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Short communication

Effects of intracerebroventricular administration of the CCK_1 receptor antagonist devazepide on food intake in rats

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Abstract

The effects of intracerebroventricular administration of devezapide, a CCK_1 receptor antagonist, was investigated on food intake in rats. In the first experiment, rats ($n=5$) were deprived of food for 17 h and injected intracerebroventricularly with either vehicle or devazepide (1, 10, 25 or 100 ng). Five minutes after vehicle or drug administration, the animals were presented with food and intake measured for 60 min. Devazepide produced a dose-related increase in food intake. Doses of 1, 10 and 25 ng significantly increased consumption (at least $P<0.01$ in each case). A second experiment was subsequently undertaken to investigate whether systemic administration of the intracerebroventricular doses used in the first experiment would affect food intake. Rats ($n=8$) that have been deprived of food for 17 h were injected intraperitoneally with either vehicle or devazepide (3, 30, 75 or 300 ng/kg). Five minutes after vehicle or drug administration, the animals were presented with food and intake was measured for 60 min. Devazepide (3–300 ng/kg, i.p.) had no significant effects on food consumption. The results show that central administration of low doses of devazepide increase food intake in rats, while similar doses, given systemically, do not affect consumption. These findings suggest the possibility that endogenous cholecystokinin (CCK), acting at central CCK_1 receptors, may play a physiological role in the control of feeding behaviour in the rat.

Keywords: Intracerebroventricular; Devazepide; Cholecystokinin; CCK_1 receptor antagonist

1. Introduction

The observation that systemic administration of the sulphated octapeptide of cholecystokinin (CCK-8S) decreases food intake in hungry animals has given rise to the hypothesis that endogenous peripheral cholecystokinin (CCK) released from the small intestine during a meal acts in a negative feedback manner to induce a state of satiety (Gibbs et al., 1973). As peripheral CCK cannot enter the brain from the systemic circulation, it is likely that its primary site of action must be outside the blood brain barrier (Ebenezer and Parrott, 1993). The results from a number of studies have suggested that systemically administered CCK probably acts on abdominal CCK_1 receptors, and that this signal is carried by vagal afferents to satiety areas in the brain to initiate behaviour consistent with satiety (Smith et al., 1981; Crawley and Schwaber, 1984). However, it still remains to be determined whether endogenous peripheral CCK is a satiety factor (Ebenezer and Baldwin, 1995; Baldwin et al., 1998). The most convincing evidence in favour of the CCK–satiety hypothesis was the demonstration that the CCK_1 receptor antagonist, devazepide, increases feeding in a number of animal species, including rat (Hewson et al., 1988; Ebenezer and Baldwin, 1995) and pig (Ebenezer et al., 1990). These findings appeared to confirm the prediction 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. However, it has been found that systemic administration of CCK_1 receptor antagonists, such as *N*-alpha-(3′-D-Glu-*N*,*N*-dipentylamide dicyclohexylammonium) (A70104) and 2-naphthalenesulphanyl-L-aspartyl-2-(phenethyl) amide (2-NAP), that do not readily penetrate the blood–brain barrier, do not stimulate feeding in pigs (Ebenezer and Parrott, 1993; Baldwin et al., 1994) and rats (Ebenezer and Baldwin, 1995; Ebenezer, 1999) under similar conditions in which devazepide stimulates food intake. However, the hypophagic effects of exogenous peripheral CCK are abolished by pretreatment with these CCK_1 receptor antagonists (Ebenezer and Baldwin, 1995; Ebenezer and Parrott, 1993; Baldwin et al., 1994). As devazepide enters the brain from the peripheral circulation, it has been sug-

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gested that the stimulation of feeding induced by systemic administration may be mediated by a central effect of the drug (Ebenezer and Parrott, 1993; Ebenezer and Baldwin, 1995). This view is further reinforced by the observation that rats that have been vagotomised still display increased food consumption after systemic administration of devazepide (Reidleberger, 1992). CCK is widely distributed in the central nervous system (CNS), and it has been found that microinjection of low concentrations of CCK into various hypothalamic and nonhypothalamic regions of the brain suppress feeding (Blevins et al., 2000a). Experiments conducted in pigs have shown that intracerebroventricular administration of devazepide increases food consumption at doses that have no effect when administered systemically (Baldwin, 1992; Baldwin et al., 1998). The present study was carried out to investigate the effects of intracerebroventricular administration of devazepide on food intake in rats to test the hypothesis that central CCK may be involved in the regulation of feeding.

