Optimal Infusion of Reverse Agonists of the GABA-Receptor Complex for Analysis of the Fast Reversible Effects of Tranquilizers and Ethanol

N. Ya. Golovenko, V. Z. Zin'kovskii, O. V. Zhuk, E. A. Stankevich, E. A. Fedorova, and D. M. Pykhteev

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A method for intravenous infusion of reverse agonists of the GABA-receptor complex (bicuculline, pentylentetrazol, and others) was used to examine the pharmacodynamics and pharmacokinetics of GABA-receptor complex agonists (benzodiazepines and barbiturates) and aliphatic alcohols in mice. A correlation was observed between the recorded indicators of convulsive seizures in intact mice and those administered ethanol or phenazepam. The dose—effect relationship and concentration ratios of the labeled analogs of the studied compounds were estimated in the brain and blood plasma to assess their distribution in the body. Examples of tests for the pharmacokinetics of ethanol, phenazepam, and their ^{14}C-analogs in mice are given.

Key Words: *aliphatic alcohols; GABA-receptor complex; intravenous infusion; rapidly reversible effects*

Several pharmacological effects of ethanol, as well as consequences of its withdrawal, can be modified by administering exogenous ligands of the GABA-benzodiazepine-receptor-ionophore complex (GABA-RC) [6,7,9,10,13]. Aliphatic alcohols, including ethanol, facilitate picrotoxin-dependent penetration of chloride ions into brain sections [14], antagonize the convulsive activity of bicuculline [5], and demonstrate a number of other pharmacological properties characteristic of GABA-RC agonists [3,5]. Analysis of the relationship between pharmacological effects of agonists and reverse agonists is necessary not only for pharmacological screening of tranquilizers [1,6] but also for investigation of the principles underlying the functioning of GABA-RC *in vivo* [2,6]. Determination of minimal effective doses for intravenously infused reverse agonists of the GABA-RC [2-4,8,11] makes it possible to estimate the dose—effect—time relationships.

Our objective was to assess temporal variations of the pharmacological effects of reverse GABA-RC agonists, ethanol, and their labeled analogs, to explore the possibility of modeling the modulation of GABA-RCs *in vivo* with exogenous ligands and alcohols, and to examine their pharmacokinetics.

MATERIALS AND METHODS

Male CBA mice weighing 18-22 g were used. The convulsants 0.01% bicuculline and 0.5-1% corazol (pentylentetrazol) were infused into the tail vein at a rate of 0.01 mg/kg/sec, and 0.5% corazol was infused at 0.01-0.1 mg/kg/sec. The minimal doses eliciting clonic convulsions (D_{CC}) and tonic extension (D_{TE}) were determined. The following benzodi-

Department of Physicochemical Pharmacology, Physicochemical Institute, Ukrainian Academy of Sciences, Kiev

