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Amphetamine-Induced Taste Aversion: A Comparison of d- Versus l-Amphetamine

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CAREY, R. J. AND E. B. GOODALL. *Amphetamine-induced taste aversion: a comparison of d- versus l-amphetamine.* PHARMAC. BIOCHEM. BEHAV. 2(3) 325–330, 1974. – A comparison of the effects in rats of four dose levels of d- and l-amphetamine (0.5, 1.0, 2.0 and 4.0 mg/kg) on development of a taste aversion to a 0.1% saccharin solution showed that d-amphetamine was approximately 4 times as potent as l-amphetamine in inducing a taste aversion to saccharin. The aversion was obtained in both forced- and free-choice tests. A 2–4 fold differential in efficacy was found when the same dose levels of both amphetamine isomers were tested for their effects in reducing water intake. The approximately 4-fold greater effectiveness of the d-isomer suggested that the taste aversion may be mediated by a dopaminergic system. In addition, it was suggested that the taste aversion behavior represented a conditioned anorexic effect rather than being indicative of a noxious or aversive consequence of the amphetamine.

Taste aversion Amphetamine Anorexia

SEVERAL recent studies [1, 2, 15] have demonstrated that amphetamine is a highly effective drug for inducing a taste aversion in the rat. This effect is of considerable importance since it indicates that amphetamine can act as a powerful negative reinforcer. Thus far, however, amphetamine-induced taste aversions have been reported primarily for relatively high dose levels of d-amphetamine (2.0 mg/kg or greater). Significantly, this dose-level range approximates the dosage levels at which amphetamine evokes stereotypy in the rat [11]. Since stereotypy effects of amphetamine have been extensively studied and appear to result from an effect on brain dopamine [12], one aspect of the present study was to determine whether the taste aversion phenomenon is similarly linked to a dopaminergic action of amphetamine. This determination was attempted by making a comparison of the relative potencies of the d- versus l-isomers of amphetamine in producing a taste aversion. As suggested by the recent studies of Harris and Baldessarini [7], the d-isomer is approximately four times more potent than the l-isomer in blocking dopamine reuptake in striatal synaptosomal preparations. Accordingly, an approximately four-fold greater potency of the d-isomer versus the l-isomer for inducing a taste aversion would be indicative of a dopaminergic effect. In addition to this attempt to identify the catacholaminergic system which underlies the taste aversion effect of amphetamine, the present study also measured the anorexic potencies of the amphetamine treatments to determine if a significant relationship exists between amphetamine anorexia and amphetamine-induced taste aversion. Finally, taste aversion testing was extended to the free-choice situation in order to evaluate the durability of this phenomenon.

METHOD

Animals

Thirty-six naive, male, Sprague-Dawley rats, 400–500 g in weight, selected out of a larger group of 40 rats, were used. The rats were individually housed in a temperature- (72°F ± 2°), humidity- (60% ± 5%), illumination- (12-hr light, 12-hr dark) controlled room. Testing was conducted between 10 a.m.–2 p.m. and all solutions were presented to the animals in their home cage.

Phase I: Saccharin Aversion Procedure

Basically, the typical saccharin aversion paradigm was used [6]. Initially, all animals were adapted to handling for 1 wk. After this acclimation period, the rats were placed on a 1-hr of water per day maintenance schedule. After 1 wk

on the water deprivation regimen, the saccharin aversion induction procedure commenced. On the first test day all rats were offered a 0.1% saccharin solution in place of water for 30 min. The saccharin presentations were temporally spaced so that each rat could be injected approximately 15 min after the saccharin solution was removed. Out of an original group of 40 rats, 9 groups of 4 each were formed that were comparably matched on the basis of their initial 30 min intake of the saccharin solution. One group received 0.9% saline, 4 groups received d-amphetamine HCl (K and K Laboratories, Jamaica, N.Y.) and 4 groups l-amphetamine sulfate (Sigma Chemical Co., St. Louis, Mo.). The d-amphetamine dose levels were 0.5, 1.0, 2.0, and 4.0 mg/kg calculated as the salt. The l-amphetamine doses were adjusted so that the doses of base of l-amphetamine were equivalent to the doses of base of d-amphetamine. The d- and l-amphetamine were dissolved in 0.9% saline and all injections were equal in volume (1 cc/kg) and were injected intraperitoneally (IP).

