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Behavioral Assessment of the Toxicity of Aspartame

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HOLDER, M. D. AND R. YIRMIYA. *Behavioral assessment of the toxicity of aspartame.* PHARMACOL BIOCHEM BEHAV 32(1) 17–26, 1989.—Six experiments with rats assessed the toxicity of aspartame with behavioral measures. The first three experiments used a conditioned taste aversion procedure since taste aversions are typically observed after a taste is followed by a toxin. Thirty min after thirsty rats drank a sweet solution they were intraperitoneally injected (Experiment 1) or intragastrically intubated (Experiment 2) with saline or 176, 352, or 704 mg/kg of aspartame. Relative to rats given saline, rats injected with 704 and 352 mg/kg aspartame showed strong and mild aversions, respectively. Rats injected with 176 mg/kg of aspartame or intubated with any dose of aspartame did not show taste aversions. In Experiment 3, rats voluntarily consumed an aspartame solution sweetened with saccharin for 7 hr each day. Consumption of the taste paired with aspartame was not reduced. When 352 mg/kg aspartame was injected (Experiment 4), but not when intubated (Experiment 5), 5 min prior to access to a running wheel, running was reduced. Wheel running was not affected by the voluntary consumption of aspartame (Experiment 6). The route of administration effect (intraperitoneal vs. intragastric) on behavior corresponded with the amino acid levels in blood plasma (Experiment 7). Aspartate, phenylalanine, tyrosine and glutamate levels increased more after the injection, than the intubation, of aspartame (176 mg/kg). Overall, the results suggest that aspartame may have adverse effects when intraperitoneally injected but not when the route of administration is oral.

Aspartame Conditioned taste aversion Rat Behavioral toxicology Wheel running Large neutral amino acids

THOUGH L-aspartyl-L-phenylalanine methyl ester (aspartame) was reapproved as an artificial sweetener by the FDA in 1981, possible adverse effects of this compound are still of concern. Though empirical work has indicated that aspartame is safe within the range of predicted consumption, concern has remained strong because of theoretical considerations, experimental studies which used high doses, consumer complaints, case histories and the increasing pervasiveness of aspartame in the food chain.

Concern surrounding aspartame has stemmed from theoretical considerations based on the metabolism of aspartame into its individual components: phenylalanine (50% by weight), aspartate (40% by weight), and methanol. Though these substances are normally found in the body, aspartame may have unwanted effects because it raises the normal levels of these substances relative to other substances in the body (55). Aspartame's metabolism was studied by orally administering the three individual components of aspartame after they had been radiolabeled (i.e., ^{14}C-labeled amino acids and [^{14}C]methanol) and comparing the excretion of these compounds in the urine, feces and expired air to excretion following oral administration of aspartame after different portions of its molecule were radiolabeled (i.e., [^{14}C-phenylalanyl]aspartame, [^{14}C-asparyl]aspartame, and [^{14}C-methyl] aspartame). Using similarly radiolabeled compounds, whole-body autoradiograms were also conducted (26). The results of both procedures were similar whether rats were given the labeled amino acids, methanol or aspartame. Aspartame was shown to be rapidly and extensively metabolized into its constituent amino acids and methanol after its consumption. This hydrolysis takes place as the aspartame passes from the intestinal lumen into the portal circulation (33).

Large doses of aspartame can increase plasma and brain levels of phenylalanine and tyrosine (12,55). Phenylalanine is a large neutral amino acid which crosses the blood-brain barrier, competing with other large neutral amino acids for a common carrier (30,34). Increased brain uptake of phenylalanine might decrease brain uptake of other large neutral amino acids because the competitive carrier is more

occupied. For example, brain levels of tryptophan can decrease after aspartame intubation (12).

By elevating the levels of plasma phenylalanine, aspartame consumption may affect motor behavior. Though the major pathway of phenylalanine metabolism is its hydroxylation to tyrosine (27), a second pathway involves decarboxylation by aromatic-L-amino acid decarboxylase to phenylethylamine (PEA). Based on the measurement of phenylalanine metabolism, earlier work suggested that its decarboxylation is an important pathway when phenylalanine is consumed in excess (8). However, later studies reported that this pathway may be trivial even with high levels of phenylalanine (20). PEA has been identified in the human and rodent brain and its structure, pharmacological effects (13) and behavioral consequences (14) are similar to the effects of the stimulant amphetamine. Injections of PEA were found to enhance various components of spontaneous motor activity: sniffing, headweaving, splayed hindlimbs, hyperreactivity and grooming in rats (10). If aspartame-induced elevation in plasma phenylalanine concentration causes PEA formation then increases in spontaneous motor activity may result.

Developmental studies have shown that the consumption of high levels of aspartame by rats during pregnancy and lactation affects these rats' offspring (4). The effects included reduced body and brain weights, increased mortality and delayed morphological and reflex development. These effects were attributed to the phenylalanine component of aspartame. Oral intake of aspartate in mice can result in neuronal necrosis of several brain regions including the hypothalamus (32,38). However, monkeys show normal hypothalamic morphology (38–40).

