THE ABILITY OF CERTAIN ANOREXIC DRUGS TO SUPPRESS FOOD CONSUMPTION DEPENDS ON THE NUTRIENT COMPOSITION OF THE TEST DIET

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Summary

The effects of five anorexic agents on food consumption were tested in rats offered single, isocaloric, isonitrogenous diets differing in carbohydrate content. Three of the test agents, d-amphetamine, benzphetamine and chlorphentermine, are sympathomimetic and cause CNS stimulation; the others, MK-212 and d-fenfluramine, are thought to facilitate serotonin-mediated neurotransmission. At ED50 doses, the sympathomimetic drugs reduced food consumption whether the test diet was rich (75% dextrin) or poor (25% dextrin) in carbohydrate. In contrast, MK-212 and d-fenfluramine failed to reduce consumption of the 25% dextrin test diet. These observations suggest that anorexic drugs like d-amphetamine and d-fenfluramine do not act via a common "amphetamine receptor," and are compatible with earlier observations, made on rats given diet pairs simultaneously, that enhanced serotoninergic neurotransmission selectively suppresses appetite for carbohydrates.

Studies on the anorexic effects of drugs usually employ single, mixed-nutrient, test diets; starved animals receive the drug before exposure to the diet, and the resulting decrease in the diet's intake is measured. One limitation of such experiments is their inability to demonstrate selective drug effects on appetites for particular macronutrients like carbohydrates (CHO) or proteins. In order to examine appetites for particular nutrients, use has been made of a two-diet paradigm in which animals are allowed to choose between isocaloric diets containing differing proportions of the single dietary component being studied (1,2). Using this paradigm, we previously showed that d,l-fenfluramine (3), or d-fenfluramine (4), serotonin-releasing drugs, selectively reduce the consumption of CHO, relative to that of protein, by rats. Similar conclusions were drawn from data on food-choice studies in humans (5).

Paul et al. (6) have suggested that fenfluramine, amphetamine, and various other anorexigenic agents produce their effect by acting at a common "amphetamine-receptor site" within the hypothalamus. Support for this concept derives from evidence that the in vitro affinities for this "receptor" of most of the drugs tested paralleled their anorexigenic potency in vivo, as previously examined in a study using rats given access to only a single diet containing 52.9% CHO and 22.8% protein (7). We now show that the proportion of CHO in a test diet determines whether or not its consumption will be suppressed by anorexic agents, like d-fenfluramine, which are thought to act at serotoninergic synapses. In contrast, the anorexia produced by sympathomimetic amines like d-amphetamine is independent of the nutrient composition of the test diet.
Methods

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were housed singly in suspended wire mesh cages at 23°C, and maintained on a reversed lighting schedule (dark period 0900 to 2100). Upon arrival animals were weighed and placed on a maintenance diet consisting of 60% CHO, 20% protein, and 5% fat (by weight). All diets used in this study contained 4% agar, 4% Rogers Harper salt mix (ICN Pharmaceuticals, Cleveland, OH), and 1 liter water per kg dry weight. The CHO source was dextrin and the protein source was casein. For the 2 weeks before experimentation, rats had ad libitum access to food and water. Animals were obtained at approximately 150 g, weighed every 3 days, and retired from the study at 300 g.

Nine separate experiments were performed, each using 10-24 rats. In some cases, individual rats were used for several experiments, each separated by at least 4 days (during which they again consumed the maintenance diet ad libitum). In any particular experiment, some animals were given the 75% CHO diet and an equal number were given the 25% CHO diet; each experiment included control rats that received no drug, and animals receiving one of the test drugs. Each drug was tested in at least 4 experiments; by the end of 9 experiments, each drug had been tested on 12-31 animals (fig. 1), and 28 animals provided control data.

On the day of an experiment, food was removed from the cages at the onset of the dark period. The animals were weighed and ED₅₀ doses of the test compounds were calculated for each animal from its weight and the drugs' published ED₅₀ values (3,7,8). After a 4-hour fast, test animals were given intraperitoneal injections of one of the five test compounds at its calculated ED₅₀ dose (Fig. 1); control animals received equivolume injections of the drug diluent.

![Graph](image)

FIG. 1

Rats received ED₅₀ doses of five anorexigenic agents, three of which are sympathomimetic CNS stimulants (7) (d-amphetamine, 1.75 mg/kg; benzphetamine, 12.5 mg/kg; chlorphentermine, 6.0 mg/kg), and two of which are thought to enhance central serotonergic neurotransmission (4,8,9) (MK-212, 1.5 mg/kg; d-fenfluramine, 2.0 mg/kg). Thirty minutes later, animals were offered single isocaloric isonitrogenous (20% protein) test diets containing either 75 or 25% CHO, and food intake was measured after 90 minutes. Control animals received equivolume doses of drug diluents. Numbers in parentheses indicate total number of animals receiving that treatment. Comparisons between drugs were tested for statistical significance by use of Duncan's multiple-range testing (10).

