Pharmacology Biochemistry & Behavior, Vol. 33, pp. 181–188. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Amphetamine Enantiomers and Rat Consummatory Behavior: A New Perspective[1]

MAXINE B. NICHOLS[2] AND ROGER P. MAICKEL

Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences
Purdue University, West Lafayette, IN 47907

Received 15 June 1988

NICHOLS, M. B. AND R. P. MAICKEL. *Amphetamine enantiomers and rat consummatory behavior: A new perspective.* PHARMACOL BIOCHEM BEHAV **33**(1) 181–188, 1989.—The anorectic actions of amphetamine have been known for over forty years, yet the precise relationship(s) between the enantiomeric forms of the drug and anorexia is not clearly understood. Previous studies have utilized primarily racemic amphetamine or its d-isomer in the analysis of feeding behavior. In the present investigation, a detailed examination of the effects of single and repeated equiactive doses of d- and l-amphetamine on food consumption by adult male rats was undertaken with emphasis on aspects of tolerance development. Weight loss and pattern of daily food intake differed depending upon the isomer, dose, and degree of tolerance. Two types of tolerance were seen with both isomers, an initial tolerance with a decrease in efficacy between days 1 and 2, and a later gradual decrease in efficacy over 12 days of repeated dosage. Rats tolerant to the anorectic effects of d-amphetamine were only minimally affected when challenged with an equiactive anorectic dose of l-amphetamine, while rats tolerant to the anorectic effects of l-amphetamine showed a significantly depressed food intake and modified eating pattern when challenged with an equiactive dose of d-amphetamine. Therefore two-way cross tolerance, as previously assumed, does not completely exist between low equiactive doses of d- and l-amphetamine.

d-Amphetamine l-Amphetamine Anorexia Tolerance

AMPHETAMINE can exert its pharmacological effects by modifying the presynaptic release and reuptake of several neurotransmitters in different neurochemical pathways at a diversity of neuroanatomic sites (6,35). Consequently, correlating specific central processes with the effects of amphetamine on behavior has been a difficult task. For many years, the generally held belief was that the only difference between the d- and l-enantiomers of amphetamine was one of quantitative potency (22). Several studies since the mid 1970's have indicated that the two isomers differ in qualitative as well as quantitative effects in eliciting some aspects of amphetamine-induced behavior (1, 13, 37). This is not surprising in view of the fact that a qualitative, as well as quantitative difference exists in the effectiveness of d-amphetamine (D-AMP) vs. l-amphetamine (L-AMP) on neurochemical processes (18,19). Although the development of tolerance to the anorexigenic actions of amphetamine has been known for many years (41), the mechanisms underlying this phenomenon remain controversial (7,8). Despite the large volume of literature on tolerance to psychomotor stimulants, relatively few studies have examined the development of tolerance to the two amphetamine isomers (26, 30, 32). It has generally been assumed that the mechanisms involved in the development of anorexigenic tolerance to each of the amphetamine enantiomers are similar, merely reflecting a difference in quantitative potency. Unfortunately, this assumption is based primarily on cross tolerance studies using relatively high chronic multiple daily injections of d- and l-amphetamine (31). Results can vary enormously depending on which drug regimens are employed. The dose, route and frequency of administration can be significant confounding variables, making the task of integrating results and forming definitive conclusions difficult.

For example, Kandel *et al.* (25) and Lewander (30) reported that no anorectic cross tolerance existed between d-amphetamine and fenfluramine. A closer examination of these data in subsequent studies (20,21) demonstrated that pretreatment with d-amphetamine did render an apparent anorectic tolerance toward fenfluramine. Enantiomeric differences in feeding behavior may exist between the two isomers of amphetamine. Recently, administration of low doses of D-AMP, but not L-AMP, was found to induce rather than suppress feeding behavior (10). Enantiomeric differences in neurochemical parameters do appear to exist following chronic exposure to d- and l-amphetamine (9,39).

In the present study, a detailed examination of the effects of single and repeated equiactive doses of d- and l-amphetamine on food consumption was undertaken with an emphasis on tolerance and cross tolerance development. The time course of tolerance

[1]Supported in Part by USPHS Training Grant GM-07095.
[2]Taken in part from a thesis submitted by M. B. Nichols in partial fulfillment of the requirements for the Ph.D. degree, Purdue University, 1983.

development, the daily temporal pattern of food and water intake, and body weight gain were determined at three different equiactive dosages. Pair-fed and weight-restricted controls were used to assess the effect of deprivation on tolerance development. The results obtained give a new viewpoint on tolerance development.

METHOD

Adult, male Sprague-Dawley rats (160–200 g) were obtained from Murphy Breeding Laboratories, Plainfield, IN and housed individually in single screen-wire cages with an ad lib supply of food (Wayne Lab Blox) and tap water for 7–10 days prior to starting experimental procedures. The animal rooms were maintained at 22–25°C with a 14/10 (lights on 0600–2000 hours) lighting cycle.

Rats were acclimated to a 3-hour limited food access cycle (0900–1200 hours) for 7–10 additional days prior to the start of drug administration. Food intake was measured each day by placing a preweighed (to the nearest 0.1 g) amount of food on the floor of each cage for the intervals 0–60, 61–120, and 121–180 minutes; at the end of each interval the remaining food was removed (along with any spillage) and reweighed. Each rat was weighed daily at 0830 hours. Water was available ad lib at all times; water intake was measured at 0900 and 1200 hours daily.

Drugs were administered, by SC injection in volumes of 0.1 ml/100 g body weight, as aqueous solutions of D-AMP (d-amphetamine sulfate, Smith Kline & French) or L-AMP (l-amphetamine phosphate, Pennwalt Corp.). Control rats were dosed SC with 0.1 ml/100 g body weight of 0.9% aqueous NaCl. All drug doses were given 20 minutes prior to the start of each 3-hour feeding period.

Preliminary studies with various doses of D-AMP and L-AMP determined relative potencies. On the basis of these studies, doses of D-AMP of 1.25, 2.50 and 5.0 mg/kg (base weight), SC were found to be equiactive to doses of L-AMP of 2.50, 5.0 and 10.0 mg/kg (base weight), SC. The dose-effect curves were parallel.

