DOI 10.1007/s10517-020-04704-0
Bulletin of Experimental Biology and Medicine, Vol. 168, No. 3, January, 2020 PHARMACOLOGY AND TOXICOLOGY

Role of $GABA_A$ Receptors in the Mechanism of *In Vivo* Psychotropic Activity of Amitriptyline in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 168, No. 9, pp. 308-312, September, 2019
Original article submitted March 20, 2019

Standard water-reinforced drug discrimination model was employed to train Wistar rats to discriminate the intraperitoneal injections of tricyclic antidepressant amitriptyline (5.4 mg/kg) and physiological saline. To examine the role of $GABA_A$ receptors in psychotropic action of amitriptyline, the substitution tests were performed with muscimol (0.1-1.0 mg/kg) and pregnenolone (30-50 mg/kg). Similar tests were carried out with amitriptyline interoceptive antagonists bicuculline (1 mg/kg), flumazenil (15 mg/kg), finasteride (5 mg/kg), and indomethacin (7.5 mg/kg). The study showed that interoceptive effects of amitriptyline depend on functional activity of $GABA_A$ receptors but not on the neurosteroid site of $GABA_A$ receptor complex.

Key Words: *drug discrimination; $GABA_A$ receptors; tricyclic antidepressant; amitriptyline; rats*

GABA is a dominant inhibitory neurotransmitter in CNS and participates in the control of emotions, behavior, memory, and higher cognitive functions. $GABA_A$ receptor is an intricate pentamer complex with a number of binding sites for benzodiazepines, barbiturates, neurosteroids, and blockers of chloride-ionophore complex [6,12]. Changes in GABA effects can be important in the pathogenesis of depression. It is well known that patients with severe depressive disorders are characterized by reduced GABA level in the cingulate gyrus region, down-regulated expression of $GABA_A$ receptors, decreased level of glutamate decarboxylase, and functional disturbances in certain subtypes of GABAergic interneurons. Reduced GABA levels in the hippocampus, cerebral cortex, and nucleus accumbens were demonstrated in behavioral models of depression in rats [2]. Experimental data attest to possible implication of GABA system in the effects of many antidepressants [8]. For instance, amitriptyline (AMT) potentiates GABA-dependent chloride ionic current [10]. In epy drug discrimination (DD) models, AMT partially reproduces the interoceptive stimulating effects of diazepam, which also attests to common elements in the mechanisms of action of tricyclic antidepressant and nonselective full agonist of $\alpha_{1,3,5}\beta_2\gamma_2$ subunit of $GABA_A$ receptor [9,14].

In vertebrates, the neurosteroid system plays the homeostatic role; additionally, it is responsible for adaptive reactions to stress [5,13]. Allopregnanolone, an endogenous metabolite of progesterone, is a positive modulator of $GABA_A$ receptors with intrinsic antidepressant properties [4]. The neurosteroid system is implicated in the realization of antidepressant effects of selective serotonin reuptake inhibitors such as fluoxetine [7]. In CNS, 3α-hydroxysteroid oxidoreductase and 5α-reductase are the major enzymes of progesterone metabolic pathway, which produces allopregnanolone. A selective inhibitor of 3α-hydroxysteroid oxidoreductase is indomethacin, while finasteride is an irreversible blocker of 5α-reductase [11].

Here we studied the role of $GABA_A$ receptors and metabolism of neurosteroids in the realization of interoceptive stimulating effects of tricyclic antidepressant AMT using DD paradigm in rats.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=12) weighing 250-300 g. The animals were maintained under standard vivarium conditions with natural day-night cycle and pelleted food (MEST) *ad libitum*. During DD training, the rats were motivated by drinking water, which was given only as reinforcement. In addition, water was given during a 30-min interval, which started in different time after training session. The rats had free access to water in calendar free days or if the experiments were not carried out within 48 h. All procedures were carried out in strict adherence to Directive No. 708n of Ministry of Health of the Russian Federation (On Establishing the Rules of Laboratory Practice; August 23, 2010). All experiments were designed to cause the least pain, suffering, distress or lasting harm.

