Pharmacology Biochemistry and Behavior, Vol. 1, pp. 265–270. ANKHO International Inc., 1973. Printed in the U.S.A.

Long-Term Aversion to a Saccharin Solution Induced by Repeated Amphetamine Injections[1]

ROBERT J. CAREY

Syracuse Veterans Administration Hospital and State University of New York Upstate Medical Center
Syracuse, New York 13210

(Received 8 June 1973)

CAREY, R. J. *Long-term aversion to a saccharin solution induced by repeated amphetamine injections.* PHARMAC. BIOCHEM. BEHAV. 1(3) 265–270, 1973.—In rats repeated 2 mg/kg injections of *d*-amphetamine given 30 min after imbibition of a 0.1% saccharin solution proved to be as effective in reducing saccharin intake as 2 mg/kg injections of *d*-amphetamine given 30 min before presentation of this saccharin solution. Treatment with alpha-methyl-para-tyrosine blocked the effect on saccharin intake of amphetamine given before, but not amphetamine given after presentation of the saccharin. After amphetamine injections were terminated, subsequent saccharin vs. water preference testing showed that the amphetamine injections given after but not the injections given before resulted in a long-term aversion to the saccharin solution. In a second experiment, the 2 mg/kg dose of amphetamine was shown to facilitate intracranial self-stimulation as well as to induce an aversion to saccharin.

d-Amphetamine Learned aversion Alpha-methyl-para-tyrosine

SEVERAL recent studies have suggested that amphetamine is an effective drug for producing learned aversions [1, 2, 3, 6]. Specifically, rats gradually learn to reject scented foods which contain amphetamine. Significantly, this rejection persists after the amphetamine is removed from the food. Also, amphetamine solutions which are initially neutral become rejected by rats after repeated imbibition. More directly, amphetamine injections given after ingestion of a saccharin solution result in a significantly decreased saccharin intake in a subsequent presentation of the saccharin. The present study was undertaken to compare directly the anorexic efficacy of repeated amphetamine injections given before vs. amphetamine injections given after presentation of a saccharin solution. In addition, a comparison was made of the susceptibility of these two injection procedures to blockage by an amphetamine antagonist, alpha-methyl-para-tyrosine (αMPT). Finally, possible long-term differences were evaluated in a two-bottle saccharin vs. water choice after injections were discontinued.

EXPERIMENT 1

Method

Animals. Twenty-four naive, male Sprague-Dawley rats, approximately 110 days old at the start of testing, were used. Throughout experimentation all rats were maintained in individual cages in a temperature- (72° ± 4° F), humidity- (60% ± 5%), and illumination- 12 hr light, 12 hr dark) controlled room. Food pellets were always available in attached feeders.

Procedure. The rats were randomly divided into four groups of six each. Two groups received subcutaneous injections of *d*-amphetamine HCL (K and K Laboratories, Jamaica, N. Y.) and the other two received saline. Initially, all animals were adapted to 23-hr of water deprivation. After the 60-min water intakes had stabilized for all groups, they were offered 0.1% saccharin for 30 min. One amphetamine group (2 mg/kg of *d*-amphetamine HCL in a concentration of 4 mg/cc) and one saline group (0.5 cc/kg) were injected 30 min before and the other amphetamine group (2 mg/kg *d*-amphetamine HCL) and saline group (0.5 cc/kg) were injected 30 min after presentation of the saccharin solutions. Subsequently, 30-min saccharin presentations were made every fourth day for a total of 13 saccharin presentations. The same injection procedure was followed for each saccharin presentation. Intakes of the saccharin solution as well as intakes of water on the noninjection days were always recorded. Two days after the 13th saccharin presentation all animals were treated for two days with *dl* alpha-methyl-para-tyrosine methylester HCL (Sigma Chemical Co., St. Louis) (100 mg/kg every 12 hr), a known amphetamine antagonist [7], and given another saccharin presentation using the same injection procedure. Following this test no more injections were given and all animals were placed on ad lib food and water. After one week on ad lib, all animals were given a two-bottle 0.1% saccharin vs. water preference test for 50 days. Bottle positions were changed every 24 hr and intakes recorded to the nearest 0.1 g.

