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Reversal of the anorectic effect of (+)-fenfluramine in the rat by the selective cholecystokinin receptor antagonist MK-329

[1]S.J. Cooper, *C.T. Dourish & D.J. Barber

School of Psychology, University of Birmingham, Birmingham, B15 2TT, and *Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR

1 Experiments were conducted to determine whether or not the effect of (+)-fenfluramine (3.0 mg kg^{-1}, i.p.) on food intake can be antagonized by the selective cholecystokinin receptor antagonist MK-239 (formerly L364,718; (3S(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1-H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide). Two feeding paradigms were employed. In the first, non-deprived rats were familiarized with eating a highly palatable, sweetened mash in a 30 min test. In the second, freely-feeding rats were trained to consume powdered chow in their home-cages, and their intake was monitored over the first 6 h of the night-period.

2 In doses of 30.0 and 100.0 μg kg^{-1}, s.c., MK-329 almost completely blocked the anorectic effect of (+)-fenfluramine in the palatable food intake test. These doses of MK-329 have previously been reported to antagonize the anorectic effect produced by exogenous cholecystokinin-octapeptide (CCK8) in rats. Both doses of MK-329 were also effective in significantly attenuating the anorectic effect of (+)-fenfluramine in nocturnal free-feeding animals over a 6 h-period.

3 MK-329 (10.0–100.0 μg kg^{-1}, s.c.) failed to antagonize the anorectic effect of either the specific dopamine D_2-receptor agonist quinpirole (0.3 mg kg^{-1}, s.c.) or the β-carboline FG 7142 (10.0 mg kg^{-1}, i.p.) in the palatable food intake test.

4 MK-329 (10.0–300.0 μg kg^{-1}, s.c.) had no effect, when administered alone, on the level of palatable food intake in non-deprived rats, even when substantial satiation was produced by a pre-feeding procedure. Furthermore, MK-329 had no effect, when administered alone, on nocturnal food intake in freely-feeding rats.

5 In conclusion, not only was MK-329 a potent antagonist of the effect of CCK8 on food intake, it also blocked the effect of (+)-fenfluramine to a significant degree. The effect of MK-329 was selective in that the anorectic effects of either quinpirole or FG 7142 remained unaffected. Administered alone, MK-329 did not affect food intake, indicating that its reversal of (+)-fenfluramine-induced anorexia was not secondary to an intrinsic hyperphagic effect. The results provide some evidence that the depressant effect of (+)-fenfluramine on food intake depends on the activity of endogenous CCK.

Introduction

(+)-Fenfluramine has been shown to induce loss of weight in obese patients (Enzi *et al.*, 1988), and to alleviate bulimic symptoms in patients with bulimia nervosa (Russell *et al.*, 1988). On the evidence of animal studies, there is considerable support for the view that the anorectic effect of (+)-fenfluramine depends upon central 5-hydroxytryptamine (5-HT) mechanisms (Samanin, 1983; Rowland & Carlton, 1986). Recent data strongly implicate the 5-HT_1 receptor subtype in the mediation of (+)-fenfluramine-induced anorexia in the rat (Neill & Cooper, 1989). This conclusion is consistent with other evidence showing that 5-HT_1-receptor mechanisms may be involved in the control of feeding responses (Kennett *et al.*, 1987; Kennett & Curzon, 1988; Hutson *et al.*, 1988; Dourish *et al.*, 1989).

The neuropeptide, cholecystokinin (CCK), is also effective in reducing food intake in obese individuals, as well as reducing food consumption in many animal species (Pi-Sunyer *et al.*, 1982; Cooper, 1985; Baile *et al.*, 1986). It has been proposed that ingestion of food during a meal causes release of endogenous CCK, which in turn leads to the termination of feeding responses (Smith & Gibbs, 1979). The recent introduction of a potent and highly selective CCK receptor antagonist (Chang & Lotti, 1986; Evans *et al.*, 1986) permits the identification of CCK-dependent actions in the control of feeding and other relevant responses. In initial studies, it has been reported by several groups that MK-329 (formerly L364,718; 3S(−)-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1-H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide), over a range of doses from 1 μg kg^{-1} to 10 mg kg^{-1} (i.p.), antagonized the anorectic effect of CCK-octapeptide, but not of bombesin (Lotti *et al.*, 1987; Dourish *et al.*, 1988b; Hewson *et al.*, 1988; Khosla & Crawley, 1988; Reidelberger *et al.*, 1988).

On the basis of the present series of experiments, we show for the first time that a component of (+)-fenfluramine-induced anorexia may be mediated by actions of endogenous CCK. In two separate feeding paradigms (palatability-induced feeding; nocturnal free-feeding), the anorectic effect of (+)-fenfluramine was reversed, to a significant extent, by the selective CCK receptor antagonist, MK-329. Control experiments indicated that in the palatability-induced feeding model, MK-329 did not attenuate either the anorectic effect of a selective dopamine D_2-receptor agonist, quinpirole, or that of the β-carboline FG 7142 which acts as an 'inverse agonist' at benzodiazepine receptors. These latter drug-induced anorectic effects occurred, therefore, independently of the actions of CCK.

