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2-Naphthalenesulphanyl-L-aspartyl-2-(phenethyl) amide (2-NAP) and food intake in rats: evidence that endogenous peripheral CCK does not play a major role as a satiety factor

[1]I.S. Ebenezer & [1]B.A. Baldwin

Neuropharmacology Research Group, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, Hampshire, PO1 2DT and *Department of Neurobiology, Babraham Institute, Cambridge, CB2 4AT

1 The demonstration that systemic administration of the CCK_A receptor antagonist, devazepide, increases food intake in rats has provided the strongest support for the hypothesis that endogenous peripherally released cholecystokinin (CCK) acts as a satiety factor. However, interpretation of these results has been confounded by the fact that devazepide can enter the brain from the systemic circulation and may increase food intake by a central action. The present study was therefore undertaken to confirm the hypothesis that endogenous peripheral CCK is a satiety factor by investigating the effects of a novel CCK_A receptor antagonist, 2-NAP, which is unlikely to cross the blood brain barrier, on food intake in rats.

2 2-NAP (1-16 mg kg^{-1}, i.p.) had no significant effects on the intake of a test meal in rats.

3 Pretreatment of rats with 2-NAP (2 mg kg^{-1}, s.c.) abolished the inhibitory effects of exogenous peripheral CCK (5 μg kg^{-1}, i.p.) on food intake.

4 In agreement with previous results, devazepide (50-200 μg kg^{-1}, i.p.) significantly increased the intake of a test meal in rats.

5 The observations that 2-NAP, which is unlikely to penetrate the blood brain barrier, had no effect on food intake, but that 2-NAP abolished the suppressant effect of exogenous peripheral CCK, suggest that endogenously released peripheral CCK is not important as a satiety factor in rats.

Keywords: Cholecystokinin; cholecystokinin receptors; cholecystokinin receptor antagonists; food intake; satiety; 2-NAP

Introduction

Gibbs and his colleagues (1973) first showed that intraperitoneal administration of the gut-brain peptide hormone, cholecystokinin (CCK), reduces food intake in hungry rats by an apparently non-aversive mechanism and have proposed that endogenous CCK released from the small intestine during a meal acts to terminate the meal and induce a state of postprandial satiety. Subsequent studies have confirmed that systemic administration of CCK inhibits food intake in a number of other species, including man (Falasco *et al.*, 1979; Savory & Gentle, 1980; Anika *et al.*, 1981; Kissileff *et al.*, 1981). The target site for this action is not known, but as the peptide cannot penetrate the blood brain barrier (Passaro *et al.*, 1982), it is likely that systemically administered CCK acts at a peripheral site to inhibit feeding (Weller *et al.*, 1990). Studies in rats have suggested that peripheral exogenous CCK activates receptors in the abdomen of this species, and that this information is relayed via vagal afferents to brain regions involved in the regulation of feeding behaviour (Smith & Gibbs, 1992). Similarly, if endogenous CCK released from the gut during a meal acts as a satiety factor, then it must also act at a similar peripheral site (Smith & Gibbs, 1992).

