processes 3 and 4 virtually did not take place. The pharmacologic effect was the result of processes 1 and 2. The process corresponds formally to the competitive type: the dose—effect curves rise in a linear manner (Fig. 1). This type of interaction is characteristic of the anticonvulsant effect of BB (antagonism with PC, ME, and BM [1, 2].

3. The complex formed by GABA-RC with E and ME as a result of processes 3 and 4 has a predominant conformation with high ionophore conductance (L_aL_c GABA-RC_A), manifested as unlimited enhancement of the anticonvulsant effect of E and lowering of the probability (blocking) of appearance of recordable parameters of the seizure following administration of high doses of ethanol (Fig. 1). This type of interaction with convulsants is not mentioned in the literature for BB and BD [2].

Essential information on the mechanisms of action of AA on the functional state of GABA-RC in vivo can be obtained by undertaking types of modification of the effect of the GABA-receptor antagonist BC and the blocker of GABA synthesis, thiosemicarbazide, by them. Administration of AA to the animals induced (Fig. 2a) a linear increase in the minimal effective doses of BD. The absence of an effect of E and acetaldehyde on the parameters of specific binding of ^{3}H-muscimol with neuron membranes [6] rules out the suggestion that AA are agonists of GABA, interacting competitively with BC (inhibiting its binding with the GABA receptor). Linear (corresponding to the competitive type [3]) interaction of the effects of AA and BC may be the result of allosteric modulation of GABA-RC functions by alcohols. In fact, a linear increase in minimal effective doses of BC was observed in a limited region of administered doses of AA. Against the background of high doses of AA, deviation of the anticonvulsant action of the alcohols from

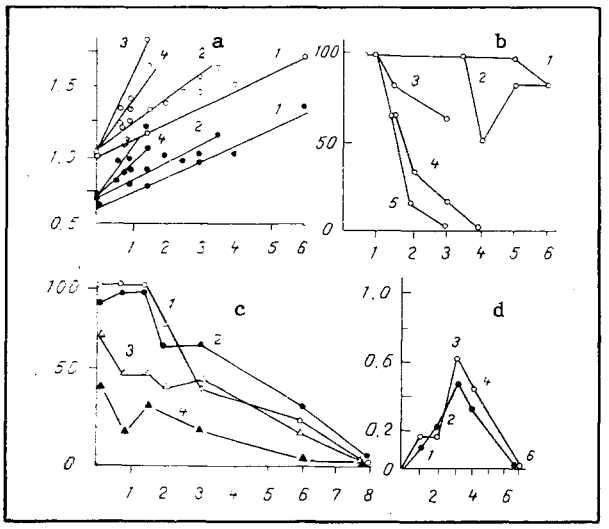


Fig. 2. Interaction of pharmacologic effects of AA — 1) methanol, 2) ethanol, 3) propanol, 4) butanol, 5) isoamyl alcohol — and GABA antagonists. a) changes in minimal effective doses of bicuculline (ED_{50}, mg/kg) inducing DCTC (filled circles) and DTE (empty circles) during peroral administration of AA (d, g/kg); b) probability of development of tonic extension (in percent) during intravenous infusion of 0.01% solution of BC in mice against the background of administration of AA (d, g/kg); c) change in intensity—probability (percent) of development — of convulsant action (tonic extension) of different doses of thiosemicarbazide — a) 20 mg/kg, b) 16 mg/kg, c) 10 mg/kg, d) 7.5 mg/kg — against the background of peroral administration of increasing doses of ethanol (d, g/kg) to mice; d) change in normalized regression coefficients of anticonvulsant effect (to the convulsant action of BC) of alcohols (filled circles indicate DCTC, empty circles DTE) depending on number of carbon atoms in their molecule (n). 6) n-heptanol.

linear (during infusion of BC) was manifested as the lower probability of development of toxic extension by the animals (Fig. 2b).

The experimental data show that AA are compounds with GABA-mimetic properties; they are allosteric modulators of the functions of GABA-RC inducing the formation of a complex with high ionophore conductance during reception of the GABA antagonist — BC. Confirmation of the GABA-mimetic properties of the effects of AA is given by the ability of E to abolish noncompetitively the manifestations of the convulsant action of TS (Fig. 2c).

Comparison of normalized (relative to the control values) regression coefficients of the anticonvulsant action of AA (antagonism to the convulsant effects of BC) shows that a change in the pharmacologic activity (with respect to both parameters of the seizure) in a series of n-aliphatic alcohols is bell-shaped (Fig. 2d). Maximal anticonvulsant activity was observed for n-propanol and minimal after administration of methanol to the animals. A significant ($p \leq 0.05$) anticonvulsant effect of n-heptanol (1.5 and 3 g/kg) was not observed during infusion of BC. The relationship found, namely competitive (linear) interaction between the effects of E and BC, and also the similarity of some elements of the structure of the propanol and picrotoxin molecules (the presence of a hydroxy group in the β-position relative to the methyl group in the structure of the molecule) lead to the conculsion that propanol is probably the ligand, or the analog of a ligand of unknown structure of the AA receptor, a subunit of the GABA-receptor complex, and that picrotoxin is a competitive antagonist of propanol or of other AA.

The data given above suggest that conductance of the GABA-RC ionophore is determined by its interaction with two series of compounds: 1) the mediator GABA and its agonists, interacting with the GABA receptor [3]; 2) AA, interacting with the putative AA receptor. Effects of BD and BB, unlike those of AA, are realized only in the presence of GABA. BC is a competitive antagonist of GABA. Antagonism of E, competitive in form, was observed only for the effect of PC.

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CYCLIC ANALOGS OF ACTH FRAGMENTS IN THE ORGANIZATION OF SELF-STIMULATION AND GROOMING BEHAVIOR IN RABBITS

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ACTH fragments without hormonal activity play an important role in behavioral adaptation in the external environment [1, 6, 9]. ACTH fragments have been shown to be involved in learning and memory [1, 6, 9], analgesia [10], sexual behavior [10], sleep processes [11], and the development of tolerance and dependence [11]. In experiments on rats [3, 4, 8] fragments $ACTH_{4-9}$, $ACTH_{4-10}$, and $ACTH_{5-8}$ intensified self-stimulation (SS) behavior during stimulation of the medial forebrain bundle, whereas intraperitoneal injection of $ACTH_{5-10}$ [2] reduced the intensity of SS of the lateral hypothalamus. ACTH analogs, injected into the cerebral ventricles, evoked intensive grooming in rats, mice, and pigeons [7].

In the investigation described below hitherto unstudied cyclic analogs of ACTH fragments, incorporating in their structure specific and nonspecific active centers:

and their role in the organization of SS and grooming behavior in rabbits were investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male chinchilla rabbits weighing 3-3.5 kg. The animals were scalped, and 1 day later bipolar nichrome stimulating electrodes were implanted in the region of the lateral hypothalamus (P-2, L-2, H-15; 15.5). To inject ACTH fragments into the animals steel cannulas 14 mm long and 0.8 mm in diameter were implanted into the lateral cerebral ventricles. On the 2nd day after the operation the animals were placed in a chamber with a fixed metal ring and testing stimulation was applied to points of the brain in order to discover the type of responses and the threshold intensity of stimulation required to evoke it. The unrestrained animals, with free access to water and food, touched a metal ring with their nose and lips, and soon learned to close the electric circuit required to obtained electrical stimulation of the brain. The parameters of the stimulating current were: volleys of square pulses with a frequency of 100 Hz and duration 0.3 sec, strength of current 40-60 μA, pulse duration 1.4 msec. Sessions of SS

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