A POSSIBLE ROLE OF SEROTONIN IN HYPOTHALAMIC SELF-STIMULATION IN DOGS[1]

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The recent demonstration of a centrally acting cholinergic system that exerts an inhibitory effect on self-stimulation (Stark and Boyd, 1963) raises the question as to what neurohormone or neurohormones may be essential for maintaining the activity of this reward system.

Sympathomimetic compounds, such as *d*-amphetamine, cause an increase in self-stimulation response rates in rats with hypothalamic electrodes (Olds, 1958; Stein, 1962a,b). The administration of reserpine to rats either causes a decrease in self-stimulation response rates or abolishes responding (Stein, 1962a; Olds *et al.*, 1957). It has also been shown that reserpine causes the depletion of the norepinephrine content of the hypothalami of cats (Holzbauer and Vogt, 1956), the serotonin content in dog and rabbit brain (Paasonen and Vogt, 1956; Pletscher *et al.*, 1956), and the dopamine content of rabbit brain (Carlsson *et al.*, 1958). The question has been raised as to whether the effects of reserpine on self-stimulation, as well as the symptoms of reserpine administration such as sedation, *etc.*, are caused by the depletion in the brain of norepinephrine, serotonin, or dopamine, or by some combination of changes in these amines.

Stein (1962a) has suggested that the inhibition of self-stimulation by reserpine is due to depletion of the brain stores of catecholamines, and that the enhancement of self-stimulation response rates by amphetamine is through a central adrenergic mechanism. This view of the mechanism of action of amphetamine is shared by others (Brodie and Shore, 1957). However, Brodie postulates that reserpine acts by depletion of the serotonin stores in the brain. To support this hypothesis he used α-methyl-*meta*-tyrosine (Brodie and Costa, 1962). The α-methyl-*meta*-tyrosine, like α-methyl-3,4-dihydroxyphenylalanine (α-methyl dopa), was found to be an effective decarboxylase inhibitor that lowers the levels of serotonin, dopamine and norepinephrine in the brains of guinea pigs, rats, pigeons and mice (Sourkes *et al.*, 1961; Hess *et al.*, 1961; Porter *et al.*, 1961; Sourkes, 1954; Hingtgen and Aprison, 1963). In these species, after administration of either α-methyl-*meta*-tyrosine or α-methyl dopa, the dopamine returns to control levels within 6 to 8 hours after administration; the serotonin levels return to control between 3½ and 20 hours; but the norepinephrine levels remain as low at 20 hours as at 3½ hours and do not return to control levels for 72 to 96 hours. Goldberg *et al.* (1960) found that α-methyl dopa lowered the brain content (caudate nucleus) of serotonin in dogs. These levels started to return toward control in 5 hours, and had returned to control within 24 hours.

In the present work some of the implications of the hypothesis that serotonin has a role in the proper functioning of the hypothalamic self-stimulation system have been examined with a view to helping establish or refute the hypothesis. First, since brain serotonin levels after α-methyl dopa return to control before the levels of catecholamines do, self-stimulation rates of dogs were studied 3½, 8 and 20 hours after administration of α-methyl dopa. Second, since JB-516 causes an elevation of brain serotonin levels in dogs without a concomitant increase in brain catecholamine levels (Maling *et al.*, 1962; Spector *et al.*, 1960), the effect of JB-516 on threshold self-stimulation response rates was studied. Third, the effects of a serotonin antagonist, BOL, were studied.

METHODS AND MATERIALS. Bipolar electrodes were chronically implanted in the hypothalami of dogs. The electrodes used were the same as previ-

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ously described (Stark *et al.*, 1962). The stimulus was a 0.45 second pulse of 60 cycle sine wave current. The current intensities used were rms values. The manipulandum was a modified Gerbrands' rat lever.

The response rates at various current intensities were determined in random order. The lowest current intensity used was below threshold and the highest was 150 μA. The equipment used in these experiments and the method for the determination of response rates have been described previously (Stark and Boyd, 1963). Two of the 3 animals used in this study (dogs no. 9 and 10) were the same animals that had been used in the cholinergic studies; thus the same reward system was involved in this aspect of the study. The control data available extend over a period of 13 months. The size of the experimental group used in this study was therefore of necessity limited.

