EFFECTOR MODELING OF THE ACTION OF GABA-RECEPTOR COMPLEX LIGANDS: FUNCTIONAL INTERACTION OF MUSCIMOL AND EXOGENOUS MODULATORS OF THE COMPLEX

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KEY WORDS: GABA-receptor complex; exogenous modulators; muscimol; pharmacological effects

Muscimol (ML) is widely used in molecular-biochemical investigations of the supramolecular GABA-receptor-ionophore complex (GABA-RC) as agonist of the GABA-receptor [4, 6, 9]. Unlike GABA muscimol can pass through the blood—brain barrier, so that it can be used in pharmacological research [6, 12, 13]. However, data obtained in vivo are quite contradictory. It has been found that ML induces a significant anticonvulsant effect when accompanied by administration of 3-mercaptopropionic acid, a compound analogous with thiosemicarbazide (TS), which competitively inhibits GABA synthesis in vivo, and protects animals, although not significantly, against seizures evoked by metrazol (MZ) and bicuculline (BC) [6]. In one study [13] ML was used as a compound potentiating the sedative effect; of barbiturates (BB); in another study [11] it was used as a convulsant, with an action antagonistic to the effects of 1,4-benzodiazepine (BD) derivatives.

The aim of this investigation was to study the functional state of GABA-RC when modified by ML and other exogenous ligands of the complex, on the level of integrity of the biosystem.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male mice weighing 18-22 mg. ML, in doses of 6 and 3 mg/kg, was injected intraperitoneally into intact animals, and in a dose of 3 mg/kg into mice of the experimental groups, which were given (30 min beforehand) injections of barbital sodium (BB) (40-200 mg/kg) and phenazepam (BD) (0.087-0.35 mg/kg). Thirty minutes after injection of ML into the mice, they and animals of the control groups received TS (6-25 mg/kg) and the probability of development of clonicotonic convulsions (CTC) and of tonic extension (TE) was recorded. MZ (1% solution) and BC (0.01% solution) was given by intravenous infusion into the caudal vein (0.01 ml/sec) of intact mice and of mice receiving preliminary injections of ML, BB, and BD, as well as of a combination of ML and BB, and ML and BD in the doses and at the times specified above. Minimal effective doses of the convulsants, inducing clonicotonic convulsions (DCTC) and tonic extension (DTE) were determined. The experimental results were analyzed with the aid of algorithms described in [5].

EXPERIMENTAL RESULTS

ML in doses of 3 and 6 mg/kg caused myorelaxation with myoclonic spasms in the mice. In a dose of 6 mg/kg a toxic action was observed, leading to death of about 20% of the animals in the course of 3 h of the experiment. The observed effects of ML developed after 10-15 min and persisted for 3 h after injection.

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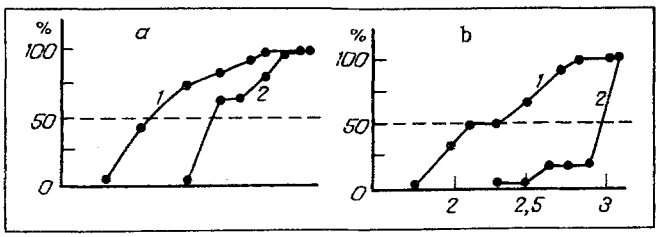

Fig. 1. Dose (ln d)—effect (%) dependence of convulsant action of thiosemicarbazide, inducing clonicotonic convulsions (a) and tonic extension (b) in animals of control groups (1) and receiving muscimol (2) in a dose of 3 mg/kg.