* = P ≤ 0.01.
Thirty minutes after injection one group was given access to a single diet containing 75% CHO, 20% protein and 5% fat, and the other was given an isocaloric, isonitrogenous diet containing 25% CHO, 20% protein, 27% fat and 28% cellulose. Animals were allowed to eat for 90 minutes, after which food consumption was measured. This test interval was chosen based on pilot studies showing that most of the food eaten on the test day — among control or treated animals — was consumed during the first 60 minutes of food presentation. Moreover experiments in which food intake was followed for a full 24 hours after treatment demonstrated the same drug effects as those using the 90-minute interval, but with more variance.) Each individual experiment followed a two-way design, with animals randomly assigned one of the two diets and also to control or one of the 5 drug treatments. The data were analyzed by three-way analysis of variance (ANOVA) with diet composition, treatment (i.e., drug), and experiment number as the three factors. Since this analysis showed a highly significant interaction between diet composition and treatment, subsequent two-way analyses (treatment and experiment number as factors) were calculated for each of the two diets. Duncan's multiple-range test was used to compare the individual treatments within each diet (see Fig. 1). The data were analyzed using a VAX 11-780 computer and the VMS statistical analysis system (SAS) release 4.07, general linear models operating system. (SAS is a copyright, 1984, of the SAS Institute Inc., Cary, NC 27511).

Results and Discussion

Animals presented with a test diet containing 75% CHO consumed significantly less food than control animals when given ED50 doses of any of the five anorexic drugs (P < .01). Differences in the effects of the five drugs were not significant (Fig. 1).

In contrast, the abilities of individual drugs to diminish food consumption varied markedly when the rats were tested using the low-CHO diet: d-amphetamine, benztropine and chlorphentermine all reduced food consumption by 54-62% (Fig. 1) (P < .01), while doses of MK-212 or d-fenfluramine which had been equipotent in suppressing consumption of the other (75% CHO) diet failed to diminish consumption of the 25% CHO diet significantly.

These observations are compatible with the hypothesis that drugs like d-fenfluramine and MK-212, which act by increasing serotonergic neurotransmission (4,8,10), selectively diminish appetite for CHO, relative to that for protein, and thus have little effect on food consumption when the CHO/protein ratio of the test food is relatively low. In contrast, d-amphetamine and its congeners, benztropine and chlorphentermine, which are not believed to act primarily at serotonergic synapses, produce a nonspecific anorexia, and diminish food consumption independent of the test food’s proportions of CHO and protein. This hypothesis has been supported by data from experiments in which rats (2,3,4,11) and humans (5) could choose from among several foods of varying composition; it has not, to our knowledge, previously been examined with the more commonly used single-food paradigm.

Our findings fail to support the view that amphetamine, fenfluramine and other structurally similar drugs produce anorexia by acting at a common receptor (6): If activation of the same receptor initiated the effects of both amphetamine and fenfluramine, then variations in the composition of the test diet would cause similar changes in the abilities of both to diminish its consumption. Our observations also indicate that unless a test diet of the proper nutrient composition is used in procedures that screen for anorexic drugs, potentially useful compounds may fail to be identified (e.g., d-fenfluramine, it tested with a diet containing a proportion of CHO that is not considerably greater than its proportion of protein (Fig. 1)).
The ability of serotonergic agents to suppress carbohydrate intake selectively, and to sustain protein intake, probably relates to the similarity between their neurochemical effects and those of dietary CHO: CHO-rich, protein-poor meals change plasma amino acid levels so as to increase brain tryptophan (12), thereby enhancing the saturation of tryptophan hydroxylase and the synthesis and release of the neurotransmitter serotonin. Protein-rich meals, which contribute relatively larger amounts of other neutral amino acids than of tryptophan to circulation, have an opposite effect on brain tryptophan and serotonin (13). Perhaps the enhancement in serotonergic neurotransmission that follows administration of d-fenfluramine or MK-212 conveys to the brain the false signal that CHO has recently been consumed. Many obese people apparently experience strong CHO cravings at characteristic times of day (5, 14); these cravings can be for sweet or for nonsweet CHOs, and can frequently be ameliorated by serotonin-releasing drugs (5). Our present observations suggest that such drugs may be acting with greater specificity than the sympathomimetic amines in treating this kind of obesity.

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References