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Development of Tolerance to the Rewarding Effects of Self-Administered S(+)-Amphetamine

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McCOWN, T. J. AND R. J. BARRETT. *Development of tolerance to the rewarding effects of self-administered S(+)-amphetamine.* PHARMAC. BIOCHEM. BEHAV. 12(1) 137–141, 1980.—Rats were implanted with chronic intravenous cannulae and trained to bar press for intravenous, self-administered S(+)-amphetamine (AMPH). After establishment of a steady baseline at 0.25 mg/kg/reinforcement, the animals were removed from the test situation and subsequently injected three times a day for four days with increasing amounts of AMPH (total=78 mg/kg). Thirty-six hours after the last injection, the animals were tested for tolerance to self-administered AMPH, and all the animals increased the amount of drug intake by at least 45% over baseline. The brain disappearance of a 10 mg/kg IV dose of AMPH was measured for the chronic AMPH and saline treated subjects to test for the possibility of enzyme induction. No differences were found. These data indicate that drug self-administration in rats is a useful paradigm to study tolerance to the rewarding effects of AMPH and may be useful in understanding the mechanisms mediating the mood elevating properties of the drug observed in humans.

Self-administration S(+)-Amphetamine Brain disappearance Tolerance Reward

THE development of tolerance to the euphoric effects of S(+)-amphetamine (AMPH) is a well documented phenomenon in human subjects [5], yet the research efforts to demonstrate tolerance to AMPH effects upon various centrally mediated behaviors in rats, after chronic AMPH administration, have proven disappointing. One exception involves tolerance to AMPH-produced facilitation of rat self-stimulation behavior [8].

Investigators have traditionally measured either AMPH-produced disruption of some behavior, such as low rates of bar pressing for food reinforcement [12], or AMPH-induced changes in locomotor activity [6, 9, 14, 15]. When appropriate control groups have been used in the former behavioral paradigm, the tolerance demonstrated on behavioral measures has proven to result from the animals' learning to adapt to the drug's disrupting effects. Thus, controls given equal amounts of amphetamine, but not tested during the period of chronic administration do not show tolerance to the disruptive effects when subsequently tested [3].

Studies employing locomotor activity to assess tolerance to AMPH's facilitatory effect have generally failed to show a diminution of activity [9, 14, 15]. Using slightly different experimental conditions, Herman *et al.* [6] reported an initial increase and subsequent decrease of activity levels when AMPH (3 mg/kg/day) was administered via the food over a period of 9 months. The increased activity was observed during weekly test sessions over the first month of AMPH intake, but during subsequent monthly test sessions, the activity decreased until it returned to control values by the fourth month. Methodological differences make direct comparison of these results, with results from studies that administered a discrete dose of AMPH prior to the test session, difficult. Additionally, Tilson and Rech [14] have suggested that the behavioral pattern reported by Herman *et al.* [6] could be attributed to conditioned activity increases, followed by extinction behavior. Studies by Segal [13] evaluated the effects of chronic AMPH upon locomotor activity by measuring both the locomotor and stereotypic effects of AMPH over a wide dosage range before and after chronic AMPH treatment. As the acute dose of AMPH increases, locomotor activity is replaced with stereotyped behavior. This investigation showed that chronic AMPH administration increases the effectiveness of a given dose of AMPH to produce increased locomotor activity and stereotyped behaviors. Thus chronic AMPH treatment actually shifts the dose-response curve to the left.

We decided to utilize the behavioral paradigm of rat intravenous self-administration to investigate the development of tolerance to the rewarding effects of AMPH. Previous self-administration studies [5, 10, 11] have demonstrated that the drug produces rewarding effects in rats as it does in man. A desirable characteristic of the self-administration paradigm is that tolerance is indicated behaviorally by an increase in bar pressing which would be opposite in direction to possible non-specific debilitating effects attributed to AMPH.

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In an attempt to further understand the changes in self-administration behavior following chronic AMPH, brain disappearance of AMPH was measured following chronic exposure to the drug. This measure tests for the possibility that repeated AMPH might cause an induction of the enzymes responsible for AMPH metabolism.

METHOD

Subjects

All subjects were naive, male rats (F-344—Microbiological Associates, Walkersville, Maryland) weighing approximately 275–325 g (90–120 days old) at the time of surgery or biochemical determinations. They were housed individually in standard laboratory conditions with *ad lib* access to food and water and with a 12-hour light-dark cycle (7:00 a.m. to 7:00 p.m.). All manipulations and testing were performed during the light phase of the cycle.