The drugs used in this study were the *l*-isomer of α-methyl-3,4-dihydroxyphenylalanine (α-methyl dopa[4]), brom-lysergic acid diethylamide as the maleate salt (BOL[5]), and phenylisopropylhydrazine as the hydrochloride (JB-516[6]).

The α-methyl dopa and BOL were administered intravenously, and the JB-516 subcutaneously. In addition, 1 cc of saline was administered to each animal as a control. All drug experiments were done at least in duplicate in each animal. Experimental trials were run 3½, 8 and 20 hours after the administration of the α-methyl dopa, 10 minutes after BOL, and 24 hours after each injection of JB-516, which was administered for up to 5 consecutive days.

The α-methyl dopa was dissolved in 0.1 N hydrochloric acid and the pH was adjusted to 3.0 with 0.1 N sodium hydroxide. The BOL was dissolved in distilled water. The JB-516 was dissolved in distilled water and the pH adjusted to 7.0 with 0.1 N sodium hydroxide. The drugs used were administered at least 1 week apart.

The thresholds for self-stimulation were unchanged throughout the experimental period except for dog no. 10 whose threshold changed between the end of the BOL studies and the studies with JB-516. Daily controls were run for 2 weeks before administering JB-516 to establish that the new base line had stabilized.

Statistical analyses were run on the response rate data using a *t* test for dependent samples (Edwards, 1954). A P value of less than .05 was taken as statistically significant. Histological verification of the electrode placement in each animal was done.

For the determination of brain amines, animals were killed with an overdose of secobarbital. The brains were removed, dissected and frozen with dry ice within 7 minutes. The analyses were made on a sample of brain that included the septum, diencephalon and midbrain. This sample was chosen for 2 reasons: (1) greater reproducibility in excision of samples is possible, and (2) the septum and midbrain areas as well as the hypothalamus represent a part of the self-stimulation reward system.

The brain samples were cut using the following anatomical landmarks: *rostral*, 2 mm rostral to the optic chiasma; *caudal*, 2 mm caudal to the mesencephalic-pontine border; *dorsal and lateral*, dorsal and lateral edges of the thalamus.

Serotonin was extracted by the method of Shore and Olin (1958) and was determined spectrofluorometrically in 3 N HCl. Activation was at 310 mμ and fluorescence was read at 550 mμ (uncorrected).

Norepinephrine was extracted by the method of Shore and Olin (1958) and determined fluorometrically by a modification of the Carlsson and Waldeck method (1958). α-Methyl dopa, α-methyl dopamine or α-methyl norepinephrine added to brain homogenates led to fluorescent products in this procedure. This precluded determination of norepinephrine levels immediately following α-methyl dopa administration. These α-methyl amines produced interfering fluorescence in other methods, involving purification by alumina adsorption, which we have tested.

RESULTS. *Effects of α-methyl dopa.* Doses of 200 mg/kg of α-methyl dopa in all 3 animals caused inhibition of self-stimulation within 3½ hours at all current intensities measured (fig. 1). There was partial recovery at 8 hours and almost complete recovery at 20 hours.

Figure 2 illustrates the effect of a dose of 200 mg/kg of α-methyl dopa on the self-stimulation response rates of all 3 animals at 150 μA. This figure shows the complete inhibition of self-stimulation at 3½ hours after drug, the start toward control values at 8 hours, and almost complete recovery at 20 hours.

Table 1 shows the range of control response rates of dog no. 11 (as an example) and the range of response rates 3½, 8 and 20 hours after 200 mg/kg of α-methyl dopa.

Brain serotonin levels after the administration of 200 mg/kg of α-methyl dopa were 53% of

[4] Supplied by Merck Sharp & Dohme, West Point, Pennsylvania.
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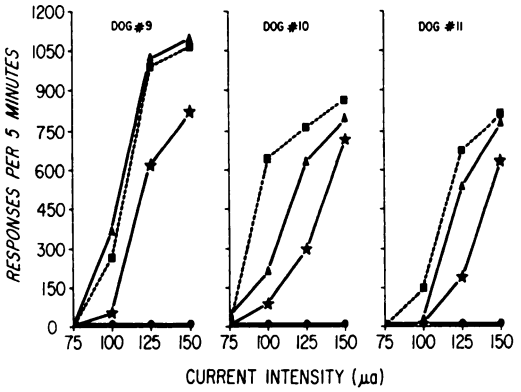

FIG. 1. Self-stimulation response rates at various current intensities for dogs no. 9, 10 and 11 before (■), and 3½ (●), 8 (★) and 20 (▲) hours after the administration of 200 mg/kg of α-methyl dopa.

