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Effect of (+)- or (−)-enantiomers of fenfluramine or norfenfluramine on nutrient selection by rats

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The effects of the (+)- and (−)-enantiomers of fenfluramine and norfenfluramine on food choice and total food consumption by rats, have been examined. Animals were trained to select their food during an 8-h daily interval from two isocaloric-isocarbohydrate (40%) diets differing in protein contents (5 or 45% casein). Low doses of (+)-fenfluramine (1·25 or 1·65 mg kg^{-1}) selectively reduced consumption of the 5%-protein diet during the hour after administration, thereby also reducing carbohydrate intake by a greater proportion than protein intake. Higher doses (2·5 or 4·0 mg kg^{-1}) diminished consumption of both test diets to an equivalent extent. (+)-Norfenfluramine, although anorectic, did not modify the relative consumptions of carbohydrate and protein at any dose tested. These observations affirm that nutrient selection as well as total food consumption can be altered by drugs affecting particular neurotransmitters.

Only recently have investigators begun to examine the neurochemical basis for nutrient selection (Wurtman & Wurtman 1977, 1979; Blundell et al 1979; Woodger et al 1979). Ashley & Anderson (1975) proposed that the ratio of plasma tryptophan concentration to the sum of the other large neutral amino acids influences protein consumption by rats allowed to choose freely among diets containing varying proportions of nutrients. When rats (Fernstrom & Faller 1978) or humans (Fernstrom et al 1979) consume meals containing both proteins and carbohydrates, this ratio changes post-prandially in inverse proportion to the diet's protein content. A rise in this ratio (after consumption of a carbohydrate-rich meal) increases brain tryptophan levels, and thereby accelerates brain 5-hydroxytryptamine (5-HT) synthesis and probably, release (Fernstrom & Wurtman 1971, 1972). For this reason, Wurtman & Wurtman (1977, 1979) examined the effects on food choice of various drugs believed to elevate intrasynaptic brain 5-HT levels. They found that such treatments selectively diminished the proportion of the next meal's calories represented by carbohydrates, and proposed that 5-HT-releasing brain neurons are a component of a neurochemical-behavioural feedback loop that serves to diminish appetite for carbohydrates after carbohydrate-rich foods have been consumed. Thus (±)-fenfluramine, an anorectic drug thought to release 5-HT (Garattini et al 1975, 1979; Blundell et al 1979; Duhault 1975a, b), selectively reduced carbohydrate intake (Wurtman & Wurtman 1977, 1979), while amphetamine, which releases catecholamines (Besson et al 1971; Chiueh & Moore 1973; Garattini et al 1975) did not alter dietary preference (Wurtman & Wurtman 1977).

We have extended these earlier studies by examining the effects of the (+) and (−)-enantiomers of fenluramine and norfenfluramine (the de-ethylated metabolite of fenfluramine) on food choice by rats. There are important neuro-chemical differences between the (+)- and (−)-enantiomers: in addition to releasing 5-HT, the (−)-enantiomers are also dopamine-receptor antagonists (Consolo et al 1979, 1980).

METHODS

Feeding and injection schedule

The procedure was similar to that described previously by Wurtman & Wurtman (1977, 1979). Male Sprague-Dawley rats (70–125 g), housed individually, were placed on a 12-h reversed-lighting schedule and trained to eat all of each day's food during the first 8 h of darkness. Animals could select food from two dishes containing isocaloric-isocarbohydrate 5 or 45% protein (casein) diets; fresh diets were presented daily. After a one week accommodation period, groups of 4–10 animals received either a drug or 0·9% NaCl (saline) intraperitoneally. Thirty min later, at the onset of the dark cycle, the two food dishes were placed in the cages. The dishes had

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been weighed before presentation and were also weighed 1, 3·5, and 8 h later. No significant reductions in food intake or consistent alterations in nutrient selection patterns were observed after the first 1-h interval; hence, data for only this initial period are presented. A particular animal may have been tested with 1–3 drugs, none given more than once; drug injections were separated by a day of either saline administration or no treatment. Food consumption on these intervening days did not differ from that exhibited by control animals which had never received the drug.

Diets

Both diets contained 2·2% vitamin mix (ICN Pharmaceuticals, Cleveland, OH), 4% Harpers-Rogers Mineral Mix (Tekland Test Diets, Madison, WI), 4% agar, and 1000 ml water per kg of dry diet mix. In addition, the 5% protein diet contained 33% fat, 40% carbohydrate, and 22% non-nutritive cellulose; the 45% protein diet consisted of 15% fat and 40% carbohydrate. The protein used in both diets was casein, the fat was vegetable shortening, and the carbohydrate was dextrin (a non-sweet substance).