Three human case histories were described in which first-time epileptic seizures followed the consumption of aspartame (54), leading to speculation that aspartame may lower the threshold for seizures. Additionally, hundreds of complaints about aspartame-containing foods have been registered including complaints about headaches and dizziness (11). However, experimental studies have not found an increase in either the incidence of seizures or these complaints following exposure to aspartame. For example, experimental work with mice has shown that pentylenetetrazol-induced and electroconvulsive shock-induced convulsant thresholds are not changed by the administration of 50 or 500 mg/kg of aspartame, intragastrically intubated 30 min prior to induced convulsion (19). Furthermore, infant monkeys did not show convulsions or seizures during a 9-month period when they were reared on high levels of aspartame and during the following year when they were fed a typical monkey diet (37). Relative to a placebo, 30 mg/kg of aspartame did not increase the incidence of headaches in humans (45).

In summary, studies showing hypothalamic damage in rodents, but not monkeys, deleterious effects of the individual components of aspartame, and consumer complaints, along with the dramatic increase in the incorporation of aspartame in popular foods, warranted continued evaluation of its possible acute adverse effects. In the studies reported here, aspartame's toxicity, if any, was measured with a conditioned taste aversion procedure and a wheel-running procedure.

Conditioned taste aversions are observed when an animal's consumption of a specific taste is reduced after the taste has been paired with illness [see (15,16)]. This procedure has been recommended as one sensitive measure to be included in test batteries designed to determine the toxicity of compounds (36,42). This recommendation is based on the finding that when any one of many known toxins is injected after an animal consumes a tasty substance, a taste aversion is typically learned. When the toxin is replaced with a nontoxic substance, a taste aversion is rarely formed. For example, known toxins such as copper sulphate, lithium chloride and strychnine sulfate all produce taste aversions, whereas agents not known to be toxic such as citric acid, glucose and quinine, do not [see (42)]. This procedure appears to be more sensitive than others. For example, after rats consumed sweetened milk or a saccharin solution and were injected with 10–20 mg/kg of lead acetate, taste aversions were observed (7). However, even with levels as high as 100 mg/kg, lead acetate did not change performance on a different learning task (3). Several agents support conditioned taste aversions at doses that do not produce behavioral indices of nausea or discomfort (17,44) and considerably below the LD_{50} dose (42).

Experiments 1–3 measured the consumption of a sweet solution by rats before and after the solution was paired with aspartame. The aspartame was intraperitoneally injected (Experiment 1), or intragastrically intubated (Experiment 2), or dissolved in the sweet solution and drank (Experiment 3). Intraperitoneal injections were used because that is the standard route of administration in conditioned taste aversion studies. Intragastric intubations were used because the route of administration is more similar to that of human aspartame consumers while maintaining control over the doses used. Voluntary consumption had the advantage of being most similar to the route of administration in humans though variability of the dose across animals and days, as well as the time course of the administration, was increased.

If aspartame has acute adverse effects, sweet consumption should decrease after it is paired with aspartame. The Center for Disease Control (CDC) contacted 517 people with complaints associated with foods that contained aspartame (11). They found that the complaints could be categorized into three groups: central nervous system, gynecologic and gastrointestinal. If the gastrointestinal complaints, which included nausea, are real and causally linked to aspartame then aspartame should support conditioned aversions. Additionally, high oral doses of monosodium L-glutamate (MSG) can support conditioned taste aversions (48). MSG, like the aspartate in aspartame, is a dicarboxylic amino acid and a food additive.

The possibility that aspartame might be behaviorally active was studied with one measure of gross motor behavior: wheel running. When rats are given access to a running wheel, they will run without being reinforced. Rate of wheel running has been shown to be sensitive to the effects of food additives. For example, red dye No. 40 decreases (49) and caffeine increases (5) wheel running in rats. Spontaneous wheel running in rats was measured before and after aspartame was intraperitoneally injected (Experiment 4), intragastrically intubated (Experiment 5), or voluntarily ingested (Experiment 6).

EXPERIMENT 1: CONDITIONED TASTE AVERSIONS AND INJECTED ASPARTAME

In Experiment 1, thirsty rats were given 10-min access to water sweetened with saccharin and then, after a 30-min delay, were intraperitoneally injected with different amounts of aspartame. A 30-min delay was used to help ensure that if a saccharin aversion developed it was the result of the sac-

charin being paired with the adverse effects of aspartame rather than aspects of the administration ritual which were not of interest (e.g., possible pain from the injection or fear). These aspects, which were not of interest, are usually not conditioned with such long delays (15). Each rat received a total of either three saccharin-aspartame pairings or three saccharin-saline pairings as a control. Changes in consumption of saccharin were measured.

METHOD

Subjects and Apparatus

Subjects were 28 male Sprague-Dawley rats weighing 271–300 g at the start of the experiment. Animals were housed individually in standard stainless-steel cages located in an animal-colony room. All training and testing were done in the animal-colony room during the first third of the light portion of the 14:10 hr, light:dark cycle. Food (Purina Rat Chow Pellets) was always available.

