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Sustained Decrease in Fat Consumption Produced by Amphetamine in Rats Maintained on a Dietary Self-Selection Regime[1]

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KANAREK, R. B., L. HO AND R G. MEADE. *Sustained decrease in fat consumption produced by amphetamine in rats maintained on a dietary self-selection regime* PHARMAC BIOCHEM. BEHAV. **14**(4)539–542, 1981.—Total daily caloric intakes and dietary self-selection of the three macronutrients, protein, fat and carbohydrate, were examined in female rats following the administration of anorectic doses of d-amphetamine sulfate (0.5, 1.0 and 2 0 mg/kg body weight, IP). Animals had access to nutrients for a six-hour period each day with food intakes measured two hours after food presentation and again at the end of the six-hour feeding period Amphetamine injections led to similar dose-related decreases in caloric intakes in animals maintained on a standard laboratory diet (ground Purina Laboratory Chow) and those maintained on the dietary self-selection regime Detailed examination of feeding patterns of animals given the self-selection regime revealed, however, that amphetamine had differential effects on the consumption of protein, fat and carbohydrate. At all three drug doses, protein and carbohydrate intakes were suppressed during the initial two-hour measurement period Intakes of these two macronutrients returned to control values by the end of the six-hour feeding period. In contrast, fat intake was initially suppressed and remained suppressed throughout the entire six-hour period following amphetamine administration Comparison of the present results with those of previous experiments indicates that the selective effect of amphetamine on fat consumption is not a general effect of drugs which reduce caloric intake.

Dietary self-selection Fat Carbohydrate Protein Caloric intake Amphetamine Anorexia

MOST studies investigating the anorectic properties of amphetamine in experimental animals have provided animals with access to only a single nutritionally complete diet (e.g. [2, 3, 4, 5]). While these experiments allow conclusions to be drawn concerning amphetamine's action on total caloric intake, they do not provide information on effects of amphetamine on specific macronutrient intakes. Recent research suggests, however, that anoretic agents may selectively affect macronutrient consumption [13,14]. For example, in an experiment in which rats were given simultaneous access to two isocaloric diets containing either 5% or 45% protein, it was found that following the administration of the anoretic drug fenfluramine animals preferentially decreased their intake of the 5% protein diet [13]. The preferential decrease in intake of the low protein diet resulted in a reduction in total caloric intake without a significant reduction in protein intake. In contrast to fenfluramine, administration of amphetamine to animals in the same situation led to proportional decreases in protein and caloric intakes [13].

Two factors in these experiments make it difficult to draw conclusions about the effects of anorectic drugs on intake of specific macronutrients. First, the two diets offered to the animals varied not only in protein content, but also in carbohydrate content. That is, the low-protein diet (5% protein) contained 80% carbohydrate, while the high-protein diet (45% protein) contained only 45% carbohydrate. Second, as the proportion of the third macronutrient, fat, was identical (15%) in both diets, no conclusions can be made about the effects of anoretic agents on the intake of this macronutrient. To overcome these difficulties, the present study examined the effects of d-amphetamine on total caloric intake and diet selection in rats provided with separate sources of the three macronutrients. Using this dietary regime, a selective decrease in fat consumption was observed following the administration of amphetamine.

METHOD

Animals

Fifteen adult female Sprague-Dawley rats (CD outbred, Charles River Laboratories, Wilmington, MA) were used.

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Animals were housed individually in standard laboratory cages in a temperature-controlled room (21 ± 1°C). Lighting in the animal room was maintained on a reversed 12-12 hour light-dark cycle, with lights on from 2100 to 0900 hours.

Procedure

Animals were assigned on the basis of body weight to one of two dietary conditions: (1) a standard laboratory diet (N=8), or (2) a self-selection regime (N=7). Rats in the standard laboratory diet group received ground Purina Laboratory Chow #5001 (caloric density = 3.60 kcal/g, calculated from the nutritive content of the diet) provided in Wahmann (Timonium, MD) LC306-A food cups. Animals in the self-selection group received three separate dietary rations: a protein ration, a fat ration and a carbohydrate ration. The protein ration (caloric density = 3.76 kcal/g) was composed of 960 g casein (ICN Pharmaceuticals, Cleveland, OH), 40 g minerals (U.S.P. XIV Salt Mixture, ICN Pharmaceuticals), and 22 g vitamins (Vitamin Fortification Mixture, ICN Pharmaceuticals). The carbohydrate ration (caloric density = 3.76 kcal/g) contained 580 g corn starch (Teklad Test Diets, Madison, WI), 280 g dextrin (Teklad Test Diets), 100 g commercial-grade sucrose, 40 g minerals (U.S.P. XIV Salt Mixture) and 22 g vitamins (Vitamin Diet Fortification Mixture). The fat ration (caloric density = 7.85 kcal/g) contained 960 g commercial-grade vegetable shortening (Crisco), 90 g minerals (U.S.P. XIV Salt Mixture) and 50 g vitamins (Vitamin Diet Fortification Mixture). Vitamins and minerals were added to the components so that the three dietary rations contained equal amounts of these micronutrients on a per kilocalorie basis. All of the dietary rations were presented in Wahmann LC306-A food cups.