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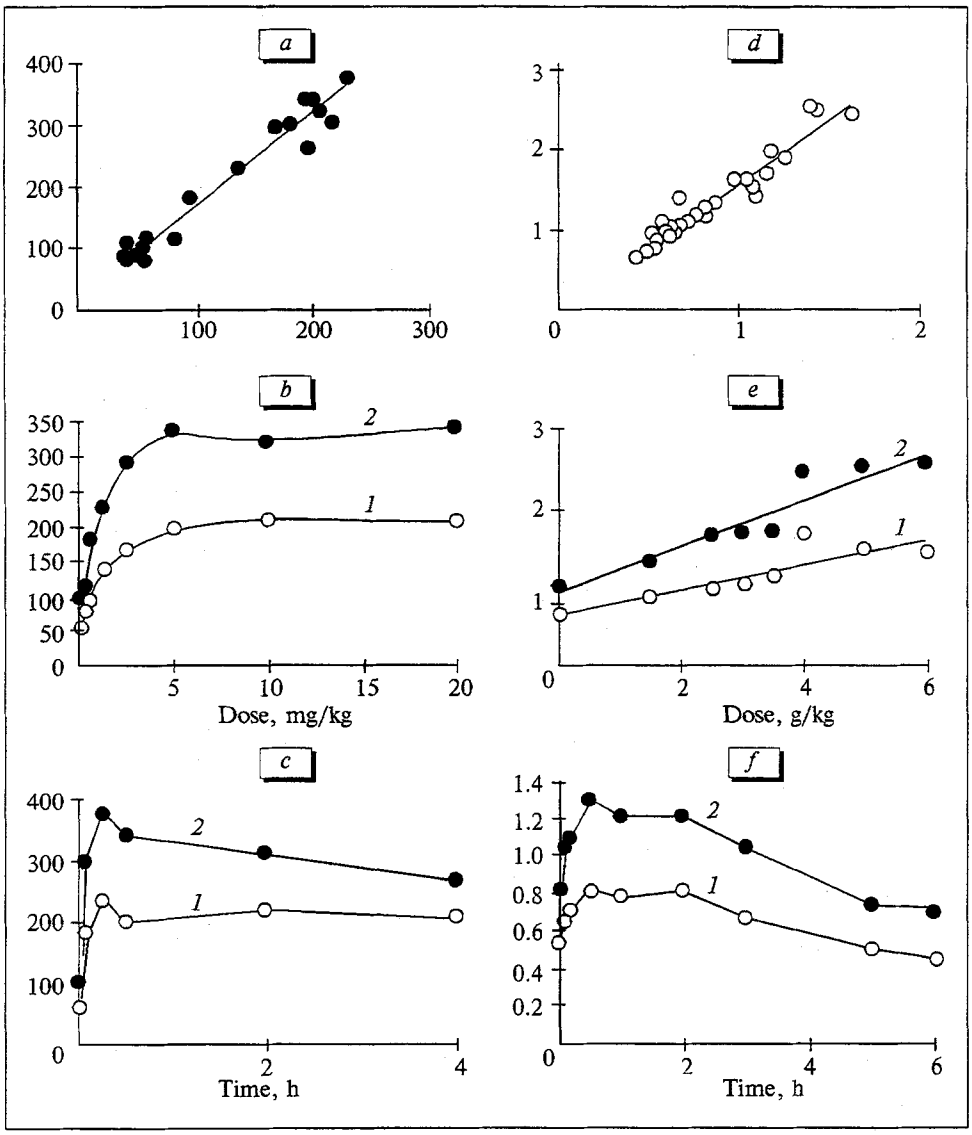

Fig. 1. Time-course of the anticonvulsive effects produced by phenazepam (*a-c*) and ethanol (*d-f*) in mice. *a*, *d*) relationship between indicators of the convulsive seizure caused by corazol at different times (5 min—4 h) in increasing doses (0.3-20 mg/kg). Abscissa: dose (mg/kg) inducing tonoclonic convulsions; ordinate: dose (mg/kg) inducing tonic extension. Changes in the minimal effective corazol doses for induction of tonoclonic convulsions (*1*) and tonic extension (*2*) in mice administered incremental doses of phenazepam (*b*) and ethanol (*e*) or at 5 mg/kg (*c* and *f*).

azepine derivatives and their labeled analogues were used, administered intraperitoneally in a Tween-80-containing emulsion: phenazepam (I) 20-0.31 mg/kg, 2-^{14}C-I (1 Ci/mol) 1.4 mg/kg, 2-^{14}C-gidazepam (II, 0.7 Ci/mol) 1.4 mg/kg, and 2-^{14}C-bromo-5-phenyl-1,2-dihydro-^{3}H-1,4-benzodiazepine-2-on (III) (0.78 Ci/mol) 1.4 mg/kg. Ethanol (aqueous solution) and 2-^{14}C-ethanol (1.3 Ci/mol) were administered intragastrally at 0.25-3 g/kg and 2 g/kg, respectively. Minimal effective doses of the convulsants were determined 5 min—24 h after the injection of I, ^{14}C-I, and ethanol. Immediately after D_{TE} was determined, the levels of ^{14}C-metabolites were measured in the brain of mice injected with ^{14}C-ethanol. The total radioactivities in the brain and blood plasma was measured 5 min—24 h after the injection of labeled compounds II and III and ethanol. Radioactivity was measured in an LKB-1215 liquid scintillation photometer (Rack-beta).

