ROLE OF THE GABA—BENZODIAZEPINE—RECEPTOR COMPLEX IN THE MECHANISM OF THE ANXIOLYTIC ACTION OF 3-HYDROXYPYRIDINES — NEW TRANQUILIZERS WITH A NONBENZODIAZEPINE STRUCTURE

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Derivatives of 3-hydroxypyridine (3-HP) are membrane-active oxidants and inhibitors of lipid peroxidation, with the ability to modify physicochemical properties of membranes, lipid–protein interconnections, activity of membrane-bound enzymes, and the receptor, metabolic, and transport functions of membranes. It has been shown that 3-HP have a broad spectrum of psychotropic action, predominantly anxiolytic and antistressor [3]. However, the molecular mechanisms of these phenomena have not yet been explained.

In the modern view the molecular mechanism of action of widely known tranquilizers of the benzodiazepine series is based on their interaction with a membrane supramolecular complex consisting of three allosterically bound protein subunits: a GABA receptor (GABAR), a benzodiazepine receptor (BDR), and a Cl^--ionophore subunit [6, 10, 11, 13]. Our knowledge of the mechanism of the anxiolytic action has been considerably increased through the discovery of atypical tranquilizers and anxiolytics with a nonbenzodiazepine structure, some of which have high affinity for BDR (Eopiclon, PK08165, Cl 218, 872), wherease others (Lonetil, Mebicar*) interact only with GABA-activated BDR. The action of Fenibut† is attributed to its effect on GABAR. Meprobamate, fenobam, and buspirone have affinity neither for BDR nor for GABAR [2, 4, 9]. Derivatives of 3-HP also are tranquilizers of a new type.

The aim of this investigation was to study the mechanism of the anxiolytic action of a water-soluble antioxidant from the 3-HP class by testing the action of pharmacologic analyzers on the various components of the GABAR-BDR-Cl^--ionophore complex.

EXPERIMENTAL METHODS

Experiments were carried out on 110 noninbred male albino rats weighing 200-240 g. To test the anxiolytic action the method of a conflict situation, created by conflict between food and defensive reflexes, caused by painful stimulation of the rat while it was drinking water, was used. The number of punished acts of drinking and approaches to the drinking bowl in 20 min was recorded.

3-HP in a dose of 200 mg/kg was injected intraperitoneally 40 min before the experiment. The following substances were used as pharmacologic analyzers: the BDR agonist phenazepam‡ (0.5 mg/kg, intraperitoneally, 1 h before the experiment), the specific BDR antagonists Ro 15-1788 (10 and 15 mg/kg, intraperitoneally, 15 min before the experiment) and CGS-8216 (3 mg/kg, intraperitoneally, 40 min before the experiment), the inversive BDR agonist β-carboline-3-carboxyethyl ester (βCCEE) (50 mg/kg, subcutaneously, 30 min before the experiment), the GABAR antagonist bicuculline (0.75 mg/kg, subcutaneously, 5 min before the experiment), the GABA antagonist picrotoxin (2 mg/kg, intraperitoneally, 30 min before the experiment), the α-dihydropicrotoxin binding blocker Ro 5-3663 (10 mg/kg, subcutaneously, 30 min before the

*Mebicar: 2,4,6,8-tetramethyl-2,4,6,8-tetra-azobicyclo-(3,3,0)-octadione-3,7.
†Fenibut: β-phenyl-GABA.
‡Soviet bromazepam analog: 7-bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

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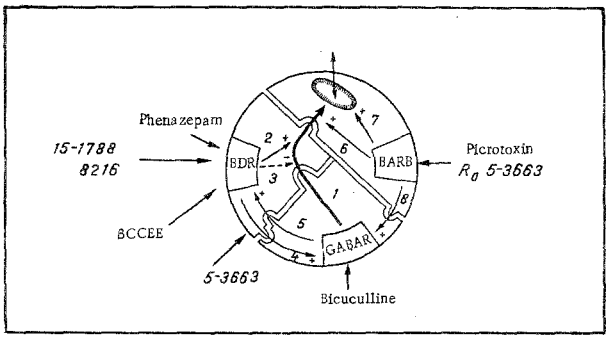

Fig. 1. Hypothetical model of a supramolecular complex according to [13]. Three interconnected components of the complex (GABAR, BDR, and Cl^--ionophore) are indicated. [BARB not explained in Russian original — Editor.]

experiment). Other analyzers used were the GABA agonist calcium valproate (200 mg/kg, intraperitoneally, 90 min before the experiment) and metrazol, an effective antagonist of most known tranquilizers (25 mg/kg, subcutaneously, 20 min before the experiment).