Despite the large amount of work carried out during the past 20 years on CCK and ingestive behaviour, there is still vigorous debate as to whether endogenous peripheral CCK acts as a satiety factor. It was predicted that if CCK does play an important role in satiety, the administration of a specific CCK antagonist should block the effects of the endogenous peptide released from the gut and increase the amount of food eaten during a meal (Hewson *et al.*, 1988; Ebenezer *et al.*, 1990). In agreement with this prediction, it has recently been shown that the CCK_A receptor antagonist, devazepide (formerly coded as L364,718 and MK329) (Chang & Lotti, 1986), increases meal size when administered systemically to several species including rat, pig, mouse, monkey, chicken and man (Hewson *et al.*, 1988; Ebenezer *et al.*, 1990; Wolkowitz *et al.*, 1990; Weatherford *et al.*, 1992; Moran *et al.*, 1993; Covasa & Forbes, 1994). Moreover, the observation that devazepide also blocks the hypophagia produced by systemically administered CCK, indicates that the inhibitory action of the peptide on feeding is mediated by peripheral CCK_A receptors (Weller *et al.*, 1990; Ebenezer & Parrott, 1993). On the basis of the results obtained mainly with devazepide, some workers have concluded that the hypothesis that endogenous peripheral CCK is an important satiety factor is proven (Smith & Gibbs, 1992; 1994). However, devazepide readily crosses the blood brain barrier to enter the brain (Pullen & Hodgson, 1987) and it remains to be demonstrated conclusively that the CCK_A receptor antagonist increases food intake by a peripheral action (see Crawley & Corwin, 1994). Indeed, recent studies in pigs have suggested that systemic administration of the CCK_A receptor antagonist probably increases food intake by a central mode of action (Baldwin & de la Riva, 1992; Ebenezer & Parrott, 1993; 1994). For example, Baldwin & de la Riva (1992) demonstrated that intracerebroventricular (i.c.v.) administration of devazepide to pigs increases food intake at doses that are too low to be effective peripherally, indicating a central site of action. In support of this view, other studies in pigs have shown that systemic administration of two novel CCK_A receptor antagonists, A70104 (Ebenezer & Parrott, 1993) and 2-NAP (Baldwin *et al.*, 1994), that do not cross the blood brain barrier, do not increase meal size, although pretreatment with these drugs blocked the hypophagic effect of intravenous CCK. Moreover, the demonstration that i.c.v. administration of A70104 increases food intake in pigs (Ebenezer & Parrott, 1994), adds further credence to the notion that systemic administration of devazepide probably stimulates feeding in this species by a central mode of action. These studies therefore suggest that endogenous peripheral CCK

[1] Author for correspondence.

does not play an important role in mediating satiety in pigs. However, it is not possible to infer from experiments on pigs that endogenous peripheral CCK does not play an important role in mediating satiety in other species, because species differences exist in the structure (Campbell *et al.*, 1991) and distribution of CCK receptors (Woodruff *et al.*, 1991), and also in the functional responses to CCK (Ebenezer & Baldwin, 1991; Baldwin, 1992).

As the initial study, and much of the subsequent work on CCK and satiety mechanisms have been carried out in the rat, we considered it essential to test experimentally whether our conclusion that peripheral endogenous CCK does not act as a satiety agent in pigs also applies to rats. In the present study we employed an argument used previously in our pig studies (Ebenezer & Parrott, 1993), that is, if a specific CCK_A antagonist that cannot cross the blood brain barrier does not increase food intake in rats, then it is unlikely that endogenous CCK released from the small intestine during a meal acts as a satiety factor in this species. The aim of the present study was to investigate the effects of systemic administration of the novel CCK_A receptor antagonist, 2-NAP [2-naphthalenesulphanyl-L-aspartyl-2-(phenethyl) amide] (Hull *et al.*, 1993), which is unlikely to cross the blood brain barrier (Hull *et al.*, 1993; Baldwin *et al.*, 1994), on food intake in rats given an oral pre-load. We also investigated the ability of 2-NAP to antagonize the inhibitory effects of intraperitoneally administered CCK on food intake in pigs.

Methods

Experiment 1

Adult Male Wistar rats ($n=8$ in each group, b.wt. 300–380 g) were deprived of food for 21 h each day prior to training sessions or drug experiments, but had free access to water at all times. The animals were given 7 training sessions during which time they were allowed free access to food in experimental cages measuring $32 \times 25 \times 19$ cm for 60 min (oral pre-load). The nutrient composition of the food was as follows: protein 20%, oil 4.5%, fibre 5%, ash, 7% plus traces of vitamins and metals. The food was presented to the rats in shallow cylindrical cups as described previously (Ebenezer, 1990). The food was then removed, and either 30 or 60 min later, they were allowed to feed for a further 60 min. During experimental sessions that followed, the rats were injected i.p. with saline or 2-NAP (see Tables 1A and 1B for doses used) immediately after the oral pre-load. 30 or 60 min later, the food was returned to the experimental cages, and the amount of food consumed by the rats during the subsequent 60 min test-meal session was measured. A repeated measures design was used in each of these experiments, and at least 2 days separated successive drug trials.