2. Materials and methods

2.1. Experiment 1. Effects of intracerebroventricular administration of devazepide

Male Wistar rats ($n=6$; body weight 280–350 g) were chronically implanted, under chloral hydrate anaesthesia, with guide cannulae directed towards the left lateral ventricle for subsequent intracerebroventricular injection, as described previously (Ebenezer, 1990). At least 5 days were allowed for recovery from surgery before experiments began. The rats were housed singly and were deprived of food in their home cages for 22 h prior to each training or experimental session. The rats received three training session when they were placed for 60 min in experimental cages that contained a weighed amount of food. During experimental sessions, the rats were injected intracerebroventricularly with either devazepide vehicle or devazepide (1, 10, 25 or 100 ng) and placed into the experimental cages 5 min later for 60 min. The volume of the intracerebroventricular injection was 5 μl administered slowly over 2 min. The amount of food eaten at the end of the 60-min period was measured. A repeated measures experimental design was used in which each rat received the vehicle and all doses of devazepide in random order. Water was available ad libitum throughout. At the end of the experiment, the rats were deeply anaesthetised and injected intracerebroventricularly with 5 μl of black India Ink. The brains were removed and dissected to confirm that the ink filled the ventricular system, as described previously (Ebenezer, 1990).

2.2. Experiment 2. Effects of intraperitoneal administration of devazepide on food intake

Rats ($n=8$, body weight 250–320 g) were housed in groups of four and were deprived of food in their home cages for 17 h prior to each training or experimental session. The experimental protocol was similar to that used in Experiment 1 except that that rats were injected intraperitoneally with devazepide vehicle or devazepide (3, 30, 75 or 300 ng/kg) and 5 min later placed in the experimental cages for 60 min. The amount of food eaten at the end of the 60-min period was measured. A repeated measures experimental design was used in which each rat received the vehicle and all doses of devazepide in a random order. At least 3 days separated successive drug trials. Water was available ad libitum throughout the experiment.

2.3. Statistical analysis

The results from were analysed by analysis of variance for repeated measures, and post hoc tests were carried out using the Tukey test.

2.4. Drugs

Devazepide was a gift from Merck Sharp and Dohme, Harlow, England. Devazepide was dissolved in dimethylsulfoxide (DSMO) and propylene glycol, as described previously (Ebenezer et al., 1990). The devazepide vehicle was administered in control experiments.

3. Results

3.1. Experiment 1. Effects of intracerebroventricular administration of devazepide

One of the rats developed a respiratory infection during the experiment, and did not receive all treatments. The partial data obtained from this animal was not included in the analysis of the feeding data. The results are shown in Fig. 1. Devazepide (1–100 ng, i.c.v.) produced a dose-related

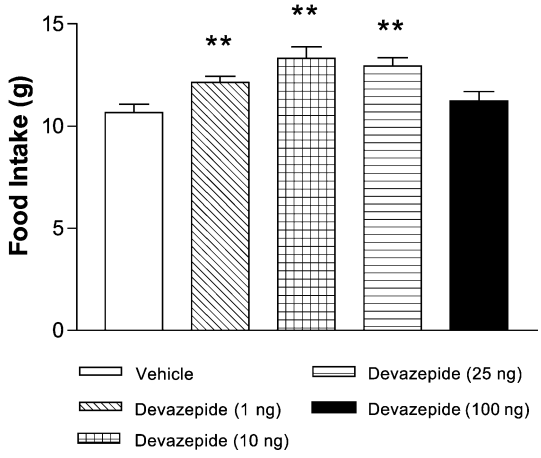

Fig. 1. Effects of devazepide (1–100 ng, i.c.v.) on food intake in 17-h food-deprived rats. Food intake was measured for 60 min. See text for experimental details. $^{**}P<0.01$ (vs. saline). Vertical line represents + S.E.M.