Each point represents the average of at least 2 experiments.

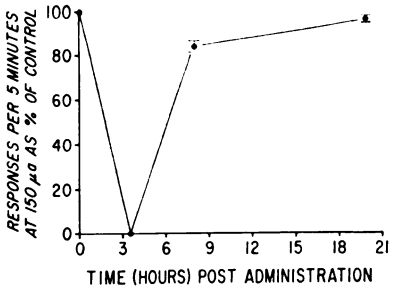

FIG. 2. The effect of 200 mg/kg of α-methyl dopa i.v. on the response rates of the 3 dogs at 150 μA, expressed as a percent of control, plotted against time.

Each point represents the average of at least 6 experiments, 2 for each dog. The standard errors of the means at 8 and 20 hours are shown; at 3½ hours the standard error was $< \pm 0.1$.

control at 3½ hours, 70% at 8 hours, and 83% at 20 hours (table 2).

Brain norepinephrine levels were measured at 20 hours after the administration of α-methyl dopa, since Hess *et al.* (1961) reported that α-methyl dopa had disappeared from tissues by this time. At 20 hours the apparent norepinephrine values are 63% of control (table 3). The true norepinephrine levels would be no higher, but, rather, could be lower than the apparent norepinephrine levels (at 20 hours) because of the reasons cited (see METHODS). This is in agreement with the findings of Sourkes *et al.* (1961) in rats, Hess *et al.* (1961) in guinea pigs and Porter *et al.* (1961) in mice. Sourkes *et al.* (1961) report the norepinephrine levels in rat brain are similar at 3 hours and 24 hours after α-methyl dopa.

Effects of a monoamine oxidase inhibitor, JB-516. The effect of JB-516 (0.5 mg/kg s.c.) on the self-stimulation response rates of dogs no. 9, 10 and 11, 48 hours after the start of daily injections, is shown in figure 3.

For dog no. 9 the response rates at 65, 75 and 100 μA were significantly greater following drug and the threshold was lowered to approximately 50 μA. The time courses for the two experiments

TABLE 1

Data of dog no. 11 showing range of control rates of self-stimulation and rates at 3½, 8 and 20 hours after 200 mg/kg i.v. of α-methyl dopa

Time after Drug	No. of Expt.	Range of Responses per 5 Min			
		75 μA	100 μA	125 μA	150 μA
Control	4	6–37	127–225	615–755	738–870
3½ hr	2	0–2	0	0	0
8 hr	2	4–5	7–9	90–298	602–678
20 hr	2	4–14	5–58	529–548	781–797

TABLE 2

Serotonin levels in dog brains[a] after 200 mg/kg i.v. of α-methyl dopa

No. of Animals	Time (hr)	Range Serotonin μg/g Wet Wt	Mean	% of Control
6	0	0.74–0.93	0.86	100
6	3.5	0.35–0.57	0.45	53
6	8	0.45–0.74	0.60	70
6	20	0.50–0.96	0.71	83

[a] Brain sample included septum, diencephalon and midbrain.

6 animals per time period, 24 total.

TABLE 3

Norepinephrine levels in dog brains[a] after 200 mg/kg i.v. of α-methyl dopa

No. of Animals	Time (hr)	Norepinephrine μg/g Wet Wt	% of Control
3	0	3.30	100
3	20	2.08	63

[a] Brain sample included septum, diencephalon and midbrain. The brain sections of each group were pooled for analysis.

3 animals per time period, 6 total.

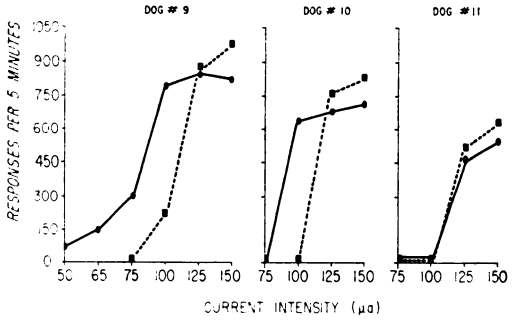

FIG. 3. Self-stimulation response rates at various current intensities for dogs no. 9, 10 and 11 before (■) and 48 hours (●) after the start of daily injections of 0.5 mg/kg of JB-516.
Each point represents the average of at least 2 experiments.

