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Dietary Influences on the Acute Effects of Anorectic Drugs[1]

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KANAREK, R. B., A. L. GLICK AND R. MARKS-KAUFMAN. *Dietary influences on the acute effects of anorectic drugs.* PHYSIOL BEHAV **49**(1) 149–152, 1991.—The effects of acute administration of d-amphetamine sulfate (0.0, 0.5, 1.0 and 2.0 mg/kg) and dl-fenfluramine hydrochloride (0.0, 1.5, 3.0 and 6.0 mg/kg) on food intake were examined in male Sprague-Dawley rats fed either a high-carbohydrate diet (carbohydrate equaled 65% of total calories) or a high-fat diet (fat equaled 65% of total calories). Animals were given ad lib access to the diets throughout the experiment. Drug injections were given at 0900 on experimental days and food intakes were measured at 1, 3 and 6 h postinjection. Amphetamine led to dose-related decreases in food intake for animals on both diets. The effects of amphetamine were most noticeable at 1 and 3 h postinjection. No differences in amphetamine's effects on food intake were found as a function of diet. Fenfluramine injections also led to dose-related reductions in food intake for animals in both dietary conditions. In contrast to amphetamine, however, fenfluramine led to greater reductions in food intake for rats fed the high-fat diet than for rats fed the high-carbohydrate diet. These data demonstrate that dietary variables must be considered when evaluating the anorectic actions of psychopharmacological agents.

Amphetamine Fenfluramine Food intake High-carbohydrate diet High-fat diet Anorectic drugs

OVER the past ten years, increasing attention has been paid to the role of pharmacological agents in the treatment of obesity. Anorectic drugs can lead to substantial reductions in body weight and body fat. However, tolerance to the anorectic and weight-reducing properties of these drugs are not uncommon and rebound weight gain may be observed following drug withdrawal. It has been suggested that combining treatment modalities may be one way of approaching these problems (3). Appropriate use of animal models can be helpful in determining the best methods of treatment combinations (1).

The majority of experiments examining the effects of anorectic agents on food intake and body weight have used only a single diet. Most frequently, this diet is laboratory chow, a high-carbohydrate, relatively low-fat (5% by weight) feed. Recent work, however, has demonstrated that dietary variables can interact with the effects of anorectic agents on food intake, body weight, and rebound weight gain. Variations in the macronutrient content of the diet can alter the actions of a number of anorectic agents, including amphetamine, naloxone and cholecystokinin [(8–11); Marks-Kaufman and Kanarek, unpublished results]. For example, work in our laboratory has demonstrated that rats fed a high-carbohydrate diet respond differently to the anorectic effects of amphetamine than rats fed a high-fat diet. When drinking equivalent amounts of an amphetamine solution, rats fed a high-carbohydrate diet decreased caloric intake less, but lost more weight than rats fed a high-fat diet. Further, rats given the high-carbohydrate diet displayed a rapid development of tolerance to the anorectic effects of amphetamine, with no tolerance to the drug's effect on body weight. In contrast, rats fed the high-fat diet suppressed caloric intake throughout the drug period, but weighed more than rats fed the high-carbohydrate diet (10).

To determine in more detail the interactions of diet with the anorectic actions of amphetamine, the present study examined the role of dietary variables on the effects of acute administration of the drug. Additionally, to assess if diet would influence the actions of other anorectic agents, the effects of acute fenfluramine administration were examined in rats fed a high-carbohydrate or high-fat diet.

EXPERIMENT 1

METHOD

Animals

Sixteen drug-naive male Sprague-Dawley rats (CD outbred, Charles River Breeding Laboratories, Wilmington, MA), weighing between 225–250 g at the beginning of the experiment, were used. Animals were housed individually in standard stainless steel cages in a temperature-controlled room (21 ± 1 °C) with a reverse 12–12-h light-dark cycle (lights on: 2200–1000 h).

