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# Fluoxetine Reduces Food Intake by a Cholecystokinin-Independent Mechanism

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COOPER, S. J., C. T. DOURISH AND D. J. BARBER. *Fluoxetine reduces food intake by a cholecystokinin-independent mechanism.* PHARMACOL BIOCHEM BEHAV **35**(1) 51–54, 1990.—The selective serotonin uptake inhibitor, fluoxetine (3.0–10 mg/kg), produced a significant dose-related suppression of palatable food consumption in nondeprived rats. The anorectic effect of fluoxetine (10 mg/kg) was not reversed by the potent and highly selective cholecystokinin receptor antagonist MK-329 [1-methyl-3-(2-indolyl) amino-5-phenyl-3H-1,4-benzodiazepin-2-one], administered in doses of 10–100 μg/kg. Fluoxetine (10 mg/kg) also significantly reduced the consumption of powdered laboratory chow in a 6-hr nocturnal free-feeding test. The anorectic effect in this paradigm was also not antagonized by MK-329. In contrast to previous data for *d*-fenfluramine (which enhances serotonin release), these results indicate that fluoxetine may suppress food intake by a mechanism which is independent of endogenous cholecystokinin.

Anorexia Cholecystokinin Fluoxetine MK-329 (L-364,718) Serotonin

FLUOXETINE is a selective inhibitor of neuronal serotonin reuptake (28,29). In agreement with the general hypothesis that serotonergic mechanisms are involved in the termination of feeding responses (2,26), Goudie and his colleagues demonstrated that 10 mg/kg of fluoxetine significantly reduced food intake in rats tested after 18 hr of food deprivation (13). Subsequently, it has been shown that fluoxetine affects food intake and nutrient selection (17,30), reduces ingestion of saccharin solution and sucrose solutions in rats (18,22), and also reduces sucrose sham-feeding in gastric-fistulated rats (22). Fluoxetine is effective clinically in the treatment of obesity (19), and reduces symptoms of bulimia (11). Sertraline, another selective reuptake inhibitor, also has an anorectic effect (21).

Fluoxetine is frequently considered in comparison with the anorectic effects of *d*-fenfluramine (3, 17, 22, 25). Both are thought to reduce food intake as a consequence of enhanced serotonergic activity. Nevertheless, as Fuller and his colleagues have made clear, *d*-fenfluramine enhances serotonergic function by an increased release of serotonin, while fluoxetine inhibits its reuptake (12). However, so far as the mechanisms involved in the control of feeding responses are concerned, there has been little or no evidence to date which readily serves to distinguish between fluoxetine's effects, on the one hand, from those of *d*-fenfluramine on the other.

Recently, we made the unexpected discovery (6) that the anorectic effect of *d*-fenfluramine in rats is significantly blocked by the potent and highly selective cholecystokinin (CCK) antagonist, MK-329 (formerly known as L-364,718) (4,10). This result strongly implies that a major part of the anorectic effect of *d*-fenfluramine is dependent upon the action of endogenous CCK at CCK receptors. Furthermore, the anorectic effect of a selective dopamine $D_2$ receptor agonist, quinpirole, was not affected by the CCK receptor antagonist, suggesting a specific CCK/5-HT interaction (6). This has led us, therefore, to investigate further the anorectic effect of fluoxetine. The aim of the studies was to determine whether or not fluoxetine-induced anorexia is also CCK-dependent.

Two feeding paradigms were employed using nondeprived animals: 1) consumption of a highly palatable, sweet mash in a 30-min test (5); 2) nocturnal free-feeding with powdered laboratory chow in a 6-hr test.

## METHOD

### *Animals*

Subjects were 70 adult, hooded rats, bred in the School of Psychology, University of Birmingham. They were housed individually in stainless steel cages, with ad lib access to standard rat food and water. Room temperature was maintained at 21–22°C. The animals weighed 250–350 g at testing.

Sixty rats were housed under normal lighting conditions (lights on at 7 a.m.; 12-hr light:12-hr dark). The remaining ten animals were adapted to a reversed lighting condition (lights off at 10 a.m.; 12-hr light:12-hr dark). This latter group was used for tests of nocturnal free-feeding.

