The Role of Neurochemical Mechanisms of Ventromedial Hypothalamus in Various Models of Anxiety in Rats

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Microinjections of glutamic acid, serotonin, and sulpiride in the ventromedial hypothalamus reduced rat anxiety in an illuminated platform avoidance task, while dopamine, apomorphine, picrotoxin, and memantine increased it. Similar injections of phenylephrine and yohimbine reduced anxiety in threatening situation task only, while GABA reduced it in both tasks. It is suggested that various emotional and stress phenotypes are realized through functionally different neurochemical mechanisms of the ventromedial hypothalamus.

Key Words: *hypothalamus; anxiety; neurochemical mechanisms*

Ventromedial hypothalamus (VMHT), the morphological and functional substrate of the cerebral aversive system [6,7,11], plays an important role in anxiety control [5,13,14]. However, the role of its transmitter systems in the realization of this adaptive behavior is unclear. Different aversive stimuli potentiating stress and anxiety produce ambiguous effect on the metabolism and synaptic release of norepinephrine and dopamine [12]. They also attenuate GABA-mediated transmission and decrease binding of ^{3}H-flunitrazepam in the median hypothalamus [4]. Electrical stimulation of VMHT potentiates fear and anxiety [6, 7,11], but they can be suppressed by local injection of diazepam or GABA-mimetics midazolam and muscimol into this limbic structure [11,13,14]. At the same time, serotonin and glutamic acid also play transmitter functions in VMHT [5,8,10], but their role in the genesis of anxious states is unclear.

In this paper, GABA, glutamic acid, monoamines, or agonists and antagonists of these neurotransmitters were directly injected into VMHT to reveal their functional significance and role in the genesis of anxious states induced by various aversive stimuli.

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MATERIALS AND METHODS

Experiments were carried out on 44 outbred male rats (body weight 260±80 g) in a box consisting of light (200-W light bulb) and dark compartments connected through a hole located 6 cm above the floor and a special section for victim rats (n=19) separated from the dark compartment by a transparent wall. Anxiety was measured in rats previously trained to avoid illuminated area (illuminated platform avoidance test) and in spectator rats trained to avoid illuminated compartment during nociceptive stimulation of victim rats (threatening situation avoidance test). Nociceptive electrical stimulation (45 V) was automatically switched off when spectator rat reached a special platform in the dark compartment. After consolidation of the avoidance reactions, the rats (n=25) were anesthetized with ether, and microinjection chemotrodes were implanted in VMHT (stereotaxic coordinates AP=2.0, L=0.7, and H=8.4) [1]. The following drugs (0.5-5%) were injected with a special microinjection system: dopamine, GABA, glutamic acid, serotonin creatinine sulfate, phenylephrine, clonidine, apomorphine, phentolamine, yohimbine, and sulpiride in doses of 5-50 μg dissolved in 1 μl. Retention of the acquired reactions was tested 2 days before the experiments (5-6 days after surgery). In further experiments, the rats with avoidance laten-

