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The Effect of 6-Aminodopamine on Electrical Self-Stimulation in Rats[1,2]

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MONASMITH, B., P. PLOTSKY, C. L. BLANK AND R. N. ADAMS. *The effect of 6-aminodopamine on electrical self-stimulation in rats.* PHARMAC. BIOCHEM. BEHAV. 5(1) 19–21, 1976. – The compound 6-aminodopamine is a powerful CNS catecholaminergic neurotoxin. Small dosages of 6-aminodopamine injected intraventricularly markedly depress electrical self-stimulation rates in rats. This 6-aminodopamine treatment produced whole brain lowering of norepinephrine to *ca.* 50% of normal while the dopamine content was unchanged. The possible use of 6-aminodopamine treatment to elucidate the relative roles of norepinephrine and dopamine pathways is discussed.

6-Aminodopamine Electrical self-stimulation Catecholamine pathways Medial forebrain bundle
6-Hydroxydopamine

THE discovery of the remarkable neurotoxicity of 6-hydroxydopamine (6-OHDA) has generated widespread interest in its use to manipulate catecholaminergic pathways and behavioral functioning in small animals [6]. The close analog 6-aminodopamine (6-ADA) is known to damage central catecholamine neurons in much the same way as 6-OHDA [1, 5, 10]. Studies of 6-ADA in which behavioral responses are a central concern are virtually nonexistent. This is understandable since 6-ADA is, in the systemic sense, more toxic than 6-OHDA. There appears to be no reason to utilize it on a widespread basis as a substitute for 6-OHDA. There is, however, a potentially significant useful difference between 6-ADA and 6-OHDA. At equivalent dosages the former compound gives long-term depletion of norepinephrine (NE) almost identical to that obtained with 6-OHDA. However, the dopamine (DA) depletion produced by 6-ADA is practically nil under the same conditions. This differential catecholamine neurotoxicity can be obtained without the use of additional pharmacological agents or special pretreatments [9].

Thus, 6-ADA could be an important tool for use in studying behavioral responses where ambiguities exist as to the involvement of NE or DA neural pathways. A particular behavioral index which has elicited much interest in this regard is electrical self-stimulation (SS) in rats. For this reason, we initiated a thorough study of the effect of 6-ADA on SS in rats and the results are summarized briefly herein.

Stein and Wise first reported that 6-OHDA markedly depressed the rate of SS in rats [11] and this has been amply verified by other laboratories [2]. In general, the present experimental procedure was intended to duplicate as closely as possible the original 6-OHDA experiment of Stein and Wise using, instead, 6-ADA as the neurotoxic agent.

METHOD

Male Charles River rats (350–400 g) were implanted with stainless steel bipolar electrodes (tip 0.7 mm, insulated except at tip). The electrodes (Plastic Products, Roanoke, Va.) were implanted in the medial forebrain bundle (MFB) with stereotaxic coordinates from bregma of 4.6 mm posterior, 1.3 mm lateral to saggital suture and 9.0 mm deep from top of skull. A stainless steel cannula and clearing wire with screw cap were implanted at the same time in the opposite LV (coordinates, 1.0 mm posterior, 1.5 mm lateral and 4.1 mm deep). Animals were allowed to recover from surgery for one week before any testing. They were then trained to bar press in a conventional SS box so as to deliver a 60 Hz sinusoidal stimulus to the electrode. The stimulus duration varied from 0.1–0.2 sec. Currents between 18–65 μa were employed. Stable baseline performances were established for each animal varying between 2000–4000 presses per hour. SS recordings were done at approximately 1600 hr daily to minimize daily activity cycle effects.

After several days of stable baseline SS rates, animals were given intraventricular injections of 100 μg of 6-ADA. This was dissolved in isotonic saline containing 1 mg/ml ascorbic acid (AA) to protect against air oxidation. Injection volumes were always 5 μl. Control injections consisted of 5 μl of saline plus ascorbate. The injections were made in the following manner. A length of polyethylene tubing was attached to a 10 μl Hamilton syringe. Saline/AA solution

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was drawn in, followed by an air space, and then the 5 μl of 6-ADA. The tubing was attached with an exact fit to the cannula. The 6-ADA was then injected, followed by approximately 1 μl of air. The tubing was severed and sealed above the cannula with a hot soldering iron to prevent escape of the drug.

RESULTS

Twenty-four hours after 100 μg of 6-ADA had been injected, the SS rates decreased in 8 of 12 animals to between 0–35% of the predrug baseline level. Three animals similarly decreased to 65–85% and one showed no significant effect (although cannula placement and injection procedure were apparently correct). Control injections showed little or no effect except an occasional slight increase in SS over baseline. A t-test of the results of 3 predrug and 3 postdrug days for all 12 animals showed $t = 8.12$, $df = 36$, $p<0.005$. Most animals returned to 50% or greater of the predrug baseline level within 5 days.

Five animals were given two or three additional injections of 100 μg of 6-ADA (at least 5 days between injections). The first injections produced results as indicated above. Following the second and subsequent injections the rats lost weight and were no longer interested in grooming. They became extremely nervous and aggressive. It became very difficult even to attach the SS cord on some animals. This behavior was undoubtedly the result of the considerable toxicity of 6-ADA which has been noted before [1,5]. It was not possible to draw any meaningful conclusions about repeated injections with such severely disturbed animals.