Animals were allowed one month to habituate to the reversed light-dark cycle and the dietary rations. Animals then were placed on a six-hour feeding schedule with food available from the end of the first hour (1000 hr) to the end of the seventh hour (1600 hr) after the onset of the dark portion of the daily lighting cycle. Animals were allowed one month to adapt to the restricted feeding schedule prior to the initiation of drug injections. Food intake and body weight data were collected every third day throughout the two-month pre-drug period. At the time drug injections were initiated, animals were 120 days of age. Mean body weight of animals in the standard laboratory diet group was 220.5 g and mean body weight of animals in the self-selection group was 225.9 g. At this time, animals on the self-selection regime were consuming 50% of their daily caloric intake as fat, 36% as carbohydrate and 14% as protein. This pattern of diet selection is consistent with that previously observed in this laboratory (e g. [8,10], Meade, unpublished results).

The procedures for drug injections and data collection were as follows. Animals were weighed to the nearest gram immediately following the onset of the dark portion of the 24-hour cycle. Intraperitoneal injections of d-amphetamine sulfate (Smith, Kline and French Laboratories, Philadelphia, PA) dissolved in physiological saline or physiological saline alone were given in a volume of 1.0 ml/kg body weight one-half hour before animals were to receive their food. Food intakes and spillage were measured to the nearest 0.1 g at two and six hours after the initiation of the feeding period. Each day of amphetamine injections was preceded by a day of saline injections. Three doses of amphetamine were given. 0.5, 1.0 and 2.0 mg/kg body weight. Animals received two injections of the 2.0 mg/kg dose of amphetamine and one injection each of the 0.5 and 1.0 mg/kg doses. Amphetamine injections were separated from each other by at least one week.

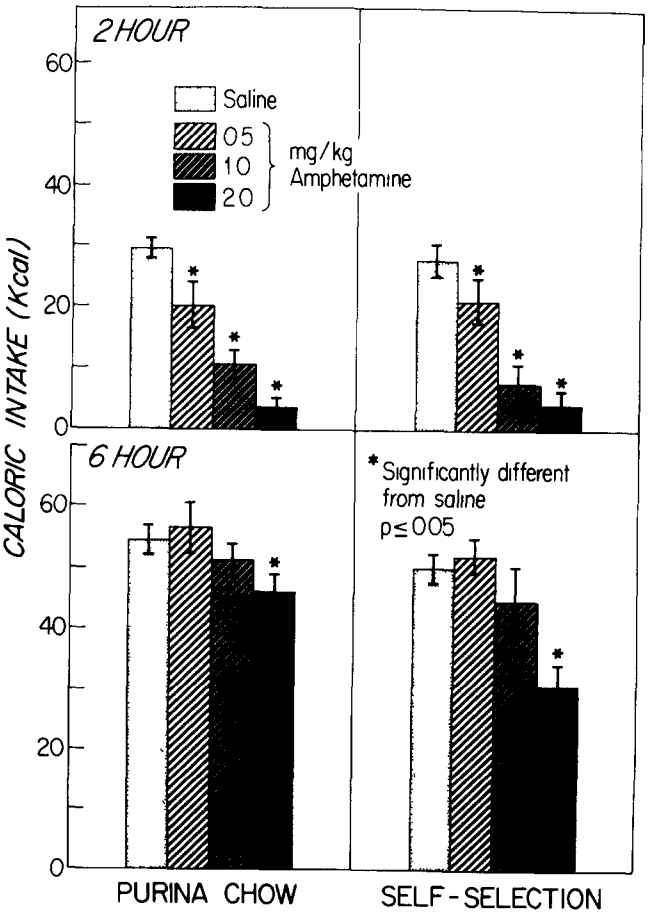

FIG 1. Cumulative two-hour (top) and six-hour (bottom) caloric intakes (mean ± SE) following the administration of saline, 0.5, 1 0 and 2.0 mg/kg amphetamine in animals maintained on either Purina Chow (left) or a dietary self-selection regime (right)

Data were analyzed by one-way analyses of variance with repeated measures, followed by a posteriori comparisons between drug doses using Scheffe's method [12]. As neither total daily caloric intakes nor nutrient selection varied across saline injection days, mean data for saline injections were used to analyze data. Similarly, as no differences in intakes were observed following the two injections of 2.0 mg/kg amphetamine, mean data were used in data analyses.