Food and/or weight restricted groups (Table 3) were accommodated to and maintained on the 3-hour limited food access schedule. Rats in the pair-fed groups were injected daily with saline and presented the average amount of food consumed by the corresponding amphetamine-treated rats for 11 consecutive days. On the 12th day, pair-fed rats were injected with the appropriate amphetamine isomer and 3-hour food and water intake measured. Weight-paired rats were presented with the amount of food necessary to maintain the body weight gain of experimental groups. On the day of greatest body weight loss (determined by the pattern of weight gain in experimental groups) weighed-paired rats were challenged with either D-AMP or L-AMP and 3-hour food intake and water intake were determined.

Data were collected over the course of the experiments and analyzed by multivariate ANOVA in post hoc Duncan's tests. The $p<0.05$ level was adopted for all tests of significance. All values are reported as mean ± S.E.M. for each group (N = 8 rats per group). Due to the large number of rats utilized in this study and limitations of space and housing (2 units:36 individual cages/unit), a group of saline controls (N = 8) was randomly placed in each of the two units to assess whether placing was a significant variable.

RESULTS

Effects of Repeated Dosage of Amphetamine Isomers on Body Weight, Food and Water Consumption

The data in Fig. 1A and B show the effects of repeated administration of various doses of D-AMP and L-AMP on the body weight of rats, as expressed in daily percent weight gain. In

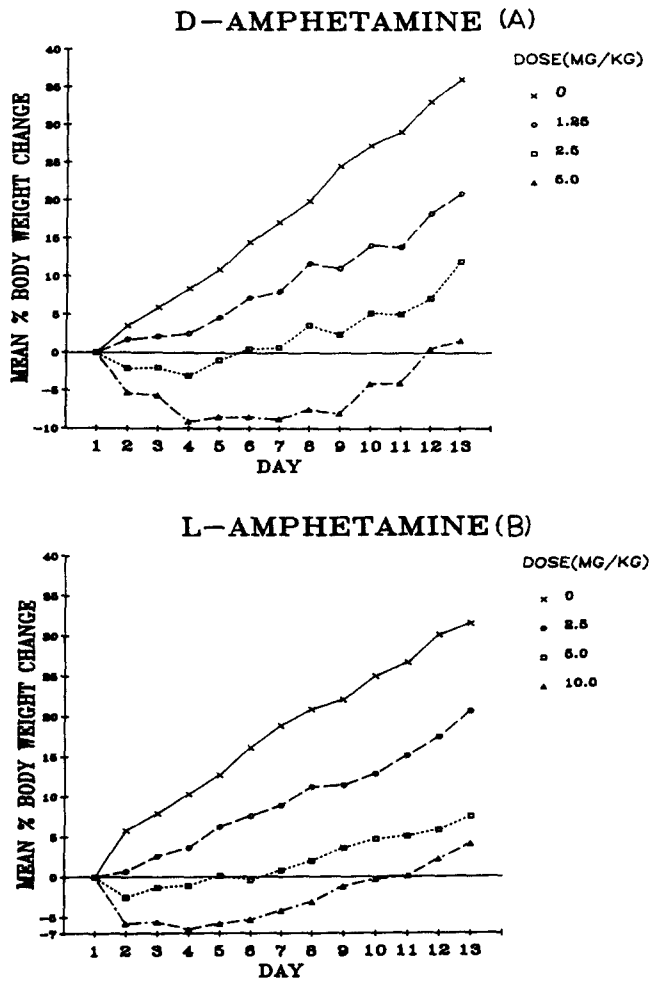

FIG. 1. Effects of repetitive dosages of D-AMP (A) or L-AMP (B) on body weight of rats. Each point is the mean of values obtained from the 8 rats in an experimental group treated as described in the Method section.

the case of D-AMP (Fig. 1A), the three doses are clearly separable. Statistical analysis of these data indicate that the animals treated with 5.0 mg/kg are significantly different ($p<0.05$) from those treated with 2.5 or 1.25 mg/kg, and all three doses differ significantly from control animals. For L-AMP (Fig. 1B) the effects of the 2.5 and 5.0 mg/kg doses are not significantly different from each other. All three doses differ significantly from control ($p<0.05$), and the 10.0 mg/kg dose differs significantly from the doses of 2.5 and 5.0 mg/kg.

The mean daily weight gain (± SEM) of the D-AMP control group over the 12 days of dosage was 2.8 ± 0.2 g/100 g per day; similar values for 1.25, 2.50, 5.0 mg/kg of D-AMP were 1.6 ± 0.2, 1.1 ± 0.3, and 0.1 ± 0.4 g/100 g per day, respectively. All of these were statistically less than control ($p<0.05$). For L-AMP, the control group mean daily weight gain was 2.6 ± 0.1 g/100 g per day, while those for 2.50, 5.0 and 10.0 mg/kg were 1.5 ± 0.3, 0.6 ± 0.4 and 0.4 ± 0.4 g/100 g per day, respectively; again, these were all significantly less than control ($p<0.05$).

The temporal pattern of daily food consumption of the rats over the course of the experiments is presented in Figs. 2 and 3. The control group of rats for the D-AMP experiment (Fig. 2A) showed no significant day effects in any of the 3 time periods or in the overall (0–180 minutes) of daily food consumption over the