[1] This study was supported in part by the Veterans Administration Research Project No. 1284-01.

Results

Over-all, the saccharin intake results for each group were quite homogeneous. However, one exception occurred in the groups which received the post-saccharin injection of amphetamine. Rather than obscure this one animal's deviancy in the over-all mean results for this group, or in conventional statistical analyses some additional insight into the effect of this drug treatment seemed afforded by presenting this animal's intake results separately. Accordingly, Fig. 1 shows the 30-min saccharin intakes over the 13 saccharin presentations for this animal as well as the mean intakes for each of the four treatment groups. As can be seen in Fig. 1, throughout the first 13 saccharin presentations, animals given amphetamine before had very low saccharin intakes of approximatley 1 cc. Five of the animals given amphetamine after the saccharin presentation gradually decreased their saccharin intake and from presentations 6 through 13 also had low intakes of approximately 1 cc. The animal which was the exception, however, exhibited a recovery of the saccharin intake. The two saline groups saccharin intakes were comparable and over the last six presentations every animal in each of the groups had an intake which was greater than 20 cc. It is important to note that no statistically significant differences occurred among groups in terms of water intake on any of the days preceding the saccharin tests. The saccharin intake results following treatment with alpha-methyl-para-tyrosine are

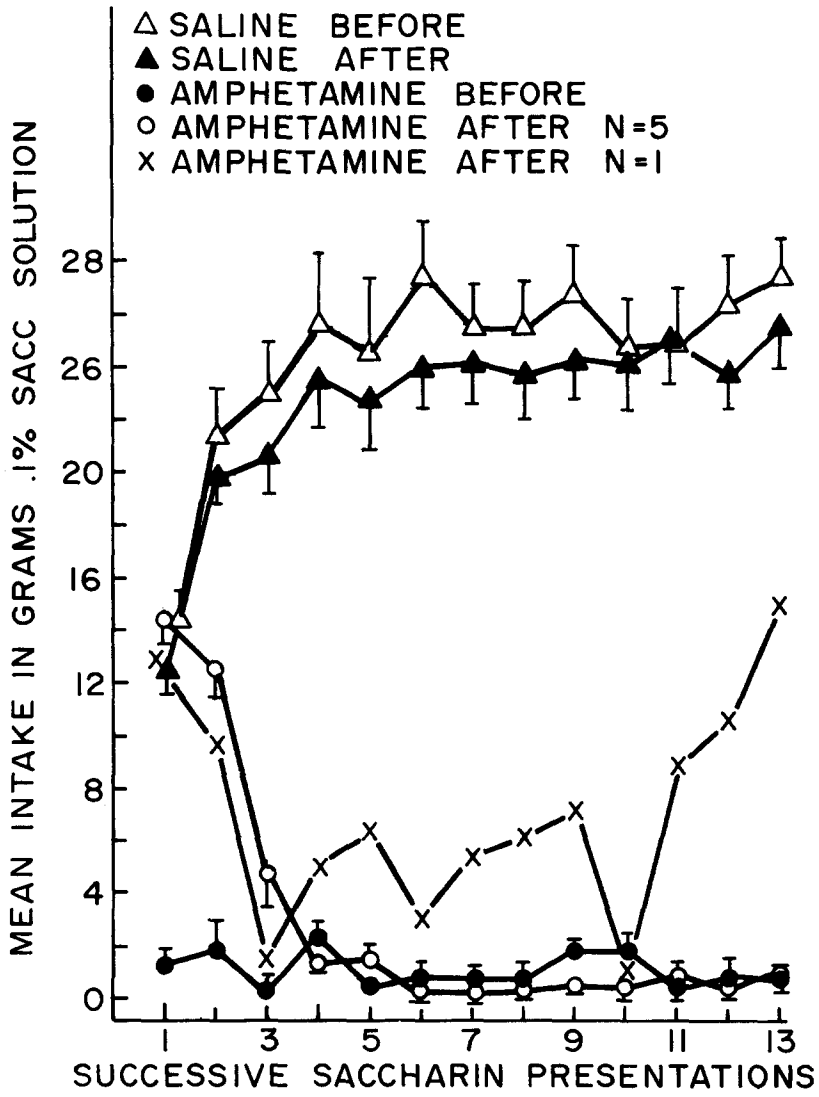

FIG. 1. Thirty-min intakes of the 0.1% saccharin solution over the first 13 injection sessions. The vertical bars indicate the standard errors of the means.

