ROLE OF CHOLINERGIC SYSTEMS IN FORMATION OF THE DISSOCIATED STATE TO CLEREGIL

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Dimethylaminoethanol N-acetyl-L-glutamic acid (CR 121, cleregil, deanol aceglumas, demanol aceglumate, otrun, risatarun) has been used successfully in medical practice in the West as a psychostimulant and psychoharmonizing agent for the treatment of asthenic states, depressions, and memory disturbances. It has been suggested that this substance plays the role of transmitter of excitation in synapses through transformation into choline and, by subsequent acetylation, into acetylcholine [7, 12].

Considering the mechanism of action of cleregil, its ability to pass through the blood-brain barrier, and also modern views on the nature of onset of the dissociated state, which is determined mainly by functioning of the cholinergic system [1], it was considered worthwhile to study the possibility of onset of dissociated behavior in animals during long-term exposure to cleregil and to investigate the mechanisms of this phenomenon.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 250-350 g. A dissociated state (reversible amnesia) was produced on the basis of the idea that it is a state dissociated from normal, in which a skill or conditioned reflex is manifested only after administration of the drug during administration of which the skill or reflex was formed [1, 2, 10, 11]. The investigation was conducted in a T maze, in which, after the habituation procedure, a conditioned reflex was formed in the rats: visiting a feeding bowl in the right compartment of the maze after the conditioned stimulus, which was a click of the door to be opened. Every day the rats visited the feeding bowl 10 times. The reflex was considered to be formed when the rat visited the bowl in under 5 sec [2]. To form a dissociated state, the animal received an injection of cleregil (150 mg/kg intraperitoneally) daily 40 min before being placed in the starting compartment of the maze, and the conditioned reflex of visiting the feeding bowl was formed again. To determine the formation of the dissociated state the time of visiting the bowl during administration of the drug and under normal conditions (24 h after its withholding) was recorded.

EXPERIMENTAL RESULTS

The time course of formation of the dissociated state during administration of cleregil consisted of several stages. During the first 4 days of the investigation conditioned reflex formation was impaired by cleregil compared with in the control rats. However, by the 19th day of administration of the drug, a lasting conditioned reflex of visiting the feeding bowl was formed in the rats, and the reflex was absent, moreover, when the drug was withheld. Until the 20th day of administration of the drug the behavior of the rats in the maze was unchanged and the time of the reflex was under 5 sec, evidence of the formation of a stable dissociated state. Starting with the 5th week of administration the animals' behavior showed a significant change: The rats' ability to pass through the maze was substantially impaired, they squeaked, behaved restlessly, became more aggressive, yet in spite of this the time taken to visit the feeding bowl during administration of cleregil still remained shorter than after

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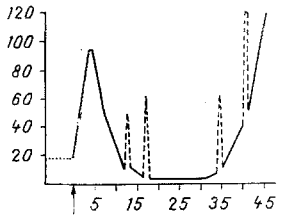

Fig. 1

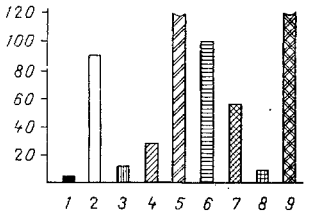

Fig. 2

Fig. 1. Time course of formation of dissociated state during administration of cleregil in a dose of 150 mg/kg. Abscissa, days of giving drug; ordinate, mean time of reflex (in sec). Dotted line — time of reflex before administration of cleregil, continuous line — time of reflex during daily administration of cleregil, broken line — time of reflex without cleregil (dissociation). Arrow marks beginning of daily injection of cleregil.

Fig. 2. Effect of various substances on dissociation induced by cleregil. 1) Cleregil (150 mg/kg); 2) withholding of cleregil; 3) benactyzine (10 mg/kg); 4) atropine (25 mg/kg); 5) arecoline (5 mg/kg); 6) β-ethyldifacil (10 mg/kg); 7) α-methyldopa (200 mg/kg); 8) pyracetam (300 mg/kg); 9) depakine (200 mg/kg). Ordinate, mean time of reflex (in sec).

withdrawal of the drug. By the 6th week of administration of the drug the rats developed spontaneous aggressiveness, they remained in the starting compartment, and the time of the reflex became equal with and without the drug (Figs. 1 and 2).