Surgery

Subjects were anesthetized with 40 mg/kg pentobarbital, using chloral hydrate supplements as needed, and a one-way, silastic infusion cannula was implanted into the external jugular vein [4]. The other end of the tubing was connected to a cannula guide (Plastic Products, Roanoke, VA) which was anchored on the skull with two screws and cranioplastic cement. One dose of bicillin (125,000 units) was injected immediately after surgery to lessen the incidence of infection. Five days were allowed for recovery prior to any testing. The incidence of cannula failure prior to the establishment of a baseline response rate was approximately 60%, due to infection, fibrotic deposits in the vein, or vein necrosis.

Apparatus

All testing occurred in grid floored Leheigh Valley operant chambers (27 cm H × 30 cm W × 24 cm L), equipped with two response levers (2×3×1 cm). AMPH reinforcements were delivered by Sage Model 341 infusion pumps at a rate of 0.84 ml/min for five seconds. The operant chambers were housed in sound attenuated cubicles, and white noise was used to mask any extraneous auditory stimuli. Electromechanical equipment housed in an adjacent room was programmed for the behavioral contingencies and recorded all responses.

Procedure

A chronic AMPH treatment group (N=5) was trained to press the left operant lever for an AMPH infusion (0.125 mg AMPH sulfate/kg body weight/reinforcement) during one or two overnight sessions. On the right operant lever responses had no programmed effect but were recorded throughout all testing sessions as an indicant of non-specific AMPH activation. Subsequent to acquisition of the operant response, subjects were tested daily during four-hour sessions until a stable baseline of AMPH self-administration (± 2 responses) was established. Testing was then suspended while four subjects were injected IP with AMPH three times a day for four days (1 mg AMPH sulfate/kg initial dose, with 1 mg/kg increments up to a final injection of 12 mg/kg) [8]. In order to determine what effect chronic injections or increased handling had, one animal received chronic saline injections instead of AMPH. Thirty-six hours after the last injection the subjects were again tested for AMPH self-administration.

Following the above procedure, an additional 4 subjects were prepared for self-administration. The purpose of testing these animals was to replicate the previous results and to independently determine for each subject what effect a known reduction in drug concentration would have on response rate prior to testing for tolerance. As with the tolerance group, the dose-response group was trained in 1 or 2 overnight sessions. Due to the large incidence of vein necrosis and subsequent cannula failure, the dose-response subjects were tested over 1.5 hour sessions, instead of 4 hour sessions to lessen the stress to the experimental preparation in hopes of prolonging the subjects' viability. After the subjects had self-administered the same number of reinforcements (0.25 mg AMPH sufate/kg/rein) per session, within a range of 2 reinforcements, over 3 consecutive test days, the concentration of AMPH was decreased by one-half. Thus, on the first and third days each response produced an infusion of 0.25 mg AMPH sulfate/kg while each response on ths second day produced an infusion of 0.125 mg AMPH sulfate/kg. Testing was then suspended while the subjects received the chronic AMPH injection regimen, as described above, and thirty-six hours after the last injection were tested for self-administration at the baseline dose (0.25 mg AMPH sulfate/kg/rein).

In order to assess the metabolism of AMPH, animals received either chronic AMPH, as described above, or equivalent chronic saline injections, and thirty-six hours after the last injection the brain disappearance of a 10 mg/kg IV dose of AMPH was determined by measuring whole brain levels of AMPH at 15, 30, 60, and 120 minutes after the IV injection using the methyl orange assay for AMPH [1]. Each time point consisted of at least four animals from a chronic AMPH group and from a saline injected control group.

RESULTS

Initially during training periods all animals made responses on both bars, but as the contingencies were learned, the response on the inactive right bar abated throughout the remaining test sessions. As can be seen in Fig. 1, baseline self-administration was very consistent. After chronic AMPH injections, each subject in the tolerance group increased the amount of self-administered AMPH by at least 50% over baseline. Figure 1 also shows that the saline injected control animal did not vary from the baseline self-administration. In order to assess the duration of tolerance, three of the four chronic AMPH-treated subjects were tested one month after the test for tolerance while one subject was tested weekly for one month after the test for tolerance. The three subjects, tested at the monthly interval, returned to baseline levels of self-administered AMPH, but the subject, tested weekly, continued to self-administer elevated amounts of AMPH until cannula failure occurred during the fourth week. Apparently, weekly AMPH intake is sufficient to maintain the tolerant state.