Statistics

A typical experiment involved 12–30 animals divided among 3–4 treatment groups and always included a group that received saline. Experimental data were expressed as the mean numbers of grams of the 5 and 45% protein diets, and of total food consumed by each of the treatment groups each day. From these data, the percent decreases from control values were calculated. Their median values are shown with mid-spreads (i.e., boundaries of 75 and 25% quartiles) on the dose-response curves. Individual curves were analysed by regression analysis ((+)-fenfluramine and (+)-norfenfluramine; Fig. 1) of the median values. Since this rigorous statistical test could not be employed for a 2-point curve, the less stringent Mann-Whitney U-test was used to compare the anorectic effects of the two doses of (±)-fenfluramine (Fig. 1). The differential effects of various doses of (+)-fenfluramine (Fig. 2) or (±)-fenfluramine were evaluated by the Wilcoxon matched-pairs test.

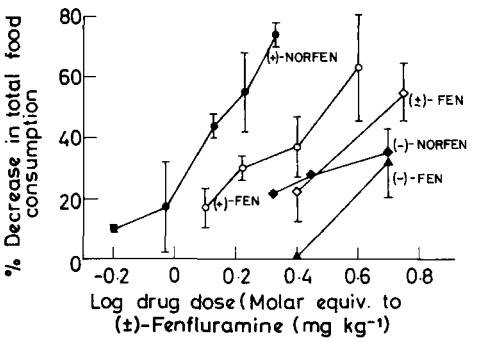

FIG. 1. Anorectic effects of fenfluramine compounds. Male rats (70–125 g) were injected intraperitoneally with various doses of (+)-fenfluramine, (±)-fenfluramine, (−)-fenfluramine, (+)-norfenfluramine, or (−)-norfenfluramine. The percent decreases in total food consumption during the subsequent hour were determined as described in the text. Individual points represent the medians of multiple experiments. Vertical bars indicate the mid-spreads.

RESULTS

The relative anorectic potencies of the five drugs were compared (Fig. 1). Since the focus of this study was the examination of changes in food choice, and since this effect was lost with increased drug dosages, incomplete dose-response curves were determined. However, the (+)-isomers of fenfluramine and norfenfluramine were more potent than the (−)-isomers, consistent with the observations of Garattini et al (1979).

The anorectic effects of (+)-norfenfluramine, (+)-fenfluramine, and (±)-fenfluramine were statistically significant ($P < 0·001$ by regression analysis for (+)-norfenfluramine and (+)-fenfluramine, n = 5 and n = 4, respectively, and $P < 0·001$ by Mann-Whitney U-test for (±)-fenfluramine n = 47). The anorectic effects of (−)-norfenfluramine and (−)-fenfluramine did not attain statistical significance possibly because too few doses in too narrow a dose range were tested.

Low doses of (+)-fenfluramine caused rats to reject selectively the 5% protein diet. Thus, there was a 42% reduction in consumption of the low-protein diet, but only a 6% reduction in that of the high-protein (45%) diet when 1·25 mg kg^{-1} of (+)-fenfluramine was administered ($P < 0·005$; n = 11; Fig. 2). When 1·65 mg kg^{-1} of the drug was given, a selective reduction in consumption of the low-protein diet ($P < 0·025$, n = 6) was again observed. The corresponding decreases in total carbohydrate and protein intakes at these doses were, respectively, 24 and 10% with the 1·25 mg kg^{-1} dose, and 28 and 21% with the 1·65 mg kg^{-1} dose. Doses of 2·5 or 4·0 mg kg^{-1} caused non-selective anorectic effects; animals rejected both diets equally (Fig. 2). A similar relationship between dose and nutrient selection was observed with (±)-fenfluramine. Thus, animals receiving 2·5 mg kg^{-1} of the drug exhibited a greater aversion for the low-protein diet than for the

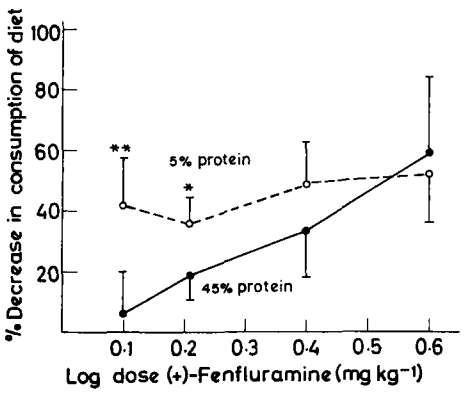

FIG. 2. Effect of (+)-fenfluramine on consumption of 45 vs 5% protein diets. The testing procedure was the same as that described in the legend to Fig. 1, except that percent decreases in consumption of the 45 and 5% protein diets are displayed individually. Vertical bars indicate the mid-spreads. ●—● represents the percent decrease in consumption of the 45% protein diet. ○ - - - ○ represents the percent decrease in consumption of the 5% protein diet. Points represent the medians of multiple experiments. * $P < 0{\cdot}025$ compared with 45% protein diet. ** $P < 0{\cdot}005$ compared with 45% protein diet.

