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Effect of Serotoninergic Drugs on Positive and Negative Reinforcing Systems in Cats

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PATKINA, N. A. AND I. P. LAPIN. *Effect of serotoninergic drugs on positive and negative reinforcing systems in cats.* PHARMAC. BIOCHEM. BEHAV. 5(3) 241–245, 1976. — Administration of tryptophan and 5-hydroxytryptophan produced inhibition of self-stimulation through hypothalamic electrodes which was accompanied by drowsiness and decrease of motor activity. In cats pretreated with Ro 4-4602, an inhibitor of peripheral decarboxylase, both drugs did not produce drowsiness and markedly activated self-stimulation. Both drugs prolonged the latency of the escape reaction and inhibited the punishment reaction. The data suggest an activating effect of serotonin on the system of reward and an inhibitory effect on the system of punishment.

Tryptophan 5-Hydroxytryptophan Serotonin Hypothalamic self-stimulation Hypothalamic reward Hypothalamic punishment

NUMEROUS data have demonstrated that serotoninergic activation inhibits systems of both reward and punishment [1, 4, 11, 13, 16]. However, these data disagree with some experimental findings on an activating role of serotonin (5-hydroxytryptamine, 5-HT) in the reward system [12] and also with clinical observations on favorable therapeutic effects of serotoninergic drugs in depression [2,10].

It is typical that in the majority of studies carried out with the method of systemic administration of serotoninergic drugs, the peripheral action of these drugs was not taken into account. There is evidence that this action may be of importance in the inhibition of behavior [9].

The aim of the present study is to observe the effects of serotoninergic drugs on reinforcing systems under conditions when peripheral inhibitory action mediated through 5-HT is limited.

METHOD

Animals

Fifteen male cats were used, ranging in body weight from 3.0–4.0 kg. They were housed in animal rooms in groups of 2 or 3 in chambers 150 × 100 × 100 cm. Food (meat, milk) and water were given ad lib. During the experimental session which lasted 5–10 min (SS trial) food and water was not present. Room temperature was 19–20°C.

Surgery

Nichrome electrodes (150–200 microns in dia.) were implanted into the hypothalamus according to the coordinates of the stereotaxic atlas [5]. Six cats had three electrodes each, eight cats had two electrodes each and two cats had only one electrode. Histological control was carried out in each cat on brain slices stained by thionine. Effects of stimulation of 36 points in the hypothalamus were studied. Twenty-nine of them were situated in the lateral hypothalamus (mainly in the zone of the medial bundle of the forebrain), 4 points in the anterior hypothalamus and 1 point in the corpus mammilare medialis and 2 points in the periventricular region. Electrical stimulation of the brain began one week after the operation. Parameters of stimulation were as follows: rectangular impulses, 100/sec; duration 0.5–1.0, msec; amplitude, 0.2–7 V at 0.1–1.0 mA.

Procedure

Positive-reinforcing effects of stimulation of the hypothalamus were studied by means of the method of pedal self-stimulation (SS). The time of closing the circuit was not limited because in previous studies it had been shown that the duration of pressing on the pedal can be a more accurate indicator of the aversive component of stimulation than the frequency of pressing and that the total time of stimulation can show a degree of the rewarding effect [17]. The method of locomotor SS was also used. An experimental chamber 170 × 150 × 110 cm was partitioned off by a barrier 25 cm high. A cat closed the circuit for brain stimulation by jumping over the barrier into one half of the chamber. It broke the current by jumping back. Duration of one session of SS was 10 min.

Negative-reinforcing effects of stimulation of the hypothalamus were studied by the method of measuring reactions that switch off brain stimulation by jumping over the barrier. During one experiment 15–20 such brain stimulations were carried out. If an animal did not switch off the current during 30 sec of stimulation, the reaction was considered as negative. Under situations of punishment,

when an animal approached meat, milk or a chosen place of the chamber, negative stimulation was switched on for 1–2 sec.

If, under brain stimulation, the SS reaction was formed, this was taken as a positive effect of stimulation. If under full intensity range the stable reaction of switching off the current was not formed, the stimulated point was considered as a pure positive. Stimulation of points which were associated with both SS and a stable reaction of switching off, were considered as ambivalent. Finally, if the reaction of SS was not observed, but stimulation was associated with permanent reaction of switching off or punishment the point was classified as a pure negative. In the present study we registered the effects of stimulation of 22 pure positive points, 12 ambivalent and 2 pure negative points.