AMT (solution for injections, Moscow Endocrine Plant) was administered intraperitoneally (5.4 mg/kg) as discriminated interoceptive stimulus during DD conditioning to distinguish the training drug (*i.e.*, AMT) from physiological saline (PS). To test for antagonistic effects against the interoceptive action of AMT, the following substances were employed: an inverse agonist at benzodiazepine site of $GABA_A$ receptor flumazenil (15 mg/kg), selective inhibitor of 3α-hydroxysteroid oxidoreductase indomethacin (7.5 mg/kg), selective inhibitor of 5α-reductase finasteride (5 mg/kg), and selective inhibitor of $GABA_A$ receptor bicuculline (1 mg/kg). All substances were from Sigma-Aldrich. To substitute AMT effects, the study used selective agonist at $GABA_A$ receptor muscimol (0.1-1.0 mg/kg) and neurosteroid precursor pregnenolone (50 mg/kg). Both substances were from Sigma-Aldrich. To test for dose-dependency in AMT-PS discrimination, AMT was used in doses of 1.35-5.40 mg/kg. All substances were injected intraperitoneally.

The experiments were carried out according to schedule developed to discriminate AMT from PS. This schedule consisted of 3 stages [3]:

1) operant conditioning according to standard FR-10 (fixed ratio) schedule, which considers 10 presses on one of two levers as elaborated conditioned response;

2) a 7-day course of daily injections of AMT (10 mg/kg), which was employed in the following as the discriminative stimulus;

3) training for DD according to modified schedule, in which FR was progressively increased from FR-2 to FR-10 with incremental step of 2 presses on the lever.

The experiments were carried out after water deprivation for 24 h. Six rats were simultaneously conditioned in 6 individual Skinner's chambers (Lafayette Instrument) operated by ABET II software. After adequate response, a drop of water (0.125 ml) was used as a single unit of reinforcement. In substitution tests and in the tests with combined administration of the substances, the responses related to administration of the training antidepressant AMT were recorded (in %), the relative number of rats choosing the levers associated with each differentiated stimuli, and the frequency of operant reactions (min^{-1}). The tests were performed 1-2 times per week; only the rats with stable operant DD were tested. Between the tests, the rats were conditioned repeatedly to maintain necessary DD level. The duration of DD test was 2-3 min. No reinforcement was used during DD testing irrespective of rat behavior.

The data were analyzed statistically using Fisher's exact test to assess the sampling fraction of variances and paired Student's t test [1]. They were presented as averaged percentage of choice of appropriate lever. The differences were significant at $p<0.05$.

RESULTS

In 11 of 12 rats, stable discrimination of AMP and PS was achieved after 6 training sessions. The mean incidences of operant reactions after administration of AMT or PS were 24.9±1.2 and 22.4±1.5 lever pressings per minute, respectively. The substitution tests with AMT (1.35-2.70 mg/kg) revealed a direct correlation between AMT dose and AMT-induced interoceptive stimulating effects, which attested to sufficient level of conditioned DD (Table 1).

In substitution tests, a selective $GABA_A$ receptor agonist muscimol induced partial and dose-dependent generalization of interoceptive effects of AMT. In experiments with 0.1 mg/kg muscimol, the rats chose AMT-appropriate lever in 9±8% of all choices. In comparison with training sessions, the percentage of rats choosing PS-appropriate lever decreased, while the percentage of rats with intermediate choice increased from 21 to 79%. When muscimol was administered in a dose of 1 mg/kg, choice of AMT-appropriate level increased to 52±14% (Table 1). The percentage of rats with intermediate choice increased, while the percentage of rats choosing PS-appropriate lever decreased (Table 1). In addition, 2 rats failed to perform the test. When testing antagonism with interoceptive effects of AMT, the selective $GABA_A$ receptor antagonist bicuculline decreased the choice of AMT-appropriate lever to 71±14%; 3 of 6 rats chose this lever. One of 6 rats demonstrated intermediate level of retrieval of AMT interoceptive effects; another rat demonstrated the PS-appropriate reactions. In this group, one rat failed to perform the test (Table 1).