Procedure

Animals were first given water in Nalgene drinking tubes for 24 hr. Then, after being deprived of water for 24 hr, the rats were given 30-min access to the drinking tubes. The next 6 days (Days 4–9) were habituation days to allow the animals to adjust to a restricted drinking schedule. This schedule consisted of giving the rats 10-min access to the drinking tubes each day and 1 hr later giving them 20-min access to tap water. On Days 10, 12 and 14 the rats' usual 10 min of unflavored water was replaced with 10 min of a 0.1% (w/v) sodium saccharin solution. Thirty min after the saccharin water was presented, the rats were injected intraperitoneally with isotonic saline or 176 (n=7), 352 (n=7), or 704 (n=7) mg/kg of aspartame. The concentration of aspartame (i.e., 88 g aspartame per liter water) was held constant and the volume was varied. The volume of the saline injection (8 ml/kg) was the same as the 704 mg/kg aspartame injection. One hour after all rats were injected they received 20-min access to tap water. There were three saccharin-aspartame acquisition trials, each separated by one day in which the rats received their normal restricted drinking schedule (10-min access to unflavored distilled water in tubes followed 1 hr later by 20-min access to tap water). Days 16, 18 and 20 were extinction days which were the same as the restricted drinking schedule except that the animals were given the saccharin solution for 10 min instead of unflavored water.

Data Analysis

Data analyses was similar for all experiments reported here. All analyses of variance (ANOVAs) treated rat as a random factor and the other factors as fixed. ANOVAs involving multiple sessions used a repeated-measures design. Probabilities associated with *t*-tests were all two-tailed.

RESULTS AND DISCUSSION

Consumption of the saccharin solution remained high for both the group given the saline injection, as well as the group given the smallest volume of aspartame. However, consumption decreased slightly for the group given 352 mg/kg of aspartame and decreased markedly for the group given 704 mg/kg. During extinction, when the saccharin was presented but not followed by aspartame injections, consumption for the groups previously given 352 and 704 mg/kg aspartame gradually increased back to the level of the other two groups. This difference between groups over trials was significant as shown by a groups by trails interaction, $F(15,50)=3.13$, $p=0.001$. The results are illustrated in Fig. 1. Overall, the results indicate that aspartame can support a conditioned taste aversion when administered intraperitoneally.

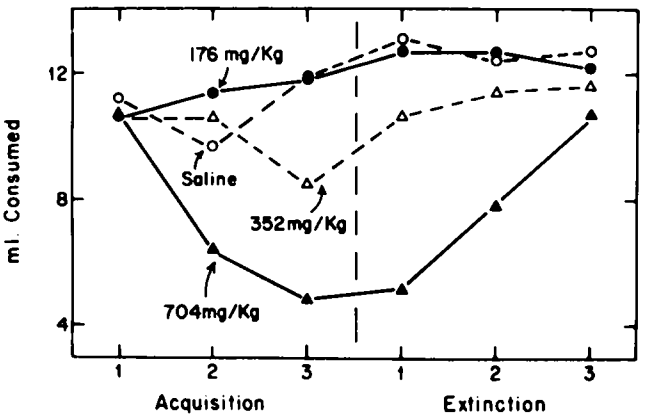

FIG. 1. Experiment 1: Mean consumption of saccharin-flavored water over days is shown for each group. During Acquisition, thirsty rats drank saccharin water for 10 min and 30 min later were intraperitoneally injected with 176, 352 or 704 mg/kg of an aspartame solution or saline as a control. Saccharin consumption remained high for the rats given saline or 176 mg/kg of aspartame, decreased slightly for the rats given 352 mg/kg of aspartame and decreased greatly for the rats given 704 mg/kg of aspartame. After three saccharin-aspartame pairings, the saccharin aversion gradually extinguished.

EXPERIMENT 2: CONDITIONED TASTE AVERSIONS AND INTUBATED ASPARTAME

The results of Experiment 1 suggested that at the doses used, aspartame has adverse properties when administered intraperitoneally. Of course, the route of administration of aspartame in humans is oral, not intraperitoneal. Therefore, Experiment 2 repeated the procedure of Experiment 1 except that the aspartame was intragastrically intubated.

METHOD

Subjects and Apparatus

The subjects were 29 rats similar to those in Experiment 1. All training and testing were done in the animal-colony room described in Experiment 1.

Procedure

The procedure was very similar to Experiment 1 so only the differences will be described here. The main difference was that the 176 (n=8), 352 (n=7), and 704 (n=7) mg/kg of aspartame and the saline (n=7) were intragastrically intubated instead of injected. To familiarize the rats with the intubation procedure, on Day 6 each rat was sham intubated (an infant feeding tube was put down their esophagus into the stomach but nothing was infused) 30 min after they were given 10-min access to the drinking tubes. There were a total of 10 habituation days followed by 3 acquisition days (Days 8, 10 and 12) and 1 extinction day (Day 14). The acquisiton

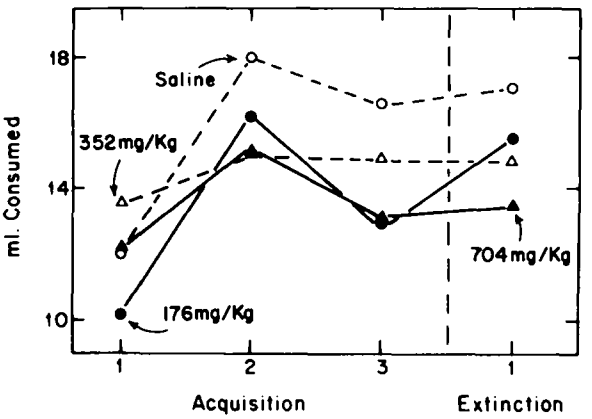

FIG. 2. Experiment 2: Mean consumption of saccharin-flavored water is shown for each group. Experiment 1 (see Fig. 1) was repeated except that the aspartame (176, 352, or 704 mg/kg) or saline was intragastrically intubated. No conditioned taste aversions were evident at any dose.

and extinction days were each separated by one habituation day. On Day 16, the final day, a two-tube test was employed. Each rat was presented with two tubes; one filled with saccharin and the other with unflavored water. For each group, 15 rats received saccharin on the left and water on the right; for the remaining rats the saccharin was on the right. After 5 min, for each rat the position of the tubes was reversed.