Drugs

Precursors of 5-HT; l-tryptophan (Trp) and 5-hydroxytryptophan (5-HTP), nialamide (N), an inhibitor of monoamine oxidase and seryl-trihydroxybenzyl-hydrazine (Ro 4-4602), an inhibitor of peripheral 5-HTP/DOPA decarboxylase, were used. All drugs, made as fresh water solutions, were administered intraperitoneally (IP). Trp was dissolved in 1 N NaOH and then solution was gradually neutralized by 1 N HCl/ ; the final pH was 7. In control tests saline was used.

RESULTS

In the control tests we did not observe any statistically significant changes in either frequency of pressings on the pedal or total time of SS. We observed the reaction of SS from points in both the lateral and anterior hypothalamus. From the anterior hypothalamus we observed only pure positive reactions, from corpus mammilare medialis, ambivalent reaction and from lateral hypothalamus all kinds of reactions, i.e. pure positive, pure negative and ambivalent reactions, even from points very close to each other. Trp in doses of 20–50 mg/kg did not affect the frequency of pressings under SS but prolongation of both total time of SS in one session and mean duration of pressings was observed (Table 1). Trp in these doses did not change spontaneous behavior of animals. After administration of Trp in doses of 100–150 mg/kg drowsiness and sedation were observed. This was associated with inhibition of SS registered in frequency of pressings and duration of being under current (Table 2). In a case of ambivalent reactions,

TABLE 1

EFFECT OF TRYPTOPHAN (20–50 mg/kg) ADMINISTERED 3 HR BEFORE SESSION (% OF INITIAL VALUES, MEAN ± SE) ON PEDAL SELF-STIMULATION

Type and number of points	Number of pressings	Changes in Total time of being under current	Mean duration of pressings
Pure positive + ambivalent (12)	−10.3 ± 8.0	+21.0 ± 2.6*	+20.9 ± 4.0*

*$p<0.05$.

TABLE 2

EFFECT OF TRYPTOPHAN (100–150 mg/kg) ADMINISTERED 3 HR BEFORE SESSION (% OF INITIAL VALUES, MEAN ± SE) ON PEDAL SELF-STIMULATION

Type and number of points	Number of pressings	Changes in Total time of being under current	Mean duration of pressings
Pure positive (22)	−27.2 ± 7.0*	−39.0 ± 13.0*	−10.0 ± 5.3
Ambivalent (12)	−24.0 ± 5.1	+20.1 ± 5.5*	+41.0 ± 11.0*

*$p<0.05$.

i.e. reactions which include positive and negative reinforcing effects, prolongation of pressings was observed. In small doses (20–50 mg/kg) Trp did not influence the latency of switching off, but it markedly elevated the threshold of punishing stimulation. In higher doses Trp inhibited both reactions of switching off and punishing effects.

In order to enhance the action of Trp pretreatment with N (20 mg/kg, 3 hr prior to Trp) was made. In the control tests (i.e. after N alone) we did not observe any remarkable behavioral effects although in a few cases minimal sedation was noticed. Pedal SS from pure positive points was either not changed or slightly inhibited. SS from ambivalent points was slightly activated, probably due to inhibition of the aversive component by N and disinhibition from the reciprocal inhibition of positive effects. This dose of N markedly increased the latency of the reaction of switching off the stimulation (mean + 110%) and elevated the threshold of punishing stimulation. Trp in a dose of 10 mg/kg after N caused even stronger inhibition of motor activity and manifestation of pleasure, i.e. cats stretched paws, showed the claws and purred. SS was remarkably more active as compared with the action of Trp alone (Fig. 1). Increase of the dose of Trp to 20 mg/kg enhanced even more the described behavioral effects, in particular the

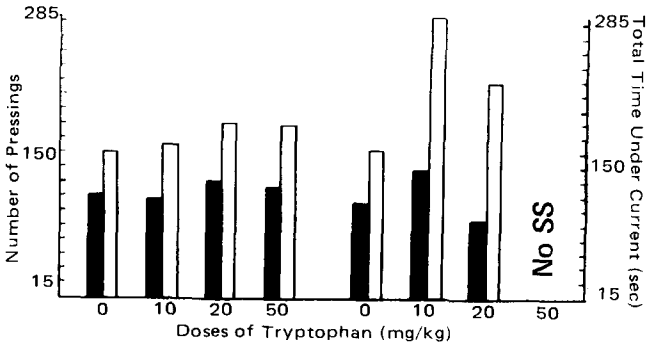

Fig. 1. Effect of tryptophan in control (to the left) and after nialamide (to the right) on pedal self-stimulation (Cat R-6). On the left ordinate – number of pressings on the pedal during 5 min of self-stimulation. On the right ordinate – total time of being under current during 5 min session of self-stimulation (open columns). On abscissa – doses of tryptophan in mg/kg.