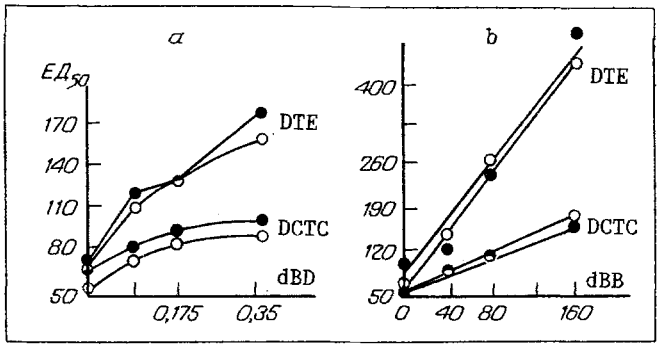

Fig. 2. Changes in values of minimal effective doses of metrazol (ED_{50}, mg/kg), inducing clonicotonic convulsions (DCTC) and tonic extension (DTE) in mice in response to injection of increasing doses of phenazepam (a) and barbital sodium (b) into intact animals (line with empty circles) and combined administration of these agonists (a and b) and muscimol (line with filled circles) in a dose of 3 mg/kg. Here and in Fig. 3: abscissa, a) dose of benzodiazepine (d BD), mg/kg; b) dose of barbiturate (d BB), mg/kg.

Injection of TS into the mice caused the development of a convulsion in animals of the control groups and those receiving ML. In a dose of 3 mg/kg ML had a significant ($p \leq 0.05$) anticonvulsant action (Fig. 1).

During infusion of MZ (Fig. 2) a significant ($p \leq 0.05$) anticonvulsant effect of ML was observed, according to both parameters recorded. After injection of increasing doses of BB and BD into the animals, the minimal effective doses of MZ increased linearly and hyperbolically respectively (Fig. 2) [1, 2]. Administration of ML to the animals did not change values of the minimal effective doses of MZ or the shape of the dose—effect curve of BB and BD.

The pharmacological action of ML in animals receiving an infusion of BC was more marked. ML had no anticonvulsant effect in animals of the control groups. Preliminary injection of ML into the mice reduced the anticonvulsant action of BB and BD (Fig. 3). Comparison of the average level and the absence of a parallel trend of the processes studied in animals of the control group (receiving and BD and BB) and after administration of ML, demonstrated the significance ($p \leq 0.01$) of differences in the mean level of change of the minimal effective doses of BC and the absence of a parallel trend between the processes ($p \leq 0.001$). Since BD (GABA-RC modulators of the metaffinoid type [1, 3] and BB (altering the probability of transition of the GABA-RC complex with mediator (GABA) into the activated form [12], realized their pharmacological effects only in the presence of GABA, the particular features of the pharmacological action of ML which were found may be interpreted as a case of partial agonism: a change in direction of the effect is observed under the influence of the "background level" of the endogenous agonist (GABA [10]). In fact, against the background of a decline of the "background" GABA level, produced by injection of TS, ML has an anticonvulsant effect. However, during the combined administration of the partial GABA-receptor agonist with modulators increasing the affinity of GABA for the

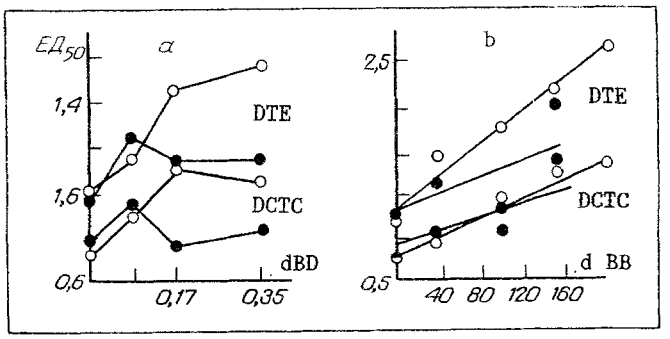

Fig. 2. Changes in values of minimal effective doses of bicueulline (ED_{50}, mg/kg), inducing clonicotonic convulsions (DCTC) and tonic extension (DTE) in mice in response to injection of increasing doses of phenazepam (a) and barbital sodium (b) into intact animals (line with empty circles) and combined administration of these agonists (a and b) and muscimol (line with filled circles) in a dose of 3 mg/kg. Here and in Fig. 3: abscissa, a) dose of benzodiazepine (d BD), mg/kg; b) dose of barbiturate (d BB), mg/kg.

GABA-receptor, namely BD [6] and BB, GABA-RC modulators of noncompetitive type [3, 7], a significant decrease was observed in their anticonvulsant action against the background of infusion of BC, a competitive GABA antagonist.

