NEUROCHEMICAL ANALYSIS OF THE MECHANISM OF ACTION OF SEROTONINERGIC DRUGS ON AVOIDANCE BEHAVIOR IN A SITUATION OF ACUTE STRESS

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The authors showed previously [3] that some serotoninergic drugs have opposite actions on avoidance behavior in a situation of acute emotional stress. Since the known serotoninomimetic and antiserotonin drugs do not act monomodally purely on serotoninergic mediation, and since complex relations exist between serotoninergic and catecholaminergic systems, further analysis of the previously established facts is necessary, and this communication is devoted to that purpose.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g, kept in cages (six animals to a cage) with free access to food and water were used. Avoidance behavior was assessed by the method described previously [2]. In all experiments the analyzer substance was injected 15 min after the drug. Testing was carried out 45 min after injection of the drug. All substances were injected intraperitoneally in aqueous solutions. The following substances were used: 5-hydroxytryptophan (5-HTP) from Serva, West Germany; zimelidine provided by Professor Ross of Astra Pharmaceuticals, Sweden; pirenperone provided by Professor Janssen of Janssen Pharmaceutica, Belgium; cyproheptadine from Serva; quipazine (All-Union Pharmaceutical Research Institute), provided by Professor N. N. Suvorov; clofelin (clonidine) from the All-Union Pharmaceutical Chemical Research Institute; haloperidol (NSD-1015) obtained from E. A. Kuznetsova, Research Institute of Pharmacology; and m-chlorophenylpiperazine (CPP) provided by Professor Maj, Krakow, Poland. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Data on the time course of the effect of the serotoninergic drugs (5-HTP, quipazine, zimelidine, CPP) on avoidance behavior under the influence of a number of neurochemical analyzers are given in Table 1 and Fig. 1.

5-HTP, a precursor in synthesis of serotonin (5-HT), optimizes parameters reflecting the goal-directedness of avoidance behavior in an acute stress situation: It shortened the latent period of avoidance and lowered the level of affective reactions and reduced the number of mistaken attempts at avoidance. Neurochemical investigations have shown that injection of the precursor causes a marked increase in the level of 5-HT (on account of activation of synthesis) and its release after only 30 min [14]. There is electrophysiological evidence that serotoninomimetic drugs decrease the firing rate of serotonin-containing neurons either because of an increase in the mediator content in them or activation of serotonin autoreceptors on neuron bodies of the nuclei raphe [5]. To determine whether the optimizing effect of 5-HTP on avoidance behavior is due to increased liberation of 5-HT from the terminals (increased synthesis) and to the action of the mediator on postsynaptic 5-HT-1-receptors or whether the cause of the behavioral effect is inhibition of tonic activity of serotonin-containing neurons of the nuclei raphe (activation of 5-HT-2-autoreceptors), NSD-1015, an inhibitor of central and peripheral decarboxylases of L-aromatic amino acids, and pirenperone and cyproheptadine, blockers of 5-HT-1- and 5-HT-2-receptors were used.

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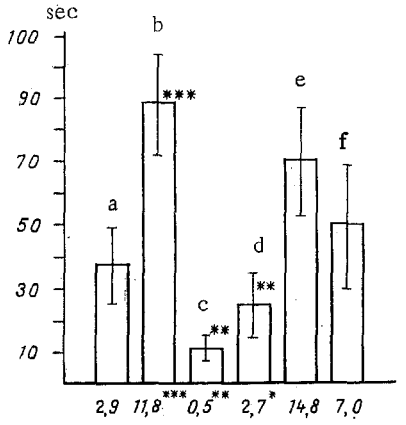

Fig. 1. Effect of combined use of quipazine and analyzer substances on avoidance behavior in acute stress situation. Columns indicate latent period of avoidance; numbers indicate number of unsuccessful attempts at avoidance: a) control testing 2.5 h before injection of substances; b) quipazine (7 mg/kg); c) quipazine + pirenperone (0.02 mg/kg); d) quipazine + cyproheptadine (0.5 mg/kg); e) quipazine + clonidine (0.05 mg/kg); f) quipazine + haloperidol (0.1 mg/kg). *P < 0.05, **P < 0.01 for effect of quipazine; ***P < 0.01 compared with control. In each experiment 7-12 animals were used. Ordinate, time (in sec).

