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Is the Release of Noradrenaline Necessary for Self-Stimulation of the Brain?[1]

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(Received 7 August 1975)

SHAW, S. G. AND E. T. ROLLS. *Is the release of noradrenaline necessary for self-stimulation of the brain?* PHARMAC. BIOCHEM. BEHAV. 4(4) 375–379, 1976. – The hypothesis that a quantity of noradrenaline released contingently on every response made to obtain brain stimulation mediates the reward produced by the stimulation was tested. An alternative hypothesis is that reward is mediated by a different system, but that a steady activation of post-synaptic receptors by noradrenaline is necessary for normal behavior. The synthesis of noradrenaline was inhibited by disulfiram, and when lateral hypothalamic self-stimulation in the rat had ceased, α-adrenergic stimulants were injected intraventricularly (IC) or intraperitoneally (IP). The directly acting receptor stimulants oxymetazoline (0.9–250 μg IC), naphazoline (20–250 μg IC), and clonidine (0.75–3 μg IC, 0.037–3 mg/kg IP) did not restore self-stimulation, but the indirectly acting stimulants amphetamine (2 mg/kg IP), methylphenidate (3 mg/kg IP) and phenylephrine (15 μg IC) did restore self-stimulation. In Experiments 2 and 3, in which either the functional noradrenaline pool was depleted with disulfiram and amphetamine, or the reserve noradrenaline pool was depleted with reserpine, the action of phenylephrine in restoring self-stimulation was shown to be indirect, probably by mobilizing a reserve pool of noradrenaline. Because only indirectly acting noradrenergic stimulants which facilitate the release of noradrenaline restored self-stimulation, it is concluded that noradrenaline must be released contingent on every response for self-stimulation to occur. Whether this released noradrenaline mediates the reward or has some other function associated with bar-pressing behavior remains to be shown.

Noradrenaline Release of noradrenaline Functional pool Reserve pool Self-stimulation

ALTERATION of activity in catecholamine systems in the brains of animals working to obtain intracranial electrical stimulation has led to the suggestion that the reinforcing properties of the stimulation depend on the release of noradrenaline (norepinephrine, NE) at synapses of the medial forebrain bundle (see [10, 16, 17]). According to this hypothesis, a quantity of noradrenaline is released from the nerve terminals contingent on a bar press, and this released amount of noradrenaline mediates the reward for that bar press. The question considered here is whether noradrenaline must be released in discrete amounts contingent upon the stimulation; or whether it is only necessary to have a certain minimal level of noradrenaline steadily activating post-synaptic structures to maintain normal behavior.

One type of experiment which poses this question of whether stimulation-elicited release of noradrenaline mediates brain-stimulation reward has been performed by Wise and Stein [17]. It was shown that the attenuation of self-stimulation produced by inhibition of the synthesis of NE (using the dopamine-β-hydroxylase inhibitors disulfiram or diethyldithiocarbamate) could be reversed by the intraventricular injection of NE. It was suggested that the norepinephrine injection allowed presynaptic terminals to take up NE so that it could be released when reward was given. The alternative, that the injected NE directly activated the post-synaptic membrane and that this steady activation reduced a general behavioral impairment (so that self-stimulation could continue) was rejected on the grounds that a similar injection of NE into an undepleted rat produced a small suppression of self-stimulation and might be expected to have mainly post-synaptic effects [17]. A clearer way to solve the problem would be to inject not NE but instead direct noradrenergic receptor stimulants to determine whether they can reverse the attenuation of self-stimulation produced by inhibition of the synthesis of NE. If a direct receptor stimulant can reverse the inhibition of self-stimulation, then it follows that the release of NE contingent upon each bar press by electrical stimulation is not necessary for brain-stimulation reward. The noradrenergic receptor agonists (α-adrenergic agonists) used in Experiment 1 were: oxymetazoline and naphazoline which probably act directly on the receptors [9,14]; clonidine, which has a direct central noradrenergic effect [1]; and phenylephrine, which has some direct noradrenergic effect [7, 8, 15].