Figure 2 shows ths data for the second group of subjects. It can be seen that reducing the concentration of AMPH results in a corresponding compensatory increase in the number of reinforcements, strengthening the interpretation that responding was contingent upon AMPH reinforcement. After the group had received chronic AMPH injections, the

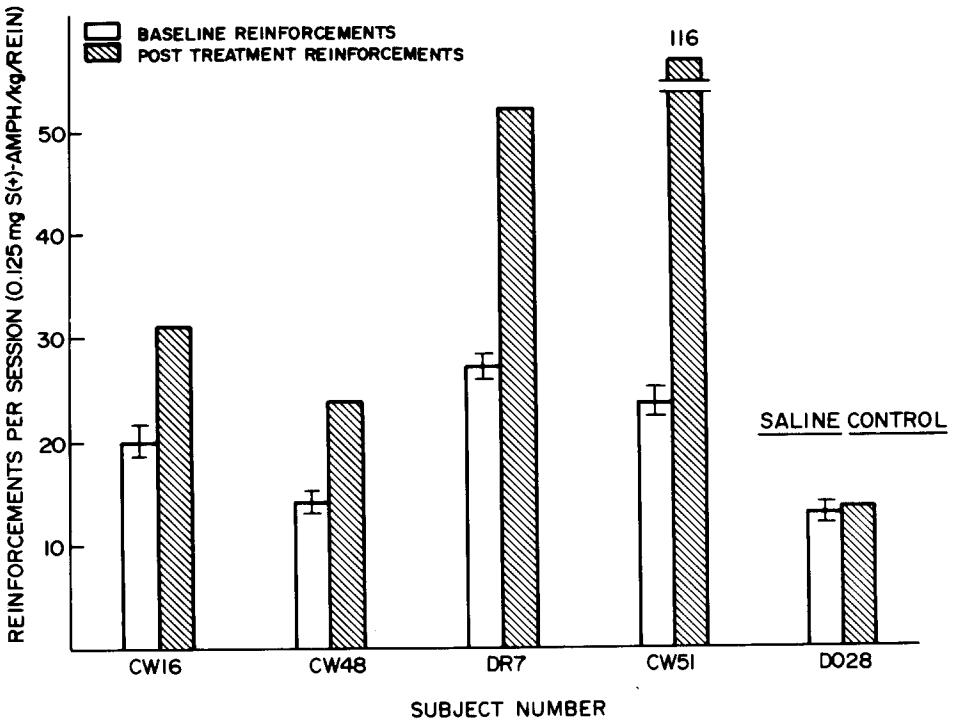

FIG. 1. The effects of chronic AMPH injections (see procedure) upon baseline level (± SEM) of self-administered AMPH over 4-hour test sessions. Each subject exhibited at least a 50% increase in self-administered AMPH after chronic treatment. A saline injected control shows no change from baseline.

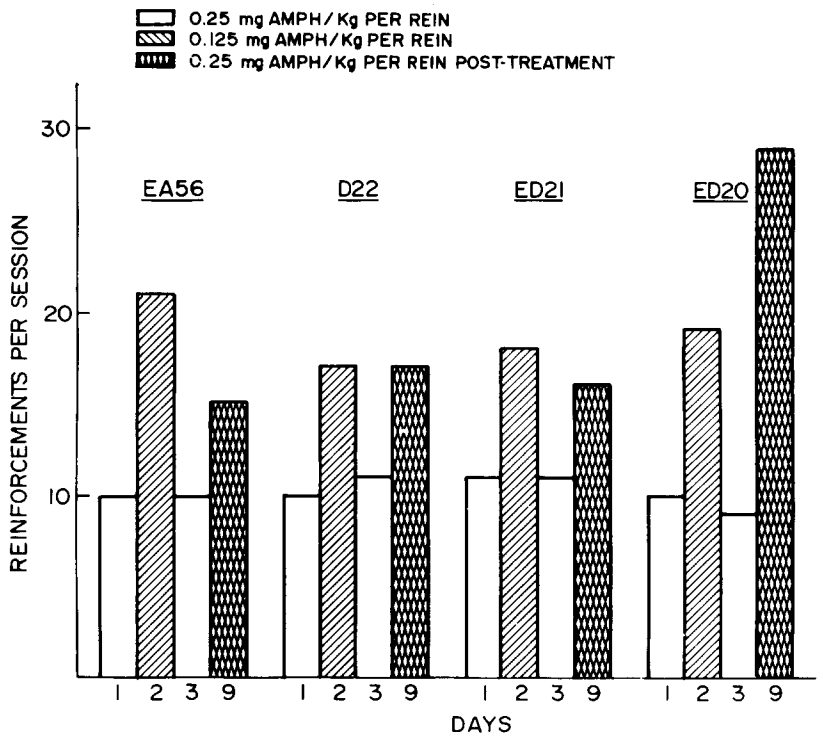

FIG. 2. Self-administration of two doses of AMPH (0.125 mg/kg/rein and 0.25 mg/kg/rein) during 1.5 hour sessions, over three days, demonstrates an inverse dose-response relationship. The test session after chronic AMPH treatment (see procedure) shows at least a 45% increase in self-administered AMPH (0.25 mg/kg/rein) over the baseline self administration.