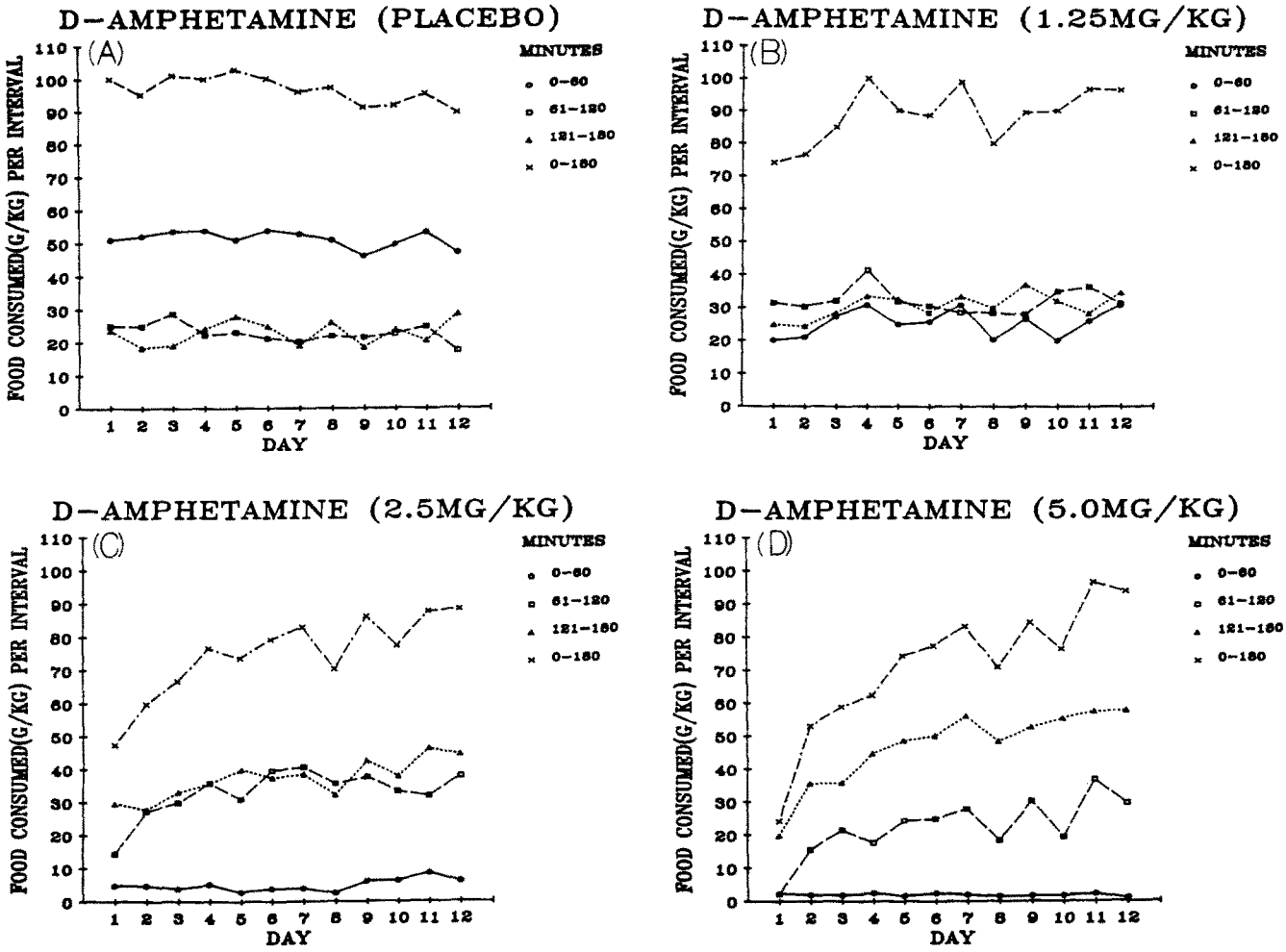

FIG. 2. Effects of repetitive dosages of D-AMP on temporal pattern of food consumption by rats. Each point is the mean of values obtained from the 8 rats in an experimental group. Data from control rats are presented in A, B, C, and D present data from rats treated daily with 1.25, 2.5 or 5.0 mg/kg of D-AMP, respectively.

12-day period. Analysis of the best fit straight lines of the data indicated all lines to have slopes that were essentially horizontal. Over the 12-day period, mean ± SEM values for daily food intake were 51.2 ± 2.7, 22.9 ± 3.3, 22.4 ± 2.8, and 97.4 ± 4.6 g/kg for the 0–60-, 61–120-, 121–180-, and 0–180-min periods, respectively. The first, second, and third hours of the total consummatory period represented 53%, 24%, and 24%, respectively of total daily consumption.

Figure 2B presents the data obtained from the rats treated daily with 1.25 mg/kg, SC of D-AMP. Over the 12-day period, mean ± SEM values for daily food intake were: 25.4 ± 2.9, 32.2 ± 2.6, 30.7 ± 4.0, and 88.8 ± 4.5 g/kg, for the 0–60-, 61–120-, 121–180-, and 0–180-min periods, respectively. When compared to the control group (Fig. 2A) the 0–60-min interval had a significantly lower mean daily consumption in the D-AMP-treated animals. Analysis of the individual time intervals over the 12 days of dosage indicated a significant day effect in the 0–180 min (total consummatory) period; post hoc analysis showed that this dose had an effect on day 1 which was significantly greater than on any other day. The results obtained in rats treated with 2.50 mg/kg of D-AMP are presented in Fig. 2C. Mean daily food intake values for the 12-day period were 4.9 ± 1.7, 32.9 ± 3.8, 37.1 ± 4.6, and 74.7 ± 5.8 g/kg ± SEM for the 0–60-, 61–120-, 121–180-, and 0–180-min intervals, respectively. When compared to the corresponding values for the control group, the amount of food consumed by the rats treated daily with 2.5 mg/kg of D-AMP was significantly lower in the 0–60- and 0–180-min intervals, but significantly higher than control animals in the 121–180-min interval, presumably reflecting "rebound" eating as the effects of the drug diminished with time. Analysis of the 12-day interval pattern indicated a significant day effect in the 61–120- and 0–180-min intervals; post hoc analysis confirmed that the effects of this dose were significantly greater on day 1 than any subsequent days.

Figure 2D contains the data obtained from rats treated with daily doses of 5.0 mg/kg of D-AMP for 12 days. Mean daily food intake values over the 12 days were 1.9 ± 0.7, 22.2 ± 4.8, 46.7 ± 4.3, and 71.1 ± 5.7 g/kg ± SEM for the intervals 0–60, 61–120, 121–180 and 0–180 min, respectively. As with the 2.5 mg/kg dose, consumption in the 0–60- and 0–180-min intervals was significantly reduced while that in the 121–180-min interval was significantly higher when compared to the control group. Analysis of the daily consummatory patterns over the 12 days of treatment showed a significant day effect in the 61–120-, 121–180-, and 0–180-min intervals; post hoc analysis confirmed this to be manifested by a greater decrease in food consumed on the first day