Since Carey [3] has shown that an amphetamine-induced taste aversion is enhanced by repeated saccharin-amphetamine pairings, a total of five saccharin presentations followed by amphetamine injections were used in this experiment in order to increase the likelihood of detecting a saccharin aversion effect. Successive saccharin-amphetamine pairings were always separated by 3 days of water intake (60 min per day). All saccharin and water intakes were measured to the nearest 0.1 g.

Phase II: Adipsic Effects of Amphetamine Injections

The saccharin-aversion paradigm assesses the conditioned inhibitory effects of amphetamine injections on drinking behavior. The next test was conducted to measure the unconditioned inhibitory effect of the amphetamine injections on drinking behavior.

After the fifth and final saccharin-amphetamine pairing, the rats continued to be maintained on the 1-hr per day water intake regimen for an additional four days. On the fifth day, 15-min before presentation of the water, each rat was given the same injection it had been given for the saccharin-aversion procedure. The subsequent 60-min water intake was recorded for each rat. The adipsic effect was assessed by comparing a rat's water intake after the injection with its mean water intake for the preceding 3 days of no injection.

Phase III: Long-term Saccharin Aversion Test

Since the saccharin-aversion in Phase I was based upon a forced saccharin choice under deprivation conditions, an additional test phase was conducted to include a free choice test under ad lib conditions. This test procedure commenced after the rats had been on ad lib water for 1 wk. Briefly, all animals were offered a two-bottle choice of the 0.1% saccharin solution versus water under ad lib conditions. Two 5-day periods of saccharin versus water choice separated by 1 wk of water ad lib were measured.

