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Reciprocal interaction of 5-hydroxytryptamine and cholecystokinin in the control of feeding patterns in rats

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1 The effect of the CCK_A receptor antagonist, devazepide (100 mg kg^{-1}) on meal parameters during the initial phase of the dark period was studied in free-feeding rats by use of a procedure for continuously monitoring feeding patterns.

2 In a second experiment, the effect of devazepide on the reduction in meal parameters induced by the 5-hydroxytryptamine (5-HT) releaser and uptake inhibitor, (+)-fenfluramine (1.5 mg kg^{-1}) in 4 h food-deprived rats was examined.

3 The hypophagic effect of an intraperitoneal injection of cholecystokinin (CCK-8, 4 μg kg^{-1}) was studied in rats treated with the 5-HT receptor antagonist, metergoline (1 and 2 mg kg^{-1}).

4 Devazepide increased the size of the first meal in free-feeding, but not in 4 h food-deprived rats and partially antagonized the effect of (+)-fenfluramine on the size and duration of the first meal. The reduction in eating rate induced by (+)-fenfluramine was not modified by devazepide. No changes in (+)-fenfluramine or (+)-norfenfluramine levels were found in the brain of rats treated with devazepide.

5 The effect of CCK-8 on meal size was completely antagonized by 2 mg kg^{-1} metergoline. A significant interaction was also found between 2 mg kg^{-1} metergoline and CCK-8 as regards their effect on the inter-meal interval.

6 The results suggest a reciprocal interaction between 5-HT and CCK-8 in enhancing the satiating effect of food in rats.

Keywords: (+)-fenfluramine; cholecystokinin; feeding patterns; satiety; 5-hydroxytryptamine

Introduction

Exogenous cholecystokinin (CCK-8) has been largely studied for its ability to reduce feeding in rats but only recently has the development of potent and selective agonists and antagonists made possible research into the roles of CCK_A and CCK_B receptors on feeding behaviour.

The anorectic effect of intracerebroventricularly or peripherally administered CCK-8 seems to be mediated by CCK_A receptors (Crawley *et al.*, 1991; Dourish *et al.*, 1989a), while the ability of CCK_A and CCK_B receptor antagonists to increase feeding in pre-satiated or free-feeding rats is still debated. One study (Dourish *et al.*, 1989b) proposed that the blockade of CCK_A or CCK_B receptors could increase food intake in pre-satiated rats, but two others subsequently reported an increase in food consumption after CCK_A but not CCK_B receptor blockade both in pre-satiated rats tested with a liquid diet (Corwin *et al.*, 1991) and in free-feeding rats (Reidelberger *et al.*, 1991).

Of particular interest were findings (Cooper *et al.*, 1990) that the CCK_A receptor antagonist devazepide (formerly known as L-374,718) (Chang & Lotti, 1986; Evans *et al.*, 1986) blocked the reduction in sweet-mash consumption and in nocturnal feeding induced by the 5-hydroxytryptamine (5-HT) releaser and uptake inhibitor (+)-fenfluramine (Garattini *et al.*, 1987). Thus 5-HT-dependent anorexia may be partially mediated by CCK_A receptors. On the other hand, the 5-HT receptor antagonist, metergoline (Hoyer, 1988) antagonized the reduction in food intake induced in food-deprived rats by an intraperitoneal injection of CCK-8 (Stallone *et al.*, 1989). There seems, therefore, to be a reciprocal functional interaction between CCK and 5-HT in the control of food intake.

Should 5-HT mediate the effect of CCK-8 on feeding, one would expect similar effects on meal patterns of CCK-8 and drugs enhancing 5-hydroxytryptaminergic transmission. (+)-Fenfluramine and CCK-8 reduce meal size, suggesting that both enhance the state of satiety in rats (Hsiao *et al.*, 1979; West *et al.*, 1987; Grignaschi *et al.*, 1992). (+)-Fenfluramine had no effect on the inter-meal interval (Grignaschi *et al.*, 1992) and the effect of CCK-8 on this parameter is not clear (Hsiao *et al.*, 1979; West *et al.*, 1987). In order to clarify the interaction between CCK and 5-HT, a meal pattern analysis of the effects of (+)-fenfluramine and CCK-8 in the presence of CCK and 5-HT receptor antagonists respectively has been carried out.