presented in Table 1. This drug treatment blocked the effect of amphetamine in the before but not the after group. The animal in the amphetamine after group which had exhibited recovery had an increased saccharin intake following alpha-methyl-para-tyrosine treatment. The saccharin intake of the saline groups was unaffected by the alpha-methyl-para-tyrosine. The two-bottle preference test results shown in Fig. 2 were dramatic and consistent. All amphetamine before animals showed an essentially complete preference for the 0.1% saccharin over water. Five amphetamine after animals showed a nearly complete aversion to saccharin but had normal water intakes over the 50 days of testing. Interestingly, the one amphetamine after animal which had demonstrated recovery during the amphetamine testing over the first 12 days also exhibited a complete aversion to the saccharin. This animal then went through a transition phase over several days in which saccharin was increasingly ingested and then exhibited a near complete saccharin preference. Although not shown in Fig. 2, all saline-injected rats showed an essentially complete saccharin preference (95–100%).

TABLE 1

MEANS AND STANDARD ERRORS OF SACCHARIN INTAKES IN GRAMS FOLLOWING ALPHA-METHYL-PARA-TYROSINE TREATMENT (SESSION 14) AND THE PRECEDING SACCHARIN PRESENTATION (SESSION 13)

Treatment Groups	Saccharin Presentation Session 13	Session 14
Amphetamine		
Before (n = 6)	0.58 ± 0.20	14.1 ± 1.7
After (n = 5)	0.76 ± 0.20	0.63 ± 0.26
After (n = 1)	15.4	19.0
Saline		
Before (n = 6)	27.8 ± 2.2	25.5 ± 1.7
After (n = 6)	25.3 ± 1.1	23.9 ± 0.8

EXPERIMENT 2

Amphetamine has been frequently reported [5] to facilitate intracranial self-stimulation (ICSS) rates in rats. Experiment 2 was undertaken to directly evaluate the effect of the 2 mg/kg dose of amphetamine on ICSS under the experimental conditions employed in Experiment 1.

Method

Animals. Nine adult, male, Sprague-Dawley rats, 400–500 g in weight, were used. Housing conditions were the same as in Experiment 1.

Surgery. A bipolar stainless steel electrode (Plastic Products, Inc., Roanoke, Va.) insulated except at the tip was implanted in each rat. Standard stereotaxic procedures were used to position and secure each electrode in the medial forebrain bundle at the level of the lateral hypothalamus. The stereotaxic coordinates used were: 1.2 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.9 mm below the skull surface. The incisor bar was fixed 3.2 mm above the interaural line. At the conclusion of the experiment, the location of the electrode tips within the medial forebrain bundle at the level of the lateral hypothal-