Methods

Animals

Adult, male, hooded rats (General strain, bred in the School of Psychology) were used. Body-weights were between 300 g and 400 g. Animals were allocated to two experimental conditions. In the first, they were housed individually in stainless-steel cages under standard laboratory conditions (room temperature: 22 ± 1°C; 12 h light-12 h dark cycle, lights on at 08 h 00 min). Nocturnal free-feeding was measured in the second condition, and for this, animals were first acclimatized to a

[1] Author for correspondence.

reversed light-dark cycle (12 light-12 h dark cycle; lights off at 10 h 00 min) over a period of 3 weeks before tests began. Animals were neither food- nor water-deprived under either condition.

Procedures

(i) *Palatable food consumption.* Non-deprived 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 food intake had reached a stable level. The composition of the mash and its presentation to the animals have been described previously (Cooper *et al.*, 1985a,b). For each experiment, animals were assigned at random to groups of 10 animals each.

In the first experiment, 50 animals were allocated to five injection conditions. Each animal was tested following two injections: (i) vehicle (0.5% methylcellulose) and vehicle (distilled water); (ii) vehicle and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$); (iii) MK-329 ($10\,\mu g\,kg^{-1}$) and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$); (iv) MK-329 ($30\,\mu g\,kg^{-1}$) and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$), (v) MK-329 ($100\,\mu g\,kg^{-1}$) and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$). The first injection was administered s.c. 30 min before the feeding test; the second was administered i.p. 20 min before the test. The amount of palatable food consumed in the 30 min test period was measured to the nearest 0.1 g.

Two subsequent experiments followed a similar design, i.e. animals were allocated at random to five equal groups. In the first experiment, quinpirole ($0.3\,mg\,kg^{-1}$) was administered alone, and also in combination with 10, 30 and $100\,\mu g\,kg^{-1}$ of MK-329. The fifth, control, group received two vehicle injections. MK-329 or its vehicle was administered s.c. 30 min before the test, and quinpirole or its vehicle was administered s.c. 20 min before the test. In the second experiment, FG 7142 ($10.0\,mg\,kg^{-1}$) was administered alone, and also in combination with 10, 30 and $100\,\mu g\,kg^{-1}$ of MK-329. A fifth, control, group received two vehicle injections. MK-329 or its vehicle was administered s.c. 30 min before the test, and FG 7142 or its vehicle was administered i.p. 20 min before the test.

Three experiments were carried out to determine whether or not MK-329 ($10.0–300\,\mu g\,kg^{-1}$) alone had any effect on palatable food intake. In the first, 50 animals were assigned at random to five equal groups and were injected with 0, 10.0, 30.0, 100.0 and $300.0\,\mu g\,kg^{-1}$, s.c., respectively, of MK-329 30 min before the feeding test. Since it has been shown previously that the hyperphagic effects of some compounds depend upon the baseline level of food consumption (Jackson & Cooper, 1985; Dourish *et al.*, 1988a), two further experiments were undertaken. In the first case, the animals were allowed to consume the palatable mash for 5 min; the food was then removed, MK-329 or its vehicle was injected, and freshly-prepared mash was returned to the animals 30 min later. The subsequent food intake in a 30 min test session was then determined. In the second case, the animals were allowed to consume the mash for 10 min before administration of MK-329 or its vehicle. After 30 min, freshly-prepared food was returned to the animals for a 30 min test session in which food intake was measured. Pilot experiments had shown that these pre-satiation procedures were sufficient to reduce the levels of food consumption to values which were similar to those which occurred following the three anorectic drug treatments ((+)-fenfluramine, quinpirole, FG 7142). Thus, these experiments were used as control procedures for the possibility of intrinsic hyperphagic effects of MK-329 under conditions of reduced levels of food consumption.

The food intake data (g per 30 min) were analysed by one-way analysis of variance for independent groups, followed by Dunnett's t test for multiple group comparisons (Winer, 1981). A value of $P < 0.05$ was considered to be significant.

(ii) *Nocturnal free-feeding.* Twenty naive rats, that had been adapted to reversed lighting conditions, 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 h 00 min, and food intake (to the nearest 0.1 g) was subsequently measured at noon, 14 h 00 min and 16 h 00 min, to cover the first 6 h of the night period.