RESULTS

In a conflict situation 3-HP in a dose of 200 mg/kg was found to have a marked anxiolytic action, increasing the number of punished acts of drinking fourfold compared with the control. Pharmacologic analyzers other than calcium valproate, in the dose used, had no significant effect on the animals' behavior in the conflict situation.

The use of analyzers affecting the benzodiazepine-receptor part of the complex (Fig. 1) showed that the specific BDR antagonist Ro 15-1788, in doses of 10 and 15 mg/kg, and the aversive agonist βCCEE, with high affinity for BDR, have no significant effect on the anxiolytic action of 3-HP, whereas the other BDR antagonist CGS-8216 caused some weakening of its activity, and the direct BDR agonist phenazepam potentiated the anxiolytic properties of 3-HP (Table 1).

Unlike the BDR antagonists, bicuculline, a GABAR antagonist, completely abolished the action of 3-HP. Picrotoxin, a GABA antagonist with a different mechanism of action, which also blocks the action of 3-HP, had a similar effect, reducing the number of punished acts of drinking to control values (Fig. 1, Table 1). Metrazol reduced but calcium valproate and Ro 5-3663 potentiated the effects of 3-HP (Table 1).

The results thus indicate that the clearest weakening of the anticonflict action of 3-HP was observed when the GABA antagonists bicuculline, blocking GABAR, andpicrotoxin, acting mainly on the Cl^--ionophore part of the complex, were used, and the effect of 3-HP was potentiated by calcium valproate, which raises the brain GABA level. GABA-negative and GABA-positive substances have a similar action on the anxiolytic effect of classical benzodiazepines [5]. However, whereas the effects of the benzodiazepines are antagonized by the specific BDR antagonist Ro 15-1788 and the aversive antagonist βCCEE [5, 6, 15], these substances do not change the action of 3-HP.

Some rather unexpected results were obtained when Ro 5-3663 was used as the analyzer: This substance has the properties of a GABA antagonist, it induces convulsions similar in appearance to those induced by picrotoxin, like picrotoxin it antagonizes the anxiolytic action of benzodiazepine, and it competitively inhibits binding of labeled α-dihydropicrotoxin [8, 9, 14]. In the present experiments Ro 5-3663 acted in the opposite way to picrotoxin and potentiated the effects of 3-HP. This can be explained on the grounds that Ro 5-3663 affects GABA-stimulated binding of benzodiazepines [7], on which picrotoxin has no effect. There is also evidence that in certain situations Ro 5-3663 acts like an agonist of an antagonist, for example, it has an inhibitory action similar to that of benzodiazepines on the avoidance reflex and it potentiates their sedative effect [1, 5].

These observations, showing the different effects of two BDR antagonists (Ro-1788 and CGS-8216) against the background of 3-HP, suggest that they have different mechanisms of action. This suggestion is confirmed by the fact that CGS-8216, unlike Ro 15-1788, antagonizes

TABLE 1. Effect of BDR and GABAR Agonists and Antagonists on the Anxiolytic Action of 3-HP (M ± m)

Experimental conditions	Dose, mg/kg	Number of punished acts of drinking	Number of approaches to drinking bowl
Control	—	1,8±0,3	19,6±2,1
3-HP	200	6,6±0,2	14,0±1,1
3-HP + Ro 15-1788	200+10	6,5±1,4	14,5±5,1
3-HP + Ro 15-1788*	200+15	7,0±1,0	19,3±6,6
3-HP + CGS-8216	200+3	3,4±0,8	18,3±0,2
3-HP + βCCEE	200+50	9,0±1,2	8,2±4,2
Phenazepam	0,5	5,3±1,8	15,4±4,1
3-HP + phenazepam	200+0,5	16,1±6,7	17,6±4,8
3-HP + bicuculline	200+0,75	1,2±0,2	2,2±0,8
3-HP + picrotoxin	200+2	1,4±0,2	11,2±2,1
Calcium valproate	200	14,8±4,2	5,1±3,2
3-HP + calcium valproate	200+200	10,0±0,7	6,7±1,5
3-HP + metrazol	200+25	3,5±0,3	18,3±0,2

*As in Russian original.

anxiolytic effects not only of benzodiazepine [5, 6], but also of some nonbenzodiazepine tranquilizers, which do not bind with BDR [12].

These investigations thus suggest that GABAR-BDR-Cl^--ionophore complex participates in the realization of the anxiolytic action of membrane-active antioxidants that are derivatives of 3-HP.

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