typical sitting posture with stretched hind paws and bent back was more clearly represented; inhibition of motor activity was more strong. Trp in a dose of 50 mg/kg after N in 40 min produced that typical posture and then cats gradually began to express symptoms of aggressive behavior in response to touching the hind part of the body. The zone of hyperaesthesia gradually expanded, but regions of the neck and head remained normal, and after touching these regions cats were quiet. Three hr after injection cats responded by hissing, growling and fierce attack not only to every touching but to a wave of the hand, approach of the experimenter or movement of the feeding rack as well. It was completely impossible to manipulate these treated animals. This aggressiveness remained one day after the administration of N and Trp. Even two and three days later an active affectionate reaction to an experimenter (approaching, fawning), which always was in the control, was absent. In a dose of 20 mg/kg Trp after N produced a weaker stimulant effect on SS. Further increase of the dose resulted in inhibition of SS. A dose of 50 mg/kg completely blocked the SS (Fig. 1). It is characteristic that inhibition of SS was developed only after doses of Trp which inhibited locomotion. After those doses of Trp locomotor SS also disappear. Trp after N suppressed the aversive effects of stimulation. The latency of the switching off-reaction was increased (mean by 15 sec) and the threshold of punishing stimulation was elevated (mean by 1–2 V).

To limit the 5-HT-mediated peripheral action of Trp, the

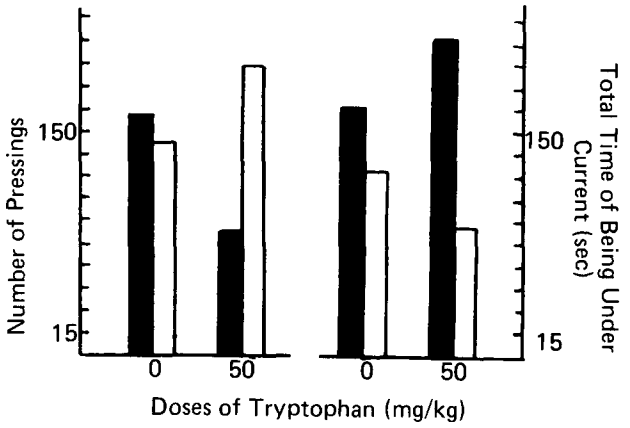

Fig. 2. Effect of tryptophan in control (to the right) and after Ro 4-4602 in a dose of 10 mg/kg (to the left) on pedal self-stimulation (Cat Mt-7). On the left ordinate – number of pressings on the pedal during 5 min of self-stimulation. On the right ordinate – total time of being under current during 5 min session of self-stimulation (open columns). On abscissa – doses of tryptophan in mg/kg.

TABLE 3

EFFECT OF TRYPTOPHAN (50 mg/kg, EXPERIMENT 1) IN CONTROL AND AFTER PRETREATMENT WITH Ro 4-4602 (10 mg/kg, EXPERIMENT 2) ON PUNISHMENT REACTION TO ELECTRICAL STIMULATION OF THE HYPOTHALAMUS APPLIED 3 HR AFTER THE INJECTION (CAT Mu-7)

No. of experiments	Intensity of stimulation (V)	Number of approaches to the rack during each min	Note
Control	1	Does not move away	Normal reaction to food
1	2	1 min-7	Alert, went away into corner
		2 min-2	
		3 min-0	
1	1	Does not move away	Eats
	2	The same	The same
	2.5	1 min-5	Alert
			Exploration
		2 min-3	
		3 min-1	
Control	1	Does not move away	Eats
2	2	1 min-6	Alert
		2 min-1	
2	1	Does not move away	Eats
	2	The same	The same
	2.5	The same	The same
	3	1 min-5	Alert. Looks at experimenter
		2 min-2	
		3 min-1	