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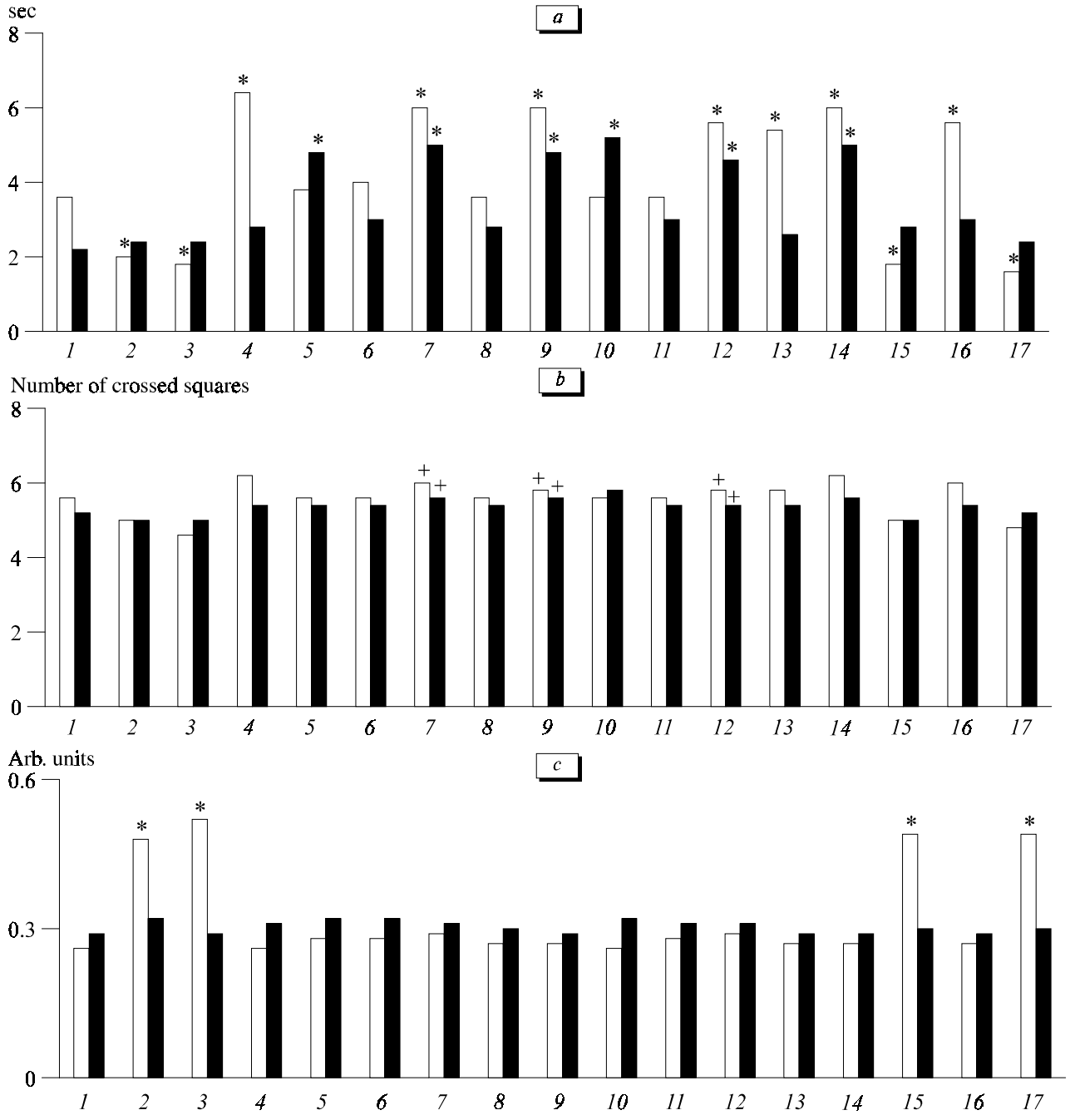

Fig. 1. Effect of neurotransmitters, their agonists, and antagonists injected into ventromedial hypothalamus on anxiety in illuminated platform (light bars) and threatening situation (dark bars) avoidance tests: *a*) time spent in illuminated compartment; *b*) motor activity; *c*) motivation to reach the dark compartment. Microinjections: *1*) 0.9% NaCl, 1 µl (control); *2*) dopamine, 10 µg; *3*) apomorphine, 10 µg; *4*) sulpiride, 50 µg; *5*) phenylephrine, 10 µg; *6*) phentolamine, 5 µg; *7*) phentolamine, 10 µg; *8*) propranolol, 10 µg; *9*) propranolol, 20 µg; *10*) yohimbine, 10 µg; *11*) clonidine, 5 µg; *12*) clonidine, 10 µg; *13*) serotonin, 20 µg; *14*) GABA, 10 µg; *15*) picrotoxin, 5 µg; *16*) glutamic acid, 10 µg; *17*) memantine, 20 µg. *$p<0.05$ compared to the control. $^{+}$Myorelaxation (40% rats slipped off the rotating rod).

cy of 2-3 sec in the illuminated platform test and 1-2 sec in the threatening situation test were used. The experiments consisted of two sessions with a 60-min interval. In each session the rats were tested in both behavioral models. In the first session, the baseline indices of anxiety were measured in both behavioral tests. In the second session (55 min later), the same indices were measured 5 min after microinjection of test drugs made outside the box. The time spent in the illuminated compartment, the number of crossed squares during this period (motor activity), and the intensity of motivation to reach the dark compartment were evaluated. The latter index was counted in arbitrary units [2] as the total force developed at the plat-

form of the dark compartment related to the time spent on it and body weight. If the tests revealed an anxiolytic effect, the rats were additionally tested for drug-induced central myorelaxation in the rotarod test.