In substitution tests with neurosteroid precursor pregnenolone, the rats did not retrieve the stimulus

TABLE 1. Effect of Tested Drugs on Interoceptive Discriminative Properties of AMT in Wistar Rats Trained to Discriminate AMT and PS

Substance	Dose	Choice of AMT-appropriate lever, %	Share of rats, %		
			AMT-appropriate lever choice	Intermediate level of AMT-appropriate lever choice	PS-appropriate lever choice
Training Conditions					
PS	0.5 ml (*n*=11)	0	0 (0/11)	0 (0/11)	100 (11/11)
AMT	5.4 mg/kg (*n*=11)	100	100 (11/11)	0 (0/11)	0 (0/11)
Substitution Tests					
AMT	1.35 mg/kg (*n*=8)	33±8.7	25 (2/8)**	37.5 (3/8)	37.5 (3/8)
	2.7 mg/kg (*n*=4)	82.4±12.8	50 (2/4)**	0	50 (2/4)
Muscimol	0.1 mg/kg (*n*=6)	9.2±7.7	0	33.3 (2/6)**	66.6 (4/6)*
	1 mg/kg (*n*=6^{+})	52.1±14.4	0	50 (3/6)	16.6 (1/6)**
Pregnenolone	30 mg/kg (*n*=6^{++})	0	0	0	66.6 (4/6)*
	50 mg/kg (*n*=5)	9.5±1.2	0	20 (1/5)**	80 (4/5)
Tests for antagonism with AMT interoceptive effects					
Bicuculline+AMT	1 mg/kg+5.4 mg/kg (*n*=6^{+})	71.4±13.7*	50 (3/6)**	16.6 (1/6)**	16.6 (1/6)**
Finasteride +AMT	5 mg/kg+5.4 mg/kg (*n*=4)	100	75 (3/4)	25 (1/4)**	0
Indomethacin+AMT	7.5 mg/kg+5.4 mg/kg (*n*=6)	100	100 (6/6)	0	0

Note. *n*: number of rats; $^{+}$2/6 (33.3%) and $^{++}$1/6 (16.6%) rats failed to perform the test; *p<0.05, **p<0.001 in comparison with 5.4 mg/kg AMT.

properties of AMT (5.4 mg/kg). In the tests for antagonism with the stimulus properties of AMT, neither indomethacin, nor finasteride changed the intermediate level of choice of the AMT-appropriate lever in contrast to bicuculline (Table 1). Moreover, both indomethacin and finasteride produced no significant effects on the percentage of rats choosing AMT-appropriate lever (Table 1).

Since selective activation of $GABA_A$ receptors resulted in partial generalization of stimulus effects of AMT, whereas selective blockade of these receptors partially inhibited the interoceptive effects of this antidepressant, it can be hypothesized that there is a certain implication of some populations of $GABA_A$ receptors in the mechanism of psychotropic activity of tricyclic antidepressants. Thus, activity of GABA system can be one of the reasons of therapeutic potency of these remedies in patients with anxiety disorders [11].

As mentioned in the above, one of the pathways of progesterone metabolism (in CNS as well) generates allopregnanolone, which could potentially engage the GABA system in realization of AMT psychotropic activity. However, pregnenolone cannot reproduce the stimulus effects of AMT even partially, whereas the enzyme inhibitors of progesterone metabolism produce no significant effect on retrieval of interoceptive effects of this popular antidepressant.

Thus, DD method showed that $GABA_A$ receptors could be implicated in the mechanism of psychotropic action of tricyclic antidepressants.

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