EXPERIMENT 1

The aim of the experiment was to determine whether noradrenergic agonists thought to act directly on the post-

[1] This work was supported by The Medical Research Council.

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synaptic receptors can reverse the attenuation of self-stimulation produced by depletion of NE using the dopamine-β-hydroxylase inhibitor disulfiram.

METHOD

Animals for this experiment were male albino Sprague-Dawley rats weighing 250–400 g. Electrodes were implanted under Equithesin anesthesia (Jensen-Salsbury Labs) in the lateral hypothalamus with level head coordinates (3.0 mm behind bregma, 1.5 mm lateral to the sagittal sinus, and 7.6 mm below the surface of the dura). A cannula was implanted at the same time into the lateral ventricle at level head coordinates 1.0 mm behind bregma, 1.7 mm lateral, and 3.5 mm down. Details of the construction of the cannula have been presented earlier [11]. Four days following surgery the animals were trained in a Skinner box to press a bar to deliver a 0.3 sec train of 0.1 msec constant current negative pulses at a frequency of 100 Hz to one of the electrodes.

The minimum current which would maintain a steady rate of bar pressing was determined and this value was rechecked and used in all subsequent test sessions. The current was monitored continuously throughout each test session. When the animals were bar-pressing at constant rates for periods of up to 5 hr, they were then given the following drug treatment. On the morning of the test the animal was allowed to self-stimulate at different current values for 30 min to check the baseline value. Disulfiram 200 mg/kg as a suspension in 1% methylcellulose was then injected IP and the animal was allowed to continue to self-stimulate. Two to 3 hr following the injection of disulfiram the rate of self-stimulation of the animals was zero and they assumed a huddled posture with their eyes closed. Priming the animals would not restore the rate of self-stimulation (see Fig. 1). Priming was continued intermittently for at least 10 min to ensure that attenuation of self-stimulation was complete.

The α-agonists used in the present experiment were oxymetazoline, naphazoline, clonidine and phenylephrine. All the compounds were dissolved in 0.9% saline. Oxymetazoline was provided by E. Merk Ltd, Surrey; naphazoline and phenylephrine by CIBA laboratories, Horsham, Sussex; disulfiram by Berk Pharmaceuticals Ltd, Guildford, Surrey, and clonidine (Catapresse) by Roche Pharmaceutical, Herts. For Experiments 2 and 3, reserpine, L-DOPA, and $RO_4$4602 were provided by Roche Pharmaceuticals; FLA 63 by A. B. Hässle, Göteborg, Sweden; and methylphenidate and amphetamine sulphate were provided by CIBA laboratories, Horsham.

Immediately following the 10 min period of priming, animals were injected intraventricularly with one of the above noradrenergic agonists. The volume injected at any one time was 5–10 μl. If the first injection failed to reinstate self-stimulation a second injection was given after 30 min, followed by a third injection if necessary after a further 30 min. If 30 min after a third injection self-stimulation had not been reinstated, the test was considered negative for that particular drug dose. The dose presented in Table 1 is the total dose that was given over the test period, and the number in brackets represents the number of separate injections given.

In addition to intraventricular injections, clonidine, which has been shown to cross the blood brain-barrier [1], was also administered (IP in doses ranging from 0.075–3 mg/kg).

RESULTS

Oxymetazoline (16 animals) and naphazoline (9 animals) in doses ranging from 0.9 μg to 250 μg did not produce any consistent reversal of the disulfiram-induced attenuation of self-stimulation. Injections of these compounds appeared to result in an increase in sedation of the animals. On being removed from the testing box some of the animals would try to bite and this was accompanied by vocalization. No reliable restoration of self-stimulation was produced by these compounds in the other animals tested (see below).