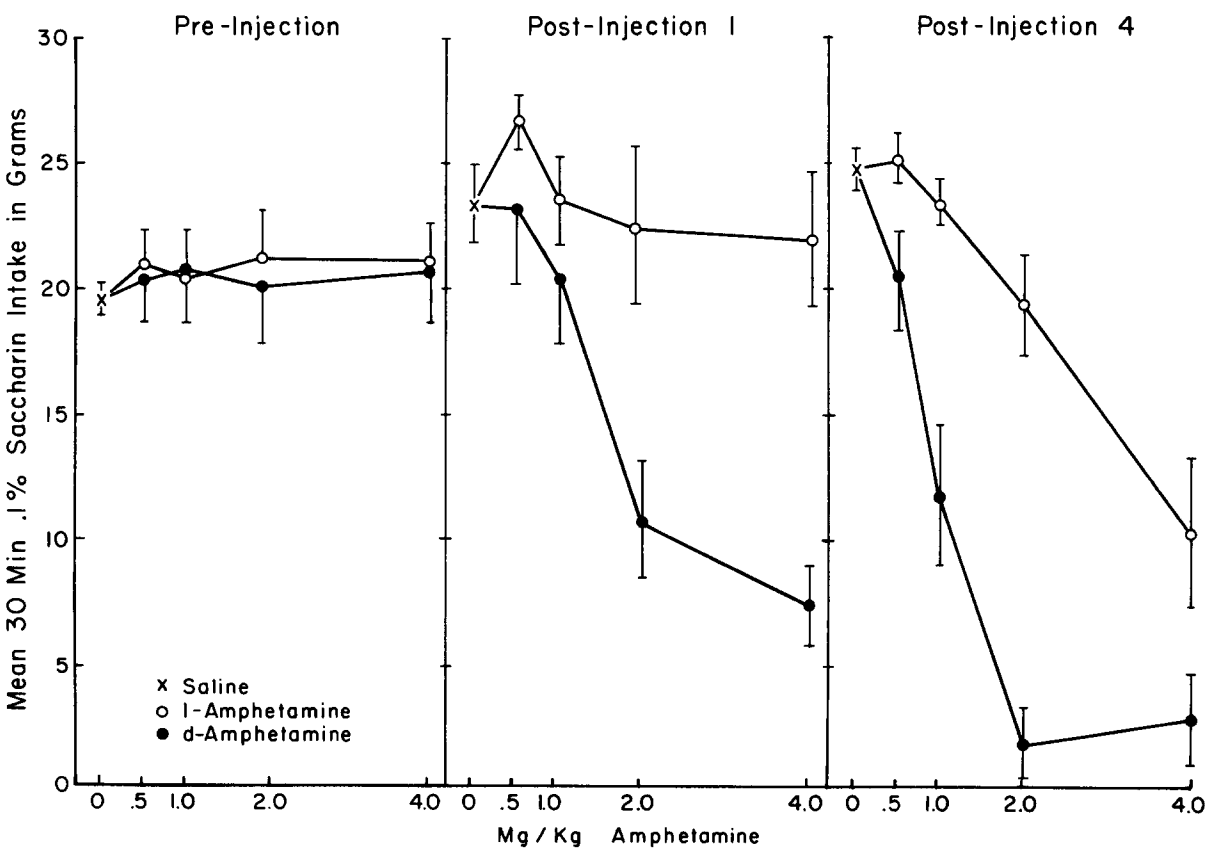

FIG. 1. Mean 30-min saccharin intakes for each treatment group on the first (pre-injection), second (post-injection 1) and fifth (post-injection 4) presentation of the saccharin solution. Vertical bars indicate the standard errors of the means.

The saccharin and water bottle positions were alternated daily and intakes were recorded to the nearest 0.1 g.

RESULTS

Figure 1 presents the 30 min 0.1% saccharin intakes on the first, second, and fifth saccharin presentations. As indicated in Fig. 1, the first saccharin presentation shows essentially equivalent saccharin intakes for all groups. This result was expected since the intakes occurred prior to the amphetamine and saline injections. The intakes on the second saccharin presentation, however, showed that a marked dose-related decrease in saccharin intake occurred for the d-amphetamine treatment groups after only one saccharin and d-amphetamine pairing. In contrast, no effect on saccharin intake was found for l-amphetamine after only one saccharin l-amphetamine pairing. By the fifth saccharin presentation (after 4 saccharin-amphetamine pairings) a dose-related decrease in saccharin intake was evident for the l- as well as d-amphetamine treatment groups. This result agrees with a previous report by Carey [3] that repeated saccharin-amphetamine pairings further decreases saccharin intakes. Over-all, the d-amphetamine isomer appears to be 4 times as potent as the l-amphetamine isomer in decreasing saccharin intake. This 4 fold differential is most apparent on post-injection day 4 where the 2.0 mg dose of l-amphetamine was approximately equivalent in effect to the 0.5 mg dose of d-amphetamine and the 4.0 mg dose of l-amphetamine was equivalent in effect to the 1.0 mg dose of d-amphetamine. Statistical comparisons of the d- and l-amphetamine treatments showed that the saccharin intakes of the groups did not differ significantly for the first saccharin presentation, $F(1,6) = 1.1$, $p>0.25$, but did differ significantly on the second, $F(1,6) = 17.56$, $p<0.01$, and fifth, $F(1,6) = 13.1$, $p<0.01$ saccharin presentations. While these differences in intake existed among treatment groups for the saccharin solution, the 60 min water intakes for all groups on days immediately preceding the saccharin presentations were essentially identical.

Figure 2 presents the effects of the sixth d- and l-amphetamine treatments given before the 60-min water presentation. Overall, both the d- and l-amphetamine isomers produced dose-related decreases in water intake with the d-amphetamine having a potency 2–4 times that of the l-amphetamine. The decrease in water intake produced by 1.0 mg of l-amphetamine was equivalent to 0.5 mg of d-amphetamine and 2.0 and 4.0 mg of l-amphetamine were equivalent to 1.0 mg of d-amphetamine. Statistical evaluation of the d- versus l-amphetamine indicated that the differences were highly significant statistically $F(1,6) = 53.2$, $p<0.01$.