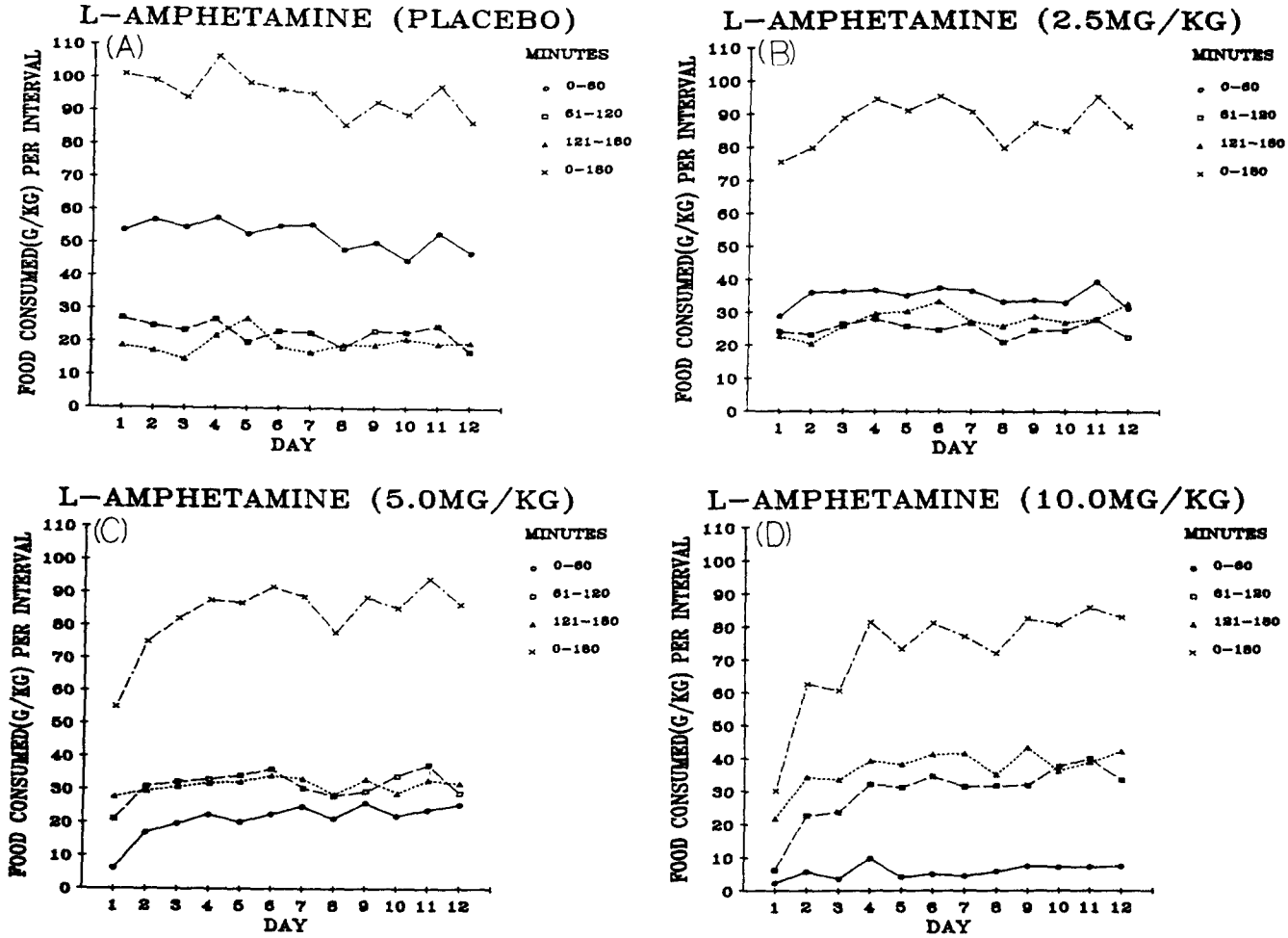

FIG. 3. Effects of repetitive dosages of L-AMP on temporal pattern of food consumption by rats. Each point is the mean of values obtained from the 8 rats in an experimental group. Data from control rats are presented in A, B, C, and D present data from rats treated daily with 2.5, 5.0, or 10.0 mg/kg of L-AMP, respectively.

of treatment. In addition, day 2 of treatment was also significantly lower, and days 11 and 12 were significantly higher for the 0–180-min interval.

The data obtained from the control group of rats for the study with L-AMP are shown in Fig. 3A. As with the D-AMP control group (Fig. 2A), consummatory behavior of these rats was consistent over the 12-day study. When rats were given daily doses of 2.5 mg/kg of L-AMP, the results seen in Fig. 3B were obtained. The mean values for food consumption over the 12 days were: 34.8 ± 2.4, 24.9 ± 2.8, 27.6 ± 4.1, and 87.4 ± 4.4 g/kg $\pm$ SEM for the 0–60-, 61–120-, 121–180-, and 0–180-min intervals, respectively. The 0–60-min interval represented a significantly lower mean daily food intake than that of control rats. Analysis of the daily interval consumption over the 12-day period showed no significant day effect.

Figure 3C presents the data obtained when rats were treated daily for 12 days with 5.0 mg/kg, SC of L-AMP. Mean values for food consumption over the 12 days were: 21.4 ± 3.2, 31.4 ± 3.0, 31.3 ± 3.3, and 83.2 ± 4.8 g/kg for the 0–60-, 61–120-, 121–180-, and 0–180-min interval mean daily consumption was significantly higher than that of the control group. Analysis of the daily consummatory pattern indicated a significant day effect in the 0–60- and 0–180-min intervals. Post hoc analysis confirmed this to reflect the fact that in these intervals, the day 1 dose of L-AMP was significantly more effective in decreasing food consumption.

Figure 3D contains the data from rats treated daily with 10 mg/kg of L-AMP. Mean daily food consumption values were: 5.8 ± 2.1, 29.6 ± 3.3, 37.0 ± 3.5, and 72.4 ± 4.8 g/kg for the 0–60-, 61–120-, 121–180-, and 0–180-min intervals, respectively; the 0–60- and 0–180-min intervals were significantly less than control, while the 121–180-min intervals were significantly greater than that of the control group. Analysis of the daily temporal pattern showed a significant day effect in all time periods except 0–60 min; this effect was associated with a significantly greater potency of the l-AMP on day 1 in the 61–120-, 121–180-, and 0–180-min intervals.

Figure 4 displays the data for mean daily food consumption in each interval as the percentage of total food consumed in the 3-hour period. Both control groups are similar in that approximately 50% of the total food was consumed in the 0–60 min interval, with the remainder consumed almost equally in each of the remaining two intervals. With increasing doses of either D-AMP or L-AMP, the percent of total consumption was reduced in a dose-related manner in the first interval, and an inverse dose-related "rebound" was seen in the third interval.