RESULTS

Experimental models based on intravenous infusion of reverse agonists have been used in a number of pharmacological studies of the functions performed by the GABA-RC and other transmitter systems [8,11]. In our previous studies where the pharmacokinetics of benzodiazepines, barbiturates, and aliphatic alcohols and GABA-RC functioning were examined *in vivo*, we used intravenous infusion of several convulsants that are reverse agonists of the GABA-RC and glycine receptor, including corazol (0.2-2% solution), bicuculline (0.0025-0.02%), picrotoxin (0.3-0.5%), bemegride (0.5%), and strychnine (0.05%) [2-6]. Their minimal effective doses inducing convulsive seizure components were recorded, usually the dose inducing false clonic twitches (D_{FCT}), D_{CC}, and D_{TE} [6]. Since the dose-dependences of these agents were linear (Fig. 1, *a*, *d*), only one of the convulsive

activity indicators (i.e., D_{FCT}, D_{CC}, or D_{TE}) is sufficient. The minimal effective doses depend on the state of the organism at a given time period (0.5-1 min), being optimal for analysis of temporal variations of the anticonvulsive effect and determination of the dose—effect relationships for GABA-RC agonists (Fig. 1). It was demonstrated that a convulsive state is established when the concentration of an anticonvulsant (corazol) exceeds a certain threshold in the "action biophase" (brain) [12]. Minimal effective concentrations of corazol in the studied range did not depend on the infusion rate. The concentrations and, consequently, volumes of infused convulsants (corazol and bicuculline) had no effect on the minimal effective doses in intact and GABA-RC agonist-treated animals [6]. The rate of infusion did not influence the minimal effective dose of corazol (Fig. 2). These findings illustrate the great potential of this method for the investigation of the dose (concentration)—time—effect relationships. Effector analysis of the GABA-RC agonist pharmacokinetics establishes a relationship between the pharmacokinetic profile of the studied agonist and the dynamics of its anticonvulsant activity. For this purpose it is necessary to estimate the following relationships.

Dose—concentration relationship. The concentration of the agonist in the plasma and in brain should be related to its dose. As shown in Fig. 3, *a*, the concentration ratio of radioactive label in the plasma and brain of mice administered compound III (physiologically active metabolite of gidazepam) is stationary (the two dose—concentration relationships are linear), indicating that the plasma and brain represent a single (central) compartment (in contrast to the situation with the original drug gidazepam). After infusion of a radiolabeled compound (Fig. 3, *b*), the plasma—brain concentration ratio changes with time because the plasma now functions as the central compartment and the brain as the peripheral one in the kinetic scheme of ^{14}C-II distribution. After ^{14}C-ethanol is administered (Fig 3, *c*), the dose—concentration relationship and the brain—plasma concentration ratio are linear (as they are in Fig. 3, *a*), which simplifies the modeling of ethanol pharmacokinetics in mice.

The dose—response relationship. In mice injected with increasing doses of phenazepam, its anticonvulsant effect increased in a hyperbolic manner (Fig. 1, *b*). For ethanol, the dose—response relationship was linear (Fig. 1, *e*).

The effector compartment—action biophase relationship in the kinetic scheme of agonist distribution is based on temporal changes in the concentration—effect relationship [6]. In the absence of significant hysteresis in the experimental data, it is