The first group of 10 animals were tested, following drug treatments, on four occasions. On each occasion they received two injections: (i) vehicle (0.5% methylcellulose) and vehicle (distilled water); (ii) vehicle and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$); (iii) MK-329 ($30\,\mu g\,kg^{-1}$) and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$); (iv) MK-329 ($100\,\mu g\,kg^{-1}$) and (+)-fenfluramine ($3.0\,mg\,kg^{-1}$). The first injection was administered s.c. 30 min before the 6-h feeding test began; the second was administered i.p. 20 min before the test. The order of injection was counterbalanced across animals, and an interval of at least 72 h separated consecutive injections. On intervening days, powdered food was presented in a similar manner and intakes were measured routinely over the first 6 h of the night period.

The second group of animals was also tested on four occasions. They were injected with 0, 10.0, 30.0 and $100.0\,\mu g\,kg^{-1}$, respectively, of MK-329. The injection was administered s.c. 30 min before the 6 h feeding test. As with the first group, the order of injection was balanced, and an interval of at least 72 h separated consecutive injections, with routine measurement of intake continued on intervening days.

The cumulative food intake data were analysed by one-way analysis of variance (repeated-measures design) at each time-point, followed by multiple comparisons using Dunnett's t test. A value of $P < 0.05$ was considered to be significant.

Drugs

MK-329 was obtained from Merck Sharp & Dohme Research Labs., West Point, PA, U.S.A., courtesy of Dr V.J. Lotti. It was dissolved in 0.5% methyl-cellulose and injected subcutaneously. The range of MK-329 doses used in the present experiments was chosen on the basis of these doses previously found to antagonize CCK-induced anorexia in rats (Dourish *et al.*, 1988b; Hewson *et al.*, 1988; Reidelberger *et al.*, 1988). (+)-Fenfluramine hydrochloride was obtained from Institut de Recherches Internationales Servier, Neuilly-sur-Seine, France. It was dissolved in distilled water and injected intraperitoneally in a dose of $3.0\,mg\,kg^{-1}$, chosen on the basis of earlier work (Neill & Cooper, 1989). Quinpirole hydrochloride (LY 171555) was obtained from Lilly Research Laboratories, Indianapolis, IN. It was dissolved in distilled water and injected subcutaneously. The dose of $0.3\,mg\,kg^{-1}$ was chosen on the basis of pilot experiments to produce a suppression of palatable food intake similar to $3.0\,mg\,kg^{-1}$ of (+)-fenfluramine. FG 7142 (N'-methyl-β-carboline-3-carboxamide) was obtained from Ferrosan Group, Soeborg, Denmark. It was suspended in distilled water to which a drop of Tween 80 had been added, and the suspension sonicated, and injected intraperitoneally. The dose of $10\,mg\,kg^{-1}$ was chosen on the basis of earlier studies (Cooper, 1986; Cooper *et al.*, 1985a) to produce a suppression of palatable food intake similar to $3.0\,mg\,kg^{-1}$ of (+)-fenfluramine.

Results

Palatability-induced feeding

Following vehicle injections, the control level of sweetened mash consumption lay between 17 and 19.5 g in a 30 min test period. (+)-Fenfluramine, administered in a dose of $3.0\,mg\,kg^{-1}$, produced a highly significant reduction (67%) in palatable food intake (Figure 1). When (+)-fenfluramine was given in conjunction with $10.0\,\mu g\,kg^{-1}$ of MK-329, there was still a significant anorectic effect, although it was slightly reduced (51%). However, when (+)-fenfluramine at $3.0\,mg\,kg^{-1}$ was given in conjunction with either $30\,\mu g\,kg^{-1}$ or $100\,\mu g\,kg^{-1}$ of MK-329, it no longer produced a significant

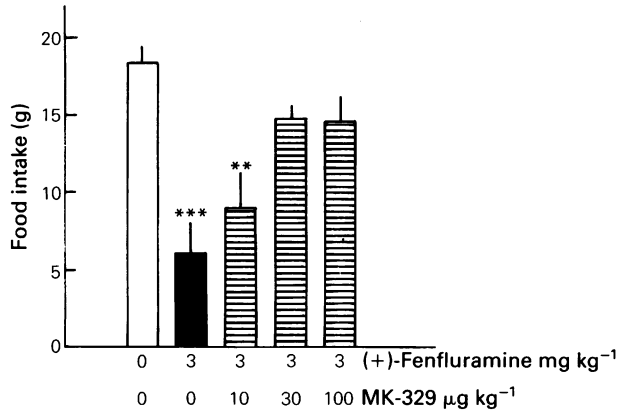

Figure 1 The anorectic effect of 3.0 mg kg^{-1} of (+)-fenfluramine (solid column) was blocked by the selective cholecystokinin receptor antagonist MK-329 at 30.0 and 100.0 μg kg^{-1}. The results are shown in terms of mean (s.e.mean shown by vertical lines) intake of a sweetened mash in a 30 min test. $n = 10$ per condition. Levels of significance for comparisons of treatments against the control condition (open column): $^{**}P < 0.01$; $^{***}P < 0.005$ (Dunnett's t test).