### *Drugs*

Fluoxetine hydrochloride (LY 110140) was provided courtesy of Eli Lilly and Company, Indianapolis. It was dissolved in distilled water, and injected intraperitoneally. MK-329 (L-364,718) [1-methyl-3-(2-indolyl)amino-5-phenyl-3H-1,4-benzodiazepin-2-

one] was suspended in 0.5% methylcellulose and injected subcutaneously.

*Procedure*

*Palatable food consumption.* Nondeprived rats were familiarized over a period of ten days to eating a sweetened mash in their home-cages in daily 30-min tests, by which time the level of intake had reached asymptote. The composition of the mash has been described previously (7,23).

Ten of the animals were used to provide a fluoxetine dose-response function. Each animal was injected with 3, 5.6 and 10 mg/kg of fluoxetine, and vehicle (distilled water), 30 min before the 30-min test of palatable food intake. The doses were chosen on the basis of an earlier study (23). The order of injection was counterbalanced across animals, and an interval of at least 72 hr separated consecutive injections to avoid possible carry-over effects (18). The amount of food consumed in the test was measured to the nearest 0.1 g.

The other 50 animals were assigned at random to five equal groups. Each animal was tested once only, following two injections: 1) vehicle (0.5% methylcellulose) and vehicle (distilled water); 2) vehicle and fluoxetine (10 mg/kg); 3) MK-329 (10 μg/kg) and fluoxetine (10 mg/kg); 4) MK-329 (30 μg/kg) and fluoxetine (10 mg/kg); 5) MK-329 (100 μg/kg) and fluoxetine (10 mg/kg). The first injection was administered SC 30 min before the feeding test; the second was administered IP 20 min before the test. The dose of fluoxetine (10 mg/kg) was chosen to produce a degree of suppression of food intake which was comparable with that produced by 3 mg/kg of *d*-fenfluramine in a previous study (6). The doses of MK-329 (10–100 μg/kg) were chosen on the basis of previous reports, which show that over this range of doses, MK-329 antagonizes the anorectic effects of exogenously administered CCK (9, 15, 16, 24). We have reported data elsewhere showing that MK-329 (10–100 μg/kg), by itself, has no effect on palatable food consumption under these conditions (6), and, therefore, the experiment was not repeated for the present study.

*Nocturnal free-feeding.* The ten rats, adapted to a reversed lighting condition, were trained to eat powdered laboratory chow in spill-proof jars placed in their home-cages. Each jar was replenished with fresh food at 10 a.m., and intake (to the nearest 0.1 g) was subsequently measured at noon, 2 p.m. and 4 p.m. Each animal was tested, following drug treatments, on four occasions. On each occasion they received two injections: 1) vehicle (0.5% methylcellulose) and vehicle (distilled water); 2) vehicle and fluoxetine (10 mg/kg); 3) MK-329 (30 μg/kg) and fluoxetine (10 mg/kg); 4) MK-329 (100 μg/kg) and fluoxetine (10 mg/kg). The first injection was administered SC 30 min before the 6-hr feeding test began; the second was administered IP 20 min before the test. The order of injection was counterbalanced across animals, and an interval of at least 72 hr separated consecutive injections. We have reported data elsewhere showing that MK-329 (30 and 100 μg/kg), by itself, had no effect on nocturnal free-feeding (6). Therefore the experiment was not repeated for the present study.

*Statistical Analyses*

Data were analysed using one-way analysis of variance (repeated-measures design or independent groups). Comparisons between the results for individual injection conditions and the corresponding control condition were made using Dunnett's *t*-test.

## RESULTS

*Palatable Food Consumption*

As Fig. 1 shows, fluoxetine (3.0–10 mg/kg) significantly reduced the level of palatable food consumption, $F(3,27) = 5.99$, $p<0.005$. Feeding was suppressed at 5.6 and 10 mg/kg, respectively. The 10 mg/kg dose was used in the subsequent combination experiment with MK-329.