RESULTS AND DISCUSSION

The results are shown in Fig. 2 and are not similar to Experiment 1. No group developed a taste aversion. In fact, average consumption for each group increased slightly after the first acquisition trial. There was no group effect, $F(3,25)=1.2$, $p>0.3$, or group by trials interaction, $F(9,56)=1.9$, $p=0.07$. Consumption by aspartame-intubated rats was the same as that of saline-intubated rats even on the test day after 3 saccharin-aspartame pairings. During the more sensitive two-bottle preference test, there was still no difference between groups, $F(3,25)<1$.

When aspartame is administered by gavage to rats, brain and blood levels of several neural transmitter precursors change (12). For example, a dose of aspartame (200 mg/kg) within the range of doses used here, administered by gavage, increased phenylalanine and tyrosine levels and decreased tryptophan levels. Smaller doses (50 and 100 mg/kg) increased phenylalanine and tyrosine levels but did not change tryptophan levels. Despite that gavaged aspartame can change blood and brain biochemistry, the taste aversion demonstrated in Experiment 1 with intraperitoneal injection was eliminated when the same amounts of aspartame were intragastrically intubated.

EXPERIMENT 3: CONDITIONED TASTE AVERSIONS AND VOLUNTARY CONSUMPTION OF ASPARTAME

Aspartame supported a conditioned taste aversion when intraperitoneally injected (Experiment 1) but not when intragastrically intubated (Experiment 2). The validity of the use of intragastric intubations has been brought into question (1, 31, 52). Therefore, the possible adverse effects of aspartame, as estimated with conditioned taste aversions, were investigated in nondeprived rats that voluntarily consumed aspartame using a procedure similar to that employed to assess toxins with delayed effects (41).

Conditioned taste aversions have been found in other situations involving voluntary consumption of diets that are not balanced for amino acids (2,56). These studies found that rats will develop aversions to the taste of a diet that lacks the amino acid L-histidine. If an amino acid imbalance results from the ingestion of aspartame, an aversion to the taste paired with aspartame may be evident.

METHOD

Subjects and Apparatus

The subjects were 20 rats similar to those in Experiment 1. All training and testing was done in the animal-colony room described in Experiment 1. Food (Purina Rat Chow Pellets) was always available.

Procedure

When the rats arrived in the home cage room they were housed in individual cages and given unflavored water. After 18 days, the unflavored water was removed for 7 hr each day and each rat was presented with a drinking tube of flavored water. Half the rats were assigned to Group Asp and received saccharin-flavored water containing aspartame (0.1% saccharin + 0.8% aspartame, w/v). The remaining rats were assigned to Group NoAsp and received only saccharin-flavored water (0.1% saccharin, w/v). Though rats can taste aspartame (46), it does not taste similar to saccharin to them (29,46). Therefore, the saccharin taste was a distinct and different taste paired with the aspartame. The group assignments were counterbalanced for water consumption on the day before the flavored water was introduced. If aspartame has adverse properties that can be associated with taste, then Group Asp should consume less saccharin-flavored water than Group NoAsp. This phase, called Acquisition, lasted 8 days, after which the rats were given a two-bottle preference test. For 7 hr, all rats were given one bottle containing the saccharin solution and one containing unflavored water. The locations of the two bottles were reversed after 1 and 5 hr.

RESULTS AND DISCUSSION

Consumption during Acquisition was similar between groups. Group Asp drank slightly less than Group NoAsp but neither this difference, $F(1,18)<1$, nor the group by trials interaction, $F(7,12)<1$, was significant. Each rat in Group Asp consumed an average of 114 mg of aspartame each day which was approximately 400 mg/kg. After Acquisition, the two groups showed almost identical preferences for the saccharin solution, $t(18)<1$. Consumption for both groups during Acquisition and the taste-preference test is shown in Fig. 3.

EXPERIMENT 4: WHEEL RUNNING AND INJECTION OF ASPARTAME

Experiment 4 investigated the effects of intraperitoneal injections of aspartame on a measure of spontaneous general activity. In order to determine the effects on motor behavior, rats were given access to a running wheel for 30 min each day. After several days of experience with the running wheels, half the rats were injected with aspartame and the other half were injected with saline and subsequent running