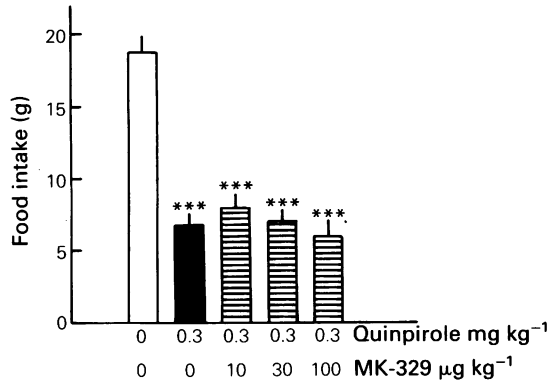

Figure 2 The anorectic effect of 0.3 mg kg^{-1} of quinpirole (solid column), a selective dopamine D_2-receptor agonist, was not antagonized by the cholecystokinin receptor antagonist MK-329 (10.0–100.0 μg kg^{-1}). The results are shown in terms of mean intake of palatable food (s.e.mean shown by vertical lines). $n = 10$ per condition. Level of significance for comparisons against the control condition (open column): $^{***}P < 0.005$ (Dunnett's t test).

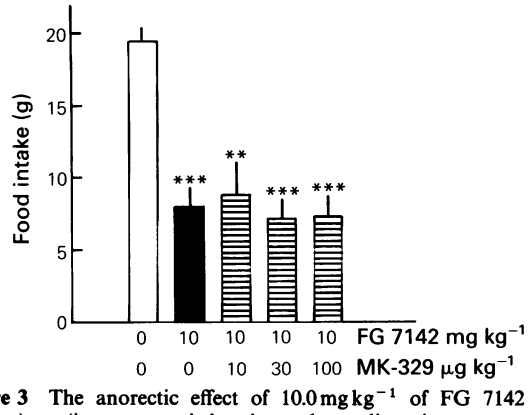

Figure 3 The anorectic effect of 10.0 mg kg^{-1} of FG 7142 (solid column), an 'inverse agonist' active at benzodiazepine receptors, was not antagonized by the cholecystokinin receptor antagonist MK-329 (10.0–100.0 μg kg^{-1}). The results are shown in terms of mean intake of palatable food (s.e.mean shown by vertical lines). $n = 10$ per condition. Levels of significance for comparisons against the control condition (white bar): $^{**}P < 0.01$; $^{***}P < 0.005$ (Dunnett's t test).

anorectic effect (Figure 1). The reduction in palatable food intake produced by (+)-fenfluramine was almost completely reversed by the two higher doses of MK-329.

The reversal of (+)-fenfluramine-induced anorexia was not a consequence of an intrinsic hyperphagic effect of MK-329 in the palatable food intake test. Over the dose-range of 10.0–300.0 μg kg^{-1}, MK-329 had no significant effect on consumption when given alone (Table 1). Furthermore, when animals were allowed to feed before administration of the drug in order to produce some prior satiation, MK-329 also failed to have any significant effect on food intake (Table 1).

The reversal of (+)-fenfluramine-induced anorexia by MK-329 was not due to the non-specific attenuation of anorexia following any drug treatment. Thus, MK-329 (10.0–100.0 μg kg^{-1}) had no effect on the anorectic effect of quinpirole, a selective dopamine D_2-receptor agonist (Figure 2). The dose of quinpirole (0.3 mg kg^{-1}) had been chosen to match its anorectic effect to that of (+)-fenfluramine. Similarly, MK-329 did not antagonize the anorectic effect of FG 7142, a β-carboline which acts as an 'inverse agonist' at benzodiazepine receptors (Figure 3). Hence, MK-329 acted specifically in its reversal of the effect of (+)-fenfluramine on food intake.

Table 1 Palatable food consumption by non-deprived rats following the administration of MK-329 (10.0–300.0 μg kg^{-1})

	MK-329 (μg kg^{-1})					
	0	10	30	100	300	F ratio
Standard procedure	17.2 ± 0.8	17.6 ± 1.2	17.8 ± 0.6	19.3 ± 1.4	20.0 ± 1.8	0.58
Pre-satiation (5 min)	8.0 ± 0.8	8.2 ± 1.0	7.3 ± 1.0	8.1 ± 0.9	9.3 ± 1.1	1.04
Pre-satiation (10 min)	5.3 ± 0.9	4.6 ± 0.6	6.1 ± 1.0	6.3 ± 1.2	5.9 ± 1.2	0.48

The food intake results are shown in terms of mean ± s.e.mean intake (g) in a 30 min test. ($n = 10$ per group). The three test procedures are described in the Methods section. The F-ratios are taken from the ANOVAs for the results obtained in each of the three feeding procedures. MK-329 treatments had no significant effect under any test condition.