By use of a procedure for continuously monitoring feeding patterns in free-feeding and 4 h food-deprived rats (Blundell *et al.*, 1976; Grignaschi *et al.*, 1992), the changes in meal parameters induced by devazepide in free-feeding rats and the drug's effect on the reduction in meal parameters induced by (+)-fenfluramine in 4 h food-deprived rats (Grignaschi *et al.*, 1992) have been studied. Finally, the effect of an intraperitoneal injection of CCK-8 on meal parameters was studied in 4 h food-deprived rats treated with the 5-HT receptor antagonist, metergoline.

Methods

Animals

Male Sprague-Dawley rats (CD-COBS, Charles River, Italy), housed in groups of four in an animal colony maintained at 21 ± 1°C and 60% relative humidity, with a 12 h light-12 h dark cycle (light off at 18 h 00 min) and water and food *ad libitum* were used for testing. At the beginning of the experiment each animal, weighing 220–250 g, was housed in an individual box and allowed seven days' habituation to the pelleted food.

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Apparatus

The apparatus consisted of eight cages (Perspex boxes, 280 × 210 × 210 mm) and a cage supervisor apparatus connected to a MS-DOS computer for long-term data storage and analysis (Microsystem, Milan, Italy). The cages were equipped with a food hopper and a drinking bottle fixed to one wall. A single 45 mg food pellet (Noyes improved formula A) was always available in the hopper. Removal of the pellet activated an infra-red photo-beam system and another pellet was delivered in 3 s. Removal of each pellet was recorded by the cage supervisor apparatus with a timing resolution of 1 s and stored on a computer hard disc for long-term data storage.

Analysis

Feeding parameters were analysed essentially as described previously (Grignaschi *et al.*, 1992). A meal consisted of two or more pellets and 10 min was adopted as the minimum inter-response interval separating two meals. These choices were based on preliminary experiments showing that the pattern of meals did not differ when the inter-response interval was between 2 and 15 min and a minimum of one or two pellets was adopted as the criterion for a meal. Meal size (g), duration (min), eating rate (g min^{-1}) and inter-meal interval (min) were measured over different periods.

Effect of devazepide on meal patterns of free-feeding rats

During the seven days' habituation the animals were handled gently, weighed and injected subcutaneously (s.c.) with saline at 17 h 50 min, 10 min before the dark period. On the test day, devazepide (0.1 mg kg^{-1}, s.c.) was injection at 17 h 50 min and the test started at 18 h 00 min. The dose of devazepide was chosen on the basis of previous studies (Dourish *et al.*, 1989a,b; Reidelberger *et al.*, 1991) and preliminary experiments indicated it to be the most appropriate.

Effect of devazepide on the changes in meal parameters induced by (+)-fenfluramine in 4 h food-deprived rats

Since previous studies (Grignaschi & Samanin, 1992) had shown that the effect of (+)-fenfluramine is studied better in 4 h food-deprived rats, at the beginning of the seven days' habituation, delivery of pellets was stopped from 14 h 00 min to 18 h 00 min. During the habituation period before this study the animals were handled gently, weighed and injected intraperitoneally (i.p.) with saline at 17 h 30 min, 30 min before food was made available. On the test day, devazepide (0.1 mg kg^{-1}, s.c.) was injected at 17 h 20 min, 10 min before (+)-fenfluramine (1.5 mg kg^{-1}, i.p.). The test started at 18 h 00 min.

Measurement of brain concentrations of (+)-fenfluramine and (+)-norfenfluramine

Each animal was housed individually and allowed seven days' habituation with food available from 18 h 00 min to 14 h 00 min. On the test day the animals were injected with devazepide (0.1 mg kg^{-1}, s.c.) at 17 h 20 min, 10 min before (+)-fenfluramine (1.5 mg kg^{-1}, i.p.). The animals were killed 30, 60 or 90 min after (+)-fenfluramine and the brains were immediately removed and stored at − 20°C.