Table 1 displays the different stages of tolerance observed with

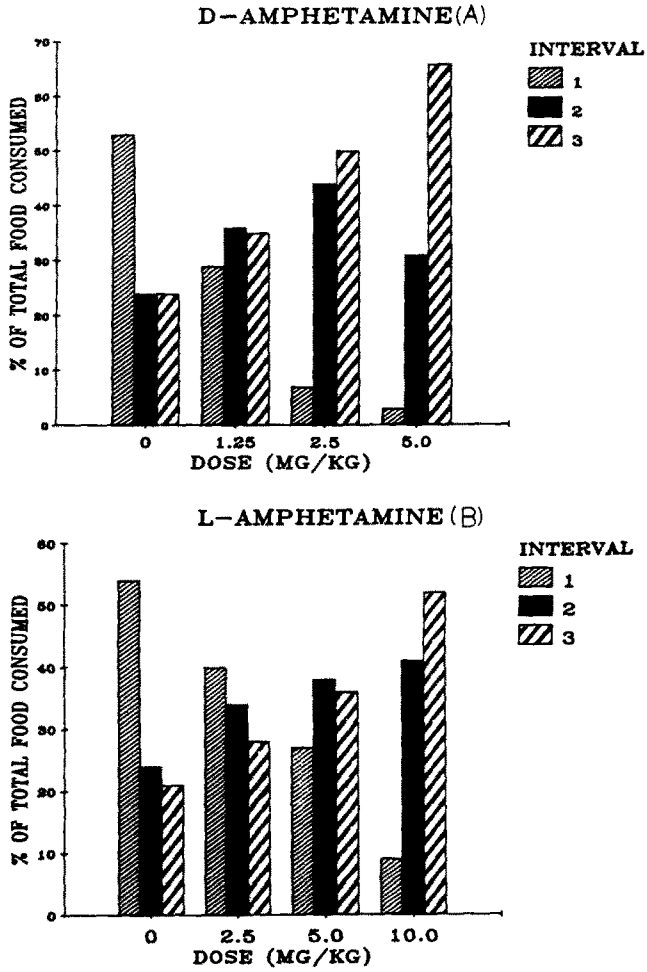

FIG. 4. Mean daily food consumed in each time period following D-AMP (A) or L-AMP (B) administration. Each bar represents the mean values obtained from the 8 rats in each experimental group, calculated as percent of total food consumed in the 0–60-min (1), 61–120-min (2), and 121–180-min (3) intervals.

repetitive dosage of 5 mg/kg D-AMP over 12 days followed by dosage of 5 mg/kg D-AMP on days 13 and 14 to rats which had been treated with 10 mg/kg L-AMP for days 1–12. At the dose shown and the intermediate (2.50 mg/kg) dose (data not shown) an "initial" tolerance was seen in that the drug was significantly more effective in decreasing food consumption on day 1 than on day 2 in all time intervals except 61–120 min. No such tolerance was seen with the lowest dose of D-AMP (1.25 mg/kg, data not shown). A significant "later" tolerance was also seen with the highest dose (Table 1) and the intermediate dose (data not shown) in that the efficacy of the drug was reduced on day 12 as compared to days 1 and 2. A similar effect was seen at the lowest dose of D-AMP (1.25 mg/kg) when day 1 was compared to day 12.

Cross tolerance was examined by giving D-AMP on days 13 and 14 to rats treated for the previous 12 days with L-AMP. Data for the highest dose is presented in Table 1. The "initial" tolerance seen on days 1 and 2 with D-AMP in naive rats was completely abolished in the L-AMP-treated rats. At the high dose of D-AMP (5.00 mg/kg), day 2 was not significantly different from day 14. When days 13 and 14 were compared to days 1 and 2 respectively, the low dose of D-AMP was similarly effective. However, at the intermediate dose of D-AMP (2.50 mg/kg), a significant decrease in efficacy was seen in both comparisons (1 vs. 13, $p<0.05$; 1 vs. 14, $p<0.05$).

Similar studies with L-AMP yielded somewhat different results. Again, comparison of day 1 vs. day 2 showed development of "initial"tolerance at the two higher doses (5.00 and 10.0 mg/kg) of L-AMP. Data for the highest dose is presented in Table 2. At all three dosage levels, L-AMP was significantly more effective on day 1 than on day 12. However, no "later" tolerance was seen at any of the doses of L-AMP when days 2 and 12 were compared.

Cross tolerance studies of L-AMP on days 13 and 14 in rats treated with D-AMP for 12 days showed that the "initial" tolerance seen on days 1 and 2 with the two higher doses of L-AMP was not seen in rats that had been given D-AMP on days 1–12. When day 13 was compared to day 1, all 3 doses of L-AMP were significantly less effective in the D-AMP-treated rats. However, when days 14 and 2 were compared, only the highest dose of L-AMP (10.0 mg/kg) showed a similar effect.

To further evaluate the effects of the amphetamine enantiomers on consummatory behavior, water intake was measured in half of each group of rats over the 3-hour period of food consumption and over the 24-hour period consisting of the food consummatory period and the 21 hours following. A significant reduction of water intake was caused by D-AMP during the 3-hour food consummatory period on days 1, 3, 4, and 5 at the intermediate dose and on days 1, 3–7, and 10 with the high dose, while the low dose was without significant effect. In contrast, when 24-hour water consumption was measured the only significant reduction was seen on day 1 with the high and intermediate doses; this effect did not occur with the lowest dose. L-AMP significantly reduced water consumption during the 3-hour food consummatory periods with all three doses, but only on day 1. A significant reduction in 24-hour water intake was also noted with all three doses of L-AMP on day 1; this persisted through day 5 only with the highest dose.

The relative contribution of food and/or weight restriction to the development of tolerance was tested by utilizing both pair-fed and weight-paired control groups (Table 3). The 11-day weight gain of pair-fed rats closely followed the same pattern of body weight gain as their corresponding amphetamine-treated group. No consistent difference in 3-hour water intake of food-restricted or drug-restricted groups occurred during the time course studied. The effects of a single dose of D-AMP (5.0 mg/kg) or L-AMP (10.0 mg/kg) in both pair-fed and weight-paired rats were similar to those seen in naive rats. No differences were observed in the 24-hour weight loss of any of the experimental groups when compared to the corresponding enantiomer dosage on day 1 of the 12-day treatment period.

DISCUSSION

Some consummatory behavior in rats administered single and repeated doses of amphetamine isomers is consistent with anorectic and hypodipsic effects previously reported (5, 11, 12, 15, 38). The present data emphasize the enantiomeric difference in anorexigenic profile of the amphetamine isomers, and the lack of two-way cross tolerance. Potency ratio of anorectic effects are similar to other recent reports (2, 26, 28). The time for complete tolerance to develop to the anorectic effects of the isomers is in accordance with previous findings (16, 26, 34, 40). The differential effects of chronic D-AMP and L-AMP on fluid consumption are consistent with the observations of Lawlor (26).