Table 2 Nocturnal food intake in free-feeding rats following the administration of MK-329 (10.0–100.0 μg kg^{-1})

	MK-329 (μg kg^{-1})				
Time intervals	0	10	30	100	F ratio
0–2 h	5.9 ± 0.6	6.2 ± 0.6	6.6 ± 0.3	6.1 ± 0.4	1.50
0–4 h	9.8 ± 0.8	10.7 ± 0.7	10.1 ± 0.8	11.8 ± 0.6	1.50
0–6 h	13.0 ± 0.8	12.8 ± 1.0	13.0 ± 0.8	14.6 ± 0.7	1.18

The cumulative food intake results are shown in terms of the mean ± s.e.mean intake (g) of powdered chow over a 6 h period. ($n = 10$ per group). The F ratios are taken from the ANOVAs for the results obtained at each time interval. MK-329 treatments had no significant effect on food intake.

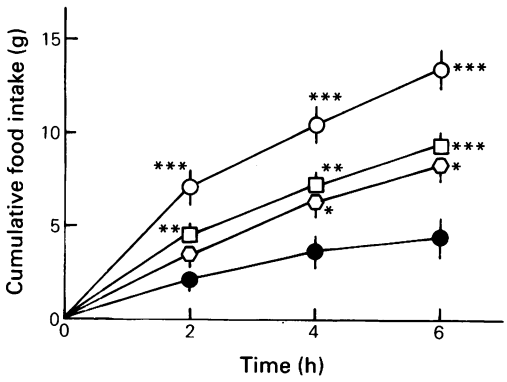

Figure 4 Cumulative intake (g) of powdered chow in freely-feeding rats over the first 6 h of the night-period. The control level of food consumption (○) was substantially reduced throughout the test following the administration of 3.0 mg kg^{-1} of (+)-fenfluramine (●). The cholecystokinin receptor antagonist, MK-329, at 100.0 μg kg^{-1} (□), significantly attenuated the anorectic effect at each time-point. At 30.0 μg kg^{-1} (○), MK 329 significantly attenuated the anorectic effect at 4 h and 6 h, respectively. The results are shown as mean cumulative intake (s.e.mean shown by vertical lines). $n = 10$ per condition. The statistical comparisons are drawn in terms of differences from the mean level of food intake after (+)-fenfluramine (3.0 μg kg^{-1}) treatment: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$ (Dunnett's t test).

Nocturnal free-feeding

Under control conditions, the free-feeding rats consumed about 13.5 g of powdered chow in a 6 h nocturnal period. During the first 2 h, the baseline level of food intake was 7.1 ± 0.7 g, and this was markedly reduced (by 69%) following administration of 3.0 mg kg^{-1} of (+)-fenfluramine (Figure 4). MK 329, at 100 μg kg^{-1}, significantly reversed the anorectic effect of (+)-fenfluramine during this period. At the two subsequent time-periods, 4 h and 6 h respectively, both 30.0 and 100.0 μg kg^{-1} of MK-329 significantly attenuated (+)-fenfluramine's anorectic effect, although the antagonism was not complete (Figure 4).

When MK-329 (30.0–100.0 μg kg^{-1}) was administered alone to free-feeding rats at the start of the night-period, there was no significant change in their subsequent food intake (Table 2).

Discussion

(+)-Fenfluramine (3.0 mg kg^{-1}) produced substantial reductions (67–69%) in the consumption of palatable food by non-deprived rats and in the nocturnal consumption of powdered chow by free-feeding rats (Figures 1 and 4). In both cases, the highly selective CCK receptor antagonist MK-329 blocked, to a significant degree, the anorectic effect of (+)-fenfluramine. These data indicate therefore that, at least in part, the anorectic effect of (+)-fenfluramine may depend upon endogenous activity of CCK at CCK receptors. In the case of palatable food consumption, the CCK-dependent component of (+)-fenfluramine anorexia appears to account for the major part of the suppression of food intake (Figure 1). About half of the anorectic effect of (+)-fenfluramine in the nocturnal free-feeding situation could be accounted for in terms of a CCK-dependent mechanism (Figure 4).

It is interesting that there is evidence for reversal of the suppressant effect of (+)-fenfluramine on feeding by MK-329 in two different feeding paradigms. As a result, several factors can be eliminated as being critical to the interaction between (+)-fenfluramine and MK-329. For example, taste and palatability were not crucial to the interactions between the two compounds. The type or texture of food, or the rate of food consumption, were also unlikely to be of great importance (rate of ingestion was very high in the case of palatability-induced feeding, and much lower in nocturnal free-feeding).

The control studies conducted in the palatability-induced feeding model argue against two possible accounts of the interaction between (+)-fenfluramine and MK-329. Firstly, MK-329 did not antagonize the anorectic effect of either quinpirole (Figure 2) or FG 7142 (Figure 3). Hence, MK-329 did not produce a non-specific reversal of drug-induced anorexia. It appears that neither quinpirole nor FG 7142 suppress food consumption through a CCK-dependent mechanism. The anorectic effect of FG 7142 can, however, be blocked by the benzodiazepine receptor antagonists Ro15-1788 and ZK 93426 (Cooper *et al.*, 1985a; Cooper, 1986), while that of quinpirole is blocked by specific dopamine D_2-receptor antagonists (Cooper, unpublished data). MK-329 acted selectively, therefore, in reversing (+)-fenfluramine-induced anorexia.