Brain concentrations of (+)-fenfluramine and (+)-norfenfluramine were extracted and analysed by electron capture gas liquid chromatography as previously described (Fracasso *et al.*, 1988; Spinelli *et al.*, 1988).

Effect of metergoline on the changes of meal parameters induced by CCK-8 in 4 h food-deprived rats

Since preliminary studies had indicated that the action of exogenous CCK-8 was rapid and short-lasting, delivery of pellets was stopped from 14 h 00 min to 18 h 00 min to facilitate analysis of drug effects at early times. During the habituation period the animals were handled gently, weighed and injected i.p. with saline at 17 h 45 min, 15 min before food was made available. On the test days, metergoline (1.0 and 2.0 mg kg^{-1}, i.p.) was injected at 15 h 00 min, 2 h 45 min before CCK-8 (4 μg kg^{-1}, i.p.). The test started at 18 h 00 min.

Drugs

Devazepide (Merck Sharp & Dohme Research Laboratories, West Point, PA, U.S.A.) was suspended in 0.5% carboxymethylcellulose and injected s.c. 10 min before the test or 10 min before (+)-fenfluramine. Metergoline base (Farmitalia Carlo Erba, Milan, Italy) was dissolved in 1% ascorbic acid and injected i.p. 3 h before the test. (+)-Fenfluramine hydrochloride (Servier, Neuilly-sur-Seine, France) and CCK-8 sulphate (Sigma, St. Louis, U.S.A.) were dissolved in saline and injected i.p. respectively 30 min and 15 min before the test. All drugs were injected in a volume of 2 ml kg^{-1}.

Statistical analysis

The effect of devazepide in free-feeding animals was analysed by Student's *t* test; a two-way analysis of variance (ANOVA 2 × 2) followed by Tukey's test was used to analyse the effect of (+)-fenfluramine in devazepide-treated rats and the effect of CCK-8 in metergoline-treated rats. Brain concentrations of (+)-fenfluramine and (+)-norfenfluramine in vehicle and devazepide treated rats were analysed by Student's *t* test.

Results

Effect of devazepide on meal patterns of free-feeding rats

As shown in Table 1, 100 μg kg^{-1} devazepide significantly increased the size of the first meal without modifying the eating rate. Devazepide increased the duration of the first meal, although the effect was not statistically significant. The CCK_A antagonist had no effect on the interval between the first and the second meal or any parameter of the second meal.

Effect of devazepide on the changes in meal parameters induced by (+)-fenfluramine in 4 h food-deprived rats

As shown in Table 2, devazepide when injected alone did not modify any parameter of the first meal but partially antagonized the reduction in meal size and duration caused by (+)-fenfluramine (F interaction $(1,23) = 6.4$ and 6.7 respectively; $P < 0.05$). It did not significantly modify the reduction in eating rate induced by (+)-fenfluramine (F interaction $(1,23) < 2.1$; $P > 0.05$). The parameters of the second meal were not affected by any treatment (data not shown).

There were no significant differences between (+)-fenfluramine and devazepide + (+)-fenfluramine groups in the brain levels of (+)-fenfluramine and (+)-norfenfluramine 30, 60 and 90 min after injection of (+)-fenfluramine (results not shown).

Effect of metergoline on the changes in meal parameters induced by CCK-8 in 4 h food-deprived rats

Preliminary studies showed that CCK-8, 4 μg kg^{-1}, reduced the size of the first meal and the interval between the first

Table 1 Effect of devazepide on meal parameters of free-feeding rats

	Vehicle	*Devazepide*
First meal:		
Size (g)	2.49 ± 0.59	4.09 ± 0.43*
Duration (min)	11.0 ± 3.2	14.1 ± 1.60
Eating rate (g min^{-1})	0.27 ± 0.03	0.30 ± 0.01
Second meal		
Size (g)	3.02 ± 0.75	3.17 ± 0.77
Duration (min)	12.4 ± 3.64	12.1 ± 2.91
Eating rate (g min^{-1})	0.27 ± 0.02	0.28 ± 0.02
Inter-meal interval (min)	55.4 ± 10.8	51.5 ± 8.90

Mean ± s.e.mean of eight animals per group.
Devazepide (100 μg kg^{-1}) was injected subcutaneously 10 min before the test.
*$P<0.05$ vs vehicle; Student's t test.

and the second meal. The other parameters were not modified, although the meal duration was clearly shorter in CCK-8-treated rats (data not shown).