TABLE 4

Data of dog No. 9 showing range of control rates of self-stimulation and rates 48 hours after the start of daily injections of 0.5 mg/kg s.c. of JB-516

	No. of Expt.	Range of Response Rates per 5 Min					
		50 μA	65 μA	75 μA	100 μA	125 μA	150 μA
Control	4	—	2-5	2-7	155-291	851-906	933-992
JB-516	2	61-81	105-208	292-313	716-856	762-942	808-856

on dog no. 9 with JB-516 were almost identical. Twenty-four hours after the first injection there was some indication of a lowering of threshold, while 24 hours after the second daily injection the threshold was clearly lowered. Within 96 hours after the last injection of JB-516, the response rates were the same as control.

Table 4 shows the range of control response rates of dog no. 9 (as an example) and the range of response rates 48 hours after the start of daily injections of JB-516.

For dog no. 10 the threshold was lowered, and the response rate at 100 μA was significantly greater than the control. The time courses for the effect of JB-516 on dogs 9 and 10 were the same. The second of the 2 experimental trials with dog no. 10 involved the daily administration of the drug for 5 consecutive days. The onset of effect was noted within 24 hours, became maximal within 48 hours and continued for the remainder of the period of drug administration. Nevertheless, response rates at control levels were obtained within 96 hours after withdrawal of the drug. This is illustrated in figure 4.

For dog no. 11 the drug was without effect on the self-stimulation response rates 48 hours after the start of daily injections, as shown in figure 3.

Brain serotonin levels (table 5) 24 hours after the second daily injection of JB-516 had risen to 154% of control in 3 nonelectrode dogs; at this time, the thresholds for electrical self-stimulation had been lowered in the experimental animals. At 96 hours, when threshold had returned to control levels in the experimental animals, the serotonin levels had dropped to 118% of control in 3 nonelectrode dogs.

Effects of a serotonin antagonist, BOL. It was noted in preliminary experiments with BOL that the duration of action of the drug was very short. Therefore, the 20-minute experimental period was broken down to an early (10 to 20 minutes post drug), and a late (20 to 30 minutes post drug) time period, and the results are presented separately.

For dog no. 9 a dose of 0.6 mg/kg of BOL resulted in a marked (and significant) inhibition of self-stimulation response rates for the early

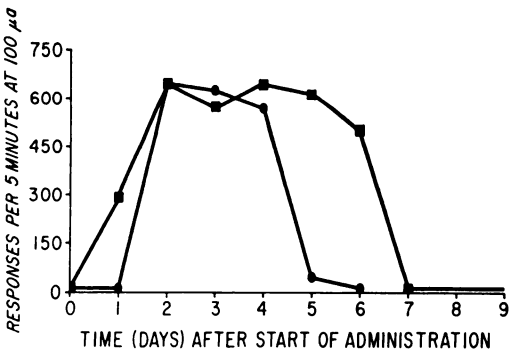

FIG. 4. Comparison of the effect of 2-day (●) and 5-day (■) administrations of JB-516 (0.5 mg/kg s.c.) on the self-stimulation response rates of dog no. 10 at 100 μA.

TABLE 5

Serotonin levels in dog brains[a] after 0.5 mg/kg s.c. of phenylisopropylhydrazine (JB-516)

No. of Animals	No. of Injections	Time after Last of 2 Inject. (hr)	Range Serotonin μg/g Wet Wt	Avg.	% of Control
3	0	0	0.76-0.92	0.86	100
3	2	24	1.05-1.61	1.33	154
3	2	96	0.98-1.07	1.02	118

[a] Brain sample included septum, diencephalon and midbrain.

time period. There was a marked recovery in the late time period, the curve for this period being close to the control curve (fig. 5). The effects of a dose of 0.21 mg/kg of BOL at an early and a late time period are shown in figure 6. The response rate curve for the early period follows the control curve to 100 μA, but is lower at 125 and 150 μA. The curve for the late period shows a lowering of threshold with increases in response rates at 75 and 100 μA. These differences are statistically significant at the 95% confidence level.