Secondly, MK-329 did not reverse (+)-fenfluramine-induced anorexia as a consequence of its own intrinsic hyperphagic effects (Tables 1 and 2). There was no evidence for intrinsic activity of MK-329 in either the palatable food consumption test or in nocturnal free-feeding. The most plausible explanation, therefore, for the interaction between MK-329 and (+)-fenfluramine is that (+)-fenfluramine enhanced endogenous CCK activity, which in turn led to the suppression of food intake. This indicates that (+)-fenfluramine-anorexia is a CCK-dependent effect on feeding, to an important degree. Since the anorectic effect of (+)-fenfluramine appears to be mediated through 5-HT_1-like receptors (Neill & Cooper, 1989), there may therefore be an involvement of this 5-HT receptor in the control of endogenous CCK activity. This possibility requires further study.

The question of intrinsic effects of selective CCK antagonists on food intake is an important one, since it has a bearing on the hypothesis that endogenous CCK plays a part in the physiological regulation of meal size (Smith & Gibbs, 1979). The weak non-selective CCK antagonist, proglumide, did not increase food consumption when administered alone (McLaughlin *et al.*, 1983; Crawley *et al.*, 1986), although Shillabeer & Davison (1984) reported an increase in food intake in food-deprived rats given a preload of liquid food. This effect, however, was not replicated by Schneider *et al.* (1986). In the case of MK-329, Hewson *et al.* (1988) reported that it increased consumption of a palatable sweet mash in doses of 10–100 μg kg^{-1}. Dourish and colleagues (1988b) reported a small increase in 24 h food-intake in freely-feeding rats, following administration of a large dose of MK-329 (1.0 mg kg^{-1}). A more robust hyperphagia was observed in 17 h food-deprived rats given a large food preload prior to injection of Mk-329 (Dourish *et al.*, 1988c). Mk-329 has also been reported to increase operant responding for food in pigs (Ebenezer *et al.*, 1989). In the present series of experiments, however, no increase in food intake was detected following the administration of MK-329 alone. In rats trained to eat a palatable sweet mash, MK-329 was ineffective, even though rats were also tested after satiation prior to the administration of the drug. It is also particularly significant that MK-329 did not affect the rats' nocturnal food intake, since this type of feeding response reflects their natural feeding pattern (Le Magnen, 1985).There are other examples of lack of effect of MK-329 alone on food intake (Schneider *et al.*, 1988). The evidence to date, therefore, fails to provide strong support for the view that antagonism of endogenous CCK should block the satiety effect of CCK and hence lead to enhanced food intake. Nevertheless, the present results indicate that when feeding is suppressed by (+)-fenfluramine, endogenous CCK may mediate to a significant extent the anorectic effect.

One possible peripheral mechanism underlying the observed MK-329-(+)-fenfluramine interaction on food intake is the control of gastric emptying. Fenfluramine reduces the rate of gastric emptying, and it has been proposed that this is responsible for its suppressant effect on food intake (Booth *et al.*, 1986). Similarly, CCK inhibits gastric emptying, and this effect has been invoked to explain its effect on food

intake (Moran & McHugh, 1982). In both cases, the reduction in gastric emptying rate is said to enhance satiety, and so lead to a reduction in meal size. Since the effect of CCK on gastric emptying can be antagonized by MK-329 (Green *et al.*, 1988), the hypothesis would require that the effect of fenfluramine on gastric emptying should be antagonized by MK-329. Even if this were confirmed, however, there are difficulties with the view that their effects on gastric emptying are sufficient to explain the anorectic effect of either (+)-fenfluramine or CCK. For example, the anorectic effect of (+)-fenfluramine was not blocked by xylamidine, a peripheral 5-HT receptor antagonist (Neill & Cooper, 1989), although it did antagonize the effect of fenfluramine on gastric emptying rate (Baker *et al.*, 1988). Furthermore, surgical removal of the pyloric sphincter, in which CCK receptors are densely localized (Smith *et al.* 1984), did not affect the ability of CCK to suppress food intake in rats (Smith *et al.*, 1988).

The antagonism of (+)-fenfluramine-induced anorexia by MK-329 may involve direct effects on mechanisms involved in appetite and the control of feeding responses. At present, more research is needed to address this possibility, and central, as well as peripheral, sites of action will have to be taken into consideration.

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References

BAILE, C.A., McLAUGHLIN, C.L. & DELLA-FERA, M.A. (1986). Role of cholecystokinin and opioid peptides in the control of food intake. *Physiol. Rev.*, **66**, 172–234.