TABLE 4

EFFECT OF 5-HYDROXYTRYPTOPHAN (20–30 mg/kg) ADMINISTERED 3 HR BEFORE SESSION (% OF INITIAL VALUE, MEAN ± SE) ON PEDAL SELF-STIMULATION

Changes in		
Number of pressings	Total time of being under current	Mean duration of pressing
−26.3 ± 5.0*	+10.0 ± 6.5	+38.0 ± 9.0*

*$p<0.05$.

latter was administered 1 hr after Ro 4-4602 (10 mg/kg), an inhibitor of peripheral 5-HTP/DOPA decarboxylase. This inhibitor, when injected alone in control experiments, did not change the studied forms of behavior and reactions but slightly inhibited SS. Combination of Ro 4-4602 and Trp did not alter spontaneous behavior of cats. SS was activated much more than after Trp alone (Fig. 2). It is of interest that whereas activation of SS after Trp alone was manifested mainly by prolongation of total time of being under current, after combination of Ro 4-4602 and Trp the frequency of pressings was significantly increased because the motor activity was not inhibited. The latency of the return reaction under locomotor SS was shortened (mean by 10 sec) and the latency of switching off the stimulation was prolonged (mean by 18 sec). The threshold of punishing effects was elevated. In Table 3 protocols of experiments demonstrating changes of the threshold of punishment after Trp alone and combination of Ro 4-4602 and Trp are presented.

Five-HTP in a dose of 20 mg/kg produced significant inhibition of the frequency of pressings under pedal SS (Table 4). Total time of being under current was decreased less intensively and therefore mean duration of pressings was significantly longer. Cats were sedated, drowsy and inert. Five-HTP (20 mg/kg) prolonged the latency of the switching off reaction. Return to active compartment was inhibited. After placement into the experimental chamber to determine the threshold of the punishment reaction, animals looked sedated and inert. This may be a reason for avoiding food. However, after a cat began to eat, it continued to approach the feeding rack, in spite of punishing stimulation. The threshold of the punishing effect was also elevated by 1–2 V. Five-HTP in a dose of 50 mg/kg produced considerable sedation, inhibition and relaxation of muscles, particularly of the hind legs which complicated movements. In this dose 5-HTP completely inhibited both pedal and locomotor SS and blocked the reaction of switching off the stimulation. When 5-HTP (20 mg/kg) was injected after Ro 4-4602 (10 mg/kg), no inhibition, sedation or drowsiness was observed. Cats often had a characteristic serotonin posture as described above. This posture was the most marked 1,5–2 hr after injection of 5-HTP. At the same time cats expressed spontaneous manifestations of pleasure, considerable miosis and relaxation and decrease in the motility of the hind legs. SS was considerably activated after 5-HTP and Ro 4-4602 (Fig. 3). During SS from pure positive points the frequency of stimulation and the total time of being under current were increased. During SS from ambivalent points the total time of being under current was prolonged whereas the frequency of pressings was decreased (Fig. 4A). However,

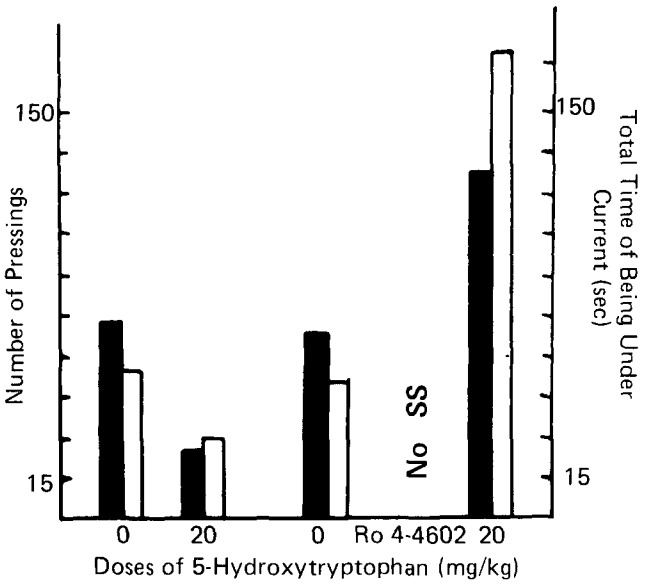

Fig. 3. Effect of 5-hydroxytryptophan in control (left) and after Ro 4-4602 in a dose of 10 mg/kg (right) on pedal self-stimulation (Cat La-7). On the left ordinate – number of pressings on the pedal during 5 min of self-stimulation. On the right ordinate – total time of being under current during 5 min session of self-stimulation (open columns). On abscissa – doses of 5-HTP in mg/kg.