As a control for experimental design, we examined the effects of devazapide on food intake in rats (male Wistar; body wt. 320–390 g; $n=8$ in each group) using similar protocols as those used for the 2-NAP experiments (see Figures 2a and 2b for doses of devazepide used).

Experiment 2

Adult male Wistar rats ($n=8$, body wt. 350–400 g) were housed in cages in groups of 4. The animals were deprived of food in their home cages for 22 a day, but had free access to water at all times. The animals were handled regularly prior to drug studies, and given three 2 h-training sessions on separate days during which time they were placed singly in experimental cages where they were allowed free access to their normal food pellets and water. During experimental sessions that followed, each rat was injected with either saline solution followed by saline, saline followed by CCK (5 $\mu g\ kg^{-1}$), 2-NAP (2 $mg\ kg^{-1}$) followed by saline, or 2-NAP (2 $mg\ kg^{-1}$) followed by CCK (5 $\mu g\ kg^{-1}$). The first injection was given s.c. while the second injection was given i.p. A period of 30 min separated the 2 injections. Immediately after the second injection, the rats were placed in the experimental cages, and the amount of food consumed by each animal measured after 30 and 60 min. A repeated measures design was used with each animal receiving all 4 treatments.

Drugs used

2-NAP (a gift from Dr V.P. Gerskowitch, James Black Foundation, U.K.) was dissolved in physiological saline solution (0.9%$^w/_v$) to give an injection volume of 0.1 ml per 100 g body weight. Cholecystokinin octapeptide sulphated (Calbiochem-Novabiochem, Nottingham, U.K.) was dissolved in physiological saline solution (0.9%$^w/_v$) to give an injection volume of 0.1 ml per 100 g body weight. Physiological saline was used as the control for 2-NAP and CCK. Devazepide (a gift from Dr L.L. Iversen, Merck Sharp and Dohme, U.K.) was dissolved in a vehicle containing dimethylsulphoxide (DMSO) and propylene glycol, as described previously (Ebenezer *et al.*, 1990), to give an injection volume of 0.1 ml per 100 g body weight. The devazepide vehicle (Ebenezer *et al.*, 1990) was used in control experiments.

Statistics

The results from these experiments, with the exception of those shown in Figure 1a were analysed by analysis of variance (ANOVA) for repeated measures, and *post-hoc* comparisons were carried out using the Tukey test. The data shown in Figure 1a were analysed by the paired *t*-test.

Results

Experiment 1

Effects of 2-NAP on food intake 2-NAP (1–16 $mg\ kg^{-1}$, i.p.) administered immediately after the oral preload, and 60 min prior to the 60 min test meal, did not significantly alter food consumption in the rats during the test meal ($F_{(5,35)}=0.6399$, NS) (Table 1A). The amount of food eaten during the oral preload (data not shown) was relatively constant from trial to trial (mean intake ± s.e.mean = 14.24 ± 0.95 g), and there were no significant differences amongst the trials ($F_{(5,35)}=1.6540$, NS).

The effects of 2-NAP (1–8 $mg\ kg^{-1}$, i.p.) administered immediately after the oral preload, and 30 min prior to the

Table 1 Effect of 2-NAP on food intake in rats given an oral preload

(**A**) The rats ($n=8$) were injected with saline (vehicle) or 2-NAP (1–16 $mg\ kg^{-1}$, i.p.) immediately after the oral preload, and 60 min prior to the 60 min test meal.

Dose of 2-NAP ($mg\ kg^{-1}$)	*Food Intake (g)*
0	10.2 ± 0.73
1	9.97 ± 1.01
2	9.65 ± 1.00
4	11.24 ± 1.10
8	10.11 ± 1.14
16	9.96 ± 0.97

(**B**) The rats ($n=8$) were injected with saline (vehicle) or 2-NAP (1–8 $mg\ kg^{-1}$, i.p.) immediately after the oral preload and 30 min prior to the 60 min test meal.