Finally, Fig. 3 shows the saccharin and water intakes of the saline and amphetamine treatment groups when offered a choice between saccharin and water under ad lib conditions. The first and ninth days of saccharin versus water

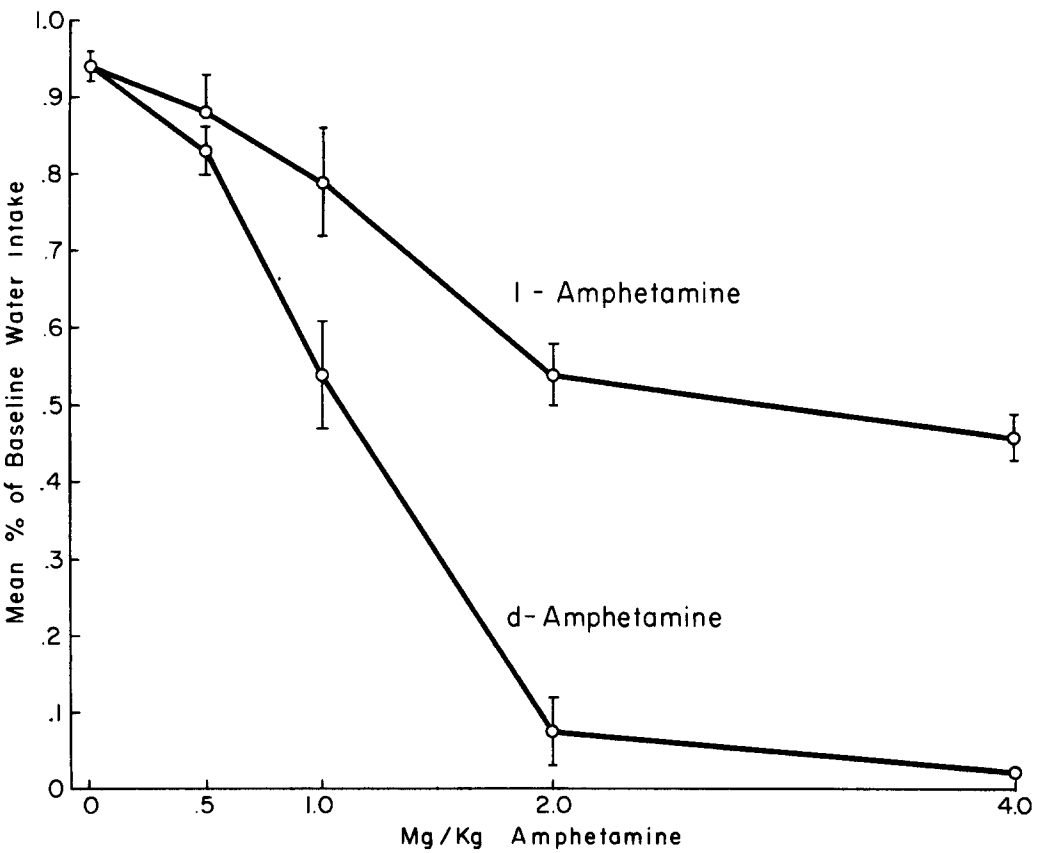

FIG. 2. Mean 60-min water intakes expressed as a percentage of baseline intake following saline and amphetamine injections. Vertical bars designate the standard errors of the means.

