THE EFFECT OF INTRAHYPOTHALAMIC SEROTONIN REINFORCEMENT ON DIRECTIONAL PREFERENCE IN RATS

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Abstract. Reinforcing properties of serotonin administration to the lateral hypothalamus were tested on the experimental model of the T-maze learning in 19 rats. The results did not definitely prove either positive or negative reinforcement produced by the central serotonergic stimulation, although the rats modified their behavior in the maze in the response to intrahypothalamic serotonin. It seems that the central serotonergic neurons do not play a decisive role in the mediation of the reinforcing phenomena but they may interact with the activity of some other reward neurons.

INTRODUCTION

Considerable evidence has already been gathered to support the catecholaminergic hypothesis of reward (9, 21, 28, 29). The participation of the brain serotonergic neurons in the central mechanism of reinforcement was also considered mainly on the basis of self-stimulation experiments. However the results obtained were not conclusive. Exogenous serotonin (30) as well as its precursor 5-hydroxytryptophane (3) and Lilly 110140 — a specific presynaptic reuptake inhibitor (15) were found to inhibit self-stimulation. Para-chlorophenylalanine, a blocker of serotonin biosynthesis increased (2, 22, 25), decreased (10, 27) or had no effect (1, 5) on this behavior. On the other hand increased serotonin release

1 Dr. Jerzy Cytawa died on 15th June 1979.
was demonstrated during highly rewarding brain stimulation (12). Serotonin was therefore postulated to have an inhibitory (23, 24, 25) or excitatory (26) influence on the positive reinforcement and even to be a neurotransmitter of the punishment system (30).

The present experiment was our another attempt to clarify the role of serotonin in the mediation of reinforcing phenomena. We tried to check if any reinforcement, positive or negative, could be produced by the direct application of serotonin into the rat's lateral hypothalamus. For our purpose we applied the procedure of T-maze learning reinforced with intrahypothalamic serotonin injections. Such an approach appeared to be useful in the evaluation of rewarding properties of intracerebral delivery of noradrenaline (6–8).

MATERIAL AND METHODS

The experiment was carried out on 19 male hooded rats, 2–3 mo old. During the whole experimental period the animals were housed individually in their home cages, in natural day-night conditions, with food and water ad libitum. All rats were stereotaxically implanted under i.p. Nembutal anesthesia (20 mg/kg) with chronic cannulas aimed at the perifornical region of the lateral hypothalamus and then allowed a one week recovery from the operation.

The whole experiment consisted of two phases. In the first phase all animals were given ten choices in the T-maze, one a day, in order to find their spontaneous preference for one of the two arms of the maze. The T-maze consisted of a stem of 55 X 15 cm and two arms each of 55 X 15 cm. At the end of each arm there was a goal box of 15 X 15 cm. In this stage of the experiment the animals did not receive any intrahypothalamic injections after entering the goal box.

After establishing the spontaneous preference in the maze, for the second experimental phase in which serotonin reinforcement was introduced, the rats were divided into three groups: two experimental (NP-group, n = 7 and P-group, n = 6) and a control group (n = 6). In the NP-experimental group the possible positive reinforcing properties of the central serotonergic stimulation were tested, so each choice of the previously nonpreferred arm of the maze was rewarded in this group with 40 µg (15.4 nmoles) of serotonin-creatine sulphate dissolved in 0.5 µl of 0.9% NaCl and injected directly into the hypothalamus. The choice of the other arm was followed by an injection of the same volume of 0.9% NaCl. In the P-experimental group the choice of the previously preferred arm of the T-maze was reinforced to test the possible negative reinforcement produced by intrahypothalamic serotonin. The
control rats were given injections of 0.9% NaCl irrespectively of the chosen arm. The results of this group were considered in two ways: with the reference to preferred (P-control) or nonpreferred (NP-control) arm of the maze. Serotonin and saline injections were administered as soon as the animal reached the goal box. The rat was taken out from the maze by the experimenter and then was injected through the cannula connected by a polyethylene drain with a Hamilton syringe. There was one trial a day (11 a.m.) so each animal was given only one injection daily for 17 days of the second phase.

During the both phases of the experiment the running time in the T-maze was measured for each animal, starting from the moment the rat was put into the maze till it reached the goal box.

For a statistical analysis of the results Student’s t-test for independent samples was applied.

After the whole experiment was completed the animals were anesthetized and perfused by 10% formaline. Brains were removed from the skull and sectioned frontally at 60 μm on a freezing microtome. Every fifth section was stained with a cresyl violet by the Nissl method. The positions of the cannula tips were located by the direct projection of the sections into the König and Klippel atlas (18).

