ROLE OF CENTRAL MUSCARINIC CHOLINERGIC SYSTEMS IN FOOD MOTOR CONDITIONING

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After complete blocking of the central muscarinic cholinergic systems by benactyzine in doses of 5 and 40 mg/kg, food motor conditioning was impossible in rats. In the case of incomplete blocking (benactyzine 1 mg/kg) or unblocking of these systems (5–5.5 h after administration of benactyzine in a dose of 40 mg/kg), conditioning was possible but slow. Galanthamine (2.2 mg/kg) weakened the effect of benactyzine on training. Blocking the central nicotinic cholinergic and adrenergic systems, and depressing the function of sertoninergic systems by administration of pediphen, haloperidol, and p-chlorophenylalanine has no significant effect on memory formation.

Investigations have shown [3, 4, 8–11, 13, 16, 18, 20] that food and defensive conditioning is impaired (retarded), but is possible, in mice, rats, cats and monkeys after administration of atropine, scopolamine, benactyzine, and other muscarinic cholinolytics. Buresova et al. [12] showed that defensive conditioning does not take place in rats if training is carried out at the height of manifestation of the cholinolytic reaction. However, Sugrue [19] has observed that defensive conditioning can be accelerated by the action of the cholinolytic dithran (4 mg/kg). The results regarding the role of muscarinic cholinergic systems of the brain in conditioning are thus conflicting. Determination of the role of muscarinic cholinergic systems of the brain during training has been based on experiments using cholinomimetics [8, 9, 17] and, in particular, with central muscarinic cholinolytics. Nevertheless, the latter, besides blocking muscarinic cholinergic receptors of the brain, can also interfere with serotonin [21, 22] and noradrenalin [6, 7, 15] metabolism.

The role of the various brain mediator systems in food motor conditioning in rats was investigated.

EXPERIMENTAL METHOD

Food motor conditioning was carried out in male albino rats kept in a narrow box 2 m long. On the day of training the animal was allowed to run 5 or 6 times in succession from its starting chamber into the final chamber (at the other end of the box) where it could obtain reinforcement in the shape of a piece of bread soaked in milk. The rats were deprived of food for 20 h before each experiment. To determine the role of the muscarinic cholinergic receptors of the brain in training, benactyzine was used. The animals of group 1 were trained after complete blocking of their central muscarinic cholinergic receptors by benactyzine hydrochloride in doses of 40 or 5 mg/kg body weight (in these doses the benactyzine completely blocks cholinergic receptors for more than 30 min). The animals of group 2 were trained after partial blocking of their cholinergic receptors (5–10 min after administration of benactyzine in a dose of 1 mg/kg, or 50–75 arecoline tremor units). The rats of group 3 were trained after blocking of their brain cholinergic receptors (5–5.5 h after administration of benactyzine in a dose of 40 mg/kg). The degree and duration of the blocking of the central muscarinic cholinergic receptors were determined in parallel experiments using the muscarinic cholinolytic drug arecoline, which, in a dose of 17 mg/kg, induces tremor (excitation of central) and salivation (excitation of peripheral cholinergic receptors) in intact rats [1]. In addition,

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TABLE 1. Effect of Various Compounds on Food Motor Conditioning in Rats

Drug and dose (in mg/kg	Number of rats trained	Number of days of training	P	Notes
a. Control	5 out of 6	11 ± 2.5	< 0.05	Not trained
Benactyzine 40	1 out of 6	21		
b. Control	7 out of 9	7 ± 2.2	< 0.05	
Benactyzine 5	1 out of 10	12		
Benactyzine 5 + Galanthamine 2.5	3 out of 9	7.3 ± 0.4	> 0.05	
c. Control	7 out of 9	7.7 ± 1.4		
Benactyzine 1	8 out of 10	9.0 ± 0.84	> 0.05	
Benactyzine 1 + Galanthamine 2.5	10 out of 10	5.8 ± 1	> 0.05	
d. Control	5 out of 6	10.4 ± 2.7		
Benactyzine 40	1 out of 6	15	< 0.05	
Benactyzine 40 + Galanthamine 5	0 out of 6	21	< 0.05	
e. Control	10 out of 10	9.5 ± 0.73		
Benactyzine 40	7 out of 10	23 ± 1.1	< 0.05	Trained after blocking
f. Control	9 out of 9	9 ± 2.2		
Pediphen 10	7 out of 9	16 ± 2	< 0.05	
Haloperidol 0.1	8 out of 9	15 ± 1.4	< 0.05	
g. Control	7 out of 10	5.6		
p-chlorophenylalanine 316	7 out of 10	5.6		