BAKER, B.J., DUGGAN, J.P., BARBER, D.J. & BOOTH, D.A. (1988). Effects of *dl*-fenfluramine and xylamidine on gastric emptying of maintenance diet in freely feeding rats. *Eur. J. Pharmacol.*, **150**, 137–142.

BOOTH, D.A., GIBSON, E.L. & BAKER, B.J. (1986). Gastromotor mechanism of fenfluramine anorexia. *Appetite*, **7** (Suppl), 57–69.

CHANG, R.S.L. & LOTTI, V.J. (1986). Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4923–4926.

COOPER, S.J. (1985). Neuropeptides and food and water intake. In *Psychopharmacology and Food.* ed. Sandler, M. & Silverstone, T. pp. 17–58. Oxford: Oxford University Press.

COOPER, S.J. (1986). Hyperphagic and anorectic effects of β-carbolines in a palatable food consumption test: comparisons with triazolam and quazepam. *Eur. J. Pharmacol.*, **120**, 257–265.

COOPER, S.J., BARBER, D.J., GILBERT, D.B. & MOORES, W.R. (1985a). Benzodiazepine receptor ligands and the consumption of a highly palatable diet in non-deprived male rats. *Psychopharmacology*, **86**, 348–355.

COOPER, S.J., MOORES, W.R., JACKSON, A. & BARBER, D.J. (1985b). Effects of tifluadom on food consumption compared with chlordiazepoxide and kappa agonists in the rat. *Neuropharmacology*, **24**, 877–883.

CRAWLEY, J.N., STIVERS, J.A., HOMMER, D.W., SKIRBOLL, L.R. & PAUL, S.M. (1986). Antagonists of central and peripheral behavioral actions of cholecystokinin octapeptide. *J. Pharmacol. Exp. Ther.*, **236**, 320–330.

DOURISH, C.T., COOPER, S.J., GILBERT, F., COUGHLAN, J. & IVERSEN, S.D. (1988a). The 5-HT_{1A} agonist 8-OH-DPAT increases consumption of palatable wet mash and liquid diets in the rat. *Psychopharmacology*, **94**, 58–63.

DOURISH, C.T., COUGHLAN, J., HAWLEY, D., CLARK, M. & IVERSEN, S.D. (1988b). Blockade of CCK-induced hypophagia and prevention of morphine tolerance by the CCK antagonist L-364,718. In *Cholecystokinin Antagonists.* ed. Wang, R.Y. & Schoenfeld, R. pp. 307–325. New York: Alan R. Liss.

DOURISH, C.T., RYCROFT, W. & IVERSEN, S.D. (1988c). Blockade of CCK receptors postpones the appearance of the behavioural satiety sequence: evidence for an involvement of endogenous CCK in satiety. *J. Psychopharmacol.*, **2**, 2.

DOURISH, C.T., CLARK, M.L., FLETCHER, A. & IVERSEN, S.D. (1989). Evidence that blockade of postsynaptic 5-HT_1 receptors elicits feeding in satiated rats. *Psychopharmacology*, **97**, 54–58.

EBENEZER, I.S., DE LA RIVA, C. & BALDWIN, B.A. (1989). Intravenous administration of the cholecystokinin (CCK) antagonist MK-329 increases operant responding for food in pigs. *Br. J. Pharmacol.*, **98**, 632P.

ENZI, G., CREPALDI, G., INELMEN, E.M., BRUNI, R. & BAGGIO, B. (1988). Efficacy and safety of dexfenfluramine in obese patients: a multicenter study. *Clin. Neuropharmacol.*, **11**, Suppl. 1, S173–S178.

EVANS, B.E., BOCK, M.G., RITTLE, K.E., DIPARDO, R.M., WHITTER, W.L., VEBER, D.F., ANDERSON, P.S. & FREIDINGER, R.M. (1986). Design of potent, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin. *Proc. Natl. Acad. Sci., U.S.A.*, **83**, 4918–4922.

GREEN, T., DIMALINE, R., PEIKIN, S. & DOCKRAY, G.J. (1988). Action of the cholecystokinin antagonist L364,718 on gastric emptying in the rat. *Am. J. Physiol.*, **255**, G685–G689.

HEWSON, G., LEIGHTON, G.E., HILL, R.G. & HUGHES, J. (1988). The cholecystokinin receptor antagonist L364,718 increases food intake in the rat by attenuation of the action of endogenous cholecystokinin. *Br. J. Pharmacol.*, **93**, 79–84.

HUTSON, P.H., DONOHOE, T.P. & CURZON, G. (1988). Infusion of 5-hydroxytryptamine agonist RU 24969 and TFMPP into the paraventricular nucleus of the hypothalamus causes hypophagia. *Psychopharmacology*, **96**, 550–552.