RESULTS

Figure 1 shows the results of the T-maze experiment. During the first phase of the experiment when rats had free but unrewarded choice of the arm of the maze they showed a distinct preference for one of the two arms. The rats of the NP-group chose spontaneously the nonpreferred arm of the maze in 30.0 ± 4.9% of all the choices while the rats of the P-group ran to the preferred arm in 73.3 ± 3.5% of trials. In the second phase of the experiment when each choice of the previously nonpreferred arm in the NP-group and the previously preferred arm in the P-group was reinforced with serotonin intrahypothalamic injections the animals of both groups modified their behavior in the maze. The rats of the NP-group increased the number of choices of the previously nonpreferred arm now running to this arm in 50.0 ± 7.9% of all the choices (P < 0.05). In fact, in the second phase of the experiment, they showed indifference in the maze, choosing each arm in about 50% of trials.

On the other hand the rats of the P-group decreased their tendency to choose the previously preferred arm of the T-maze as soon as this arm became associated with serotonin injections from 73.3 ± 3.5% to
Fig. 1. Choice of one arm of T-maze before (I phase, white bars) and during (II phase, dashed bars) serotonin stimulation (mean ± SE).

Fig. 2. Running time in T-maze before (I phase) and during (II phase) serotonin stimulation. Circles, P-group; triangles, NP-group; black squares, control group.

60.0 ± 10.0% of all the choices. This change of preference was only slight and insignificant however.

The rats of the control group kept their preference unchanged during the whole experimental period.

Figure 2 shows the measurements of the running time in the T-maze prior and after the application of serotonin reinforcement. In the con-
trol group receiving only saline injections the time of running to the goal box was prolonged on successive experimental days from mean 22.0 ± 7.1 s. to mean 112.5 ± 24.0 s (data for the first and the last day of the whole experiment). In comparison to controls the animals of the NP and P-experimental groups were significantly faster (P < 0.01) in the T-maze and on the last day of the experiment their running time was mean 25.4 ± 13.0 s and 21.5 ± 8.2 s respectively.

Figure 3 shows the example of the cannulas/placement in a representative rat chosen as the most characteristic one. The cannulas went down through the cortex and the thalamus up to the perifornical region of the lateral hypothalamus where most of the cannula tips were located.

Fig. 3. Localization of the cannulas in the representative rat (S-27). Black points — the placement of cannula tips.

DISCUSSION

The results obtained did not definitely prove either positive or negative properties of serotonin delivered intracerebrally. In fact, the serotonergic stimulation of the hypothalamus did influence the rats' preference in the T-maze as well as their running time but in a way difficult to interpret. Intrahypothalamic serotonin showed simultaneously some features of both positive (NP-group) and negative (P-group)
reinforcement but in any case it was a real reinforcer i.e. it caused a change of the rats' spontaneous preference.

A very similar picture was observed in the experiments in which the cholinergic stimulation of the hypothalamus was tested on the same experimental model (19). On the contrary, the direct application of noradrenaline into the perifornical region of the lateral hypothalamus (6–8) or to the dorsomedial amygdala (14) appeared to be highly rewarding in the T-maze situation.

These findings as well as a bulk of self-stimulation data point to noradrenaline as a main neurotransmitter in the brain reinforcement system. It seems that the central serotonergic neurons similarly as the cholinergic ones do not play any decisive role in the mediation of the reinforcing phenomena. Their function may be rather secondary, i.e., in some way they may influence the activity of real reward neurons, probably noradrenergic ones. Such an assumption may explain to some degree inconsistent results of the experiments on effects of serotonin on self-stimulation as well as our data.

Numerous facts indicate the existence of noradrenaline-serotonin interaction within the brain: there are anatomical connections between the locus coeruleus and the dorsal raphe nucleus (20), the intravenous delivery of 5-hydroxytryptophane decreases brain noradrenaline content (4), lesions of the midbrain raphe nuclei increase the turnover of brain noradrenaline and vice versa (17), amphetamine induced behavior is potentiated by raphe lesions or pharmacological inhibition of serotonin (11, 13, 24). On that basis it is postulated (16) that serotonin neurons of the raphe complex and noradrenaline neurons of the locus coeruleus reciprocally inhibit one another.

As concerns neurochemical reinforcement mediation it seems that it can be attributed to several transmitting systems, among which serotonin neurons take some part, but their exact role is still far from the complete clarification and needs further experiments.

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