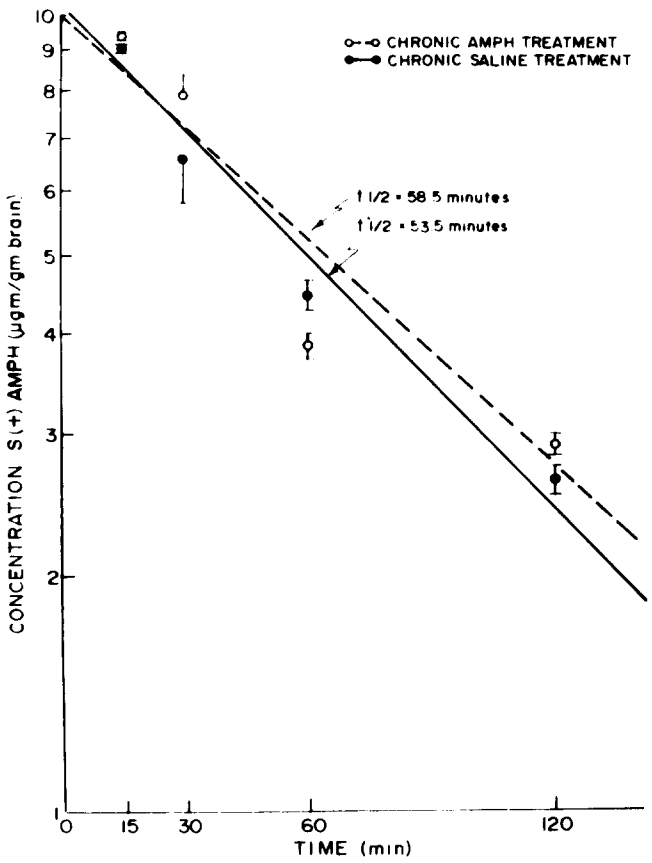

FIG. 3. The brain disappearance of a 10 mg/kg IV dose of AMPH sulfate for chronic AMPH and chronic saline treatment groups, as measured by the methyl orange assay. Each time point determination consists of at least 4 animals. No difference in $t^1/_2$ was found (analysis of variance, 2 (chronic AMPH × saline) × 4 (15, 30, 60, 120 minutes), $F<1$).

self-administration of AMPH increased by at least 45% above baseline levels.

The metabolic fate of AMPH was assessed by the measurement of brain disappearance of AMPH for AMPH-treated and saline-treated groups. There was no significant difference in brain disappearance of the test dose of AMPH between the two groups (analysis of variance, 2 (chronic AMPH × saline) × 4 (15, 30, 60, 120 minutes), $F<1$), as shown in Figure 3. The half-life of AMPH was 58.5 minutes for the AMPH-treated group and 53.5 minutes for the saline-control group.

DISCUSSION

The increased self-administration of AMPH indicates the development of tolerance to the drug's reward properties. Since tolerance to the rewarding effects of AMPH in this paradigm results in increased behavioral output, the interpretation of the data is not confounded by possible nonspecific debilitating effects of chronic AMPH, which would be expected to reduce responding. The compensatory increase in responding which occurred when the dose of AMPH per reinforcement was reduced demonstrates that the response is contingent upon AMPH delivery, not some random activity effect AMPH infusions might produce. If AMPH facilitation of activity were responsible for responding, a change in the drug dose per reinforcement would produce a direct dose-response relationship, however, an inverse dose response relationship was observed. These results verify that responding was contingent on AMPH reinforcement.

Since the brain disappearance of AMPH is similar following chronic AMPH or saline treated subjects, one can conclude that the chronic AMPH injections have no significant effect upon AMPH metabolism. Thus, the tolerance to AMPH's rewarding properties is probably mediated by some central nervous system adaptation brought about by chronic AMPH exposure. Additionally, Barrett [2] has recently reported the development of tolerance to AMPH in a two-lever drug discrimination task.

The mechanism of this tolerance phenomenon could provide insight into central nervous system adaptive mechanisms in the reward system which would be of interest in understanding affective disorders in man. Further delineation of this CNS adaptation is currently being investigated.

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