Similarly for intraventricular injections of clonidine (6 rats) in doses ranging from 0.75–3 μg no consistent reversal of self-stimulation was seen. The rats, however, did show marked aggression and also increased motor activity charac-

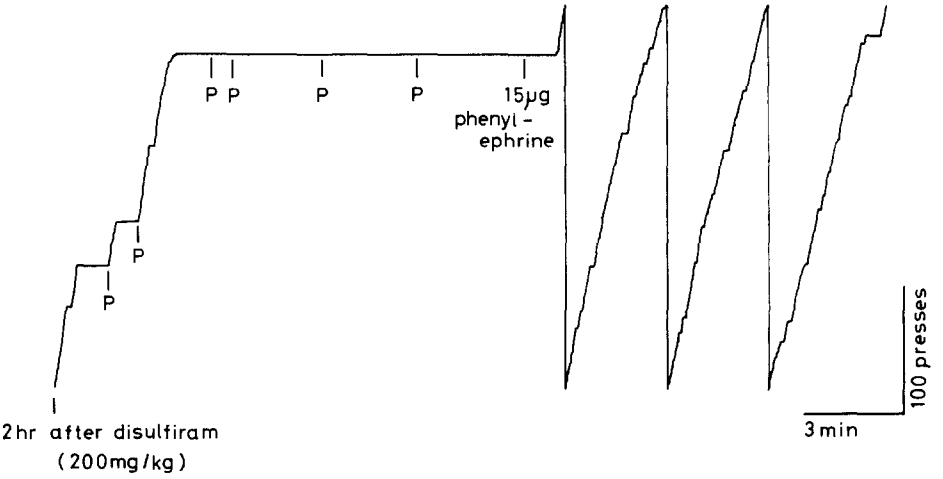

FIG. 1. Two hours after treatment with disulfiram (200 mg/kg IP) this rat finally stopped self-stimulation. Self-stimulation could not be reinstated by priming (P) bursts of stimulation given by the experimenter. When 15 μg of phenylephrine hydrochloride (in 3 μl) was injected intraventricularly, vigorous self-stimulation was reinstated.

TABLE 1

THE EFFECTS OF INTRACRANIAL (IC) AND INTRAPERITONEAL (IP) NORADRENERGIC-RECEPTOR AGONISTS ON SELF-STIMULATION AFTER INHIBITION OF THE SYNTHESIS OF NORADRENALINE BY DISULFIRAM (200 MG/KG IP)*

Phenylephrine μg IC			Oxymetazoline μg IC			Naphazoline μg IC			Clonidine μg IC			Clonidine mg/kg IP		
Rat No.	No Effect	Restoration	Rat No.	No effect	Apparent Restoration	Rat No.	No effect	Apparent Restoration	Rat No.	No effect	Apparent Restoration	Rat No.	No effect	Restoration
15	—	15(1)	20	0.9(3)	—	56	—	20(1)	138	0.75(1)	—	126	0.0375	—
17	—	15(1)	23	0.9(3)	—	55	—	22(1)	21	—	1.5(2)	129	0.0375	—
43	—	15(1)	50	—	0.9(3)	54	—	37(2)	132	1.5 (3)	—	AS2	0.075	—
47	—	15(1)	83	—	0.9(3)	17	50(3)	—	133	1.5 (2)	—	AS4	0.075	—
48	—	15(1)	90	—	0.9(3)	47	50(3)	—	135	1.5 (2)	—	26	0.075	—
51	—	20(2)	85	—	1.0(3)	56	50(3)	—	134	—	1.5(1)	27	0.075	—
55	—	30(2)	51	1.0(3)	—	15	90(3)	—	137	1.5 (2)	—	128	0.075	—
49	—	42(3)	76	1.3(2)	—	52	100(3)	—	139	—	2.0(3)	130	0.075	—
52	—	45(3)	84	2.5(3)	—	51	—	125(3)	138a	—	3.0(3)	131	0.075	—
54	—	45(3)	116	10.0(3)	—	52	200(3)	—	140	3.0 (3)	—	130	0.15	—
50	—	50(3)	21LV	15.0(3)	—	47	150(3)	—				128	1.0	—
56	50(3)	—	91	15.0(3)	—	50	250(3)	—				126	3.0	—
			117	18.0(3)	—	56	250(3)	—				129	3.0	—
			20LV	—	20.0(3)									
			23LV	—	20.0(3)									
			74	20.0(3)	—									
			117	—	40.0(3)									
			119	100.0(3)	—									
			180	100.0(3)	—									
			180	—	250.0(3)									
			191	15.0(3)	—									
			192	15.0(3)	—									
			193	0.9(3)	—									
			194	0.9(3)	—									