Dose of 2-NAP ($mg\ kg^{-1}$)	*Food Intake (g)*
0	7.32 ± 0.44
2	7.23 ± 0.50
4	7.96 ± 0.90
8	7.20 ± 0.50

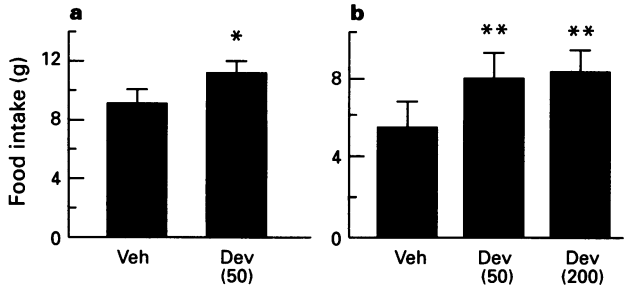

Figure 1 Effects of devazepide on food intake in rats given an oral preload. (a) The rats ($n=8$) were injected with vehicle (Veh) or devazepide (Dev, 50 $\mu g\,kg^{-1}$; i.p.) immediately after the oral preload and 60 min prior to the 60 min test meal. $^*P<0.02$ (paired t test) (b) The rats ($n=8$) were injected with vehicle (Veh) or devazepide (Dev, 50 or 200 $\mu g\,kg^{-1}$; i.p.) immediately after the oral preload and 30 min prior to the 60 min test meal. $^*P<0.05$, $^{**}P<0.01$ comparison to vehicle (Tukey test).

60 min test meal on food intake in the rats are illustrated in Table 1B. Statistical analysis of the data showed no significant effect of drug treatment on meal size at any of the doses tested ($F_{(3,21)}=0.8073$, NS). The amount of food eaten during the oral preload (data not shown) was relatively constant from trial to trial (mean intake ± s.e.mean = 14.17 ± 0.82 g) and there were no significant differences amongst the trials ($F_{(3,21)}=0.8550$, NS).

The effects of devazepide on food intake The effect of devazepide (50 $\mu g\ kg^{-1}$) administered immediately after the oral preload and 60 min prior to the 60 min test meal on food intake in rats is shown in Figure 1a. Devazepide produced a significant increase in feeding compared to vehicle ($P<0.02$,2-tailed paired t test) during the test meal.

The effects of devazepide (50 and 200 $\mu g\ kg^{-1}$, i.p.) administered immediately after the oral preload and 30 min prior to the 60 min test meal on food intake in rats are shown in Figure 1b. Analysis of the data showed that devazepide caused a significant increase in food intake during the test-meal ($F_{(2,14)}=11.2000$, $P<0.01$). *Post-hoc* tests revealed that both doses of devazepide produced significant increases in feeding ($P<0.01$, in each case). The amount of food eaten during the oral preload (data not shown) was relatively constant from trial to trial (mean intake ± s.e.mean = 15.0 ± 0.84 g) and there were no significant differences amongst the trials ($F_{(2,14)}=3.110$, NS).

Experiment 2

Effect of 2-NAP pretreatment on CCK-induced hypophagia The results are illustrated in Figure 2. Statistical analysis of the data showed that there were significant effects of drug treatment on food intake ($F_{(3,21)}=43.7010$, $P<0.01$ at 30 min and $F_{(3,21)}=18.6480$, $P<0.01$ at 60 min). *Post hoc* tests revealed that while CCK (5 $\mu g\ kg^{-1}$, i.p.) significantly decrease cumulative food intake 0–30 min 0–60 min after administration ($P<0.01$ in each case), pretreatment with 2-NAP (2 mg kg^{-1}) abolished the depressant effect of CCK on feeding. 2-NAP had no effect on food intake on its own.

Discussion

In the present study, the rats were given oral preloads prior to assessing the effects of the CCK_A receptor antagonists, 2-NAP or devazepide, on food consumption during a subsequent test meal. We believe that oral preload experiments have the merit that if endogenous peripheral CCK is a satiety factor, then a CCK_A receptor antagonist should block (a) the satiating effects of CCK released during the 1st meal and therefore increase the size of the 2nd meal, and (b) block the satiating effects of CCK released during the 2nd meal and further increase the size of the 2nd meal. The results obtained clearly show that 2-NAP had no effect on food intake in the rats when administered 30 or 60 min prior to the test meal. By contrast however, we found that in general agreement with previous results obtained in the rat (Dourish *et al.*, 1989; Garlicki *et al.*, 1990; Corwin *et al.*, 1991) devazepide increased food intake when administered 30 or 60 min before the test meal.

