

Mechanisms of Amnestic Effect of Ergot Alkaloid Agroclavin

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Neurochemical mechanisms of the effect of agroclavin on spatial memory in the Morris water maze in mice were studied by analyzing the effect of neurotransmitter receptor ligands on the amnestic effect of this alkaloid. D₁-receptor agonist SKF-38393 and calcium channel blockers verapamil and nimodipine abolished the negative effect of agroclavin on spatial memory. The role of intracellular calcium in the mechanisms of amnestic effect of agroclavin is discussed.

Key Words: *agroclavin; spatial memory; receptor ligands; neurotransmitters*

Agroclavin belongs to the family of ergot alkaloids. Activity of this compound and mechanisms of its effects on the behavior and higher nervous activity are poorly studied. It is known that agroclavin is a dopamine receptor ligand. Treatment with this preparation causes stereotyped behavior [8]. This alkaloid modulates activity of the serotonin- and noradrenergic systems of the brain acting as antagonist and partially agonist of 2A-type serotonin receptors (5-HT_{2A} receptors) and as α_1 -adrenoceptor antagonist [7].

Agroclavin specifically modifies spatial memory in mice by impairing reproduction of conditioned navigation reflex in the Morris water test [1]. Neurotransmitters play a special role in synaptic processes underlying the formation of memory traces. On the other hand, neurotransmitter synthesis, neuronal activity, receptor sensitivity, and memory processes in general are calcium-dependent functions [5].

We studied neurochemical mechanisms of the effect of agroclavin on memory. To this end we analyzed the effect of ligands of different neurotransmitter receptors on mnemotropic activity of this alkaloid.

MATERIALS AND METHODS

Experiments were carried out on 190 random-bred male mice (20-25 g).

The animals were kept in cages (10 animals per cage) under conditions of natural day/night regimen with free access to water and food.

Morris water maze [6] in our modification [1] was used. The effects of 14 drugs affecting the neurotransmitter systems and calcium channels on the amnestic effect of agroclavin were studied: β_1 -adrenoreceptor blocker atenolol (1 mg/kg), D₁ and D₂ receptor antagonist haloperidol (1 mg/kg), D₁ receptor antagonist R(+)-SCH-23390 (0.7 mg/kg), D₁ receptor agonist SKF-38393 (3 mg/kg), D₂ receptor agonist quinpirole (0.5 mg/kg), peripheral D receptor blocker domperidone (0.3 mg/kg) (all from RBI); GABA_A receptor blocker bicuculline (1.5 mg/kg), 5-HT/5-HT_{1c} receptor antagonist ketanserin (3 mg/kg) (Sigma); nonspecific 5-HT receptor antagonist mianserin (1 mg/kg), serotonin reuptake blocker trazodone (2 mg/kg) (RBI); 5-HT_{1c} and 5-HT₂ receptor antagonist cyproheptadine (5 mg/kg, Serva); μ -receptor blocker naloxone (2 mg/kg), and calcium channel blockers verapamil (5 mg/kg) and nimodipine (10 mg/kg) (Sigma). Agroclavin was synthesized microbiologically using *Cla-viceps* sp. BKM F-2609 strain [3].

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These compounds were injected 60 min and agroclavin (10 µg/kg) 40 min before memory testing in experiments with combined treatment; for studies of the isolated effects of agroclavin and drugs that modulated its amnestic effect, they were injected in the same doses 60 min before memory testing. All compounds were injected intraperitoneally (0.1 ml). Controls were injected with the same volume of normal saline.

The results were statistically processed using factorial analysis and Student's *t* test.

RESULTS

Amnestic effect of agroclavin did not manifest after injections of D₁ receptor agonist SKF-38393 and calcium channel blockers nimodipine and verapamil (Table 1).

Agroclavin completely disturbed maze test performance in trained mice. Injections of SKF-38393, nimodipine, or verapamil 20 min before agroclavin abolished the amnestic effect of this alkaloid (Table 1). D receptor ligands haloperidol and quinpirole produced less pronounced effects. SKF-38393 did not improve test performance. A slight deterioration of detection of the training sector observed after verapamil and nimodipine injection can be explained by their side effects (inhibition of total motor activity).