RESULTS

Animals Maintained on the Single Standard Laboratory Diet

Amphetamine administration led to dose-related decreases in caloric intakes during the first two hours of the six-hour feeding period (Fig. 1, top left). Two hour caloric intakes after injections of 2.0 mg/kg amphetamine were significantly lower than intakes after injections of 1.0 mg/kg amphetamine, which in turn were significantly lower than

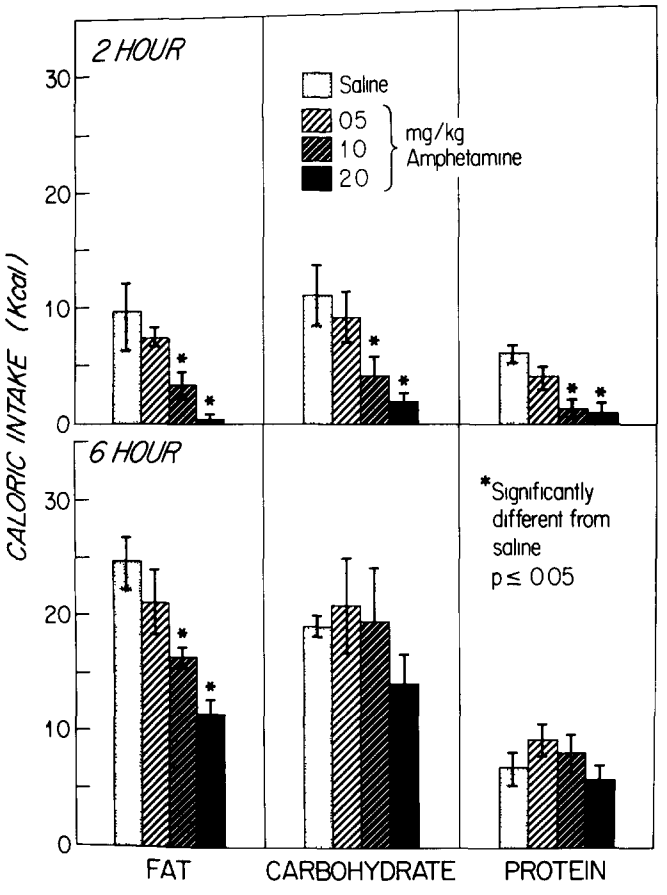

FIG. 2. Cumulative two-hour (top) and six-hour (bottom) caloric intakes of fat, carbohydrate and protein following the administration of saline, 0.5, 1 0 and 2 0 mg/kg amphetamine in animals maintained on a dietary self-selection regime

intakes after the 0.5 mg/kg dose of the drug. Further, animals consumed significantly less food after the lowest dose of amphetamine than they did after saline injections.

During the final four hours of the feeding periods following administration of the low and medium doses of amphetamine, animals compensated for initial suppressions in caloric intakes. As a result of this compensatory behavior, six-hour caloric intakes following injections of 0.5 and 1.0 mg/kg amphetamine did not differ from intakes following saline administration (Fig. 1, bottom left). In contrast, caloric intake following the highest dose of amphetamine remained significantly suppressed throughout the entire six-hour feeding period.

Animals Maintained on the Dietary Self-Selection Regime

Like animals maintained on Purina Chow, animals given the self-selection regime decreased two-hour caloric intake (calculated as the sum of caloric intakes from each of the three macronutrients) directly as a function of the dose of amphetamine administered (Fig. 1, top right). Although animals ate less following injections of 2.0 mg/kg than following injections of 1.0 mg/kg amphetamine, this difference was not statistically significant.

Analysis of individual macronutrient intakes revealed that the reduction in two-hour caloric intake was accounted for by decreases in the consumption of all three dietary components (Fig. 2, top). While macronutrient intakes following the medium and high doses of amphetamine were significantly lower than intakes after saline injections, macronutrient intakes after the lowest dose of the drug did not differ significantly from saline values.

