large enough to create conditions for massive activation of opiate-sensitive neurons in the zone of infusion. Despite this fact, selective analgesic (PGM, dorsomedial hypothalamus) and secondary reinforcing (VTR, nucleus accumbens—weaker) responses were obtained from different structures, evidence of the neuroanatomical heterogeneity of the trigger zones for these effects of morphine.

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CLONIDINE-INDUCED AGGRESSION IN MICE: ROLE OF GENOTYPE AND DOMAPMINERGIC SYSTEM

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Administration of the α-adrenoreceptor stimulator clinidine in large doses to mice leads to the appearance of characteristic manifestations of intraspecific aggressiveness (attacking, biting, assuming the side position). Investigation of the neurochemical mechanisms of clonidine-induced aggression has shown that it is manifested as a result of stimulation of postsynaptic α-adrenoreceptors [8, 9]. Meanwhile, it has been shown that certain neuroleptics and, in particular, spiperone, inhibit clonidine-induced aggressiveness in mice [8]. This suggests that the dopaminergic system is involved in the effects of clonidine. However, no investigations of this aspect of the action of clonidine have been undertaken.

It is not yet clear how characteristic of clonidine is that induced aggressiveness, for this phenomenon has been discovered virtually in only one strain of mice. There are good reasons to investigate this aspect of the problem because marked dependence of the reaction to amphetamine [13], a dopaminergic apomorphine agonist [2, 6] on genotype has been observed.

The aim of this investigation was to study genotypic differences in the role of the dopaminergic system in aggressiveness induced in mice of various strains by the Soviet preparation clofelin, which is identical to clonidine.

EXPERIMENTAL METHOD

Experiments were carried out on male inbred BALB/c, C57BL/6, CBA/Lac, DD, A/He, C3H, CC57BR, and DBA/1 mice aged 3-4 months and weighing 22-35 g. The mice were kept under standard animal house conditions, eight to a cage.

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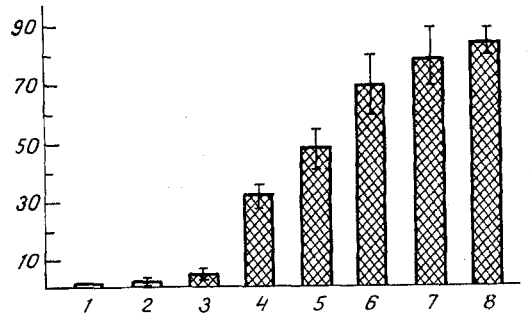

Fig. 1. Aggressive behavior in mice of different strains after injection of clonidine (10 mg/kg). Abscissa, lines of mice: 1) DD; 2) A/He; 3) C3H; 4) CBA; 5) C57BL/6j; 6) CC57BR; 7) DBA/1; 8) BALB/c; ordinate, number of attacks per test, M ± m.

Testing for aggressive behavior was carried out in glass cylinders, in which four mice of equal weight were placed immediately after receiving the drug, by recording the number of aggressive contacts in the course of 1 h.

To induce aggressive behavior, the mice were given an intraperitoneal injection of clofelin (identical to clonidine) in a dose of 10 mg/kg, but in some experiments the doses were increased to 25 and 50 mg/kg. Apomorphine ("Sigma," USA) was injected subcutaneously in doses of 1.0 and 2.5 mg/kg; flupenthixol ("Lundbeck," Denmark) and haloperidol ("Reanal," Hungary) in a dose of 0.1 mg/kg intraperitoneally; bromocriptine ("Sigma") in a dose of 10 mg/kg, intraperitoneally. Apomorphine was injected simultaneously with clonidine, flupenthixol and haloperidol 15 min before clonidine. Since the peak effect of bromocriptine is observed after 3 h [4], bromocriptine was injected 2.5 h before clonidine. All preparations were dissolved in distilled water and were freshly prepared. Injection of physiological saline served as the control for injection of clonidine.

The results were subjected to statistical analysis by Student's and Fisher's tests and by dispersion analysis.

EXPERIMENTAL RESULTS

After a single injection of clonidine the animals in most cases developed aggressive behavior. Meanwhile injection of physiological saline did not induce aggressive behavior in any of the different strains of mice. The mean duration of aggression after injection of clonidine was 40 ± 5.8 attacks per test — similar to the intensity of aggression observed in Swiss mice [7, 8] after receiving the same dose of clonidine. However, mice of different strains differed sharply in the intensity of clonidine aggression: mice of the most aggressive lines BALB/c, DBA/1, and CC57BR attacked throughout the period of testing, whereas virtually no aggressive contacts were observed betweeen DD and A/He mice (Fig. 1). Dispersion analysis revealed a highly significant influence of genotype on the manifestation of clonidine-induced aggressiveness: $F(7.40) = 29.1$ ($p < 0.001$). Differences between the lines for aggressiveness were significantly greater than intralinear variations.

In mice of the resistant strain A/He an increase in the dose of clonidine to 25 and 50 mg/kg significantly increased aggressiveness, but the intensity of aggression was low (Table 1). In the other nonaggressive line DD an effect was obtained only from a dose of 50 mg/kg, but it was 5 times weaker than that induced by clonidine in a dose of 50 mg/kg, but it was 5 times weaker than that induced by clonidine in a dose of 10 mg/kg in lines such as BALB/c and DBA/1. This indicates marked differences in the sensitivity of mice of different genotypes to clonidine. Incidentally, in an earlier study of two lines of mice, whose locomotor activity was recorded, differences also were observed in the sensitivity of the mice to clonidine [3]. Since clonidine, in the doses used, acts primarily on α_1-adrenoreceptors, there is no doubt about the fact that α_1-adrenoreceptors differ in their sensitivity. A difference in the state of the catecholaminergic system of the brain, estimated as tyrosine hydroxylase activity, have been observed in investigations on small numbers of lines [1, 12]. They showed that tyrosine hydroxylase activity of CBA mice is significantly lower than that of BALC/c mice. BALB/c mice, however, have a higher level of tyrosine hydroxylase activity than DBA/2 and C56BL/6 mice.