Diets

Two diets were used, a high-carbohydrate diet containing 3.9

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kcal/g, and a high-fat diet containing 5.7 kcal/g. On a per 1000 kilocalorie basis, the high-carbohydrate diet contained 62.5 g (250 kcal) vitamin-free casein (ICN Pharmaceuticals, Cleveland, OH), 11.25 g (100 kcal) hydrogenated vegetable fat (Crisco), 162.5 g (650 kcal) corn starch (Teklad Test Diets, Madison, WI), 5.0 g vitamin diet fortification mix (ICN Pharmaceuticals) and 10 g AIN-76 mineral mix (ICN Pharmaceuticals). The high-fat diet contained 62.5 g (250 kcal) vitamin-free casein, 72.2 g (650 kcal) hydrogenated vegetable fat, 25.0 g (100 kcal) corn starch, 5.0 g vitamin diet fortification mix, and 10 g AIN-76 mineral mix. The diets were presented to the rats in spill-proof Wahmann LC-306A stainless-steel food cups. Tap water was available ad lib throughout the experiment.

Drugs

d-Amphetamine sulfate (Smith, Kline and French, Philadelphia, PA) was dissolved in physiological saline to concentrations that allowed selected doses to be administered in a volume of 1 ml/kg.

Procedure

Eight animals were given ad lib access to the high-carbohydrate diet and eight animals, ad lib access to the high-fat diet. Body weights and nutrient intakes were measured daily for three weeks. Drug injections were then initiated. On injection days, animals received intraperitoneal (IP) injections of either physiological saline or amphetamine at 0900 h. Nutrient intakes were measured at 1, 3, and 6 h following injections. Drug doses were given in the following order: saline, 1.0 mg/kg, 0.5 mg/kg, and 2.0 mg/kg d-amphetamine sulfate. Each animal received each dose of the drug twice. A minimum of six days separated injection days.

Data Analysis

As there were no differences between the first and second injections of each drug dose, data were combined for statistical analyses. Data were analyzed using two-way analyses of variance using diet and drug dose as the independent variables. Post hoc comparisons between groups were made using Scheffe's procedure. All data reported as significant have a p value of 0.05 or less.

RESULTS

At the initiation of drug injections, rats fed the high-fat diet weighed more (387 g) than rats fed the high-carbohydrate diet (353 g).

Amphetamine led to dose-related decreases in food intake for animals on both diets (Table 1). The effects of amphetamine on food intake were most noticeable at 1 and 3 h postinjection. At 1 h, all three doses of the drug led to significant reductions in food intake on both diets. At 3 h, all but the lowest dose for rats on the high-carbohydrate diet significantly reduced food intake. By 6-h postinjection, only the higher doses of amphetamine produced a suppression in food intake. Although at 3 and 6 h following injections rats fed the high-fat diet consumed more calories than rats fed the high-carbohydrate diet across all injection conditions, no differences in amphetamine's suppressive effects on food intake were found as a function of diet.

TABLE 1

CALORIC INTAKE (kcal) AT 1, 3 AND 6 HOURS FOLLOWING ADMINISTRATION OF AMPHETAMINE (0.0, 0.5, 1.0, AND 2.0 mg/kg) FOR RATS FED A HIGH-CARBOHYDRATE OR HIGH-FAT DIET

		Dose (mg/kg)			
	Time	0.0	0.5	1.0	2.0
High-Carbohydrate	1 h	10.6	4.6* (43)	2.9† (27)	0.14† (1)
	3 h	24.3	17.2 (71)	13.6† (56)	5.9† (24)
	6 h	35.5	32.8 (93)	31.2 (88)	26.3 (75)
High-Fat	1 h	14.2	4.3† (30)	2.6† (18)	0.11† (1)
	3 h	31.7	21.2* (67)	16.5* (52)	7.7† (24)
	6 h	43.3	39.7 (92)	32.5* (75)	33.4* (77)

At each time point, within each dietary condition, if drug is significantly different from saline: *$p<0.05$; †$p<0.01$. Numbers in parentheses equal percent reduction in caloric intake.