high-protein diet ($P < 0{\cdot}005$, n = 38; data not shown), which corresponds to a 28% decrease in carbohydrate intake and a 21% fall in protein consumption. A dose of 5·0 mg kg^{-1} was ineffective in altering the dietary preference patterns of rats. Although 2·5 mg kg^{-1} of (−)-fenfluramine caused no changes either in nutrient selection or in total food consumption (data not shown), 5·0 mg kg^{-1} of the drug diminished total food intake by 33%, preferentially reducing consumption of the 5%-protein diet. Since (±)-fenfluramine is the racemic mixture of the (+)- and (−)-enantiomers, it is not surprising that the racemate was a less potent anorectic than (+)-fenfluramine or that rats were still capable of demonstrating food selectivity at a dose (2·5 mg kg^{-1}) which would have been non-selective had the drug been (+)-fenfluramine (Fig. 2).

(+)-Norofenfluramine produced a non-specific anorexia at all doses tested; this effect resembles more closely the anorexia caused by amphetamine than that evoked by the isomers of fenfluramine. In four experiments, administration of 4·5 mg kg^{-1} of (−)-norfenfluramine caused a 63% reduction in consumption of the 5% proteub diet, but only reduced intake of the 45% protein diet by 17% However, single experiments with 1·9 or 2·5 mg kg^{-1} of the drug demonstrated selective rejection of the *high*-protein diet.

DISCUSSION

Our observations that rats given access to two isocaloric diets of differing protein contents, and given low doses of (+)- or (±)-fenfluramine, preferentially reduce their intake of the low-protein diet are consistent with those reported by Wurtman & Wurtman (1977, 1979).† Our results support the view that neurochemically-distinct mechanisms control total food consumption and nutrient selection and that drugs modifying one such mechanism need not perturb the other. Thus, sub-anorectic doses of (+)- or (±)-fenfluramine selectively suppressed consumption of the diet with the high carbohydrate-to-protein ratio, while higher, anorectic doses failed to alter dietary preferences (Fig. 2). Other anorectic agents such as amphetamine (Wurtman & Wurtman 1977) or (+)-norfenfluramine were different in this regard, not altering nutrient selection patterns even when given in low doses. It is perhaps surprising that (+)-norfenfluramine failed to affect food choice, since it, like (+)-fenfluramine, is a potent 5-HT releaser (Garattini et al 1979). It is also not clear why higher doses of (−)-norfenfluramine selectively reduced consumption of the low-protein diet, while lower doses of the same drug lacked this directional effect.

Higher doses of (+)-fenfluramine (Fig. 2) or (±)-fenfluramine lacked selective effects on diet choice. Perhaps non-5-HTergic neurons are affected at these higher drug doses and counteract the 5-HT-mediated inhibiton of consumption of diets with high carbohydrate/protein ratios. Alternatively, low doses may principally affect certain 5-HTergic neurons, i.e. those involved in carbohydrate consumption, whereas higher doses may also affect other 5-HTergic neurons whose actions antagonize the food-choice effect.

Our data do not rule out the possibility that fenfluramine acted by suppressing fat intake: the 45% protein diet contined 15% fat, while the 5% protein diet was 33% fat. However, while evidence is available that carbohydrate (Wurtman & Wurtman 1979) and protein (Ashley & Anderson 1975) intakes, per se, are regulated, such evidence has apparently not yet been obtained for dietary fat content. Before inferring a drug effect on the

† In the earlier studies, anorexia was observed 1–4 h after (±)-fenfluramine, but not during the first hour; in the present study this effect was noted only during the first hour. The differences between the two studies may reflect the ways in which (±)-fenfluramine was administered: in the earlier experiments, (±)-fenfluramine was suspended in 2% methylcellulose; in the current one, the drug was dissolved in saline, thus perhaps attaining adequate plasma levels sooner.

proportion of fat in the diet that is desired, it would first be necessary to show that fat intake in the 15–33% range is, in fact, controlled.

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