TABLE 1. Aggressiveness of DD and A/He Injection of Different Doses of Clonidine

Lines of mice	Dose of clonidine, mg/kg	Number of experiments	Number of attacks per test, M ± m
DD	10	6	0
	25	6	0
	50	5	14,0±5,5
A/He	10	6	2,0±0,6
	25	6	13,5±3,7
	50	5	8,8±1,9

TABLE 2. Effect of Dopaminergic Drugs on Manifestation of Clonidine-Induced Aggressiveness in Mice of Different Lines

Line of mice	Drugs given	Dose, mg/kg	Number of experiments	Number of attacks per test, M ± m
CC57BR	Clonidine alone	10	6	70,2±6,0
	Flupenthixol + clonidine	0,1		
		10	5	22,0±4,5**
	Apomorphine + clonidine	0,1		
		10	6	0
C57BL/6	Clonidine alone	10	6	48,3±3,4
	Apomorphine + clonidine	1,0		
		10	6	82,3±10,4*
DD	Clonidine alone	10	6	0
	Bromocriptine + clonidine	10		
		10	6	0
	Apomorphine + clonidine	1,0		
		10	6	20,3±3,2**
	Apomorphine + clonidine	2,5		
		10	6	38,1±4,4**

Legend. *p < 0.05, **p < 0.01 compared with clonidine alone.

To study the role of the dopaminergic system, mice with high (CC57BR), average (C57BL/6), and low (DD) intensities of clonidine-induced aggressiveness were used (Table 2). The dopamine antagonists flupentixol and haloperidol either clearly depressed or completely prevented the appearance of aggressive behavior in CC57BR mice. Injection of clonidine (10 mg/kg) together with clonidine induced aggression of such a degree that it did not appear after injection of a very high dose of clonidine. Meanwhile bromocriptine, which stimulated D2 receptors, did not enhance the manifestations of clonidine aggressiveness.

The results indicate involvement of the dopaminergic system in the mechanisms of clonidine-induced aggressiveness; however, the role of the different types of dopamine receptors in this phenomenon is not equivalent. Stimulation of dopamine receptors by apomorphine, which is a mixed agonist of D1 and D2 receptors [11], strengthened clonidine aggressiveness. Bromocriptine [5], a specific agonist of D2 receptors, did not affect aggressiveness, and stimulation of D2 receptors evidently does not contribute to the manifestation of clonidine aggressiveness. Dopamine receptors blockade by antagonists inhibited clonidine aggressiveness and the degree of the effect depended on the spectrum of action of the drug. Flupenthixol, a specific D1-receptor antagonist, significantly inhibited, whereas the broad-spectrum neuroleptic haloperidol blocked aggressiveness of mice of this highly aggressive line. It can be postulated that D1 receptors, linked with adenylate cyclase, mainly participates in the action of clonidine, although involvement of other types of dopamine receptors cannot be ruled out. There is no doubt that clonidine, if used in large doses, acts on the dopaminergic system. In an investigation of rats clonidine increased aggressiveness induced by apomorphine [10].

Aggressiveness induced in mice by clonidine thus depends on the animal's genotype and is realized with the participation of the dopaminergic system, which exerts its effect predominantly through D1 receptors.

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EFFECT OF METRAZOL ON TIME COURSE OF MEMBRANE-BOUND CALCIUM LEVEL IN THE CEREBRAL CORTEX

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In the modern view calcium ions play an important role in the mechanisms of hyperexcitability and injury of brain neurons under the influence of convulsants [1, 7-9]. An increase in the inflow of extracellular Ca^{++} into cerebral cortical nerve cells has been found under the influence of several convulsants. Changes in intracellular Ca^{++} exchange arising under these circumstances evidently exert some influence primarily on the disturbance of regulation of ionic permeability of the plasma membrane, and activity of enzymes involved in neurotransmitter synthesis and secretion, and energy production.

Ca^{++} ions are known [2, 5, 6] to act as regulators of intracellular processes, mainly on binding with components of neuron membranes. It can be postulated that a disturbance of this process may play an essential role in the pathogenesis of convulsive states. However, no information is yet available on this problem.

The aim of this investigation was to study the time course of the membrane-bound calcium (Ca^{++}_{mb}) in structures of the cerebral cortex under the influence of metrazol, a typical convulsant.

EXPERIMENTAL METHOD

Structural elements (bodies of neurons and the surrounding neuropil) from layers II and III of a living preparation of the motor cortex taken from cats anesthetized with pentobarbital (30 mg/kg), with artificial ventilation of the lungs, served as the test object.

Intravital spectrophotometry of microregions of the cerebral cortex (diameter 60 μ), stained with chlorotetracycline (50 μM) was carried out with the LYUMAM KF contact microscope. The time course of the Ca^{++}_{mb} concentration was estimated as a change in the intensity of fluorescence of chlortetracycline–Ca^{++}–membrane complex (CTC-Ca^{++}-MC) [4, 6] and expressed as a percentage of the initial level.

Metrazol was applied to the microregions for microscopy by means of an iontophoretic current (70 nA) through one barrel of a multibarreled micropipet for various periods of time (5-120 sec). Electrical activity of the neuron was recorded extracellularly through another channel. The techniques for combined morphological and physiological study of structures of the cortical preparation with microiontophoretic application of biologically

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