A two-stage tolerance response following repetitive administration of both D-AMP and L-AMP was found. Days 1 and 2 represent an initial abrupt tolerance stage which is followed by the

TABLE 1

TOLERANCE TO REPETITIVE D-AMP DOSAGE (5.00 mg/kg) AND CROSS TOLERANCE TO D-AMP (5.00 mg/kg) IN RATS DOSED REPETITIVELY WITH L-AMP (10.0 mg/kg)

Treatment Day	Food Consumed (g/kg ± SEM) for Interval			
	0–60 min	61–120 min	121–180 min	0–180 min
1	2.3 ± 0.6	2.1 ± 1.0	19.7 ± 3.2	24.1 ± 3.9
2	1.9 ± 0.4	15.5 ± 2.6	35.6 ± 3.0	53.0 ± 4.2
12	1.0 ± 0.5	29.4 ± 5.3	57.5 ± 3.8	93.5 ± 6.0
13	2.0 ± 1.2	2.0 ± 1.2	48.5 ± 2.5	57.5 ± 2.7
14	1.7 ± 0.3	7.6 ± 3.4	47.5 ± 5.4	56.0 ± 7.4
Comparisons				
1 vs. 2	NS	1<2 ($p<0.05$)	1<2 ($p<0.05$)	1<2 ($p<0.05$)
1 vs. 12	NS	1<12 ($p<0.05$)	1<12 ($p<0.05$)	1<12 ($p<0.05$)
2 vs. 12	NS	2<12 ($p<0.05$)	1<12 ($p<0.05$)	2<12 ($p<0.05$)
13 vs. 14	NS	NS	NS	NS
13 vs. 1	NS	NS	1<13 ($p<0.05$)	1<13 ($p<0.05$)
14 vs. 2	NS	NS	NS	NS

Values for days 1, 2 and 12 are those for D-AMP (5.00 mg/kg) as presented in Fig. 2D. Values for days 13 and 14 are those from rats given D-AMP (5.00 mg/kg) after 12 days of dosage with L-AMP (10.0 mg/kg) as presented in Fig. 3D.

gradual onset of a second tolerance stage. This latter effect may be the result of accumulation of hydroxylated metabolites such as PONE in noradrenergic neurons (5,33) or POHA in dopaminergic neurons (23,24). The apparent lack of complete two-way cross tolerance between the two isomers could be explained, at least partially, by the differential metabolism and accumulation of metabolites of D-AMP and L-AMP (23,24). Recently Dougan *et al.* (9) reported the stereoselective tissue specific accumulation and persistence of hydroxylated metabolites of D-AMP and L-AMP in aminergic neurons in association with sensitization following chronic administration. The possible contribution of localized disruption induced by metabolites to the development of tolerance and/or sensitization to amphetamine actions may warrant reinvestigation. The rapid adaptation to the suppression of eating (days 1 and 2) cannot be due to this phenomenon.

Clear differences in the eating profiles produced by D-AMP and L-AMP occur during tolerance development. The contrasting temporal pattern observed with repeated injections may be associated with actions of the isomers upon differing components of the feeding process. A single injection of D-AMP or L-AMP produces a powerful initial suppression of eating, followed by the release of this suppression and the resumption of normal eating. As tolerance develops to D-AMP, the major effect remains a delay in onset of eating, with the latency between drug injection and initiation of feeding not proportional to the dose of drug administered. The delay in initiation of eating may be characterized as an

TABLE 2

TOLERANCE TO REPETITIVE L-AMP DOSAGE (10.0 mg/kg) AND CROSS TOLERANCE TO L-AMP (10.0 mg/kg) IN RATS DOSED REPETITIVELY WITH D-AMP (5.00 mg/kg)

Treatment Day	Food Consumed (g/kg ± SEM) for Interval			
	0–60 min	61–120 min	121–180 min	0–180 min
1	2.9 ± 1.1	6.2 ± 2.3	21.7 ± 3.4	30.1 ± 4.4
2	5.7 ± 1.4	22.6 ± 2.7	34.2 ± 2.6	62.5 ± 5.0
12	7.4 ± 2.7	23.6 ± 2.6	33.4 ± 3.2	60.5 ± 4.7
13	29.7 ± 8.0	47.1 ± 2.9	37.1 ± 2.6	107.6 ± 4.3
14	16.5 ± 4.7	43.5 ± 4.6	45.7 ± 4.2	100.3 ± 5.7
Comparisons				
1 vs. 2	NS	1<2 ($p<0.05$)	1<2 ($p<0.05$)	1<2 ($p<0.05$)
1 vs. 12	NS	1<12 ($p<0.05$)	1<12 ($p<0.05$)	1<12 ($p<0.05$)
2 vs. 12	NS	NS	NS	NS
13 vs. 14	NS	NS	NS	NS
13 vs. 1	1<13 ($p<0.05$)	1<13 ($p<0.05$)	1<13 ($p<0.05$)	1<13 ($p<0.05$)
14 vs. 2	2<14 ($p<0.05$)	2<14 ($p<0.05$)	2<14 ($p<0.05$)	2<14 ($p<0.05$)

Values for days 1, 2 and 12 are those for L-AMP (10.0 mg/kg) as presented in Fig. 3D. Values for days 13 and 14 are those from rats given L-AMP (10.0 mg/kg) after 12 days of dosage with D-AMP (5.00 mg/kg) as presented in Fig. 2D.

TABLE 3

EFFECTS OF SINGLE DOSES OF AMPHETAMINE ENANTIOMERS ON FOOD CONSUMPTION BY PAIR-FED OR WEIGHT-PAIRED RATS

Group/ Condition	Food Intake (g/kg) in Interval			
	0–60 min	61–120 min	121–180 min	0–180 min
Free-Feeding Control	53.6 ± 6.1	19.8 ± 2.3	13.6 ± 2.9	84.5 ± 9.9
Pair-Fed Control + D-AMP	58.2 ± 7.4	18.1 ± 3.2	26.0 ± 10.7	104.7 ± 3.7
Pair-Fed Control + D-AMP	1.9 ± 0.2*†	0.7 ± 0.3*†	17.0 ± 6.3	19.6 ± 6.3*†
Pair-Fed Control + L-AMP	51.9 ± 7.4	19.5 ± 4.9	17.6 ± 2.4	88.9 ± 9.5
Pair-Fed Control + L-AMP	3.8 ± 3.2*†	6.3 ± 4.3*†	18.1 ± 6.9	28.2 ± 13.0*†
Weight-Paired + D-AMP	2.0 ± 0.2*	1.2 ± 0.1*	11.2 ± 1.8	14.6 ± 1.8*
Weight-Paired + L-AMP	6.6 ± 4.2*	7.1 ± 5.8	16.3 ± 4.7	29.9 ± 13.5*

Each value is the mean ± SEM of values obtained from 4 rats treated as described in the Method section.