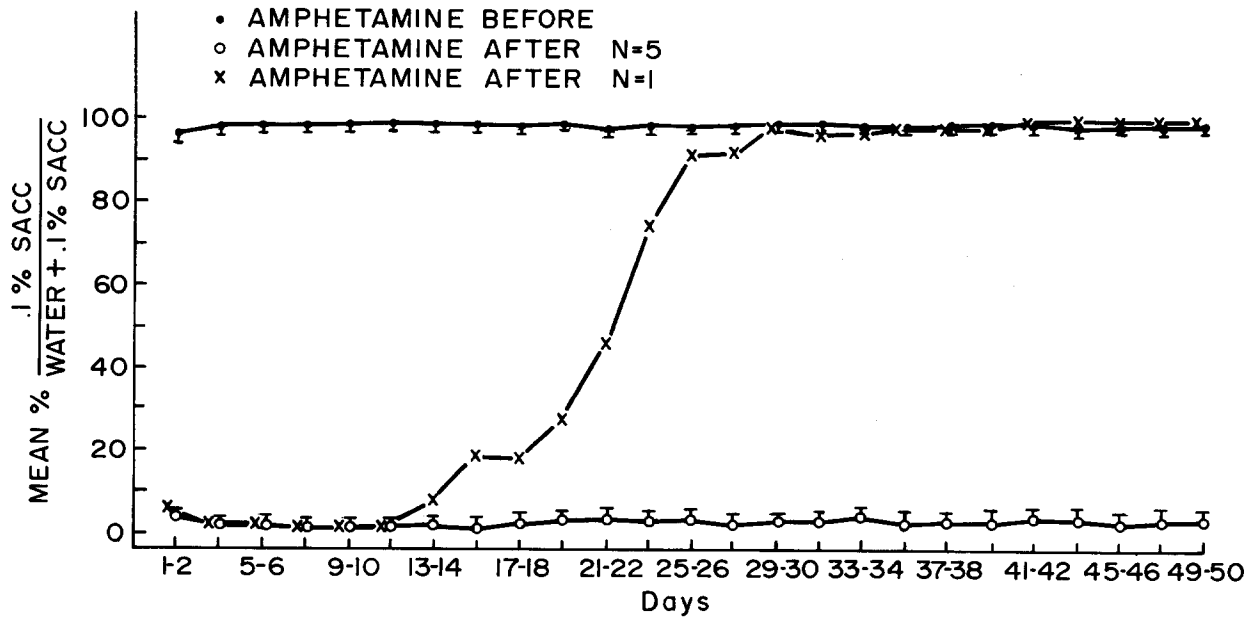

FIG. 2. The 0.1% saccharin vs water preference ratios over 50 days of testing. The vertical bars indicate the standard errors of the means.

amus were confirmed in three of the rats using standard histological procedures. The remaining six implanted rats were subsequently used in another unrelated experiment so that histological confirmation was not available.

Procedure

Saccharin testing. After it was confirmed that all rats would self-stimulate, they were divided into three groups of three each equated for ICSS performance. As in Experiment 1, all rats were first adapted to the 23-hr water deprivation schedule. After the 1-hr water intakes had stabilized, the rats were offered the 0.1% sodium saccharin solution for 30 min on each of the test days. There were a total of four saccharin test days which were separated from one another by three days on the one hr water intake schedule. The groups were treated in the following ways: one group, ICSS-A, received a 2 mg/kg subcutaneous injection of *d*-amphetamine 30 min after the presentation of the saccharin. Then 15 min after the amphetamine injection the rats were placed in the operant chamber and allowed to self-stimulate for 30 min at the same current level as had been used during baseline testing. The second group, ICSS-NA, received a subcutaneous saline injection 30 min after the saccharin presentation and then 15 min later was allowed to self-stimulate for 30 min. The third group, NICSS-A, received the 2 mg/kg injections of *d*-amphetamine 30 min after the saccharin presentation, but received no self-stimulation test.

ICSS testing. All stimulation tests were done in two 10 1/2 x 12 x 9 1/2 in. operant chambers housed individually in sound attenuating enclosures (LVE No. 1417). Each chamber was equipped with a 1 1/8 x 3/8 in. lever which required a force of 15 g to activate an attached microswitch. A mercury-swivel commutator mounted above the chamber connected the electrode to the stimulation equipment. A Grass Brief Pulse stimulator Model BPS 1 set to deliver a bipolar square wave of 60 Hz and 0.2 msec duration served as the source of current for brain stimulation. Applied voltage and current, calculated from the voltage drop across a 1/kΩ resistor in series with the rat, were monitored continuously on an oscilloscope. The stimulation duration was 0.5 sec but current, of course, depended on the particular rat's threshold. Before testing with the saccharin solutions all rats were stabilized under the 23-hr water deprivation schedule for bar pressing rates between 2,000–3,000 responses per hr. Current parameters established during this baseline testing were maintained throughout the experiment.

Results

As can be seen in Table 2 the amphetamine injections both decreased saccharin intake and facilitated self-stimulation. Although only mean self-stimulation rates are shown in Table 2, all amphetamine rats on all test sessions except the first session increased responding from between 1.75 to 2.25 times their baseline rate. On the first test session under amphetamine, one of the rats failed to make any responses, subsequently on the following three test sessions this rat under amphetamine responded at least two times its baseline rate.