To insure reliability, the entire study was repeated some five months later with a new experimenter and, of course, different animals. In this case, five rats, implanted and treated as described above were used for the 6-ADA study. In this run, each animal received a saline injection about Day 7 after reaching a stable baseline SS rate. This was followed 4 days later by 100 μg of 6-ADA as before. The SS rates were totally unaffected by the saline injection but they dropped to 14–30% of control for every animal when tested 24 hr after the 6-ADA injections. A similar return to almost predrug baseline level was again seen within 4 days after the 6-ADA injection. Some 6-ADA solutions were intentionally air-oxidized completely before injection. In four separate rats injection of 100 μg of this air-oxidized 6-ADA caused no change in SS rate (the results were essentially identical with saline injections).

Figure 1 is an example of the highly consistent results obtained. Following the experiments, the brains of all 5 animals were removed and analysed for whole brain NE and DA content. The NE was decreased to 54.9% of that of saline-injected controls (control NE = 275 ng/g ± 6.5% SD). The DA content remained constant at 100% of control (control DA = 615 ng/g ± 7.7% SD). These depletions are completely consistent with results obtained on previous injections of fairly low single dosages of 6-ADA [9].

DISCUSSION

These studies show clearly that 6-ADA drastically reduces the rate of electrical SS in rats when the drug is applied in a fashion similar to that normally used in 6-OHDA SS studies. Under these conditions, whole brain

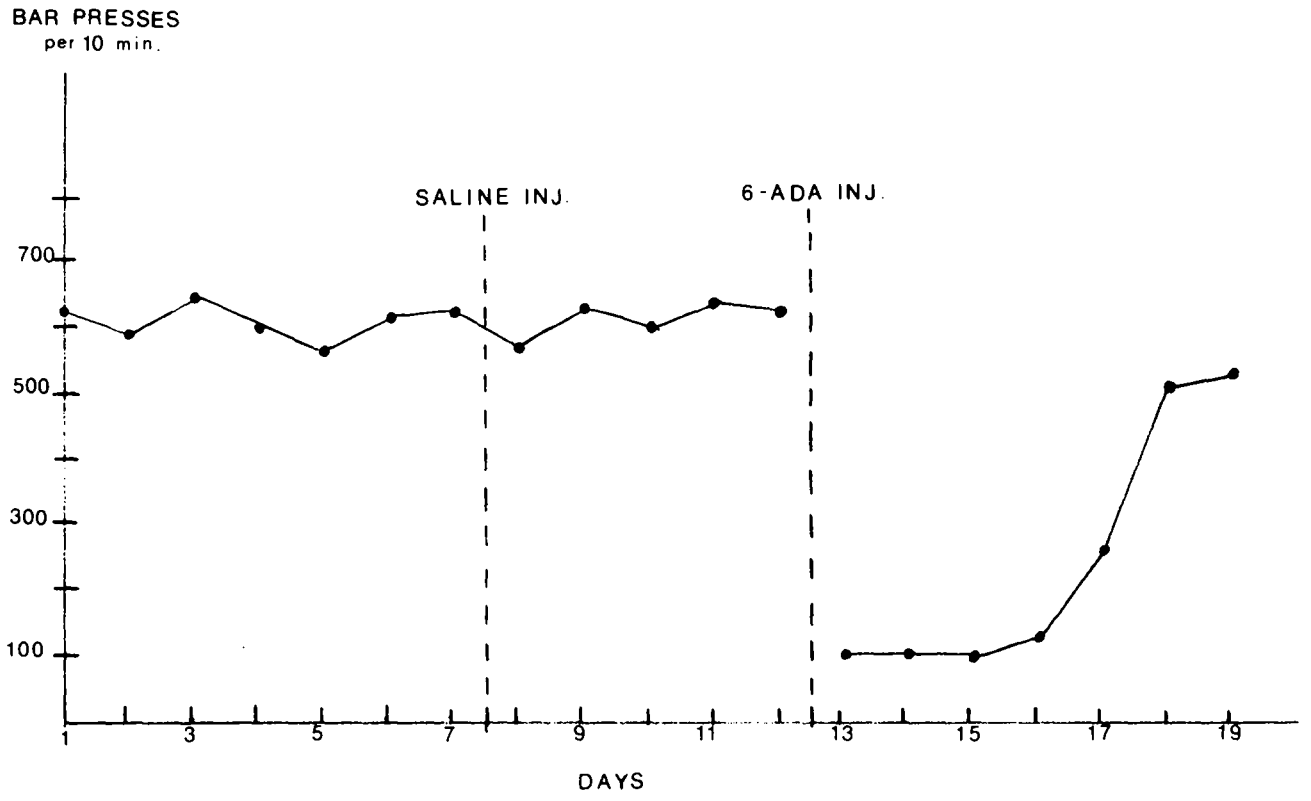

FIG. 1. Typical effects of 6-ADA on self-stimulation.

analyses indicated the 6-ADA had produced no long-term depletion of DA. These results are particularly pertinent in view of the fact that a variety of investigators have suggested that both DA and NE pathways are involved in electrical SS [3, 4, 7, 8, 12, 13].

Although the present results show that 6-ADA causes a marked decrease in SS rate with no whole brain DA depletion, they cannot be taken as evidence against the involvement of DA pathways in MFB electrical stimulation. An examination of the detailed pattern of catecholamine depletion is required. We hope to report in the future on such studies using neonatal rats injected with 6-ADA and a stereotaxic mapping of the NE and DA depletion in the brain regions of concern. Utilizing the differential effect of 6-ADA may shed additional light on this problem.

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