Table 1
Effects of devazepide (3–300 ng/kg, i.p.) on food intake in 17-h food-deprived rats

Dose of devazepide (ng/kg)	0	3	30	75	300
Food intake (g) ± S.E.M.	10.8 ± 0.5	10.6 ± 0.6	10.7 ± 0.5	11.1 ± 0.6	11.1 ± 0.5

Food intake was measured for 60 min. See text for experimental details.

increase in feeding [$F(4,16) = 31.320$, $P < 0.01$]. Post hoc tests revealed that the 1, 10 and 25 ng doses significantly increased feeding (at least $P < 0.01$, in each case). Interestingly, the 100-ng dose did not increase food intake. The dose–response feeding curve for devazepide assumed an inverted U shape, with maximal increases in feeding apparent at 10 ng. None of these doses of intracerebroventricular devazepide produced any overt abnormal behaviour.

3.2. Experiment 2. Effects of intraperitoneal administration of devazepide on food intake

The results are shown in Table 1. None of the doses of devazepide given intraperitoneally produced significant effects on food intake in the rats [$F(4,28) = 1.0701$, N.S.].

4. Discussion

The results of the study show that intracerebroventricular administration of the CCK_1 receptor antagonist devazepide increases food intake in rats. These results are in agreement with those reported in pig following intracerebroventricular administration of devazepide (Baldwin, 1992) and extends these observations to a rodent species. By contrast, Corps et al. (1997) have reported that intracerebroventricular administration of devazepide (0.5, 1.5 and 5 nmol) did not produce significant increases in food intake in rats. They found that the mean non-significant food intake (milk consumption) increases after devazepide assumed an inverted U-shaped curve. They used six rats in their study and the statistical analysis of their data (one-way analysis of variance with repeated measures) showed a P-value of 0.07, which is indicative that further studies with intracerebroventricular devazepide using more animals, different feeding protocols, food types (solid vs. liquid foods) or dosing regimes are warranted. In the present study, the dose-related effect devezapide on food intake also assumed an inverted U-shaped curve. A similar dose–response has been reported after intracerebroventricular administration of another CCK_1 receptor antagonist, A70104, in pigs (Ebenezer and Parrott, 1993). It is possible that high doses of CCK receptor antagonists are aversive to the animals when administered centrally (Corps et al., 1997; Baldwin et al., 1998).

The results obtained in Experiment 2 suggest that it is unlikely that the effects on food consumption observed after intracerebroventricular administration were due to the CCK_1 receptor antagonist entering the circulation and increasing feeding by a peripheral mode of action, because when these doses where given by intraperitoneal injection, they did not increase food consumption. Previous experiments in this laboratory and elsewhere have shown that microgram doses of devazepide are required to increase food intake when administered systemically to rats (Hewson et al., 1988; Ebenezer and Baldwin, 1995). The present findings, therefore, indicate that devazepide, administered intracerebroventricularly, acts centrally to increase intake. Moreover, it is also possible that systemically administered doses of devazepide that increases food consumption (see Hewson et al., 1988; Ebenezer and Baldwin, 1995), do so by a central mode of action.

A number of studies have shown that microinjections of CCK into the cerebral ventricles or discrete hypothalamic or nonhypothalamic regions of the brain inhibit food intake in a variety of animal species, including rat, sheep, pig and chicken (see Baldwin et al., 1998). Although some workers have argued that intracerebroventricular injection of CCK enters the circulation to produce a plasma CCK level sufficient to inhibit feeding by a peripheral mode of action (Crawley et al., 1991), there is also convincing evidence to suggest that centrally administered CCK can produce its anorexic effects by a direct central action. Thus, Blevins et al. (2000a,b) have shown that an anorectic dose of CCK microinjected into the hypothalamus of rats does not increase plasma CCK levels sufficiently to suppress feeding by a peripheral mechanism. There is also neurochemical evidence to support a role for central CCK in the control of food intake. Experiments carried out in both rats (McLaughlin et al., 1985) and primates (Schick et al., 1987) have shown that CCK levels in the hypothalamus increase during feeding. The results obtained in the present experiment suggest that blocking central CCK_1 receptors with devazepide, and presumably preventing the action of endogenous CCK, increases meal size; a result that would be expected if central CCK is involved in the regulation of food intake by some inhibitory mechanism. Devazepide may be regarded as having a disinhibiting action on this mechanism.