DISCUSSION

The results of this study dramatically highlight the effectiveness of amphetamine in inducing learned aversions. In fact, amphetamine aversion is as effective as the direct anorexic action of amphetamine in suppressing intake of a saccharin solution. In contrast to the direct anorexic action of amphetamine, the learned aversion induced by amphetamine is not blocked by alpha-methyl-para-tyrosine. This result is interesting in that it suggests that the learned aversion is not simply a conditioned anorexic effect of amphetamine. That is, it might be argued that the taste of saccharin as a consequence of conditioning comes to evoke the neurochemical effects of amphetamine (i.e., release of brain norepinephrine). Also the durability of the aversion effect of amphetamine shown in the two-bottle preference test suggests that amphetamine administration after ingestion of a particular food may be much more effective than the traditional premeal administration of amphetamine in curbing appetite. Indeed, the finding that the animal in the

TABLE 2

MEANS AND STANDARD ERRORS OF 30 MIN 0.1% SACCHARIN INTAKES AND MEANS OF 30 MIN LEVER-PRESSING SESSIONS FOR ELECTRICAL INTRACRANIAL SELF-STIMULATION (ICSS) OF THE BRAIN

Group	Sessions			
	Saccharin Intakes			
	1	2	3	4
ICSS-A (n = 3)	18.3 ± 0.82	10.3 ± 0.9	10.5 ± 2.5	7.2 ± 3.3
NICSS-A (n = 3)	19.3 ± 1.8	9.4 ± 1.9	10.0 ± 3.8	6.9 ± 3.1
ICSS-NA (n = 3)	14.3 ± 1.5	20.2 ± 2.1	22.7 ± 2.7	24.2 ± 1.9
	Self-Stimulation			
	Baseline	1	2	3	4
ICSS-A (n = 3)	1192.6	1367.3	2165.0	2493.6	2947.3
ICSS-NA (n = 3)	1564.0	1363.6	1286.0	1473.3	1530.3

amphetamine after group which exhibited recovery in the 30 min saccharin tests, but then initially showed a complete aversion to saccharin in the choice test, reinforces the efficacy of the amphetamine after treatment in altering food preference in the nondeprived state.

The results of this and other studies which indicate an aversive aspect to amphetamine appear to conflict with other animal experiments which have shown that amphetamine self-administration is positively reinforcing [4] or that amphetamine enhances positively reinforcing brain stimulation [5]. Indeed, as shown in Experiment 2, the dose of amphetamine used in this study to induce an aversion to saccharin also facilitated self-stimulation behavior. This apparent discrepancy seems most readily resolved by considering the behavioral modality measured as the critical variable. Positive reinforcement effects have been observed where the behavioral modality is motor behavior. The effect of amphetamine on motor behavior, of course, is facilitative. In contrast, the aversive effects as shown in this report are manifested in appetitive behavior. Consistently, amphetamine decreases appetitive behavior.

REFERENCES

1. Carey, R. J. Acquired aversion to amphetamine solutions. *Pharmac. Biochem. Behav.* **1**: 227–229, 1973.
2. Cappell, H. and A. E. LeBlanc. Conditioned aversion to saccharin by a single administration of Mescaline and *d*-amphetamine. *Psychopharmacologia* (Berl.) **22**: 352–356, 1971.
3. LeMagnen, J. Peripheral and systemic actions of food in the caloric regulation of intake. *Ann. N. Y. Acad. Sci.* **157**: 1126–1157, 1969.
4. Pickens, R. and W. C. Harris. Self-administration of *d*-amphetamine by rats. *Psychopharmacologia* (Berl.) **12**: 158–163, 1968.
5. Stein, L. Amphetamine and neural reward mechanisms. In: *Animal Behavior and Drug Action*, edited by H. Steinberg. Bostong: Little, Brown, 1964, 91–113.
6. Stolerman, I. P., K. Kumar and H. Steinberg. Development of morphine dependence in rats: Lack of effect of previous ingestion of other drugs. *Psychopharmacologia* (Berl.) **20**: 321–336, 1971.
7. Weissman, A., K. B. Koc and S. S. Tenen. Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmac. exp. Ther.* **151**: 339–352, 1966.