Control groups for both behavioral tests included 5+5 rats trained for the corresponding avoidance reaction. They received 1 μl isotonic NaCl though implanted chemotrodes. After the experiments, the rats were sacrificed under ether narcosis. Morphological verification showed that in 23 rats chemotrode tips were located in *n. ventromedialis hypothalami*, while in 2 rats they were placed in the ventral region of *n. dorsomedialis hypothalami*. The data were treated by Wilcoxon, Mann—Whitney, and Kolmogorov—Smirnov tests and considered significant at $p<0.05$ in all three tests.

RESULTS

Local injection of phenylephrine and yohimbine into VMHT did not affect avoidance of illuminated platform, but antagonized the development of anxiety in the threatening situation test characterized by more complicated aversive stimulation (Fig. 1). By contrast, hypothalamic microinjections of serotonin, glutamic acid, and sulpiride did not change the parameters of threatening situation avoidance test, although produced an anxiolytic effect in the illuminated platform avoidance test. At the same time, hypothalamic injection of GABA prolonged the time spent by rats in the illuminated compartment and antagonized the development of anxiety in both tests. The antiaversive effects of phenylephrine, yohimbine, glutamic acid, serotonin, GABA, and sulpiride were not accompanied by a decrease of muscular tone or inhibition of motor activity, which excludes motor deficiency in avoidance reaction (Fig. 1).

Microinjections of β-adrenoblocker propranolol (10 μg), presynaptic α_2-adrenomimetic clonidine, which blocks norepinephrine release from catecholaminergic terminals (5 μg), and blocker of postsynaptic α_1- and α_2-adrenoreceptors phentolamine (5 μg) into VMHT did not affect behavior in both anxiety models. By contrast, high doses of these adrenergic transmitters effectively reduced anxiety in both behavioral tests, *i. e.* prolonged the time spent in the illuminated compartment. However, in this case the anxiolytic effect was caused by motor deficiency, since effective doses of clonidine, phentolamine, and propranolol significantly reduced muscular tone (Fig. 1, *b*).

Enhancement of dopaminergic synaptic traffic or deficiency in GABA- and glutaminergic influences due to microinjections of dopamine and apomorphine or picrotoxin and memantine into VMHT did not affect avoidance of threatening situation, but potentiated anxiety and stimulated the mechanisms controlling innate preference of darkness: these drugs significantly ($p<0.05$) shortened the time spent in the light compartment in the illuminated platform avoidance test (Fig. 1). These data suggest that the neurotransmitter profile of VMHT dopamine-, GABA-, glutamate-, and serotonergic neurotransmitter mechanisms responsible for anxiogenic and anxiolytic effects in fear-induced avoidance reaction in the illuminated platform avoidance test [2]. This conclusion is in line with previous reports showing that local injection of apomorphine or $GABA_A$-receptor blocker bicuculline into median hypothalamus potentiates anxiety and induced behavioral changes similar to those caused by aversive stimulation [9,11], while injection of ipsapirone and muscimol inhibits avoidance reaction based on fear-anxiety emotions [5]. At the same time, our data obtained in the model of threatening situation indicates that neuronal matrix of anxiety formed by zoosocial stress aversive stimulation [2] includes not dopamine-, glutamate-, and serotonin, but adreno- and GABAergic neurotransmitter mechanisms in VMHT neuronal networks. This hypothesis is corroborated by the fact that microinjection of yohimbine (presynaptic a_2-adrenoblocker stimulating epinephrine release from adrenergic hypothalamic terminals) or muscimol ($GABA_A$-receptor agonist) into VMHT increases the aversive threshold of its electrical stimulation [3,11] or prolongs zoosocial contacts [5,14], which attests to their anxiolytic effect.

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