Consistent with data of the Purina Chow group, six-hour caloric intakes of the self-selection animals after the low and medium doses of amphetamine did not differ from intakes following saline injections. Furthermore, parallel to caloric intakes of their Purina Chow counterparts, caloric intakes of self-selection animals were significantly suppressed throughout the entire six-hour feeding period following administration of the highest dose of amphetamine (Fig. 1, bottom right).

Inspection of specific macronutrient intakes at the end of the total six-hour feeding period revealed no differences in either protein or carbohydrate intakes as a result of drug administration (Fig. 2, bottom). In contrast, amphetamine injections resulted in dose-related decreases in fat consumption across the six-hour feeding period. Total six-hour intakes of fat were significantly lower following the medium and high doses of amphetamine than after saline injections.

DISCUSSION

As previously reported (e.g. [1, 2, 3, 9]), in the present experiment, amphetamine administration resulted in initial decreases in caloric intakes in animals maintained on a single nutritionally complete diet. This suppression in caloric intake was no longer evidenced at the end of the six-hour feeding periods following injections of the medium and low doses of the drug. Similar modifications in feeding behavior were observed in animals maintained on a self-selection regime. Detailed examination revealed that amphetamine had different effects on intakes of the three macronutrients. Intakes of protein and carbohydrate were suppressed during the first two hours of the feeding period, but returned to control levels by the end of the six-hour feeding period. In contrast, fat intake was initially suppressed and remained suppressed throughout the entire six-hour period. Similar patterns of diet selection have been observed in male rats maintained under a normal light-dark cycle following amphetamine administration (Kanarek and Orthen-Gambill, unpublished results).

The present results can be contrasted with previous data from an experiment by Wurtman and Wurtman [13] which demonstrated a lack of selectivity of amphetamine on macronutrient consumption. The difference in results between the present experiment and previous work may be explained by differences in the experimental paradigms employed. In the Wurtmans' experiment, animals were not offered a separate source of fat, and therefore, the effects of amphetamine on the consumption of this macronutrient could not be assessed. In the present study, when rats were concurrently presented with separate sources of protein, carbohydrate and fat the selective effects of amphetamine were unmasked.

Not all drugs which lead to reductions in caloric intakes result in similar patterns of diet selection For example, administration of morphine to animals maintained under conditions very similar to those of the present experiment resulted in a rather different pattern of diet selection [10]. Following morphine administration, both protein and carbohy-

drate intakes were suppressed across a six-hour feeding period, while fat intake was actually elevated above levels observed following saline injections [10]. In contrast to morphine and amphetamine, administration of fenfluramine to animals offered separate sources of the three macronutrients led to dose-related decreases in protein and fat intakes with no significant modifications in carbohydrate intake across a six-hour feeding period (Kanarek and Orthen-Gambill, unpublished results). Taken together, these data indicate that the pattern of diet selection observed following amphetamine administration is not a general effect of drugs which decrease energy consumption.

It has been known for some time that drugs which produce similar general behavioral effects (e.g. a reduction in caloric intake) may produce quite distinctive effects on brain neurochemistry [1, 2, 4, 5, 9, 11]. For example, while both amphetamine and fenfluramine lead to decreases in food intake, they are hypothesized to produce their behavioral actions through different neurochemical systems. The action of amphetamine appears to be most marked on catecholamine metabolism while fenfluramine is characterized by its capacity to release and block the re-uptake of serotonin [1, 2, 4, 5]. Although the general effects of these two anoretic agents on food intake are similar, recent experiments employing more refined analyses of feeding behavior have revealed differences in the temporal patterns of eating following administration of fenfluramine and amphetamine [2,11]. Amphetamine has been reported to suppress food intake by delaying the initiation of feeding while fenfluramine permits feeding to begin normally but acts to terminate food intake prematurely [2]. The present data, in conjunction with previous work [13,14], Kanarek and Orthen-Gambill, unpublished results), indicates that these drugs also produce different patterns of nutrient selection. While these results make it tempting to conclude that different neurotransmitter systems may be involved in mediating intakes of specific macronutrients, current information makes it untenable to establish a one-to-one correspondence between neurotransmitter activity and macronutrient consumption.

Dietary self-selection experiments provide information on both quantitative and qualitative aspects of feeding behavior. Previous data from this laboratory have clearly established that hyperphagia in different forms of obese animals is associated with different patterns of diet selection [6, 7, 8]. It is now evident that drug-induced anorexia, like hyperphagia, may be associated with different patterns of nutrient choice.

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