*The dose of each drug in each rat which produced either a restoration of self-stimulation (see text) or had no effect on self-stimulation is shown in the appropriate column. In cases where the total doses shown were injected in a series of separate smaller doses, the number of such injections is shown in brackets.

terized by jumping. These observations show that the doses of clonidine used did have effects on behavior.

Intraperitoneal injection of clonidine in doses ranging from 37 μg/kg to 3.0 mg/kg produced a similar increase in motor activity, which was more marked as the dose was increased, but this was not normal coordinated locomotor activity. All animals given intraperitoneal injections of clonidine also showed marked piloerection, exophthalmos and reversal of the hypothermia induced by disulfiram. None of the animals showed any tendency to self-stimulate.

The only noradrenergic agonist to have any dramatic effect was phenylephrine (in 11 animals) which produced a consistent reversal of the disulfiram-induced suppression of self-stimulation after an average of 15 μg intraventricularly. After phenylephrine self-stimulation behaviour appeared to be normal.

It was found that in some of the rats treated with oxymetazoline, naphazoline or clonidine, self-stimulation occurred intermittently at slow rates after the injection (see Table 1). This probably occurred because these were preliminary rats in which priming stimulation had not been repeatedly given after the inhibition of self-stimulation by disulfiram first appeared. Thus it is probable in these rats that the poor self-stimulation which was seen was not produced by the injection. It was certainly not possible to obtain reliable effects with oxymetazoline, naphazoline or clonidine.

DISCUSSION

This experiment shows that intraventricular injections of the direct noradrenergic-receptor stimulants oxymetazoline, naphazoline and clonidine do not mimic the effects of norepinephrine in reversing the attenuation of self-stimulation produced by inhibition of the synthesis of noradrenaline. This suggests that when NE injections reverse the inhibition of self-stimulation, they do so by replacing noradrenaline pre-synaptically so that it can be released when a train of electrical stimulation is obtained. When phenylephrine was injected, self-stimulation was completely restored in the disulfiram-treated animals (see Table 1). To test whether this effect of the phenylephrine might be due not to a direct noradrenergic effect, but to an indirect one (by allowing the mobilization of previously unavailable NE) the remaining experiments described here were performed.

EXPERIMENT 2

Norepinephrine in nerve terminals is believed to be present in 2 separate pools, the reserve and functional pools [13]. The amphetamines release noradrenaline from a func-

tional pool which is depleted by α-methyl-p-tyrosine, and methylphenidate releases noradrenaline from a reserve pool which is depleted by reserpine [12].

The purpose of Experiment 2 was to determine whether the functional NE pool had been exhausted after disulfiram treatment (as suggested by Wise and Stein, see [17]), to try to exclude the probability that phenylephrine acts on the functional pool. (In addition the experiment tests the hypothesis that inhibition of NE biosynthesis by disulfiram does not deplete the reserve pool.)

METHOD

Eight male Sprague-Dawley rats were implanted with electrodes in the lateral hypothalamus and with a cannula in the lateral ventricle. The rats were injected intraperitoneally with disulfiram (200 mg/kg) and allowed to self-stimulate until the rate had decreased to zero and priming would not reinstate the self-stimulation. The animals were then divided into 2 groups each consisting of 4 animals.