Because of the appearance of these changes in the animals' behavior, it was necessary in the course of the experiment to determine the pain threshold and to estimate the aggressiveness of the rats, for we know that during long-term administration of drugs causing acetylcholine cumulation (physostigmine, oxotremorine, arecoline, etc.), changes arise in the emotional state of animals, with a response of fear or aggression [3, 9]. After a single injection of cleregil the parameters of aggressiveness and the pain threshold were indistinguishable from the control: The pain threshold was 0.29 ± 0.01 mA, and aggressiveness appeared in response to a current of 3.0 ± 0.1 mA. However, after administration of cleregil for 18 days a marked decrease was observed in the threshold parameters: pain to 0.24 ± 0.1 mA and aggressiveness to 0.29 ± 0.2 mA; spontaneous aggressiveness was observed after 42 days in all the animals.

To discover the role of the cholinergic system in the formation of the dissociated state to cleregil, a substitution test [10] was used. Muscarinic cholinolytics — atropine (25 mg/kg, intraperitoneally) and benactyzine (10 mg/kg, intraperitoneally), the muscarinic cholinomimetic arecoline (5 mg/kg, subcutaneously), and the nicotinic cholinolytic β-ethyldifacil (10 mg/kg, intraperitoneally) [4] were injected into animals dissociated to cleregil. In addition, as substitute drugs, the GABA-positive agent depakine (200 mg/kg, intraperitoneally), the psychotropic drug pyracetam (300 mg/kg, intraperitoneally), and the dopa-decarboxylase inhibitor α-methyldopa (200 mg/kg, intraperitoneally) were injected. Atropine and, in particular, benactyzine were found to be able to replace cleregil and to restore the conditioned reflex disturbed by cleregil withdrawal, and also to abolish the signs of aggressiveness, fear, and restlessness due to prolonged administration of cleregil. Ability to restore the dissociated state to cleregil also was possessed by α-methyldopa and pyracetam. By contrast, arecoline potentiated the restlessness of the animals, lowered the threshold of aggressiveness, and increased the time of the reflex. β-Ethyldifacil and depakine did not change the dissociated state to cleregil.

These investigations thus showed that during conditioned reflex formation against the background of cleregil a dissociated state arises, in which the animals complete their visit only after administration of the drug. We know that if acetylcholine concentration in synapses rises to an optimal level, the rate of reflex formation increases, and if the optimal acetylcholine level is exceeded, this leads to the appearance of synaptic blockade, which significantly impairs the formation of the conditioned reflex [5, 6]. Considering the mechanims of action of cleregil it can be tentatively suggested that stabilization of the dissociated state is connected with achievement of the optimal acetylcholine level, whereas disturbance of conditioned-reflex activity, increased aggressiveness of the rats, and their rest-

less behavior can be regarded as the result of overproduction of acetylcholine and its cumulation at synaptic level, with the development of synaptic blockade. This hypothesis is confirmed by the fact that injection of drugs lowering the acetylcholine level (atropine, benactyzine) abolishes the signs of aggression, lowers the intensity of the emotional background, and restores the blocked conditioned reflex, whereas arecoline, which releases additional amounts of acetylcholine from the depots [8, 13], potentiates these undesirable effects. Since administration of β-ethyldifacil did not change the rats' behavior, it can be postulated that predominantly the muscarinic rather than the nicotinic cholinergic system is involved in the mechanism of action of cleregil. Since α-methyldopa and pyracetam can partially replace cleregil under the conditions of a stable dissociated state, it can be postulated that not only the cholinergic, but also other mediator systems are involved in the mechanism of development of dissociation.

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COMPARATIVE STUDY OF MUSCARINIC ACETYLCHOLINE RECEPTORS OF HUMAN AND RAT CORTICAL GLIAL CELLS

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For the overwhelming majority of subpopulations of acetylcholine receptors in the vertebrate brain the cellular and subcellular localization has not been established and, in particular, acetylcholine receptors of glial cells have virtually not been studied. However, there is much information on the high level of enzymes for acetylcholine synthesis and breakdown in glial cells. For instance, cholinesterase [7], acetylcholinesterase [1, 4, 9], and cholineacetyltransferase [10] have been found in glial cells. Although data on choline-acetyltransferase are contradictory [11], the presence of enzymes of acetylcholine metabolism in glial cells is indirect evidence of the presence of acetylcholine receptors in these cells

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