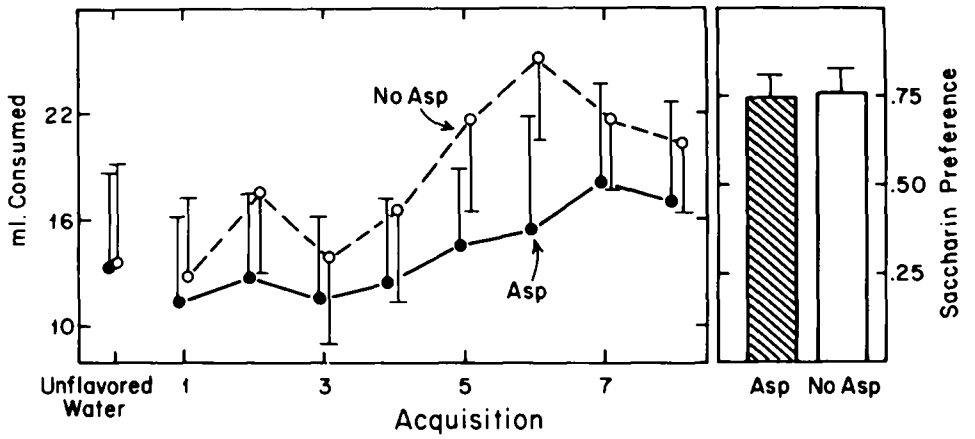

FIG. 3. Experiment 3: Mean consumption of unflavored water (first data points) and saccharin-flavored water is shown for each group in the left panel. During Acquisition, each day rats were given 7 hr access to saccharin water (Group NoAsp) or saccharin water that contained 0.8% aspartame (Group Asp). Consumption was similar for both groups during Acquisition. When given a choice between saccharin water and plain water, the saccharin preference (saccharin consumed/total consumed) was the same for both groups (right panel). (Error bars in this and all subsequent figures refer to standard errors of the means.)

behavior was measured. A few days later the procedure was repeated using a lower dose of aspartame.

METHOD

Subjects and Apparatus

Thirty Sprague-Dawley albino male rats weighing 240–363 g served as subjects. They lived in the colony room described in Experiment 1. The rats were tested in 10 similar running-wheel chambers (Wahmann manufacturer) kept in a small room. The chambers consisted of a metal rectangular cage (26×16×13 cm) with a small opening (9×7 cm) through which the rat could easily climb to a metal running wheel (36 cm diameter, 11 cm wide). A counter on the side of the wheel recorded the number of complete circles, in either direction, that each rat ran.

Procedure

Each day, during the first third of the light cycle, the rats were given 30-min access to a running wheel. Each rat was placed in the same wheel every day. On Day 7, the rats were given an injection 5 min before they had access to the running wheel. A 5-min delay was chosen because observation of the rats in Experiment 1 showed that the rats began to show signs of illness, including reduced motor activity, 5 min after the 352 and 704 mg/kg aspartame injections. The rats' behavior in the running wheel was measured for 30 min because in Experiment 1, the rats appeared to begin to recover after 30 min. Half the rats were injected with 4 ml/kg of an aspartame solution (88 g/liter distilled water, 352 mg/kg) and the other half were injected with an equivalent volume of saline. The assignments of rats to groups were arranged so that the average running rates for each group on Day 6 and the average body weights were similar. In order to measure any longer-lasting effects of aspartame, on Day 8, the rats were given the usual 30-min access to the wheels without injections. On Day 8 the running wheels for 3 rats stuck (2 from the aspartame-injected group) and data from these rats on Day 8 are not reported. On Days 9 and 10 the rats were allowed their normal 30-min access to the running wheels. On Day 11, half the rats were injected with aspartame (0.5 ml/kg, 44 mg/kg) and the remainder were injected with saline. Approximately half the rats in each group had previously been injected with saline on Day 7. In this, and Experiments 5 and 6, averages are reported as means followed by standard errors in parentheses.

RESULTS AND DISCUSSION

Relative to the saline controls, wheel running was sharply reduced for the rats injected with 352 mg/kg of the aspartame solution. For the rats injected with aspartame, the mean running rate was 13 (3) and for the rats injected with saline, the mean running rate was 62 (11), $t(26)=3.9$, $p<0.001$. Average running rates were similar between groups on Day 6 and on Day 8, $ts(26)<1$.

When the rats were injected with 44 mg/kg of the aspartame solution, there was no change in wheel running. This lack of an effect was shown by the absence of a significant group by previous injection interaction, $F(1,24)=3.09$, $p>0.09$, and group effect, $F(1,24)<1$. The effects of both the high and the low volume injections are illustrated in Fig. 4.

These results indicate that injections of a high aspartame dose reduced overall running rates. There was no evidence that this effect was long-lasting since running rates returned to normal the day after the injection. The effect may be dose dependent since the lower injection had no effect.

EXPERIMENT 5: WHEEL RUNNING AND INTUBATION OF ASPARTAME

The drop in running rates after the injection of aspartame in Experiment 4 indicates that at the doses used, aspartame has behavioral effects. Experiment 5 tested aspartame's effect on wheel running when the route of administration was

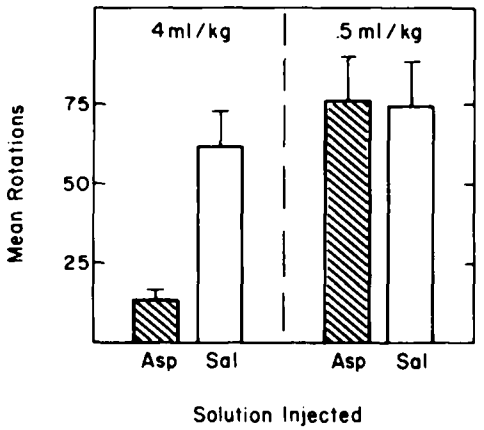

FIG. 4. Experiment 4: Mean number of wheel rotations are shown for each group after aspartame (Asp) or saline (Sal) was intraperitoneally injected. Each day the rats were given 30 min access to a running wheel. Aspartame (4 ml/kg=352 mg/kg) decreased wheel rotations compared to saline. Lower doses of aspartame (0.5 ml/kg=44 mg/kg) did not affect wheel running.