The functioning of the receptor-ionophore ensembles is determined by several parameters: their affinity for agonists, the probability of conformational transition of the agonist—receptor—ionophore ensemble complex into a state with modified (high) conductance of the ionophore, by the average time of existence of the complex in the activated state, and by conductance of the ionophore.

Affinity of ML for the GABA receptor is about 10 times greater than the affinity of GABA [3]. The average length of existence of GABA-RC in a conformational state with high conductance of the ionophore, under conditions of ML reception (60-70 msec) also exceeds the corresponding parameter during GABA reception (20-30 msec). Conductance of the ionophore in both cases is the same (15-17 pS) [8]. Accordingly, the partial agonism of ML found in vivo can be explained only by the lower probability of a conformational change of GABA-RC into a state with high conductance of the ionophore under conditions of ML reception than during interaction between the complex and the endogenous mediator (GABA).

It can be concluded from data in the literature [3, 7, 10, 12] and the results of the present investigation that during functioning of the integral biosystem (in vivo) GABA-RC is in a state of dynamic equilibrium (conformers with high and low conductance of the ionophore), determined by the endogenous GABA level. The trend of the pharmacological action (agonism/antagonism, retrograde agonism relative to the molecular mechanism of action in vitro [10]) of the partial GABA-receptor agonist ML is determined by the level of endogenous GABA and by the modulating influence of exogenous ligands in vivo, modifying affinity of the complex for GABA.

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POTENTIATION BY PROMETHAZINE OF THE HYPOTENSIVE ACTION OF ADRENERGIC DRUGS ON THE INTRAOCULAR PRESSURE

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The intraocular pressure (IOP) level is determined by dynamic equilibrium between active secretion of the aqueous humor by the ciliary epithelium and its outflow through the drainage system at the angle of the anterior chamber. Stimulators and blockers of adrenergic receptors acting on the epithelium of the ciliary body can modulate production of the humor and thus control the IOP level [6, 7]. It has recently been found that substances stabilizing membrane regions of adrenergic receptors can modify the pharmacological activity of adrenergic agents introduced into the eyes in order to depress IOP [3, 4]. It is also known that the integrity of a cell membrane depends on the potassium ion concentration in the surrounding medium; disturbance of the ionic permeability of membranes, moreover, is calmodulin-dependent in character [8]. One agent with a membranotropic action is promethazine (P; pipolphen). At the same time, this preparation is a calmodulin inhibitor. With respect to its basic pharmacological characteristics P is a histamine antagonist and blocker of H_1-receptors. The action of P on IOP and, in particular, its influence on the hypotensive (relative to IOP) action of adrenergic drugs has not been studied.

In the investigation described below the effect of P together with timolol and adrenalin on IOP and the hydrodynamics of the eyes was studied in experiments on rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 24 Chinchilla rabbits weighing 2.6-3.5 g. The animals' right eye was experimental, the left — control. IOP was measured with a Maklakov tonometer under local anesthesia with 0.5% amethocaine. The hydrodynamics of the eye was studied by tonography on an electronic tonograph [1]; a 2.5% solution of promethazine [N-(2-dimethylamino-1-propyl)-phenothiazine hydrochloride)], from "Egis" (Hungary) was diluted with Hanks' medium to a 0.5% solution (pH 6.8-7.0). Solutions of the preparations were instilled into the experimental (right) eyes of the rabbits, and Hanks' solution alone into the control (left) eyes of all the animals. After 30 min, a 1% solution of adrenalin (Isoptoepinal, from "Alcon," Belgium) was instilled into both eyes of 12 animals (group 1), and a 0.5% solution of timolol maleate (Optimol, from "Star," Finland) was similarly instilled in group 2; IOP of both eyes was measured immediately before and 30 min after instillation of P, and again 1, 2, 3, 4, and 24 h after instillation of adrenalin or timolol. Tonography was carried out before and 24 h after instillation of the drugs. Altogether 454 tonometric and 108 tonographic investigations were undertaken.

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