As 2-NAP is a novel drug and has not been tested for its CCK_A receptor antagonist efficacy in rodent behavioural models before, it was considered possible that the doses of 2-NAP used in the 1st experiment were insufficient to block adequately the effect of endogenous CCK, resulting in the lack of effect on food intake. To test this posibility, we carried out another experiment to investigate whether the inhibitory effects of exogenous peripheral CCK on food intake in hungry rats could be blocked by pretreatment with 2-NAP. We chose a dose of 2-NAP for this experiment (i.e. 2 mg kg^{-1}) which was on the lower end of the dose-range used in the 1st experiment. The results show that pretreatment with 2-NAP abolished the hypophagia induced by i.p. CCK (5 $\mu g\ kg^{-1}$) in 22 h-fasted rats and provide further confirmation that peripherally acting CCK acts on CCK_A receptors to inhibit food intake in rats (Dourish *et al.*, 1989; Weller *et al.*, 1990; Smith & Gibbs, 1992). Moreover, these results indicate that the dose range of 2-NAP used in the 1st experiment would have been sufficient to block the effects of endogenously released peripheral CCK and increase food intake during the test meal if endogenous peripheral CCK acts as a satiety factor. The fact that 2-NAP did not increase feeding argues strongly against the hypothesis that endogenous peripheral CCK is important in the mediation of satiety. As devazepide readily enters the brain, we propose that peripherally administered devazepide increases food intake by a central mode of action. Recent studies in pigs have shown that i.c.v. administration of devazepide at doses that are too low to be effective when given systemically, increases food intake in these animals (Baldwin & de la Riva, 1992), and preliminary experiments in rats have suggested a similar effect (unpublished results).

In summary, therefore, the results of this study have shown that a peripherally acting CCK_A receptor antagonist does not stimulate food intake in rats given an oral preload. These findings provide strong evidence against the view that endogenous peripheral CCK acts as a satiety factor in rats. Similar results have also been reported in pigs with 2-NAP

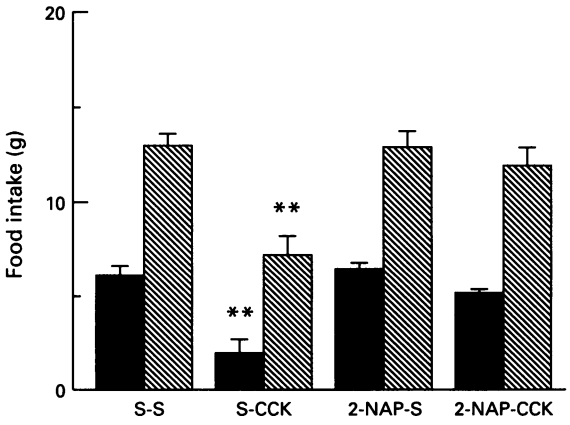

Figure 2 Effect of 2-NAP pretreatment on CCK-induced hypophagia. Rats ($n=8$) were pretreated with 2-NAP (2 mg kg^{-1}) 30 min prior to i.p. administration of CCK (5 $\mu g\,kg^{-1}$). Food intake was measured 30 min (solid columns) and 60 min (hatched columns) after the second injection. S-S = saline + saline; S-CCK = saline + CCK (5 $\mu g\,kg^{-1}$); 2-NAP-S = 2-NAP (2 mg kg^{-1}) + saline; 2-NAP-CCK = 2-NAP (2 mg kg^{-1}) + CCK (5 $\mu g\,kg^{-1}$). $^{**}P<0.01$ comparison to saline control (Tukey test).

(Baldwin *et al.*, 1994) and another CCK_A receptor antagonist, that cannot enter the brain from the systemic circulation, A70104, (Ebenezer & Parrott, 1993). As we have obtained comparable results in two different mammals, we tentatively conclude that endogenous peripheral CCK is not a major satiety factor in mammalian species.

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