A dose of 0.6 mg/kg of BOL caused a lowering of threshold for the early time period in dog no. 10 (fig. 5). The difference between the control rate and the early period rate at 75 μA is statistically significant, P = .001. At 100 and 150 μA, the early period curve is essentially the same as the control curve. The late time period curve is almost identical to the control curve. A higher dose (1.2 mg/kg) of BOL in this dog caused agitated behavior which did not permit an evaluation of the effect of this dose on self-stimulation response rates.

For dog no. 11, a dose of 0.6 mg/kg of BOL at the early time period significantly depressed the response rates. The later period curve, while still below the control curve, clearly shows the rapid recovery from the inhibitory effect of this dose of BOL. The differences in the response rates between the early period and the late period at 125 and 150 μA are statistically significant, P = .04 and .01, respectively. Lower doses of

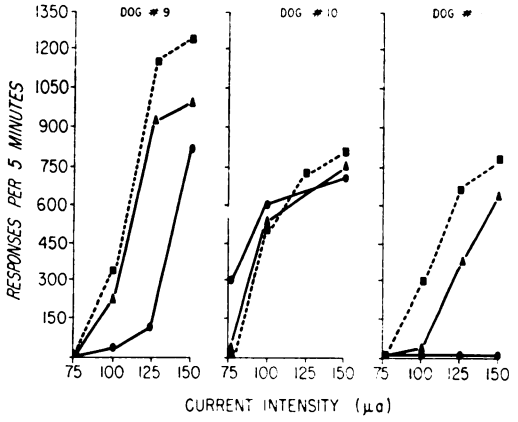

FIG. 5. Self-stimulation response rates at various current intensities for dogs no. 9, 10 and 11 before (■) and 10 to 20 minutes (●) and 20 to 30 minutes (▲) after the administration of 0.6 mg/kg of BOL.
Each point represents the average of at least 2 experiments.

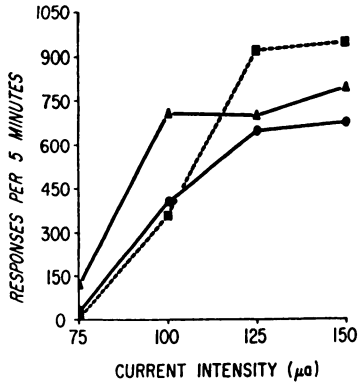

FIG. 6. Self-stimulation response rates at various current intensities for dog no. 9 before (■), 10 to 20 minutes (●) and 20 to 30 minutes (▲) after the administration of 0.21 mg/kg of BOL.
Each point represents the average of at least 2 experiments.

0.3, 0.15, 0.075, and 0.42 mg/kg of BOL were tried but none of these doses lowered the threshold for self-stimulation.

Thus, in two of the animals a "high" dose of BOL was found to inhibit self-stimulation, and in two of the animals, a "low" dose of BOL was found to lower threshold for self-stimulation.

Effects of saline. The administration of 1 cc of saline was without effect on self-stimulation response rates in all three animals.

Histology. Histological examination of the animals' brains for the placement of the electrodes showed the following: *Dog no. 9*—The electrode tip was between the medial mammillary nucleus and the lateral mammillary nucleus, being closer to the former. *Dog no. 10*—The electrode tip was between the medial mammillary nucleus and the lateral mammillary nucleus, being closer to the latter. *Dog. no. 11*—The electrode tip was at the caudal-lateral edge of the medial mammillary nucleus.

DISCUSSION. For the purpose of discussion, a lowering of threshold or an increase in submaximal response rates will be taken to indicate excitation, and elevation of threshold or a decrease in submaximal response rates will be taken to indicate inhibition.

The results with α-methyl dopa provide some indications. The almost complete inhibition of self-stimulation at 3½ hours post drug and the marked recovery of response rates after 20 hours suggest that norepinephrine may not be an essential mediator of this reward system, since norepinephrine levels were no higher than 63%

