ROLE OF CHOLINERGIC AND SEROTONINERGIC PROCESSES IN MODULATION OF ACTIVITY OF THE NEGATIVE REINFORCEMENT SYSTEM

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The effect of cholinergic and serotoninergic drugs on the latency of blocking of central aversive stimulation was studied. Physostigmine and fluoxetin increased the latency of avoidance. Hyoscine and n-chloramphetamine could either increase or decrease the time of active avoidance. Fluoxetin reduced the activating and reversed the depriming effects of hyoscine. A combination of fluoxetin with physostigmine potentiated the depressant effect of the latter. n-Chloramphetamine weakened inhibitory effect of physostigmine and potentiated the action of hyoscine. An inhibitory role is suggested for cholinergic and serotoninergic mechanisms in the activity of the negative reinforcement system. Functional interconnection between these neuromediator systems is postulated.

KEY WORDS: *physostigmine; hyoscine; fluoxetin; n-chloramphetamine; negative reinforcement system.*

It is suggested that activation of cholinergic processes leads to increased activity of the negative reinforcement system [8]. Experimental data on the role of serotoninergic mediation in the activity of the so-called punishment system are contradictory, for both an inhibitory [3] and an activating [6, 10] role has been postulated for serotonin in the mechanisms of aversive effects. Meanwhile definite functional synergism has been demonstrated between inhibitory cholinergic and serotoninergic mechanisms of the limbic system and midbrain in relation to certain types of behavioral activity [11]. However, such interaction, if it extends to other forms of motivated behavior also, must lead to qualitatively similar disturbances during activation of the negative reinforcement system also.

To test this hypothesis the influence of cholinergic and serotoninergic drugs and combinations of them during stimulation of mesencephalic and diencephalic structures forming part of the morphofunctional "punishment" system [9], was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 7 male Wistar rats weighing 250-310 g with monopolar nichrome electrodes 180 μ in diameter implanted into the region of the posterior zones of the medial hypothalamus (MH) and the central gray matter of the midbrain (CGM) in accordance with the coordinates of König and Klippel's atlas [7]. The operation was carried out under pentobarbital anesthesia (50 mg/kg, intraperitoneally). One week after the operation, the animals were taught to block central aversive stimulation (square pulses, frequency 100 Hz, duration 1 msec, intensity 30-100 μA by running in a shuttle chamber (50 × 40 × 21 cm) into the safe compartment through a passage 7 cm wide. In the course of the experiment 15 to 20 such stimulations were applied. After a period of stabilization of the habit (the stable latency of avoidance was between 3 and 5 sec over a period of 5 days) the next series of experiments was carried out under different conditions: At 8.30 a.m. the initial latency of blocking was determined (ten tests). The solvent (sterile water for injection) or the corresponding drugs was then injected and the experiment repeated at 1 p.m. The significance of the effect was determined by Student's t-test [2]. Activity of cholinergic processes was modulated by the use of the muscarinic cholinolytic hyoscine and the anticholinesterase drug physostigmine, which were injected subcutaneously 30 min before the

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TABLE 1. Effect of Cholinergic and Serotoninergic Drugs on Latency of Avoidance of Central Aversive Stimulation (M ± m)

Drug	Dose, mg/kg	Number of experiments	Change in latency of avoidance, in percent of control
Physostigmine	0,1	12	+64,7±16,3*
Fluoxetin	10	13	+23,0±8,0*
Hyoscine	0,5	10	+17,2±4,0*
		7	—42,5±10,0*
n-CHA	5	6	+48,2±14,4*
		4	—37,5±3,9*
Fluoxetin + hyoscine	10±0,5	7↑	—12,2±4,6*
		6↓	—16,7±15,7*
Fluoxetin + physostigmine	10±0,1	18	—127,6±21,7*
n-CHA + hyoscine	5±0,5	4↑	+43,5±28,0
		4↓	—36,4±10,9*
n-CHA + physostigmine	5±0,1	6	+17,2±11,4

Legend: 1. Effect of n-CHA shown 4 h after injection. 2. Arrows pointing upward or downward denote observations in animals with initial increase or decrease in latency of avoidance compared with hyoscine or with n-CHA.