*Significantly less ($p<0.05$) than corresponding interval for Free-Feeding Control.

†Significantly less ($p<0.05$) than corresponding interval for Pair-Fed Control.

effect upon hunger which is not susceptible to tolerance with repeated exposure to L-AMP.

In contrast, tolerance development to L-AMP appears to allow eating to begin and proceed, but at a depressed level, and with the duration of the initial depression of eating approximately proportional to the dose injected. This raises the possibility that L-AMP partially exerts inhibition over feeding by enhancing feeding signals from the consumption of food via satiation mechanisms, with subsequent tolerance development involving a decrease in L-AMP's ability to produce satiety.

This differential pattern also suggest that different neurochemical substrates may be involved in the development of tolerance to the isomers of amphetamine. Newly emerging neurochemical models of feeding regulation have tended to replace neural foci with specific neurochemical systems deemed responsible for the initiation of hunger (noradrenergic), for the maintenance of feeding (dopaminergic), or for satiation (serotonergic) (3, 17, 27). The lack of cross tolerance between D-AMP and L-AMP observed in the present study may provide additional, although indirect, evidence that the development of tolerance to the two isomers is subserved by different neurochemical mechanisms. Steranka (39) has shown a stereoselective differences in the biochemical adaptation of striatal dopaminergic pathways following chronic administration of D-AMP or L-AMP.

The observed lack of two-way cross tolerance is not inconsistent with the other known interactions of the isomers. A lack of cross tolerance between D-AMP and L-AMP has been observed with other drug-induced effects. Tilson and Sparber (40) reported a lack of cross tolerance between the isomers in producing self stimulation. Jori *et al.* (23) reported a lack of cross tolerance in chronically-induced alterations of striatal dopamine metabolism. These results apparently contradict previous reports by Lewander (30–32) on tolerance to amphetamine anorexia. One possible explanation for this discrepancy is most likely the selection of doses. Lewander (30–32) based his observations of cross tolerance between D-AMP and L-AMP on studies using very high doses of 20 mg/kg and 40 mg/kg, respectively. Such large doses may produce other behavioral effects which could interfere or compete with the initiation of feeding behavior. In rats, doses of 16 mg/kg or more of d,l-amphetamine, D-AMP, or L-AMP have been reported to be lethal or to cause one or more of the following: convulsions, bizarre backward movements, and a profound state of behavioral disturbance. High doses, therefore, may have rendered the experimental protocol used by Lewander (31) insensitive to certain drug effects, masking qualitative aspects of the feeding processes involved. In the present investigation, care was taken in choosing the dose range to avoid such extremely high toxic doses which could produce false "anorexigenic" effects. Effects on activity are found within the dose ranges utilized in this study. No direct assessment was made of the potential interference. Such activity might play a role in determining differences in anorectic profiles of d- and l-amphetamine. However, the difference between profiles at lower doses tested, which are unlikely to produce a significant effect on activity, suggest that this may be a minor confounding variable.

Tolerance induced by both D-AMP and L-AMP appears to be mediated, at least in part, by a physiological adaptation to chronic exposure. No experimental evidence was obtained to support the hypothesis that anorexigenic tolerance results from increased motivational drive. Acquisition of tolerance under the present experimental conditions could not be accounted for simply in terms of weight loss. No decrease in anorexic potency or change in temporal pattern of food intake was observed in either food- or weight-restricted animals. A significantly greater anorexic effect was observed in the weight-restricted group receiving D-AMP.

Although these results support the view that tolerance is not totally a behavioral artifact (7,31), it cannot be argued that food deprivation or other behavioral adaptation mechanisms play no part in amphetamine-induced anorexia (8).

REFERENCES

1. Aulakh, C. S.; Bhattacharyya, A. K.; Pradhan, S. N. Correlation of behavioral and neurochemical effects of d- and l-amphetamine. Drug Dev. Res. 2:65–72; 1982.
2. Baez, L. A. Role of catecholamines in the effects of amphetamine in rats. Psychopharmacologia 35:91–98; 1974.
3. Blundell, J. E.; Latham, C. J. Characterization of adjustments to the structure of feeding behavior following pharmacological treatment: Effects of amphetamine and fenfluramine and the antagonism induced by pimozide and methergoline. Pharmacol. Biochem. Behav. 12: 717–722; 1980.
4. Brodie, B. B.; Uno, A. K.; Gessa, G. G. Possible role of p-hydroxynorephedrine in the depletion of norepinephrine induced by d-amphetamine and in tolerance to this drug. In: Costa, E.; Garattini, S., eds. International symposium on amphetamine and related compounds. New York: Raven Press; 1970:217–230.
5. Carlisle, H. J. Differential effects of amphetamine on food and water intake in rats with lateral hypothalamic lesions. J. Comp. Physiol. Psychol. 58:47–54; 1974.
6. Cole, S. O. Brain mechanisms of amphetamine-induced anorexia, locomotion, and stereotypy. Neurosci. Biobehav. Rev. 2:89–100; 1978.
7. Demellweek, C.; Goudie, A. J. An analysis of behavioral mechanisms