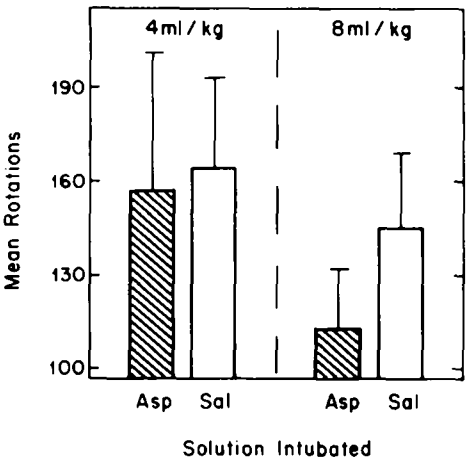

FIG. 5. Experiment 5: Mean number of wheel rotations during a 30 min access period to running wheels are shown after aspartame (Asp) or saline (Sal) was intragastrically intubated. One dose of aspartame (4 ml/kg=353 mg/kg) did not change wheel running though a higher dose (8 ml/kg=106 mg/kg) decreased running rates slightly.

more similar to that of humans. Experiment 5 repeated the procedure of Experiment 4 except that the aspartame was intragastrically intubated.

METHOD

Subjects and Apparatus

The subjects were 28 rats similar to those in Experiment 4. All training and testing was done in the running wheels described in Experiment 4.

Procedure

The procedure was very similar to Experiment 4 so only the differences are described here. The main difference was that the aspartame and saline were intragastrically intubated instead of injected. In order to familiarize the rats with the intubation procedure, on Day 8 all the rats were sham intubated 5 min before they were placed in the wheel-running apparatus. When aspartame was intubated for the second time, the volume was doubled to 8 ml/kg. The first intubation corresponded to 352 mg/kg aspartame and the second to 706 mg/kg.

RESULTS AND DISCUSSION

Unlike when the aspartame was injected in Experiment 4, wheel running was not reduced when rats were intubated with 352 mg/kg of the aspartame solution. When intubated with aspartame, the rats' mean running rate was 157 (42) and for the rats intubated with saline, the mean running rate was 164 (29), $t(26)<1$.

When 706 mg/kg of the aspartame solution was intubated running rates decreased. However, this decrease was not statistically significant as shown by the absence of a significant group by previous injection interaction, $F(1,24)<1$, and group effect, $F(1,24)=1$. The effects of both the low and high volume intubations are illustrated in Fig. 5.

These results indicate that aspartame's effect on running rates depends on the route of administration. Though reduction in running rates was apparent when the aspartame was injected (Experiment 4), the same levels of aspartame had no effect when intubated. The running rates were greater in Experiment 5 compared to Experiment 4. This may reflect the fact that the rats in Experiment 5 had more prior experience, and therefore, more exericse, with the running wheels.

EXPERIMENT 6: WHEEL RUNNING AND VOLUNTARY CONSUMPTION OF ASPARTAME

In Experiment 6, a group of 10 rats lived in the running wheels with continuous access to the wheels, water and food. After the animals had become familiar with the wheels, their water was replaced with an aspartame solution, then an aspartame/glucose solution and later a glucose solution. The effect of voluntary consumption of aspartame and glucose on wheel running was then measured.

Glucose was used in this study because it may potentiate any effect of aspartame. Previous work has shown that when aspartame is consumed with carbohydrates the levels of phenylalanine and tyrosine in the rat brain are much greater than when aspartame is consumed alone (53).

METHOD

Subjects and Apparatus

Subjects were 10 male rats weighing 276–296 g at the start of the experiment. The running wheels were the same as those used in Experiment 4. Food was always available in the small rectangular cage of the running wheel. Water was continuously available in a Nalgene tube attached to each cage.

Procedure

The animals were placed in the running wheel cages where they remained for the entire experiment. At the same

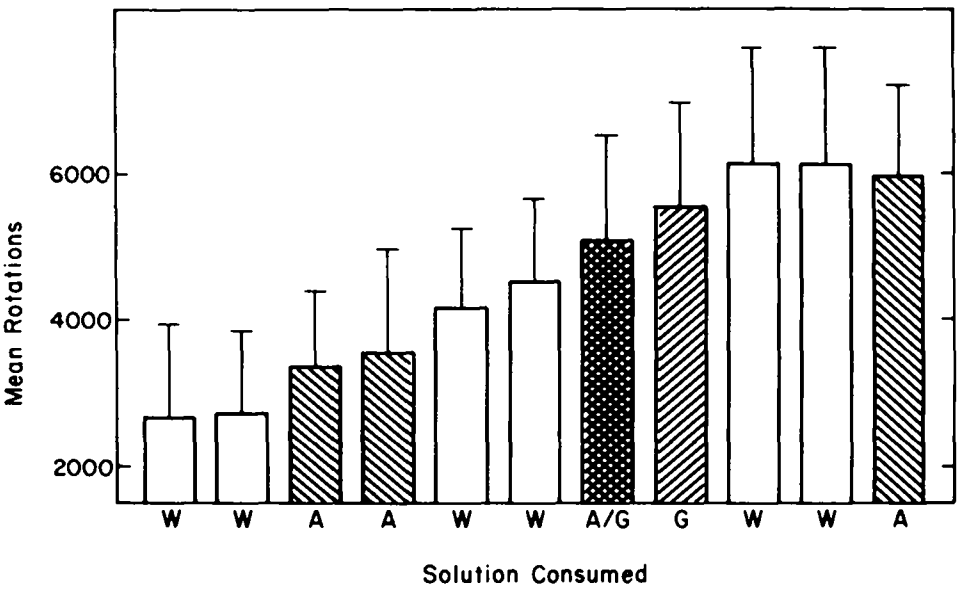

FIG. 6. Experiment 6: Mean number of wheel rotations are shown over days. Rats lived in small cages attached to running wheels. There was a steady increase in running rates over days, but no apparent change that was attributable to replacing the water (W) solution with aspartame (A), glucose (G), or both (A/G).