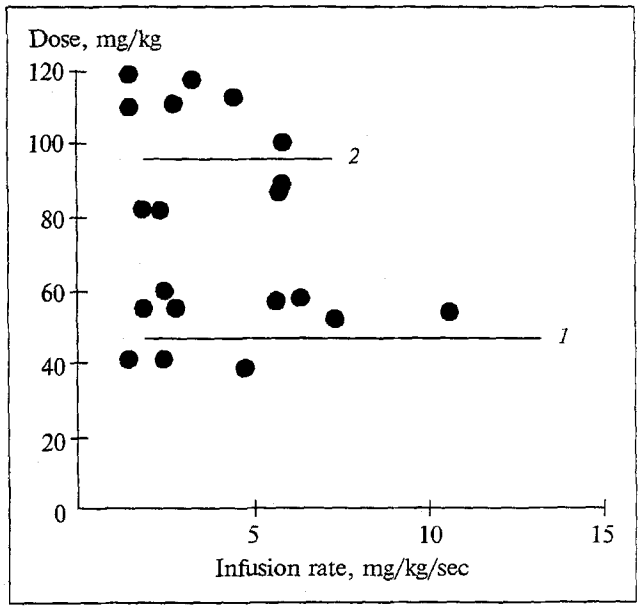

Fig. 2. Relationship between the anticonvulsive effect and infusion rate in mice after intravenous infusion of corazol. *1*) minimal effective dose inducing tonoclonic convulsions; *2*) dose inducing tonic extension.

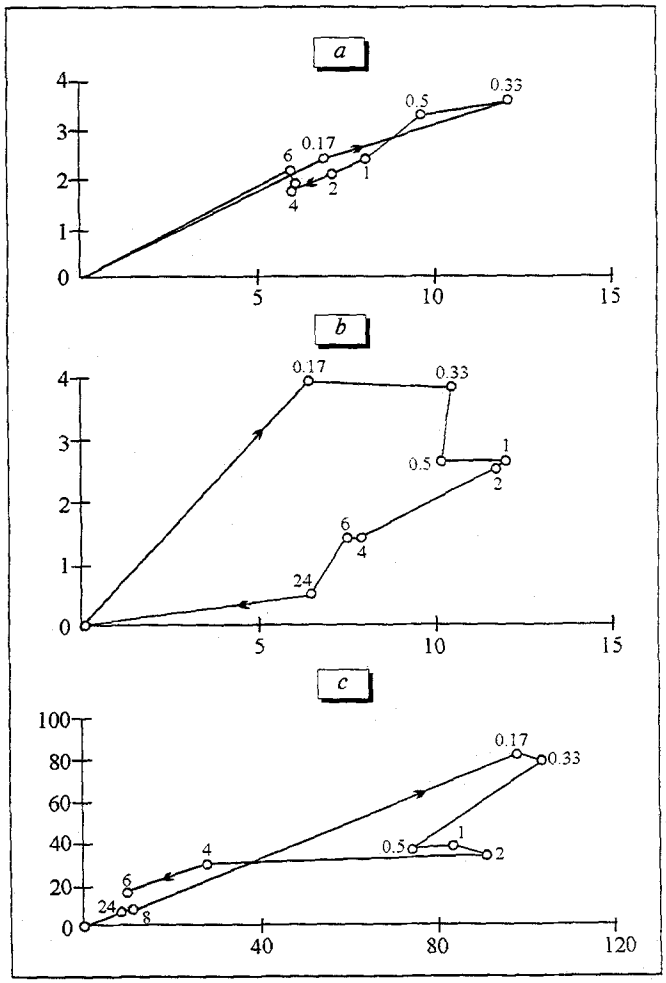

Fig. 3. Concentration ratios of ^{14}C-III (*a*), ^{14}C-II (*b*), and ^{14}C-ethanol (*c*) in mouse brain (abscissa, counts×10^3/min×g) and plasma (ordinate, counts×10^3/min×g).

possible to regard the "effector compartment" and "action biophase" as a single compartment in the kinetic scheme of phenazepam distribution and to describe the anticonvulsant action of this drug as being "concentration-central."

The use of fast reversible effects for the investigation of the interaction between ligands and the GABA-RC enabled us to analyze subsequently temporal variations in the anticonvulsant effects of various aliphatic alcohols, to design deterministic models of ethanol pharmacokinetics, and to identify specific features of the interaction between ethanol and the GABA-RC *in vivo*.

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