JACKSON, A. & COOPER, S.J. (1985). Effects of *kappa* opiate agonists on palatable food consumption in non-deprived rats, with and without food preloads. *Brain Res. Bull.*, **15**, 391–396.

KENNETT, G.A., DOURISH, C.T. & CURZON, G. (1987). 5-HT_{1B} agonists induce anorexia at a postsynaptic site. *Eur. J. Pharmacol.*, **141**, 429–435.

KENNETT, G.A. & CURZON, G. (1988). Evidence that hypophagia induced by mCPP and TFMPP requires 5-HT_{1C} and 5-HT_{1B} receptors; hypophagia induced by RU 24969 only requires 5-HT_{1B} receptors. *Psychopharmacology*, **96**, 93–100.

KHOSLA, S. & CRAWLEY, J.N. (1988). Potency of L-364,718 as an antagonist of the behavioral effects of peripherally administered cholecystokinin. *Life Sci.*, **42**, 153–159.

LOTTI, V.J., PENDLETON, R.G., GOULD, R.J., HANSON, H.M., CHANG, R.S.L. & CLINESCHMIDT, B.V. (1987). In vivo pharmacology of L-364,718, a new potent nonpeptide peripheral cholecystokinin antagonist. *J. Pharmacol. Exp. Ther.*, **241**, 103–109.

LE MAGNEN, J. (1985). *Hunger.* Cambridge University Press.

McLAUGHLIN, C.L., PEIKIN, S.R. & BAILE, C.A. (1983). Feeding behavior response of Zucker rats to proglumide, a CCK receptor antagonist. *Pharmacol. Btochem. Behav.*, **18**, 879–883.

MORAN, T.H. & McHUGH, P.R. (1982). Cholecystokinin suppresses food intake by inhibiting gastric emptying. *Am. J. Physiol.*, **242**, R491–R497.

NEILL, J.C. & COOPER, S.J. (1989). Evidence that *d*-fenfluramine anorexia is mediated by 5-HT_1 receptors. *Psychopharmacology*, **97**, 213–218.

PI-SUNYER, F.X., KISSILEFF, H.R., THORNTON, J. & SMITH, G.P. (1982). C-terminal octapeptide of cholecystokinin decreased food intake in obese men. *Physiol. Behav.*, **29**, 627–630.

REIDELBERGER, R.D., O'ROURKE, M.F. & SOLOMON, T.E. (1988). Comparative effects of the CCK antagonist L364,718 on food intake and pancreatic exocrine secretion in rats. *Soc. Neurosci. Absts.*, **14**, 1196.

ROWLAND, N.E. & CARLTON, J. (1986). Neurobiology of an anorectic drug: fenfluramine. *Prog. Neurobiol.*, **27**, 13–62.

RUSSELL, G.F.M., CHECKLEY, S.A., FELDMAN, J. & EISLER, I. (1988). A controlled trial of *d*-fenfluramine in bulimia nervosa. *Clin. Neuropharmacol.*, **11**, Suppl. 1, S146–S159.

SAMANIN, R. (1983). Drugs affecting serotonin and feeding. In *Biochemical Pharmacology of Obesity.* ed. Curtis-Prior, P.B. pp. 339–356. Amsterdam: Elsevier Science Publishers.

SCHNEIDER, L.H., GIBBS, J. & SMITH, G.P. (1986). Proglumide fails to increase food intake after an ingested preload. *Peptides*, **7**, 135–140.

SCHNEIDER, L.H., MURPHY, R.B., GIBBS, J. & SMITH, G.P. (1988). Comparative potencies of CCK antagonists for the reversal of the satiating effect of cholecystokinin. In *Cholecystokinin Antagonists.*

ed. Wang, R.Y. & Schoenfeld, R. pp. 263–284. New York: Alan R. Liss.

SHILLABEER, G. & DAVIDSON, J.S. (1984). The cholecystokinin antagonist, proglumide, increases food intake in the rat. *Regul. Peptides*, **8**, 171–176.

SMITH, G.P., FALASCO, J., MORAN, T.H., JOYNER, K.M.S. & GIBBS, J. (1988). CCK-8 decreases food intake and gastric emptying after pylorectomy or pyloroplasty. *Am. J. Physiol.*, **255**, R113–R116.

SMITH, G.P. & GIBBS, J. (1979). Postprandial satiety. In *Progress in Psychobiology and Physiological Psychology*. ed. Sprague, J.M. & Epstein, A.N., vol. 8, pp. 179–242. New York: Academic Press.

SMITH, G.T., MORAN, T.H., COYLE, J.T., KUHAR, M.J., O'DONAHUE, T.L. & McHUGH, P. (1984). Anatomic localization of cholecystokinin receptors to the pyloric sphincter. *Am. J. Physiol.*, **246**, R127–R130.

WINER, B.J. (1971). *Statistical Principles in Experimental Design*. New York: McGraw-Hill.

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