TABLE 1. Effect of Combined Administration of Drugs on Avoidance Behavior in Acute Stress Situations (M ± m)

Time of testing	Substances injected	Latent period of avoidance, sec	Number of unsuccessful attempts at avoidance
Control		39,0±9,0	4,9±2,5
After 45 min	5-HTP (10 mg/kg)	15,5±3,3**	1,7±1,7
	5-HTP + pirenperone	11,0±2,1	0,0*
	5-HTP + cyproheptadine	12,8±3,7	4,2±1,9
	5-HTP + NSD-1015	41,8±17,0	11,8±7,7
Control		36,8±10,2	4,5±1,9
After 45 min	CPP (5 mg/kg)	62,8±6,5**	2,3±0,7
	CPP + pirenperone	18,2±3,8*	1,2±0,6
	CPP + cyproheptadine	93,0±32,4	10,8±6,4
Control		38,0±8,3	3,5±2,0
After 45 min	Zimelidine (15 mg/kg)	46,7±11,9	10,6±4,8
	Zimelidine + clonidine	80,7±10,6*	26,7±4,1*

Legend. *P < 0.05 compared with effect of substance itself; **P < 0.05 compared with control. In each experiment 7-12 animals were used. Doses of analyzer substances the same as in Fig. 1. NSD-1015 was given in a dose of 50 mg/kg.

As Table 1 shows, NSD-1015 reversed the optimizing effect of 5-HTP on behavior. After administration of the inhibitor of 5-HT synthesis, behavior in a situation of emotional stress was disturbed by an even greater degree than in the control. The selective blocker of 5-HT-2-receptors, pirenperone [7] not only did not weaken the effect of 5-HTP, but actually potentiated its positive action on behavior (a tendency for the latent period of avoidance to decrease further and complete suppression of incorrect responses). Cyproheptadine, which blocks both 5-HT-1- and 5-HT-2-receptors, abolished the modulating action of 5-HTP on behavior developing in a stress situation: The number of unsuccessful attempts at avoidance increased considerably. It can be concluded from a comparison of these facts that the optimizing action of small doses of 5-HTP (10 mg/kg) on avoidance behavior in a stress situation is due to intensification of synthesis of 5-HT and its release from terminals, and activation of postsynaptic 5-HT-1-receptors. Pirenperone potentiates the effect of 5-HTP evidently by inhibiting the mechanism of negative feedback in the system of serotonin-containing neurons (blockade of 5-HT-2-autoreceptors). This conclusion is in agreement with data in the literature [5] showing that 5-HTP, in small doses (10-40 mg/kg), without the peripheral decarboxylase blocker, does not change the firing pattern of serotonin-containing neurons of the nuclei raphe. Inhibition of activity of serotonin-containing neurons was observed after injection of large doses of 5-HTP (100-500 mg/kg).

The effect of quipazine, an activator of postsynaptic serotonin receptors [10] was directly opposite in its action on avoidance behavior in a stress situation to that of 5-HTP (Fig. 1). Quipazine increased the latent period of avoidance and the number of incorrect responses, and also raised the level of affective manifestations in the animal. However, it had not only a serotoninomimetic, but also a dopaminomimetic effect, and also blocked presynaptic inhibitory α-adrenoreceptors, leading to increased release of noradrenalin [6, 13]. Preliminary injection of cyproheptadine (15 min before quipazine) abolished behavioral hyperactivity and contributed to the realization of avoidance behavior. Pirenperone had a similar effect. This was evidently due to blocking not only of serotonin, but also of dopamine receptors, for cyproheptadine and, in particular, pirenperone have sufficiently high affinity for dopamine receptors: The dissociation constant (K_d) is 31 and 16 nM, respectively [8]. Haloperidol, which blocks dopamine receptors, did not, however, exhibit such a definite action. Clonidine, which stimulates presynaptic α_2-adrenoreceptors, had no positive effect.

On the whole it can be concluded from these results that the negative action of quipazine on behavior of the rats in a situation of emotional stress was due more to activation of serotonin 5-HT-2-receptors, which leads to inhibition of tonic activity of neurons of the nuclei raphe, for both cyproheptadine and pirenperone acted in the same direction (unlike their action on the effect of 5-HTP). The dopaminomimetic effect of quipazine made a definite contribution toward behavioral hyperreactivity.