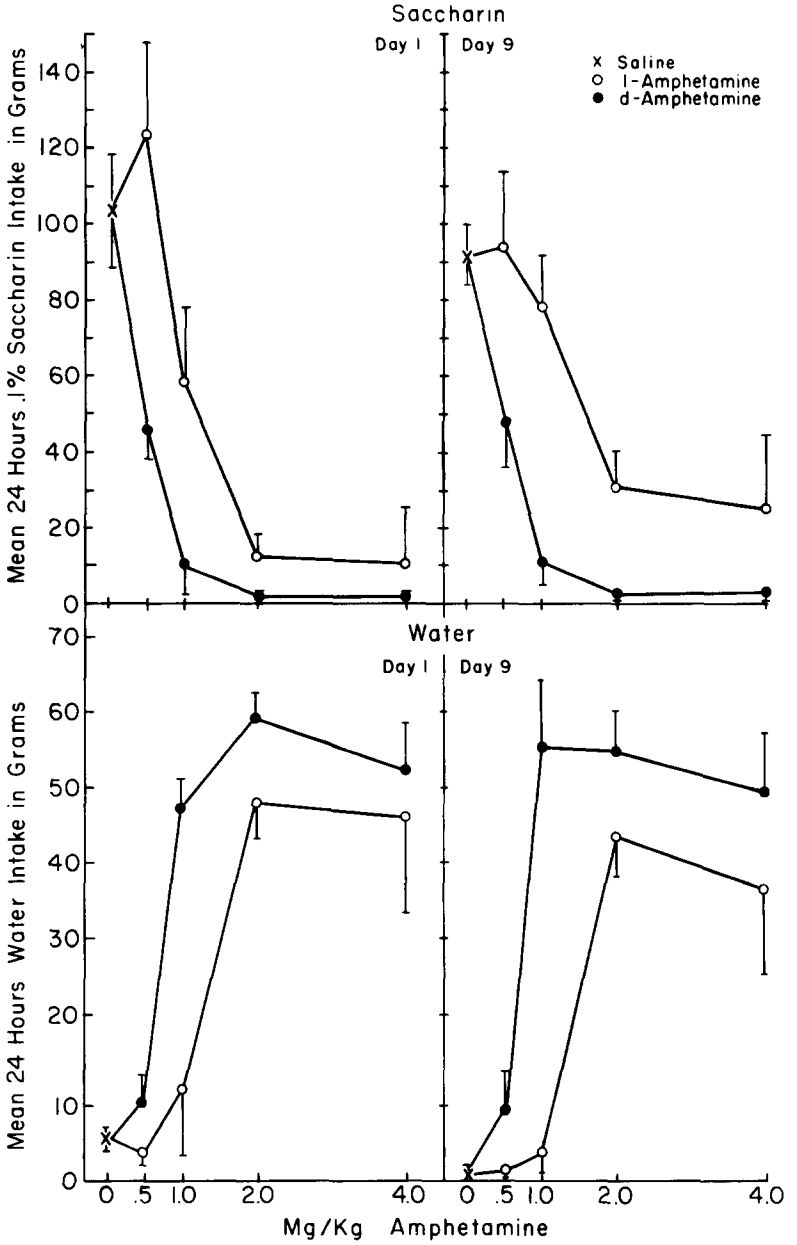

FIG. 3. Mean saccharin and water intake on the first and ninth days of the two-bottle saccharin versus water choice. The vertical bars indicate the standard errors of the means.

choice are presented to indicate the initial and more long-term intake preferences. Dose-related decreases in saccharin intake with complementary increases in water intake are apparent for both the d- and l-amphetamine groups. Again, the d-amphetamine appears to be 2–4 times as potent as the l-amphetamine particularly with regard to saccharin intakes. On the first day, the effects of d- and l-amphetamine closely resembled the results on water intake shown in Fig. 2. That is, the 1.0 mg dose of l-amphetamine was equivalent to the 0.5 mg dose of d-amphetamine and the 2.0 and 4.0 mg doses of l-amphetamine were equivalent to the 1.0 mg dose of d-amphetamine. On Day 9, the d- and l-amphetamine differential shifted somewhat closer to a 4 fold difference. It is of interest to observe that even the lowest dose levels of d-amphetamine (0.5 mg/kg) had a marked effect in reducing saccharin intake. Interestingly, this treatment group still preferred saccharin to water, but differed from the saline treatment group in not exhibiting the high levels of saccharin intake which are typical for this highly preferred saccharin concentration. Statistical analyses of the d- and l-amphetamine treatments on saccharin and water intake indicated that the two treatments were significantly different statistically. For saccharin intakes on Days 1 and 9 the levels of statistical significance were

$F(1,6) = 11.5$, $p<0.01$ and $F(1,6) = 11.3$, $p<0.01$, respectively. Similarly, differences in water intakes on Day 1, $F(1,6) = 43.1$, $p<0.01$, and Day 9 $F(1,6) = 20.7$, $p<0.01$, were statistically significant.