* $P<0.05$.

experiment in doses of 0.5 and 0.1 mg/kg respectively. n-Chloramphetamine (n-CHA), which inhibits the function of serotoninergic neurons [13], and fluoxetin (Lilly 110140*), a selective inhibitor of serotonin reassimilation [12], which prolongs the action of mediator on postsynaptic membranes [5], were tested as serotoninergic substances. Both drugs were injected intraperitoneally 4 h before the experiment in doses of 5 and 10 mg/kg respectively. The effect of n-CHA also was determined after 24 and 48 h. When a combination of cholinergic and serotoninergic drugs was used, hyoscine and physostigmine were injected 3.5 h after fluoxetin and 24.5-27 h after n-CHA.

EXPERIMENTAL RESULTS

The effect of stimulation of 11 points was studied. Physostigmine and fluoxetin significantly increased the latency of active avoidance by 64.7 and 23.0% respectively (Table 1). Hyoscine and n-CHA had opposite actions in a situation of blocking central aversive stimulation. In some cases (six points, ten experiments) hyoscine increased (by 17.2%), but in other experiments (five points, seven experiments) it reduced (by 42.5%) the time of active avoidance. n-CHA 4 h after injection in six experiments (six points) increased on average by 48.2%, and in the four cases (four points) reduced on average by 37.5% the latency of avoidance. The time of avoidance of negative-reinforcement stimulation 24 and 48 h after injection of n-CHA did not differ appreciably from its initial value.

As Table 1 shows, preliminary injection of fluoxetin into animals in which hyoscine increased the avoidance time reversed the depriming effect of the cholinolytic. Under these circumstances there was significant difference between these groups ($P < 0.001$). In rats with an initial activating effect of hyoscine, fluoxetin prevented the reduction in the blocking latency. The action of hyoscine in this case ceased to be significant. The depriming effect of the cholinolytic against the background of n-CHA showed a tendency to increase. In the group of animals in which hyoscine by itself reduced the blocking time, preliminary injection of n-CHA had no effect. Its injection after fluoxetin and physostigmine was accompanied by a considerable, on average almost twofold, increase in the intensity of the inhibitory effect of the latter (Table 1) and a significant difference between the effect of physostigmine and that of a combination of fluoxetin with physostigmine. Preliminary administration of n-CHA, on the other hand, reduced the depriming effect of physostigmine. The difference between these groups (physostigmine alone and physostigmine with n-CHA) was statistically significant.

The increase in the blocking latency after injection of fluoxetin and physostigmine could indicate inhibition of the perception of central aversive stimulation, and this could

*The sample of Lilly 110140 was kindly provided by Dr. R. Fuller (Research Laboratories, Eli Lilly and Co., USA).

be interpreted as a reduction in the activity of the negative reinforcement system under conditions of stimulation of cholinergic and serotoninergic mechanisms. In a situation of a rapid decline in the serotonin level following injection of n-CHA [4] or blocking of the cholinergic receptors by hyoscine [1], in the present experiments not only a decrease, but sometimes an increase in the latency of avoidance was observed. This fact cannot be satisfactorily explained by the ordinary histological analysis of the location of the electrodes, for opposite effects were often observed even when the electrodes were in the identical position in different animals. It may be that the increase in the avoidance time depended on activation of latent positive components in the structure of perception, triggered by stimulation of each concrete zone. The possibility of such pharmacological transformation of negative points into ambivalent ones in principle has been demonstrated [3]. Strengthening of the inhibitory effect of physostigmine against the background of potentiation and the reduction of this effect when the activity of serotoninergic processes was depressed suggest the existence of definite interaction between cholinergic and serotoninergic mediator mechanisms during stimulation of the negative reinforcement system at the MH and CGB level. Reversal of the inhibitory action of hyoscine by fluoxetin is not fully clear from this point of view. Meanwhile the decrease in the activating effect of the cholinolytic when given together with a serotonin-positive drug and the strengthening of the depriming effect of hyoscine during a fall in the serotonin level correspond to the suggestion that functional interconnection may take place between cholinergic and serotoninergic processes.

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