As shown in Table 3, metergoline did not modify the size of the first meal but significantly antagonized the effect of 4 μg kg^{-1} CCK-8 on this parameter (F interaction (2,47) = 3.3; $P<0.05$). Further analysis by Tukey's test showed that only 2 mg kg^{-1} metergoline completely antagonized the effect of CCK-8. The interval between the first and the second meal was significantly reduced by CCK-8 ($P<0.05$, Tukey's test) and there was a statistically significant interaction (F interaction (2,47) = 6.5; $P<0.01$) between metergoline and CCK-8. Metergoline, at the dose of 2 mg kg^{-1}, significantly reduced the inter-meal interval ($P<0.01$, Tukey's test) and the treatment with CCK-8 did not modify this measure in the animals treated with the higher dose of metergoline.

Discussion

The CCK_A antagonist devazepide, at the dose of 100 μg kg^{-1}, increased the size of the first meal consumed during the initial phase of the dark period in free-feeding rats, with no effect on subsequent meals. Cooper *et al.* (1990) found that devazepide did not increase nocturnal feeding by free-feeding rats but the longer period considered in that study (2, 4 and

Table 2 Effect of devazepide on the changes of first-meal parameters induced by (+)-fenfluramine in 4 h food-deprived rats

	Saline	*(+)-fenfluramine*
Size (g)		
Vehicle	8.1 ± 0.7	1.4 ± 0.4**
Devazepide	6.7 ± 0.4	3.5 ± 0.8** [b]
Duration (min)		
Vehicle	28.2 ± 3.7	6.7 ± 1.5**
Devazepide	20.6 ± 2.6	14.0 ± 3.0 [b]
Eating rate (g min^{-1})		
Vehicle	0.30 ± 0.02	0.19 ± 0.02*
Devazepide	0.34 ± 0.03	0.25 ± 0.03*

Mean ± s.e.mean of at least six animals per group.
(+)-fenfluramine (1.5 mg kg^{-1}) was injected intraperitoneally 30 min before the test session.
Devazepide (100 μg kg^{-1}) was injected subcutaneously 10 min before (+)-fenfluramine.
F interaction (1,23) = 6.4 and 6.7 respectively for meal size and meal duration.
**$P<0.01$; *$P<0.05$ vs their respective saline group: Tukey's test.
[b]$P<0.05$ F interaction: 2-way ANOVA.

Table 3 Effect of metergoline on the changes in meal parameters induced by cholecystokinin (CCK-8) in 4 h food-deprived rats

	Dose (mg kg^{-1})	*Saline*	*CCK-8*
First meal (g)			
Vehicle		6.3 ± 0.5	3.1 ± 0.4*
Metergoline	1.0	6.4 ± 1.1	3.0 ± 0.6*
Metergoline	2.0	5.7 ± 0.6	6.0 ± 1.3[b]
Inter-meal interval (min)			
Vehicle		84.6 ± 8.5	52.9 ± 8.3*
Metergoline	1.0	78.8 ± 10.8	41.3 ± 4.5**
Metergoline	2.0	41.6 ± 6.4°°	54.8 ± 6.2[a]

Mean ± s.e.mean of eight animals per group.
Metergoline (1 and 2 mg kg^{-1}) and CCK-8 (4 μg kg^{-1}) were injected intraperitoneally respectively 3 h and 15 min before the test.
F interaction (2,47) = 3.3 and 6.5 respectively for meal size and inter-meal interval.
[b]$P<0.05$; [a]$P<0.01$: 2-way ANOVA
*$P<0.05$; **$P<0.01$ vs saline treated group: Tukey's test.
°°$P<0.01$ vs vehicle: Tukey's test.