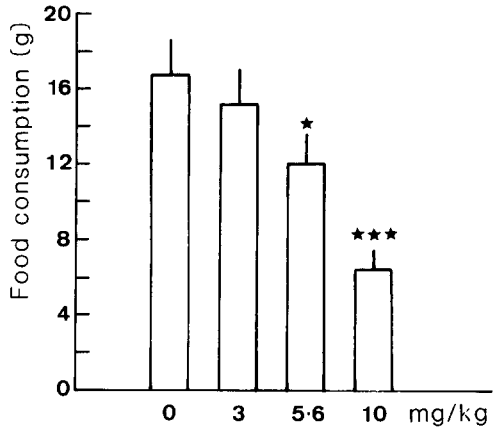


FIG. 1. Fluoxetine (3.0–10 mg/kg) produced significant anorectic effects in a test of palatable food consumption, using nondeprived rats. Results are shown in terms of mean intake (g)+S.E.M. N = 10 per group. Levels of significance for comparisons with the control level of intake: ★$p<0.05$; ★★★$p<0.005$ (Dunnett's *t*-test).

In this study, fluoxetine (10 mg/kg) reduced intake of the palatable food from 18.8 to 5.2 g (a reduction of 72% ) (Fig. 2). When it was administered in combination with MK-329 (10–100 μg/kg), the pronounced anorectic effect was unaffected, $F(4,45) = 6.78$, $p<0.001$. Thus, the highly selective CCK antagonist did not attenuate the reduction in feeding produced by fluoxetine.

*Nocturnal Free-Feeding*

In the first 2-hr period of the feeding test, under control conditions, rats consumed 7.0 g of powdered chow. Following the administration of fluoxetine (10 mg/kg), the level of food intake

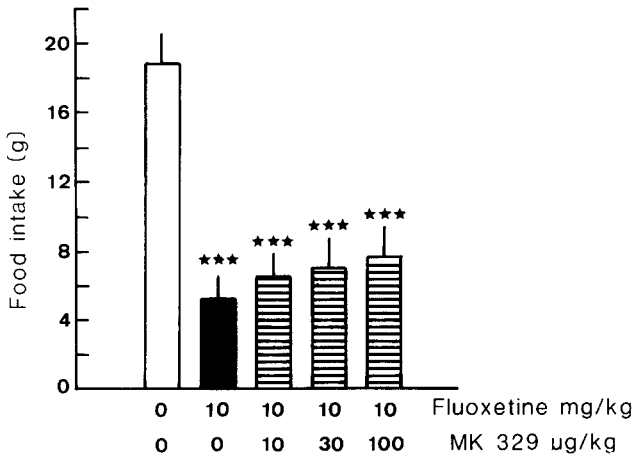


FIG. 2. Fluoxetine (10 mg/kg) significantly reduced the level of palatable food consumption by nondeprived rats in a 30-min test. MK-329 (10–100 μg/kg), a highly selective CCK receptor antagonist, did not affect fluoxetine-induced anorexia. Results are shown in terms of mean intake (g)+S.E.M. N = 10 per group. Level of significance for comparisons with the control level of intake: ★★★$p<0.005$ (Dunnett's *t*-test).

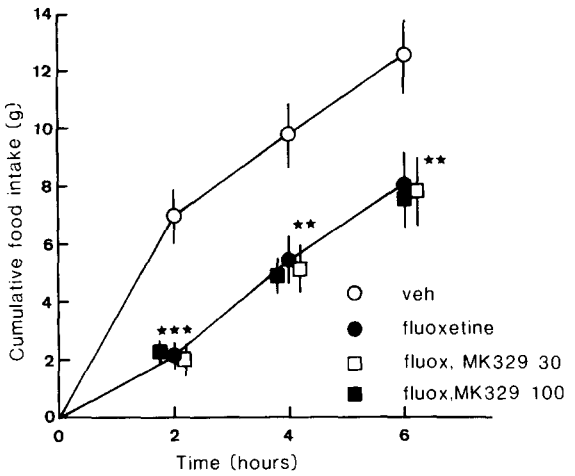


FIG. 3. Fluoxetine (10 mg/kg) significantly reduced the level of nocturnal consumption of powdered chow in free-feeding rats in a 6-hr test. MK-329 (30 and 100 μg/kg) had no effect on the anorectic effect of the serotonin uptake inhibitor. N = 10 per condition. Results are shown as mean cumulative food intake (g)+S.E.M. Levels of significance for comparisons with the control level of intake: $**p<0.01$; $***p<0.005$ (Dunnett's *t*-test).

was reduced to 2.2 g (a reduction of 69%) (Fig. 3). Once again, this anorectic effect remained unchanged when fluoxetine was administered in combination with MK-329 (30 or 100 μg/kg), $F(3,27)=26.34$, $p<0.0001$. There was no antagonism of fluoxetine's effect by the CCK receptor antagonist.