DISCUSSION

Overall the results of this study showed that d-amphetamine is significantly more effective than l-amphetamine in inducing a taste aversion. The 2–4 fold greater potency of d-amphetamine compared with l-amphetamine in inducing a taste aversion found in the present experiment fits well into the schema of Harris and Baldessarini [7] and is suggestive of a dopaminergic mediated effect of amphetamine. A dopaminergic action would also appear to be consistent with numerous reports [16] which indicate that feeding behavior is mediated by dopamine. Specification of a catecholaminergic mechanism, however, is speculative. First of all, the evidence presented in this experiment is indirect being based only on a d- versus l- difference in behavioral efficacy. Furthermore, the original observation of Coyle and Snyder [5] on the comparative effects of d- versus l-amphetamine on synaptosomal reuptake of dopamine and norepinephrine had indicated that the d-isomer was 10 times more effective than the l-amphetamine in blocking norepinephrine reuptake and that both isomers were about equal in blocking dopamine reuptake. Thus, if one chose to use pharmacological data of Coyle and Snyder a quite different catecholaminergic mechanism could be proposed.

That amphetamine can induce a taste aversion is firmly established. Furthermore, the dose effect relationship found in this experiment between d-amphetamine and saccharin-aversion closely approximates the results obtained by Martin and Ellinwood [9] using methamphetamine to induce a saccharin-aversion. Given that an amphetamine-induced taste aversion is a replicable dose-dependent phenomenon, it is necessary to also consider what interpretation can be given to this effect of amphetamine. LeMagnen [8] who first demonstrated the effectiveness of amphetamine in a learned aversion paradigm interpreted this finding as a demonstration that amphetamine anorexia could be conditioned. The close correspondence found in this study between the magnitude of the amphetamine-induced taste aversion and the magnitude of amphetamine adipsia as measured by water intake inhibition is consistent with this interpretation. In general, the efficacy of amphetamine in suppressing intake when given before ingestion of a solution corresponds well with the magnitude of the suppression of intake observed when the amphetamine is given after ingestion of a particular solution. Carey [3] found that amphetamine treatments given before ingestion of a saccharin solution markedly and persistently reduced saccharin intake and correspondingly, the same amphetamine injections given after ingestion of the saccharin solution gradually reduced intake of the saccharin to the same extent as the before treatments. On the other hand, Carlton and Wolgin [4] observed only a transient suppression of intake of sweetened milk when amphetamine was given before the presentation of the milk and correspondingly observed only a small suppressant effect of the same amphetamine treatments on intake when given after ingestion of the milk. Alternatively the occurrence of an amphetamine-induced taste aversion might be interpreted as showing that amphetamine has a noxious or nausea-type side effect which can be conditioned. This argument can be made on the grounds that other types of treatments which induce a taste aversion (e.g., x-radiation, lithium chloride and apomorphine) [13] all induce obvious distress to an animal. Amphetamine anorexia, however, is not considered to be secondary to a drug-induced nausea. While such an aversive side effect of amphetamine or probably any drug at a sufficiently high dose level would not be too surprising, this possibility seems unlikely with regard to the dose levels used by LeMagnen [8] and the lower dose levels used in this study. Furthermore, the lower dose levels used in this study have been shown by others to reliably facilitate intracranial self-stimulation in the rat [14], and to be self-administered intravenously by rats [10]. Thus, although both interpretations are possible, a conditioned anorexia rather than a conditioned noxious effect appears to best fit with the known behavioral effects of amphetamine.

An important difference exists, however, between a conditioned versus an unconditioned amphetamine anorexia. The unconditioned anorexia is manifested as a suppression of intake generally, whereas the conditioned anorexia is selective to the food object paired with the amphetamine administration. The selectivity of the conditioned anorexia is apparent in the saccharin versus water choice in the present experiment in that intake of the saccharin solution but not water was decreased. Possibly a conditioned anorexic effect of amphetamine might have some therapeutic usefulness where reduction in intake of a specific type of food or solution is important. This possibility, of course, is speculative and any amphetamine treatment must be viewed with caution.

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