EXPERIMENT 2

METHOD

Animals

Sixteen drug-naive male Sprague-Dawley rats (CD outbred, Charles River Laboratories, Wilmington, MA), weighing 225–250 g at the beginning of the experiment, were used. Animals were housed as described in Experiment 1.

Drugs

dl-Fenfluramine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in physiological saline to concentrations allowing selected doses to be administered in a volume of 1.0 ml/kg.

Procedure

Eight rats were given ad lib access to the high-carbohydrate diet described in Experiment 1, and eight rats, ad lib access to the high-fat diet. The procedure was the same as Experiment 2 with the exception that drug injections were administered in the following order: saline, 3.0 mg/kg, 1.5 mg/kg and 6.0 mg/kg fenfluramine. Each animal received each dose of the drug twice with a minimum of six days between drug injections.

Data Analyses

Data were analyzed as described in Experiment 1.

RESULTS

At the initiation of drug injections rats fed the high-fat diet weighed more (371 g) than rats fed the high-carbohydrate diet (351 g).

Fenfluramine injections led to dose-related reductions in food intake for animals in both dietary conditions. Fenfluramine significantly suppressed feeding behavior throughout the 6-h feeding period (Table 2). Dietary variables did interact with the anorectic effects of fenfluramine. Fenfluramine led to greater reductions in food intake for rats fed the high-fat diet than those fed the high-carbohydrate diet. This effect was most noticeable following injections of the low dose of fenfluramine. For animals fed the high-fat diet, this dose of fenfluramine reduced caloric intake to 50–60% of saline levels at all time points. In comparison, for

TABLE 2

CALORIC INTAKE (kcal) AT 1, 3 AND 6 HOURS FOLLOWING ADMINISTRATION OF FENFLURAMINE (0.0, 1.5, 3.0, AND 6.0 mg/kg) FOR RATS FED A HIGH-CARBOHYDRATE OR HIGH-FAT DIET

		Dose (mg/kg)			
	Time	0.0	1.5	3.0	6.0
High-Carbohydrate	1 h	16.5	16.1 (98)	4.8† (29)	2.6† (16)
	3 h	35.1	34.7 (99)	13.3† (38)	8.3† (24)
	6 h	49.4	45.3 (92)	22.9† (46)	13.7† (28)
High-Fat	1 h	23.2	11.9* (51)	13.3 (57)	6.0† (26)
	3 h	36.5	19.9†[a] (55)	14.5† (40)	6.9† (19)
	6 h	54.9	32.0†[a] (59)	17.7† (32)	7.5† (14)

At each time point, within each dietary condition, if drug is significantly different from saline: *$p<0.05$; †$p<0.01$. Superscript indicates that reduction in intake of the high-fat diet was significantly ($p<0.05$) greater than reduction in intake of the high-carbohydrate diet under corresponding conditions. Numbers in parentheses equal percent reduction in caloric intake.

animals fed the high-carbohydrate diet, this dose of fenfluramine reduced caloric intake to 93–99% of saline values.

DISCUSSION

The results of the present studies demonstrate that the anorectic potency of fenfluramine is altered by dietary conditions. Acute administration of a low dose of fenfluramine led to significantly greater reductions in caloric intake in rats fed a high-fat diet than in rats fed a high-carbohydrate diet. These results correspond well with data from previous studies examining the effects of fenfluramine and other serotonergic agents on patterns of dietary self-selection. In these studies, following injections of fenfluramine or serotonin, rats offered a choice of separate sources of fat, protein and carbohydrate suppressed fat intake to a greater degree than either protein or carbohydrate intake (7,12).

In previous studies investigating the effects of fenfluramine and serotonin on diet selection, rats consumed a greater proportion of their calories as fat than as protein or carbohydrate. Thus it could be argued that serotoninergic agents act to reduce the intake of the most preferred dietary item. In the present study, caloric intake of rats consuming the high-fat and high-carbohydrate diets did not differ prior to drug administration. However, preference tests between the two diets were not performed, so it is possible that the high-fat diet was the more favored diet. The contribution of nutrient content of the diet versus palatability or preference for the diet is an important consideration in studies assessing the effects of pharmacological and physiological treatments on food intake and food choice (1, 6, 10). Unfortunately, this contribution has not yet received adequate attention.