time each morning the number of rotations and the amount of solution consumed were recorded. Also at this time the drinking tubes were refilled and the bedding for each cage was changed. On Days 7 and 8, the rats' water was replaced with an aspartame solution (0.4%, w/v). After 17 more days with water only, on Day 26, the rats' water was replaced with an aspartame/glucose solution (0.8% aspartame + 3.5% glucose, w/v). On Day 27 the rats were given a glucose solution (3.5%, w/v) followed by two days of water only. Finally, on Day 30 all rats were given an aspartame solution (0.8%, w/v).

RESULTS AND DISCUSSION

Running rates on days when the rats consumed the aspartame/glucose solution and the aspartame alone solution were similar to rates on days the animals drank water or glucose alone. Figure 6 illustrates these results. There was a steady increase in running rates over days but this increase was not attributable to the solution consumed. There was no significant effect of solution consumed, $F(8,2)<1$.

Unlike when the aspartame was injected, the rats' general motor activity, as measured by wheel running, was unaffected by voluntary consumption. The actual dose of aspartame self-administered in this experiment was greater than the dose injected or intubated in the previous experiments though it was spread across a 24-hr period. Each rat consumed 45 ml of liquid each day (the capacity of the drinking tubes). This 45 ml contained 360 mg of aspartame at the higher dose which converts to an average dose of 1259 mg/kg. Even with this very high dose, there was no effect of voluntarily-consumed aspartame on wheel running.

EXPERIMENT 7: AMINO ACIDS IN BLOOD PLASMA

Experiments 1–6 showed conditioned taste aversions and decreases in spontaneous wheel running after an intraperitoneal injection of aspartame but not after the oral administration of aspartame. This route of administration effect may reflect differences in the amino acid levels in the blood as a result of differences between metabolism and absorption in the intraperitoneal cavity and the stomach and intestines. Experiment 7 focused on the route of administration effect from a biochemical perspective. Amino acid and aspartame concentrations in blood plasma were measured after aspartame or saline were intraperitoneally injected or intragastrically infused.

METHOD

Subjects

Subjects were 24 male rats weighing 350–446 g maintained on ad lib Purina Rat Chow and water in plastic tub home cages.

Procedure

Each rat was assigned to one of four groups that differed on whether a slurry of aspartame (176 mg/kg, n=16) or an equal volume of isotonic saline (n=8) was intubated (n=12) or intraperitoneally injected (n=12). Each rat was injected or intubated and then deeply anesthetized with pentobarbital (65 mg/kg and then as needed). Blood samples were taken from the abdominal aorta 11.5 to 17.7 min after the aspartame or saline was administered. One rat died before its sample was obtained and, therefore, no data were recorded for this rat. All animals were in the absorptive state when blood samples were taken.

Blood was immediately deproteinized with an equal amount of 10% sulfosalicyclic acid, the precipitated proteins removed by centrifugation, and the supernatant adjusted to pH 2.2 with LiOH. Amino acids were determined on a Beckman model 121-MB amino acid analyser by a modifica-

TABLE 1
EXPERIMENT 7: AMINO ACID LEVELS IN BLOOD PLASMA (MEANS)

	Amino Acid Concentration (nmol/ml)			
	Intraperitoneal Injection		Intragastric Intubation	
	Saline (4)	Aspartame (8)	Saline (4)	Aspartame (7)
Threonine	218	221	232	196
Serine	169	187	182	175
Glutamine	511	553	557	532
Aspartate	19	305†	14	18
Proline	168	178	170	151
Glutamate	80	198‡	153	123
Half-Cystine	54	55	54	53
Glycine	185	188	170	199
Alanine	409	447	454	423
Valine	163	172	178	178
Methionine	48	58	55	50
Isoleucine	65	67	73	70
Leucine	114	118	130	126
Tyrosine	80	160*	72	89
Phenylalanine	59	205‡	65	75
Ornithine	48	50	46	43
Lysine	390	414	375	441
Histidine	58	63	61	61
Tryptophan	79	89	97	85
Arginine	159	167	148	148
Aspartame	ND	ND	ND	ND

Values in parentheses are number of rats in each group. Symbols, *, † and ‡, refer to probability levels, $p=0.05$, $p<0.01$, and $p<0.001$, respectively, of route of administration by substance given interaction determined by a two (intraperitoneal vs. intragastric) by two (saline vs. aspartame) ANOVA. ND refers to not detectable.

tion (Beckman technical bulletin 121M-TB-013) of the method of Lee (22).