6 h) may account for this difference. In 4 h food-deprived rats devazepide did not modify the parameters of the first meal, confirming that the animals' deprivation status may be important for revealing the ability of CCK_A antagonists to increase feeding.

Devazepide significantly antagonized the reduction in meal size and duration induced by (+)-fenfluramine confirming that endogenous CCK appears to mediate partially the reduction in food intake induced by (+)-fenfluramine (Cooper *et al.*, 1990). This antagonism could not be attributed to an interference of devazepide with the distribution and metabolism of (+)-fenfluramine since the brain levels of (+)-fenfluramine and its main metabolite (+)-norfenfluramine were not modified. Since the reduction in meal size by (+)-fenfluramine seems to be mediated by 5-HT_{1B} receptors (Grignaschi & Samanin, 1992), these receptors probably interact with CCK to reduce feeding.

5-HT appears to act as a potent releaser of CCK-like immunoreactivity in the rat through activation of the 5-HT_3 receptor type (Paudice & Raiteri, 1991). In that study the $5\text{-HT}_1/5\text{-HT}_2$ receptor antagonist methiothepin (Hoyer, 1988) did not modify the release of CCK-like immunoreactivity caused by 5-HT but the study employed synaptosomes prepared from rat cerebral cortex and nucleus accumbens. It therefore remains open whether stimulation of 5-HT_{1B} receptors enhance the release of CCK or increases the sensitivity of CCK receptors in the hypothalamus, a region playing a major role in monoamine- or peptide-induced changes in feeding (Leibowitz, 1989).

The decrease in eating rate by (+)-fenfluramine was not modified by devazepide, suggesting that the effect of (+)-fenfluramine on meal size and eating rate can be separated. This agrees with previous findings that the reduction in eating rate disappears more rapidly than the effect on meal size after repeated treatment with (+)-fenfluramine (Grignaschi *et al.*, 1992), and (±)-cyanopindolol antagonized the effect of (+)-fenfluramine on meal size but not on eating rate (Grignaschi & Samanin, 1992). It seems therefore that after changes in 5-HT transmission the duration rather than the rate of eating varies in relation to changes in meal size.

The hypothesis of an interaction between 5-HT and CCK in the control of feeding behaviour is further supported by the fact that, in agreement with previous findings on 3 h food-deprived rats (Stallone *et al.*, 1989), the 5-HT receptor antagonist metergoline completely antagonized the reduction in the size of the first meal induced by CCK-8. In rats used

to eating for 5 h daily, no interaction between metergoline and CCK-8 has been found (Garattini et al., 1992) confirming the importance of animals' deprivation status in feeding studies.

Dense CCK-8-like immunoreactivity has been found in the rat dorsal raphe (Vanderhaegen et al., 1980; Van Der Koy et al., 1981), an important site of origin of 5-HT-containing neurones innervating the forebrain, and a recent study (Boden et al., 1991) showed that CCK-8 excited some 5-HT-containing cells in this region. Possibly therefore part of the satiating effect of CCK-8 is due to its ability to activate 5-HT neurones in the raphe nuclei.

While (+)-fenfluramine reduced meal size with no effect on inter-meal interval, suggesting that it prolongs the satiating effect of a given amount of food (Samanin et al., 1991; Grignaschi et al., 1992), CCK-8 did not prevent the compensatory decrease in inter-meal interval which usually follows the reduction in meal size. The relatively short-acting effect of CCK-8 in our conditions may have contributed to this since when administered by continuous infusion CCK-8 had no effect on inter-meal interval (West et al., 1987). However, the fact that the rats ate more frequently to compensate for the CCK-8-induced reduction of meal size (West et al., 1987) suggests that the main action of CCK is to hasten the onset of satiety.

In conclusion, this study confirmed that the CCK_A antagonist, devazepide, at the dose of $100\,\mu g\,kg^{-1}$ increases the size of the first meal in free-feeding rats. That 5-HT and CCK interact reciprocally was shown by the finding that devazepide and metergoline attenuated the reduction in meal size caused respectively by (+)-fenfluramine and CCK-8.

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