the effect of the anticholinesterase drug galanthamine, in doses of 2.5 and 5 mg/kg, given simultaneously with benactyzine (1.5 and 40 mg/kg), on conditioning was investigated. Finally, the possibility of conditioning after complete blocking of the central nicotinic cholinergic receptors was studied. Such a block was produced by pediphen [2] in a dose of 10 mg/kg. The role blocking the central adrenergic receptors in training in rats was demonstrated by experiments in which haloperidol was given in a dose of 0.1 mg/kg. The possibility of conditioning against the background of a reduced serotonin concentration in the rat's brain, produced by a single administration of 316 mg/kg p-chlorophenylalanine [14], also was investigated.

All the compounds tested (except p-chlorophenylalanine) were injected intraperitoneally, daily except on Sundays. Conditioning was regarded as successful if the rats completed three or more runs out of six attempts. The latent period did not exceed 30 sec and the duration of the run 60 sec.

EXPERIMENTAL RESULTS

The results showed (Table 1a, b, d) that after complete blocking of the muscarinic cholinergic systems of the brain, food conditioning did not take place (in some animals after administration of benactyzine in a dose of 5 mg/kg, sometimes individual conditioned-reflex responses appeared, but they were unstable: they were not observed next day or in the future). Conditioning was successful if the training took place after incomplete blocking of the muscarinic cholinergic receptors or after their unblocking (Table 1c, e). After simultaneous administration of galanthamine (2.5 mg/kg) and benactyzine in doses of 1 and 5 mg/kg, the inhibitory action of the cholinolytic on conditioning was weakened (Table 1b, c). Administration of galanthamine in a dose of 5 mg/kg, together with benactyzine in a dose of 40 mg/kg, was ineffective despite a long period of training (Table 1d).

After complete blocking of the nicotinic cholinergic receptors and adrenergic systems of the brain, and also after considerable (by 90%) exhaustion of the brain serotonin content, the rats could be trained, but slowly (Table 1f, h).

The view that the ability to undergo training is lost through the action of benactyzine entirely because of blocking of the muscarinic cholinergic receptors and not to the effect of benactyzine on metabolism, of noradrenalin for example [6, 7], was supported by the next observations. A decrease in the content of catecholamines in the rat brain following administration of benactyzine was observed only during the first

6 days of daily administration of the cholinolytic, while training was unsuccessful for more than 20 days, i.e., after restoration of the normal catecholamine content in the central nervous system. The following fact, however, must be noted. On the average, 7-12 days of training was required for successful food motor conditioning by this method. If memory formation is connected with the influence of cholinolytics on catecholamine metabolism during the first 6 days, during the next 8 days of the experiments on conditioning, when there were no longer any changes in the catecholamine content, at least a tendency toward successful conditioning would appear.

The results also show that disturbances of memory formation produced by benactyzine cannot be explained by general toxic effects, by disturbance of motor activity, or disturbance of food excitability, for starting with the 9th day of daily administration of the cholinolytic, no visible disturbances of motor activity or of food motivation could be observed: the rats of the experimental and control of groups immediately after the training period ate their normal ration. The weight of the animals was unchanged. These results suggest that neither nicotinic cholinergic nor adrenergic and serotoninergic systems of the brain play a significant role in formation of the condition-reflex memory (in training), for against the background of their complete blocking (or of marked exhaustion of the serotonin content) training was possible.

The writer's earlier investigations on dogs showed that disturbance of the training process is due to the effect of benactyzine on short-term memory of objects: after complete blocking of muscarinic cholinergic receptors the animals were unable to retain information in their memory.

The present results also suggest that the conflicting results cited at the beginning of this paper concerning the action of muscarinic cholinolytics on training of animals are evidently attributable to the absence of a reliable control in these investigations of the degree of blocking of the central muscarinic cholinergic structures.

Analysis of all the results presented in this paper thus provides support for Kalyuzhnyi's [5] opinion that food motor conditioning is based on cholinergic processes, and it emphasizes the exceptional importance of competence of the muscarinic cholinergic structures of the brain neurons in conditioning (in the training of animals).

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