The first group received amphetamine sulphate IP (2 mg/kg) and the second group received methylphenidate IP (3 mg/kg).

RESULTS

Within 20 min following injection of either drug the self-stimulation rate of all rats had been restored from zero to the normal baseline levels. In those rats which had received amphetamine the restoration was of shorter duration (40 min) than that produced by methylphenidate (5 hr). This indicates the presence of at least some NE in both pools after self-stimulation had been completely attenuated following treatment with disulfiram, and it would appear that there is NE available in both the functional and the reserve pools for phenylephrine to act on.

Two of the animals from the amphetamine-treatment group were given a second injection of amphetamine (2 mg/kg) approximately 45 min following the first injection. By this time, the rate of self-stimulation had decreased again to half the normal baseline value. No increase in rate had occurred 25 min following this second injection of amphetamine. Phenylephrine (20 μg) was then injected intraventricularly into both rats and within 5 min the self-stimulation rate had increased to normal. The restoration lasted for a comparable time (20–30 min) to that observed for intraventricular injection of phenylephrine into animals treated only with disulfiram.

DISCUSSION

This latter finding suggests that phenylephrine and amphetamine act by different mechanisms to reverse the disulfiram-induced attenuation of self-stimulation. Since amphetamine is known to affect release of NE only from a functional pool it is tentatively proposed that phenylephrine reverses the disulfiram-induced attenuation of self-stimulation by an ability to mobilize NE from a reserve pool. This proposal is tested in Experiment 3.

EXPERIMENT 3

To test further the proposal that phenylephrine mobilizes NE from a reserve pool, phenylephrine was injected intraventricularly into animals selectively depleted of reserve pool NE. Reserpine was used to produce the depletion, but reserpine also reduces brain dopamine (DA) concentration by 90%. To avoid confusion in the interpretation of the present experiment, animals were injected with 5–10 mg/kg reserpine, 19 hr prior to the experiment, and DA was then selectively replaced by L-DOPA after inhibition of peripheral decarboxylase with $RO_4$4602 and pretreatment with FLA 63 (method of Ahlenius and Engel, see [3]).

METHOD

The order of drug treatments was as follows: Reserpine, 5–10 mg/kg, was given 19 hr prior to the experiment; FLA 63, 10 mg/kg, was given 10 min before the peripheral DOPA-decarboxylase inhibitor; and $RO_4$4602, 50 mg/kg, followed by L-DOPA, 100 mg/kg, were given 30 min later. Phenylephrine, 8–10 μg, was then injected intraventricularly 30 min and 1 hr after the injection of L-DOPA. Rats were prepared for this experiment as described under Experiment 1.

RESULTS

Eleven male Sprague-Dawley rats were tested according to the above procedure and in no case did intraventricular injection of phenylephrine reinstate self-stimulation [2]. In the 3 rats which were injected with amphetamine sulphate (4 mg/kg) after the sequence of compounds listed above, self-stimulation was reinstated within 15 min to normal baseline rates, indicating that the failure of phenylephrine to reinstate self-stimulation was not due to nonspecific effects of the previous drug treatment (except perhaps for impaired dopamine release). One animal which was incompletely reserpinized (judged from the observation that it did not show a large degree of peripheral ptosis, and that it would self-stimulate at a low rate) was given intraventricular phenylephrine (15 μg). This produced a restoration of self-stimulation (25–30 min duration).

DISCUSSION

It is concluded that phenylephrine restored self-stimulation by acting on a reserve pool of NE because phenylephrine is ineffective in animals depleted of the reserve pool of NE with reserpine (even though these reserpinized animals could self-stimulate if the functional pool of NE was mobilized by amphetamine).