CPP, an active metabolite of trazodone, activates postsynaptic 5-HT-receptors and blocks reuptake of the mediator [8]. Under conditions of acute stress CPP has a sedative action and lengthens the latent period of the avoidance reaction sharply. Under these circumstances, the number of incorrect reactions did not increase, but had a tendency to decrease (Table 1). Preliminary injection of pirenperone caused a considerable degree of abolition of the inhibitory effect of CPP. Cyproheptadine had no such effect. On the contrary, it potentiated the inhibitory action of CPP on the rate of the avoidance reaction. The number of incorrect reactions also increased in this case.

These results do not support the various opinions which have been expressed regarding the common or similar mechanisms of action of CPP and quipazine [4, 9], since cyproheptadine abolishes the behavioral effects of quipazine — both serotoninomimetic and dopaminomimetic [11]. It has been shown that cyproheptadine blocks the behavioral effects of CPP such as the serotonin syndrome in rats, connected with activation of postsynaptic 5-HT-receptors. However, it does not prevent accumulation of 5-HTP (on administration of the precursor) [11], which points to depression of 5-HT synthesis in the terminals. Blocking of 5-HT-2-autoreceptors evidently also lies at the basis of the mechanism of the antagonistic action of pirenperone on the inhibitory effect of CPP in relation to avoidance behavior.

According to previous observations [3], zimelidine, a selective inhibitor of 5-HT reassimilation, in a small dose (2.5 mg/kg), like 5-HTP, had an optimizing effect on avoidance behavior in a stress situation. In a large dose (15 mg/kg), however, it disturbed avoidance behavior. Since zimelidine in such doses blocks uptake not only of 5-HT, but also of noradrenalin [12], clonidine was used for analysis. In a dose of 0.05 mg/kg, clonidine activates presynaptic α_2-adrenoreceptors, which inhibits release of noradrenalin and, consequently, weakens its postsynaptic effects. However, preliminary injection of clonidine (15 min before zimelidine) caused a considerable increase in the avoidance behavior deficit (Table 1). The adrenergic component is evidently not determinant in the effect of a large dose of zimelidine. Clonidine itself (without premedication) likewise did not improve the parameters of avoidance behavior in a stress situation [1]. When administered systemically (but not by iontophoresis) clonidine has been shown to inhibit discharges of serotonin-containing neurons of the nuclei raphe [15].

This analysis showed that the optimizing effect on avoidance behavior in a stress situation develops in response to factors stimulating synthesis and release of 5-HT and activation of postsynaptic 5-HT-1-receptors. Activation of 5-HT-2-receptors ought not to take place under these circumstances, for this would lead to inhibition of tonic firing of serotonin-containing neurons of the nuclei raphe and to limitation of mediator release from the terminals. From this point of view the use of selective blockers of 5-HT-2-receptors of the pirenperone type, activating the serotoninergic system through depression of inhibitory autoreceptors, is promising.

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EFFECT OF ACUTE ALCOHOLIC INTOXICATION ON ANTIGENIC COMPOSITION OF SOLUBLE RAT-BRAIN PROTEINS

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Animals preferring ethanol solution or water when allowed free choice differ in many parameters characterizing the state of their tissue metabolism [4, 7]. Preferential consumption of ethanol by animals, it can be tentatively suggested, is connected with the specific character of their metabolism, including their brain tissue metabolism, whose specificity is largely determined by protein composition. Investigation of the brain protein composition of animals consuming water or ethanol solution when allowed free choice is therefore an important aspect of the study of mechanisms of ethanol preference.

Data in the literature invariably indicate depression of protein synthesis in the brain in acute alcoholic intoxication [6]. However, the effect of a single dose of ethanol on brain protein composition has not been studied.

The aim of this investigation was to study the antigenic composition of soluble brain proteins of rats preferring water or ethanol solution. At the same time, the effect of acute alcohol intoxication was studied on the brain protein composition of rats similar in character of preference (intermediate group).

EXPERIMENTAL METHOD

Male rats weighing 250-300 g were used. The animals were divided into three groups with respect to voluntary consumption of water or 15% ethanol solution by preference. The separation was effected in 10 days. Animals preferring water, those preferring ethanol, and rats of the intermediate group consumed not more than 8%, not less than 50%, and 16-35%, respectively of ethanol solution relative to the total fluid intake. Rats of the intermediate

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