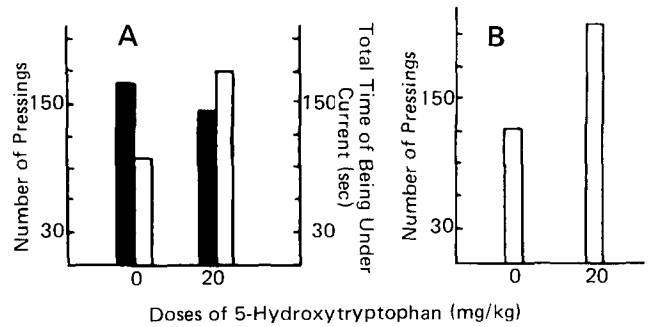

Fig. 4. Effect of 5-hydroxytryptophan after Ro 4-4602 on pedal self-stimulation (Cat La-6) under regimen of free duration (A) and fixed duration of bundles of impulses; 0.5 sec (B). On abscissa – doses of 5-HTP in mg/kg. (A) on the left ordinate – number of pressings on the pedal during 5 min of self-stimulation. On the right ordinate – total time of being under current during 5 min session of self-stimulation (open columns). (B) on ordinate – number of presses on pedal during 5 min session of self-stimulation.

TABLE 5

EFFECT OF SEROTONINERGIC DRUGS ON MOTOR ACTIVITY, AWAKEFULNESS AND HYPOTHALAMIC SELF-STIMULATION IN CATS (↑–ACTIVATION, ↓—INHIBITION, 0—WITHOUT EFFECT. ONE ARROW MEANS SLIGHT OR MODERATE EFFECT, TWO ARROWS MEAN STRONG EFFECT)

Drugs mg/kg	Effect on motor activity	awakefulness	switching off reaction	punishment	self-stimulation
Tryptophan (50–150)	0 ↓	↓	0 ↓	↓	↑ 0 ↓
Nialamide (20)	↓	↓	↓	↓	↑ 0 ↓
Tryptophan (10-50) + nialamide (20)	↑	↑	↓	↓	↑ ↓
Ro 4-4602 (10)	0	0	0	not tested	↓
Ro 4-4602 (10) + tryptophan (50)	0	0	↓	↓	↑ ↑
5-hydroxytryptophan (20)	↓ ↓	↓ ↓	↓	↓	↓
Ro 4-4602 (10) + 5-hydroxytryptophan (20)	↓	0	↓	↓	↑ ↑

based on the considerable increase of the total time of being under current, we conclude that the combination of Ro 4-4602 and 5-HTP have an activating effect on SS. This effect was also shown by the method of fixed bundle impulses (0.5 sec). Under these circumstances the frequency of SS from the same ambivalent points was markedly increased (Fig. 4 B). After Ro 4-4602 and 5-HTP the latency of the reaction of switching off was increased and the threshold of the reaction of punishment was elevated.

DISCUSSION

Cumulative Table 5 shows that all tested serotoninergic drugs decrease activity of the system of punishment. There is a tendency of activation of the system of positive reinforcement. The activating action of small doses of Trp (10 mg/kg) on SS was markedly increased by N. These data are in accordance with the favorable therapeutic effect of Trp and IMAO in depressions [2,10]. It is probable that the inhibitory effect of large doses of Trp and 5-HTP on SS is related to peripheral, mediated through 5-HT, action because Ro 4-4602, an inhibitor of peripheral decarboxylase, diminished that effect. It prevented drowsiness and inertness and activated SS. This observation suggests the effectiveness of combination of precursors of 5-HT and Ro 4-4602 in the treatment of depressions. Thus, central effects of serotoninergic mediation include activation of the system of reward and inhibition of the system of punishment. These results agree with the hypothesis on the important role of a serotoninergic component in the action of antidepressants and antimanic drugs [6,7]. It is of interest that in our experiments the reaction of SS from points of different localization but of the same rewarding characteristics, was similarly changed by the used drugs.

It can be suggested that the antistress action of 5-HT [3, 6, 14, 15] is related to the central inhibitory action of 5-HT on the system of punishment and to the central stimulating effect of 5-HT on the system of positive reinforcement as well. It has been reported elsewhere that activation of the system of reward diminishes the reaction to stressors [8].

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