DISCUSSION

The main finding described here is that intracranial injections of the directly acting noradrenergic receptor stimulants clonidine, oxymetazoline and naphazoline do not reinstate self-stimulation when it is attenuated by inhibition of the synthesis of norepinephrine (Experiment 1). In contrast, similar intraventricular injections of NE do restore the self-stimulation [17]. This suggests that self-stimulation only occurs when NE is available in presynaptic terminals and can be released when the animal obtains a train of electrical stimulation. It does not appear to be sufficient just to have a normal steady excitation of the post-synaptic receptors, such as would be produced by the direct receptor stimulants, even though this may reverse at least to some extent the general impairment of behavior produced by NE depletion. (The rats treated with the receptor stimulants were affected by the stimulants, as described above, but

normal locomotor activity, for example, was not restored.)

Whether the release of NE contingent on neuronal activity which thus appears to be necessary for self-stimulation actually mediates the reward is still not known. It may be, for example, that some other behavior associated with self-stimulation, such as bar-pressing, requires the release of NE from nerve terminals in a time related or phasic manner by neuronal activity.

The finding that intracranial injections of phenylephrine can restore self-stimulation after inhibition of the synthesis of NE (Experiment 1) was investigated in the subsequent experiments. First it was shown that there was some NE in both the functional (amphetamine treatment) and reserve (methylphenidate treatment) pools after self-stimulation had been attenuated by disulfiram (Experiment 2). To determine on which, if either of these pools phenylephrine acted to restore self-stimulation after disulfiram, each of these pools was dispersed. The observation that after depletion of the functional pool of NE by disulfiram and repeated injections of amphetamine, self-stimulation could be restored by phenylephrine suggests that phenylephrine acts by mobilizing a reserve pool of NE (Experiment 2). This was confirmed by the observation that after depletion of the reserve pool of NE in Experiment 3, phenylephrine was no longer effective in restoring self-stimulation (although self-stimulation could occur, in that amphetamine, acting on the functional pool, did restore self-stimulation) (Experiment 3). Thus it appears that phenylephrine restores self-stimulation, which has been attenuated by decreasing the synthesis of NE, by mobilizing NE from a reserve pool, and not by a direct action on noradrenergic receptors. It is of pharmacological interest that this mobilization of the reserve pool must allow the NE to be released mainly during firing of the presynaptic neuron, otherwise self-stimulation would not occur.

In a preliminary but comparable experiment on whether the direct stimulation of DA receptors and NA receptors either alone or simultaneously is necessary for self-stimulation, we have found that neither apomorphine (3 mg/kg) which directly activates dopamine-receptors, nor clonidine (0.15–3 mg/kg) which directly activates NA receptors, nor a combination of apomorphine and clonidine restore self-stimulation which has been attenuated by reserpine (6 mg/kg). Although the drugs had some effect on the receptors, in that the clonidine produced jumping, and the apomorphine produced gnawing, no restoration of self-stimulation was ever seen.

The results described here in addition suggest that 2 pools of NE can affect self-stimulation, a functional pool from which the release of NE is facilitated by amphetamine, and a reserve pool from which NE can be mobilized by methylphenidate or phenylephrine. The functional pool of NE is reduced when the biosynthesis of NE is inhibited by disulfiram, and the reserve pool is depleted by reserpine treatment. Those views are consistent with those of Franklin and Herberg [5], who performed experiments on self-stimulation using injections of α-methyl-p-tyrosine to inhibit the enzyme tyrosine hydroxylase, and showed that mobilization of NA from a reserve pool can reinstate self-stimulation. One additional observation made in the course of these experiments was that in the normal (undepleted) rat, intraventricular injections of clonidine in doses of 1–10 μg attenuated self-stimulation, whereas doses of up to 20 μg phenylephrine did not. This probably reflects a predominantly direct action of clonidine, and at least in part an indirect action of phenylephrine. The attenuation of self-stimulation produced in the normal rat by clonidine may be due to overstimulation and post-synaptic depolarization leading to functional blockade, in that intraventricular infusions of low doses of NE facilitate locomotor activity, but higher doses lead to sedation [6].

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