RESULTS AND DISCUSSION

The biochemical results observed in this experiment complement those of the previous six experiments and are summarized in Table 1. The concentration of amino acids related to aspartame metabolism increased following intraperitoneal injection of aspartame compared to the concentrations following intubation of aspartame or the administration of saline. Following administration of aspartame, concentrations of phenylalanine increased, $F(1,19)=19.1$, $p<0.001$, and this increase was much larger when the aspartame was injected as evidenced by a significant interaction between the substance injected and the route of administration, $F(1,19)=14$, $p<0.001$. Phenylalanine is a precursor of tyrosine and following administration of aspartame concentrations of tyrosine also increased, $F(1,19)=10.8$, $p<0.005$, and this increase was larger when the aspartame was injected as evidenced by a significant substance injected by route of administration interaction, $F(1,19)=4.3$, $p=0.05$. Following administration of aspartame, concentrations of aspartate increased when the aspartame was injected but not when it was intubated, $F(1,19)=11.6$, $p<0.001$. Glutamate increased when the aspartame was injected but not when it was intubated, $F(1,19)=4.3$, $p=0.05$. One possible explanation for the increase in glutamate involves the fact that aspartate and glutamate compete for a common transport mechanism for absorption into tissue (6). Aspartame caused an increase in aspartate levels, and because of this increase the common transport mechanism was more occupied with aspartate. Therefore, the uptake of glutamate by the tissue was slowed resulting in an increase in glutamate in the plasma. Aspartame was not detected supporting other work showing that it is rapidly metabolized (26).

GENERAL DISCUSSION

When aspartame was intraperitoneally injected into rats following consumption of sweet water, rats consumed less of the sweet water when it was next encountered (Experiment 1). The conditioned taste aversion procedure has been suggested as one measure of toxicity (36,40), and our result suggests that intraperitoneally administered aspartame may be toxic by this measure. However, when Experiment 1 was repeated but the aspartame was intubated (Experiment 2) or voluntarily consumed (Experiment 3), no conditioned taste aversions were observed. This is consistent with the idea that aspartame can support a conditioned taste aversion through a blood-borne pathway but not through vagal afferents (18). Alternatively, the route of administration effect may be attributable to differences in the rate of absorption. Aspartame may increase amino acid levels in the blood more rapidly when intraperitoneally injected than when it is orally administered. The rapidity of this increase, and not only the amount of the increase, may be responsible for the route of administration effect. Therefore, aspartame does not show toxic properties as measured with a conditioned taste aversion procedure when the route of administration is oral. This preliminary conclusion should be stated with caution. Of the known toxins that have been tested with the conditioned taste aversion procedure, a few do not support taste aversions. For example, aluminum chloride and sodium cyanide when paired with a taste do not support aversions (28,52). However, variations in the amino acid content of rats' diets have been shown to result in conditioned taste aversions (2,56).This finding indicates that the taste aversion procedure is sensitive to changes in amino acid intake and, therefore, is an appropriate procedure to assess the toxicity of the dipeptide aspartame.

Wheel running was decreased when aspartame was intraperitoneally injected (Experiment 4) but not when the aspartame was intubated (Experiment 5) or freely consumed (Experiment 6). The doses used were 9 times greater than one estimate for human consumption (35) and up to 27 times greater than the generally accepted projection of the 99th percentile level of humans (43).

The phenylalanine in the aspartame is mainly hydroxylated to tyrosine but can also be decarboxylated to PEA. PEA has been shown to affect many behaviors: feeding (9), self-stimulation of the brain (47), and motor activity (9). However, since only about 2% of phenylalanine can be converted to PEA (21), the maximum dose of PEA associated with our studies was 7 mg/kg which is lower than the 50 mg/kg typically needed to affect behavior. Furthermore, our results suggest that any effect of aspartame on motor activity does not involve PEA formation since low doses of PEA usually increase motor activity and we found that aspartame, when effective, had the opposite result.

The route of administration effect with conditioned taste aversions and spontaneous wheel running may be related to differences in rate of absorption. Levels of phenylalanine, tyrosine, aspartate and glutamate were much higher soon after intraperitoneal compared to oral administration of aspartame (Experiment 7). This route of administration effect suggests that the alteration of diet selection by the intraperitoneal administration of amino acids (23,24) may also be due to the route of administration. The fact that the amino acid concentrations following intraperitoneal aspartame increase so quickly and to such high levels, compared to oral administration, makes it particularly difficult to generalize from intraperitoneal administration of amino acids to factors that govern normal diet selection.

Our findings of differential effects as a function of the route of administration of aspartame are not uprecedented. Unpublished work indicates that hypothalamic damage from aspartame is greater following subcutaneous injection than when given orally (J. W. Olney, personal communication, January 15, 1986). Olney suspected that this difference could be attributed to the presence of proteolytic enzymes in the subcutaneous tissues. Additionally, 200 mg/kg of aspartame intraperitoneally injected was found to reduce systolic arterial pressure in spontaneously hypertensive rats (25) but when the same amounts were administered orally, no changes in blood pressure or heart rate were found (50). However, this latter study failed to replicate the intraperitoneal effect of aspartame. Overall, these results emphasize the importance of considering the route of administration when toxicological studies are conducted and evaluated.

Even with levels of aspartame that exceeded high estimates of daily human intake, no effects were observed when aspartame was administered orally. These results are consistent with the literature suggesting that aspartame is safe for use as a low-calorie sweetener.

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