It has been hypothesized that the anorectic effects of serotoninergic agonists, such as fenfluramine, may be related to the actions of these drugs on gastric motility (2, 4, 5, 14, 15). Administration of fenfluramine or serotonin leads to a reduction in gastric emptying which has been hypothesized to serve as a satiety signal (2,13). The greater reduction in intake of high-fat diets than high-carbohydrate diets produced by these drugs may also be related to gastric emptying. Fat, itself, prolongs gastric emptying relative to protein and carbohydrate. Consumption of a high-fat diet in association with administration of serotoninergic agonists could have an additive effect on gastric emptying. The decrease in rate of gastric emptying produced by these two stimuli could lead to a reduction in intake of additional fat.

Further evidence of the role of dietary variables in altering the effects of fenfluramine was garnered in a preliminary study examining the effects of chronic drug administration. Rats, fed either the high-fat or high-carbohydrate diet used in this study, were given saline injections for two days, followed by daily injections of 20 mg/kg fenfluramine for six days, and then two additional days of saline injections. No differences in caloric intake were noted as a function of diet when animals received saline. However, diet did influence the rats' responses to fenfluramine. Caloric intake decreased to an equivalent degree on the first day of drug administration. For rats fed the high-carbohydrate diet, caloric intake rapidly increased with subsequent injections of fenfluramine. In comparison, fenfluramine continued to suppress caloric intake throughout the drug injection period in rats fed the high-fat diet. Caloric intake of rats fed the high-fat diet was significantly less than intake of rats fed the high-carbohydrate diet from the second to sixth day of fenfluramine injections (Kanarek, Glick and Marks-Kaufman, unpublished results). These data suggest that diet may influence the development of tolerance to the anorectic actions of fenfluramine.

In contrast to our previous study in which diet modified the effects of chronic oral amphetamine administration on caloric intake, body weight, and feed efficiency (10), in the present study, diet did not alter the anorectic actions of acute amphetamine injections. In our earlier work, we found that amphetamine led to significantly greater reductions in caloric intake in rats fed a high-fat diet than in rats fed a high-carbohydrate diet. Several factors may help to explain the differences in the results of these two studies. First, the method of drug administration was different. Chronic oral administration of amphetamine could produce a relatively steady level of drug exposure. In comparison, acute amphetamine injections would produce a rapid increase in drug levels. Second, the doses of the drugs to which animals were exposed differed between the two studies. In our earlier study, rats received between 1.9 and 2.0 mg of amphetamine per day or approximately 6 mg/kg body weight, a higher dose than employed in the present study (10). A final related factor is that in our earlier study, rats were self-administering amphetamine, while in the present study, the drug was administered by the experimenter.

There have only been a small number of other experiments investigating the role of the macronutrient content of the diet on the effects of anorectic drugs. Maggio et al. (9) recently reported that cholecystokinin was more effective in reducing caloric intake in both obese and lean Zucker rats fed a high-fat diet than in animals fed either an isocaloric low-fat diet or chow. While limited in number, studies examining the effect of diet composition on the actions of anorectic drugs suggest that the macronutrient content of the diet significantly alters the effects of a number of anorectic agents. In studies comparing the effects of anorectic agents in rats consuming high-carbohydrate or high-fat diets, the drugs invariably lead to greater reductions in intakes of high-fat diets than of high-carbohydrate diets.

Understanding the role of diet in the actions of anorectic drugs could provide insights into methods of combining therapeutic strategies in the clinical treatment of obesity. Diets which are associated with greater reductions in caloric intake, less tolerance and less rebound weight gain when used in conjunction with anorectic drugs would obviously be of benefit and a simple adjunct to pharmacological treatment.

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