There was no compensation for fluoxetine's initial anorectic effect over the remaining 4-hr of the 6-hr feeding test (Fig. 3). Furthermore, no evidence of antagonism by MK-329 emerged later in the test.

## DISCUSSION

### *Anorectic Effect of Fluoxetine*

Fluoxetine produced a dose-related reduction in the consumption of a palatable diet in nondeprived rats. Previously, Leander suggested that palatability-induced intake may be more sensitive to the action of the serotonin uptake inhibition than deprivation-induced intake (18). In his studies, 10 mg/kg of fluoxetine reduced ingestion of a 0.001 M sodium saccharin solution by nondeprived rats in a 1-hr test by about 65%, and ingestion of a 0.01 M solution by a similar percentage (estimated from Fig. 1 of his paper). The present results indicate a reduction of up to 72% in the consumption of a sweetened diet following administration of fluoxetine. For comparison, Goudie *et al.* found a 54% reduction in the intake of standard laboratory chow by 18-hr food-deprived rats in a 1-hr period (13). Rowland and colleagues reported a 33% suppression of food intake in 24-hr food-deprived rats given a 1-hr test (25). At first sight, therefore, the data appear consistent with Leander's proposal that palatability-induced intake is more sensitive than deprivation-induced feeding following fluoxetine administration. Nevertheless, we should like to propose an alternative view.

The present results also demonstrated a long-lasting reduction in laboratory chow consumption during nocturnal free-feeding, as a consequence of fluoxetine administration. During the first 2-hr period, fluoxetine (10 mg/kg) reduced food intake by 69%. This type of feeding response is clearly, therefore, as sensitive as fluoxetine's action on palatability-induced ingestional responses. An important factor in determining the potency of fluoxetine may therefore be the presence or absence of food deprivation. Food deprivation appears to attenuate fluoxetine-induced anorexia quite markedly. The factors involved in this attenuation are not clear at the present time, but it is known that food deprivation induces changes in brain 5-HT metabolism [e.g., (8)]. It is not an isolated example since adaptation to a food-deprivation schedule has previously been reported to attenuate the anorectic effect of naloxone (27). Thus, for fluoxetine-induced anorexia, free-feeding and palatability-induced feeding responses are more sensitive than deprivation-induced consumption. It has been noted before that feeding in starved rats is not an appropriate model of over-eating in obese human individuals (25). The present data have a direct bearing, therefore, upon the potential clinical efficacy of fluoxetine (11,19).

### *Fluoxetine and MK-329*

MK-329 is a highly potent and selective CCK receptor antagonist (4,10), which antagonizes the effects of exogenous CCK on food intake and gastric emptying (9, 14–16, 20). In doses of 30 and 100 μg/kg, MK-329 also significantly antagonized the anorectic effects of *d*-fenfluramine in tests of palatable food consumption and nocturnal free-feeding (6). This result suggested that *d*-fenfluramine increases serotonin release, stimulates 5-$HT_1$-like receptors (23), and brings about, in turn, increased activity of endogenous CCK at CCK receptors, which leads finally to a reduction in food intake.

In the present experiments, MK-329 (10–100 μg/kg) did not significantly attenuate the anorectic effect of fluoxetine (10 mg/kg). Hence, it appears that fluoxetine may achieve its anorectic effect by means which are independent of endogenous CCK activity. Although behavioural evidence favours the possibility that CCK (1) and fluoxetine (5) enhance satiety, the fluoxetine effect could be CCK-independent. If fluoxetine enhances satiety as a result of selective 5-HT uptake inhibition, then the effect of the uptake inhibition may not, in turn, enhance endogenous CCK activity.

The implication of these data is that differences could exist between the underlying mechanisms for fluoxetine- and *d*-fenfluramine-induced anorexia, respectively. Hence, anorectic effects of "serotonergic" agents do not reflect a simple, homogeneous neurochemical action. We propose, therefore, that CCK-dependent and CCK-independent anorexia may provide a valuable means with which to analyze further the mechanisms which underlie drug-induced anorexias involving serotonergic systems.

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