In summary, the results of this study extend previous observations in pigs and show that intracerebroventricular administration of the CCK_1 receptor antagonist, devezapide, increase food intake in a rodent species. These results suggest that central CCK may play a physiological role in the regulation of food intake.

References

Baldwin, B.A., 1992. CCK as a putative satiety factor in farm animals. In: Dourish, C.T., Cooper, S.J., Iversen, S.D., Iversen, L.L. (Eds.), Multiple Cholecystokinin Receptors in the CNS. Oxford Univ. Press, Oxford, pp. 206–221.

Baldwin, B.A., De la Riva, C., Gerskowitch, V.P., 1994. Effect of a novel CCK_A receptor antagonist (2-NAP) on the reduction of food intake produced by CCK in pigs. Physiol. Behav. 55, 175–179.

Baldwin, B.A., Parrott, R.F., Ebenezer, I.S., 1998. Food for thought: a critique on the hypothesis that cholecystokinin is a physiological satiety factor. Prog. Neurobiol. 55, 477–507.

Blevins, J.E., Stanley, B.G., Reidelberger, R.D., 2000a. Brain regions where cholecystokinin suppresses feeding in rats. Brain Res. 860, 1–10.

Blevins, J.E., Hamel, F.G., Fairbairn, E., Stanley, B.G., Reidelberger, R.D., 2000b. Effects of paraventricular injection of CCK-8S on plasma CCK-8 level in rats. Brain Res. 860, 11–20.

Corps, E.S., Curcio, M., Gibbs, J., Smith, G.P., 1997. The effect of centrally administered CCK-receptor antagonists on food intake in rats. Physiol. Behav. 61, 823–827.

Crawley, J.N., Schwaber, J.S., 1984. Abolition of the behavioural effects of cholecystokinin following bilateral radiofrequency lesions of the parvocellular division of the nucleus tractus solitarius. Brain Res. 295, 289–299.

Crawley, J.N., Fiske, S.M., Durieux, C., Derrien, M., Roques, B.P., 1991. Centrally administered cholecystokinin suppresses feeding through a peripheral-type receptor mechanism. J. Pharmacol. Exp. Ther. 257, 1076–1080.

Ebenezer, I.S., 1990. The effect of intracerebroventricular administration of baclofen on food intake in rats. NeuroReport 1, 73–76.

Ebenezer, I.S., 1999. A70104 and food intake in rats: evidence against a role for endogenous peripheral CCK as a satiety factor. Br. J. Pharmacol. 128, 296P.

Ebenezer, I.S., Baldwin, B.A., 1995. 2-Naphthalenesulphanyl-L-aspartatyl-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. Br. J. Pharmacol. 116, 2371–2374.

Ebenezer, I.S., Parrott, R.F., 1993. A70104 and food intake in pigs: implications or the CCK satiety hypothesis. NeuroReport 7, 495–498.

Ebenezer, I.S., De la Riva, C., Baldwin, B.A., 1990. Effects of the CCK receptor antagonist MK329 on food intake in pigs. Physiol. Behav. 47, 145–148.

Gibbs, J., Young, R.C., Smith, G.P., 1973. Cholecystokinin decreases food intake in rats. J. Comp. Physiol. Psychol. 84, 488–495.

Hewson, G., Leighton, G.E., Hill, R.G., Hughes, J., 1988. The cholecystokinin receptor antagonist L364, 718 increases food intake in the rat by attenuating the action of endogenous cholecystokinin. Br. J. Pharmacol. 93, 79–84.

McLaughlin, A., Baile, C.A., Della-Fera, M.A., Kasser, T.G., 1985. Meal-stimulated increased concentrations of CCK in the hypothalamus of Zucker obese and lean rats. Physiol. Behav. 35, 215–220.

Reidleberger, R.D., 1992. Abdominal vagal mediation of the satiety effects of exogenous and endogenous cholecystokinin in rats. Am. J. Physiol., R1354–R1358.

Schick, R.R., Reilly, W.M., Roddy, D.R., Yaksh, T.L., Go, V.L.W., 1987. Neuronal cholecystokinin-like immunoreactivity is postprandially released from primate hypothalamus. Brain Res. 418, 20–26.

Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R., Simansky, K.L., 1981. Abdominal vagotomy blocks the satiety efects of cholecystokinin in the rat. Science 213, 1036–1037.