involved in the acquisition of amphetamine anorectic tolerance. Psychopharmacology (Berlin) 79:58–66; 1983.
8. Demellweek, C.; Goudie, A. J. Behavioral tolerance to amphetamine and other psychostimulants: The cases for considering behavioural mechanisms. Psychopharmacology (Berlin) 80:287–307; 1983.
9. Dougan, D. F. H.; Duffield, A. M.; Duffield, P. H.; Wade, D. N. Stereoselective accumulation of metabolites of amphetamine in rat striatum and hypothalamus. Br. J. Pharmacol. 88:285–290; 1986.
10. Evans, K. R.; Vaccarino, F. J. Effects of d- and l-amphetamine on food intake: Evidence for a dopaminergic substrate. Pharmacol. Biochem. Behav. 27:649–652; 1987.
11. Epstein, A. Suppression of eating and drinking by amphetamine and other drugs in normal and hyperphagic rats. J. Comp. Physiol. Psychol. 52:37–45; 1959.
12. Falk, J. L.; Burnidge, G. K. Drug antagonism and water intake. Physiol. Behav. 5:193–198; 1970.
13. Franklin, K. B.; Vaccarino, F. J. Differential effects of amphetamine isomers on self stimulation: Evidence for DA neuron subtypes. Pharmacol. Biochem. Behav. 18:747–751; 1983.
14. Ghosh, M. N.; Parvathy, S. Tolerance pattern of the anorexogenic action of amphetamine, fenfluramine, phenmetrazine and diethylpropion in rats. Br. J. Pharmacol. 56:479–485; 1976.
15. Glick, S. P.; Greenstein, S. Pharmacological inhibition of eating, drinking and prandial drinking. Behav. Biol. 8:55–61; 1973.
16. Gotesman, K. G.; Lewander, T. The duration of tolerance to the anorexigenic effect of amphetamine in rats. Psychopharmacologia 42:41–45; 1975.
17. Grossman, S. P. Correlative analyses of ingestive behavior and regional amine depletions after surgical transections of neuronal pathways in the mesencephalon, diencephalon, and striatum. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:43–51.
18. Heikkila, R. E.; Oralnsky, H.; Mytillineous, C.; Cohen, G. Amphetamine:evaluation of d- and l-isomers as releasing agents and uptake inhibitors for (^{3}H) dopamine and norepinephrine in slices of rat neostriatum and cerebral cortex. J. Pharmacol. Exp. Ther. 194:47–56; 1975.
19. Holmes, J. C.; Rutledge, C. O. Effects of d- and l-isomers of amphetamine on uptake, release and catabolism of norepinephrine, dopamine and 5-hydroxytryptamine in several regions of the rat brain. Biochem. Pharmacol. 25:444–451; 1976.
20. Hunsinger, R. N.; Barnes, T. B.; Kapeghian, J. C.; Wilson, M. C. Alteration of the descriptive effect of fenfluramine on food consumption in rat by repeated post-session administration of d-amphetamine. Psychopharmacology (Berlin) 25:358–362; 1981.
21. Hunsinger, R. N.; Wilson, M. C. Alteration of the neurochemical effect of fenfluramine by previous treatment with d-amphetamine. Pharmacol. Biochem. Behav. 22:127–134; 1985.
22. Innes, I. R.; Nickerson, M. Norepinephrine, epinephrine and the sympathomimetic amines. In: Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics. New York: Macmillan; 1975: 496–499.
23. Jori, A.; Caccia, S.; Dolfini, E. Tolerance to anorexic drugs. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:179–198.
24. Jori, A.; Caccia, S.; Guiso, A.; Ballabio, M.; Garattini, S. Selective storage of p-hydroxy-d-amphetamine in the dopaminergic nerve terminals. Biochem. Pharmacol. 28:1205–1207; 1979.
25. Kandel, D.; Doyle, D.; Fischman, M. W. Tolerance and cross tolerance to the effects of amphetamine, methamphetamine, and fenfluramine on milk consumption in rat. Pharmacol. Biochem. Behav. 3:705–707; 1975.
26. Lawlor, R. B.; Trivedi, M. C.; Yelnosky, J. Determination of the anorexigenic potential of d,l-amphetamine, l-amphetamine, d-amphetamine and phentermine. Arch. Int. Pharmacodyn. Ther. 179: 401–407; 1969.
27. Leibowitz, S. F. Neurochemical systems of the hypothalamus in control of feeding and drinking behavior and water and electrolyte excretion. In: Morgane, P.; Panksepp, J., eds. Handbook of the hypothalamus. New York: Marcel Dekker; 1980:299–437.
28. Leibowitz, S. P. Amphetamine: Possible site and mode of action for producing anorexia in the rat. Brain Res. 84:160–167; 1975.
29. Lesses, M. F.; Meyerson, A. Human autonomic pharmacology. XVI. Benzedrine sulfate as an aid in the treatment of obesity. N. Engl. J. Med. 218:119–194; 1938.
30. Lewander, T. Experimental studies on anorexigenic drugs: Tolerance, cross tolerance and dependence. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorexic drugs. New York: Raven Press; 1978:347–355.
31. Lewander, T. On food deprivation in relation to amphetamine tolerance. In: Ellinwood, E. H., Jr.; Kilbey, M. M., eds. Advances in behavioral biology. vol. 21. New York: Plenum Press; 1977:201–215.
32. Lewander, T. Effect of chronic treatment with central stimulants on brain monoamines and some behavioral and physiological functions in rats, guinea pigs, and rabbits. In: Usdin, E., ed. Neuropharmacology of monoamines and their regulatory enzymes. New York: Raven Press; 1974:221–239.
33. Lewander, T. Effects of chronic amphetamine intoxication on the accumulation in the rat brain of labeled catecholamines synthesized from circulating tyrosine-^{14}C and dopa-^{3}H. Naunyn Schmiedebergs Arch. Pharmacol. 271:211–233; 1971.
34. Lewander, T. A mechanism for the development of tolerance in rats. Psychopharmacologia 21:17–31; 1971.
35. McCabe, J. T.; Leibowitz, S. F. Determination of brainstem catecholamine fibers mediating amphetamine anorexia. Brain Res. 311: 211–224; 1984.
36. Magour, S.; Coper, H.; Fahnchrich, C. H. The effects of chronic treatment with d-amphetamine on food intake, body weight, locomotor activity and subcellular distribution of the drug in rat brain. Psychopharmacologia 34:45–54; 1974.
37. Maickel, R. P.; Light, K. E.; Zabik, J. E. Differential activity of enantiomers of amphetamine in rats. Neuropharmacology 21:1323–1327; 1982.
38. Neil, D. B.; Grossman, S. P. Interactions of the effects of reserpine and amphetamine on food and water intake. J. Comp. Physiol. 76:327–336; 1971.
39. Steranka, L. R. Stereospecific long-term effects of amphetamine on striatal dopamine neurons in rats. Eur. J. Pharmacol. 76:443–446; 1981.
40. Tilson, H. A.; Sparber, S. B. The effects of d- and l-amphetamine on fixed interval and fixed ratio behavior in tolerant and non-tolerant rats. J. Pharmacol. Exp. Ther. 187:372–379; 1973.
41. Tormey, J.; Lasagna, L. Relation of thyroid function to acute and chronic effectors of amphetamine in the rat. J. Pharmacol. Exp. Ther. 128:201–209; 1960.