Hence, only D receptor ligands and calcium channel blockers verapamil and nimodipine modulated the amnestic effect of agroclavin. It can be hypothesized that the dopaminergic system and calcium channels play an important role in the mediation of the mnemotropic effect of agroclavin. Other authors also reported the presence of a dopamine-stimulating component in the spectrum of agroclavin activities [9]. The effect of D₁ receptor agonist SKF-38393 can be explained by its higher affinity for these receptors compared to agroclavin. Agroclavin injected 20 min after SKF-38393 cannot replace it from receptors and exhibited no negative effect on spatial memory. In addition, the sensitivity of D₁ receptors can decrease after injection of agonist in high doses or after blockade of these receptors with antagonists.

The mechanism underlying the effect of calcium channel blockers seems to be more intricate. D₁ receptors belong to the metabotropic receptor family. They change their conformation after binding of neurotransmitter or agonist (in our case agroclavin), which stimulates cAMP production [2]. The increase in cAMP content under the effect of agroclavin was confirmed experimentally [4]. Accumulation of cAMP stimulates Ca²⁺ entry into the cell. The increase in intracellular Ca²⁺ concentration due to opening of calcium channels promotes activation of Ca-calmodulin-dependent phosphodiesterase and the concentration of cAMP de-

TABLE 1. Effects of Combined Treatment with Agroclavin and Test Drugs on Spatial Memory of Mice Tested in Morris Water Maze (Time Spent in the Sectors, sec; *M*±*m*)

Group	Factor F; <i>p</i>	Sector			
		1	2	3	4
Control	103.18; 0.000...	12.34±0.73*	10.19±0.66*	27.78±1.09	9.97±0.81*
Agroclavin	1.669; 0.179	15.65±0.56	13.50±0.77	16.10±1.08	14.80±1.03
+SKF-38393	16.542; 0.000...	11.33±1.51***	11.26±1.26**	25.00±2.26	12.06±1.58*
+nimodipine	10.114; 0.000...	12.80±2.43***	7.60±1.12***	26.80±2.95	13.00±3.28***
+verapamil	19.074; 0.000...	16.42±1.55**	8.42±1.52**	26.42±2.51	10.14±1.68**
+atenolol	1.563; 0.229	18.00±2.40	12.16±2.18	13.16±1.40	16.66±2.69
+naloxone	1.908; 0.16	15.33±4.16	9.83±1.55***	19.50±3.02	15.33±2.02
+ketanserin	2.510; 0.073	19.00±2.13	13.70±1.82	13.90±1.15	12.90±1.60
+domperidone	2.744; 0.089	20.00±1.35	13.00±1.35	17.00±1.08	15.00±2.85
+bicuculline	3.806; 0.026	15.66±2.04	11.66±1.89	12.83±0.87	19.83±2.31***
+haloperidol	6.839; 0.002	15.85±1.47	9.14±1.48***	21.42±2.70	13.71±1.87
+SCH-23390	0.354; 0.79	15.50±1.18	14.08±1.68	16.00±0.97	13.67±2.53
+quinpirole	4.55; 0.017	14.20±1.39***	12.00±1.38***	20.00±0.95	13.80±2.42
+mianserin	0.74; 0.54	13.40±3.88	11.40±3.80	18.18±3.50	17.20±4.90
+cyproheptadine	1.55; 0.24	15.40±3.99	17.80±4.42	19.60±5.40	9.20±4.20
+trazodone	7.655; 0.0016	15.83±1.70	8.66±1.47***	17.83±1.85	17.66±1.11

Note. **p*<0.01, ***p*<0.01, ****p*<0.05 compared to sector 3 (training).

creases [5]. Hence, maintenance of a dynamic equilibrium between these two systems of secondary messengers is essential for normal functioning of the neuron. We assume that agroclavin disturbs this balance, which results in accumulation of intracellular calcium level and modification of many functions of the neuron. That is why both decreased sensitivity of D₁ receptors and blockade of calcium channels abolished the negative effects of agroclavin on memory. The protective effects of D receptor ligands and calcium channel blockers preventing the amnestic effect of agroclavin can be due to these phenomena.

Hence, the mechanism of the effect of agroclavin on spatial memory in mice is mediated by interactions of this alkaloid with D receptors and alteration of calcium channel permeability.

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