of control at 20 hours in dogs, and in other species the norepinephrine levels are as low (approximately 60% of control) at 20 hours after administration of α-methyl dopa as at 3 hours (Sourkes *et al.*, 1961). Similarly, the fact that brain dopamine levels in other species return to control values in 6 to 8 hours after administration of α-methyl dopa would suggest that dopamine also is not an essential mediator in this reward system, since response rates were only partially recovered at this time. In contrast, brain serotonin levels do parallel self-stimulation response rates at 3½ and 8 hours, and both brain serotonin levels and response rates have returned almost to control values 20 hours after α-methyl dopa. This would indeed suggest that serotonin may be an essential mediator in this reward system. Additional evidence implicating serotonin was obtained by administration of JB-516, a monoamine oxidase inhibitor that raises brain serotonin levels but not catecholamine levels in dogs (Spector *et al.*, 1960; Maling *et al.*, 1962). Administration of JB-516 lowered the threshold for self-stimulation in 2 out of 3 animals. Maling *et al.* (1962) showed that serotonin levels, following chronic administration of JB-516, are as high at the end of 1 week as they are at the end of 14 weeks. However, the interpretation that an elevated serotonin level is responsible for the lowering of thresholds in these experiments in 48 hours requires that the serotonin levels be elevated within 48 hours after the start of daily injections and that the serotonin levels be back to control values 96 hours after the last administration of JB-516. This was shown to be the case. A possible explanation for the lack of effect of JB-516 on the response rates of the third dog (no. 11) could be the difference of the electrode placement.

A high dose of BOL inhibited self-stimulation in 2 out of 3 dogs. The third dog showed agitated behavior from higher dose levels, precluding the accurate determination of response rates. BOL exhibited a short duration of action, partial recovery being seen within 20 minutes after administration.

The data also show that a low dose of BOL caused a lowering of threshold in 2 out of 3 dogs. This would be compatible with a mechanism of competitive inhibition. BOL has been shown to be a potent serotonin antagonist in peripheral organ systems (Sollero *et al.*, 1956; Gaddum and Hameed, 1954; Gaddum, 1957). Further, a dual action has been shown (Little, 1957) in that low doses potentiated knee jerk and flexion, while high doses inhibited knee jerk and flexion. Little (1957) also showed that BOL would block the effect of serotonin on spinal cord reflexes. Therefore BOL has some effects in the central nervous system. Further, the work of Sollero *et al.* (1956) with *in vitro* systems in several species shows that BOL is relatively specific in that BOL inhibits the effects of serotonin but not those of acetylcholine, histamine, epinephrine, or norepinephrine.

A possible explanation of the failure to lower the threshold in the third dog might be the fact that the electrodes were in a slightly different location. Another possibility is related to the fact that the dose necessary to elicit a lowering of threshold was critical. It should be pointed out that dog no. 11 was also the only animal in which JB-516 did not cause a lowering of threshold. The apparently normal coordination of the animals indicates that the elevation of threshold by high doses of BOL was not due to a depression of spinal reflexes.

Our results do not confirm those reported by Olds (1958) for rats. He reported that BOL was without effect on the self-stimulation response rates of rats with electrodes just rostral to the mammillary nucleus. In his experiments there was a great variability in results for each 8-minute test period, and his results were reported for the second 8-minute period. It is possible Olds may have interpreted his data differently if he had compared early and late periods following administration of BOL.

Our BOL data are compatible with the hypothesis that serotonin is a neurohormone of at least equal importance to that of a catecholamine in this reward system.

SUMMARY

Electrodes were chronically implanted in dogs in the area of the medial mammillary nucleus of the posterior hypothalamus. These animals were trained to press a lever for electrical stimuli at various current intensities. Drugs were administered which altered the brain levels of serotonin and catecholamines. The effects of these drugs and a serotonin antagonist on the thresholds for self-stimulation response rates were measured.

The administration of α-methyl dopa, which lowers the brain levels of norepinephrine, dopamine and serotonin in several species, resulted

in a marked and significant depression of intracranial self-stimulation response rates in dogs. This inhibitory effect correlated better with brain levels of serotonin than of norepinephrine or dopamine.

A low dose of BOL, a serotonin antagonist, lowered the threshold for self-stimulation, and a high dose raised the threshold, in 2 of 3 animals in each case.

The administration of JB-516, a monoamine oxidase inhibitor that causes an elevation of brain serotonin levels in dogs without causing a concomitant elevation of norepinephrine levels, resulted in a lowering of threshold for self-stimulation in 2 of 3 animals.

Thus the evidence suggests that serotonin may be a neurohormone of importance in maintaining the activity of this reward system of the posterior hypothalamus. This does not preclude a role of catecholamines, and the fact that some sympathomimetic agents can affect this system under different conditions would suggest that more than one neurohormone may be involved in maintaining the activity of hypothalamic reward systems.

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