action potential; only the 600-ms data are reported here because the 200-ms data showed similar results and were redundant. Duration measures were obtained by setting a straight line across the oscilloscope screen along the top of the baseline noise. AHP duration was calculated from the third Na^+ action potential to the point at which the top of the AHP tracing returned asymptotically to the line set along the baseline. Estimates of this point rarely varied more than 20 ms between two investigators, or between on-line and off-line values. AHP half-decay times were calculated from the maximal amplitude of the slow AHP to the point at which the amplitude had decayed to half this maximal value.

22. W. W. Schlaepfer and M. B. Hasler, *Brain Res.* **168**, 299 (1979); W. G. Nayler, P. A. Poole-Wilson, A. Williams, *J. Mol. Cell Cardiol.* **11**, 683 (1979); J. L. Farber, *Life Sci.* **29**, 1289 (1981); B. K. Siesjö, *J. Cereb. Blood Flow Metab.* **1**, 155 (1981); D. P. Perl, D. C. Gajdusek, R. M. Garruto, R. T. Yanagihara, C. J. Gibbs, Jr., *Science* **217**, 1053 (1982).

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Postponement of Satiety by Blockade of Brain Cholecystokinin (CCK-B) Receptors

C. T. DOURISH, W. RYCROFT, S. D. IVERSEN

Exogenous cholecystokinin (CCK) decreases food intake and causes satiety in animals and man. However, it has not been established that endogenous CCK causes satiety or whether the response is mediated by peripheral-type (CCK-A) or brain-type (CCK-B) receptors. The development of potent and selective antagonists for CCK-A (MK-329) and CCK-B (L-365,260) receptors now allows these issues to be addressed. The CCK-A antagonist MK-329 and the CCK-B antagonist L-365,260 increased food intake in partially satiated rats and postponed the onset of satiety; however, L-365,260 was 100 times more potent than MK-329 in increasing feeding and preventing satiety. These results suggest that endogenous CCK causes satiety by an agonist action on CCK-B receptors in the brain.

THE NEUROPEPTIDE CCK DECREASES food intake in a large number of animal species and in lean and obese humans (*1*). Intraperitoneal injection of CCK causes a specific decrease in the meal size of food-deprived rats but has no effect on the water intake of water-deprived rats (*2*). Furthermore, CCK inhibits sham-feeding in rats (*3*) and monkeys (*4*), a condition in which food-deprived animals do not satiate spontaneously. In addition, CCK elicits the sequence of behavior in rats (feeding → activity → grooming → resting) that characterizes the onset of postprandial satiety (*5*). The above effects of exogenous CCK on feeding, together with the observation that CCK is released in the gut and in the brain during a meal, have led to the hypothesis that endogenous CCK is an important mediator of postprandial satiety (*1*). However, this hypothesis is controversial (*1*), and a definitive test of the involvement of endogenous CCK in satiety has been hindered by a lack of potent, selective CCK antagonists. The identification of potent, selective CCK antagonists (*6*) has now enabled us to examine the potential role of endogenous CCK in satiety.

It is also not clear if CCK decreases feeding by a peripheral or central mechanism (*1*). It has been suggested that CCK stimulates receptors in the gut that activate the vagus nerve; this signal is then thought to be relayed via the nucleus tractus solitarius (NTS) (either directly or through the parabrachial nucleus) to the hypothalamus (*7*). Thus, injection of CCK or the related decapeptide cerulein into the lateral ventricles, the ventromedial hypothalamus (VMH), and the paraventricular nucleus of the hypothalamus (PVN) has been claimed to decrease feeding, although not in all studies (*1, 8*). Furthermore, the satiety effect of CCK is blocked by vagotomy and by lesions of the NTS, VMH, and PVN (*9*). However, the blockade of CCK-induced satiety by VMH lesions has not been reproduced in all studies (*1, 9*). Ligand binding studies in rodents have shown that brain (CCK-B) receptors (which are found, for example, in the PVN, VMH, and lateral NTS) can be differentiated from peripheral (CCK-A) receptors (found, for example, in the gut, stomach, pancreas, and medial NTS) (*10*). The discovery of selective antagonists for CCK-A and CCK-B receptors (*6*) has enabled us to examine the respective roles of these receptor types in mediating CCK-induced satiety.

In the first experiment we determined the effects of access to food for periods of 0, 10, 20, 30, or 40 min on the behavioral satiety sequence in rats that had been starved overnight (*11*). As expected, increasing the length of the prefeeding period decreased food intake and accelerated the onset of the satiety sequence during the test period. A 40-min prefeeding period induced satiety almost immediately in the test period, and this condition was used to examine the potential antisatiety effects of the CCK antagonists. Doses of MK-329 [1-methyl-3-(2-indoloyl) amino-5-phenyl-3*H*-1,4-benzodiazepin-2-one] of 10 ng, 100 ng, 1 μg, 10 μg, 100 μg, or 1 mg per kilogram of body weight or an equal volume of 0.5% carboxymethylcellulose vehicle were injected subcutaneously immediately after a 40-min prefeeding period in rats that had been deprived of food for 17 hours. The animals were observed 30 min later for a period of 60 min. MK-329 significantly increased the frequency of feeding and, consequently, delayed the onset of resting (Fig. 1) (*12*). The effects of MK-329 (10 μg/kg) on the satiety sequence after a 40-min feeding period are compared to the effects of a 20- or 40-min

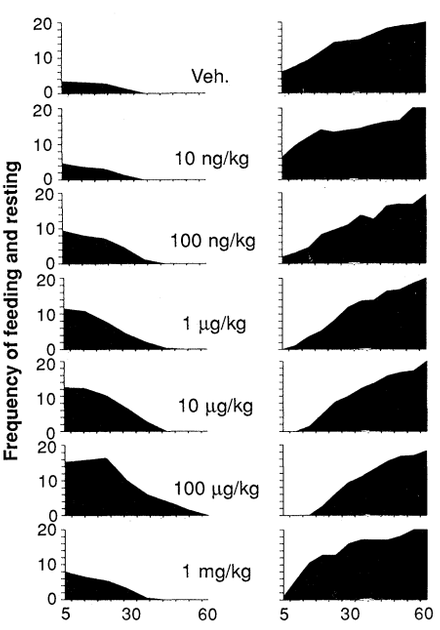

Fig. 1. Effect of MK-329 on the frequency (number of times the response is observed out of a total of 20 observations per 5-min period) of feeding (left panel) and resting (right panel) in rats deprived of food for 17 hours and then given a 40-min prefeeding period. The data are the mean of at least 12 rats per group. MK-329 significantly increased the frequency of feeding and postponed the onset of resting; two factor analysis of variance (ANOVA) with repeated measures indicated a significant main effect of MK-329 on feeding [$F(6,101) = 12.25$, $P < 0.00001$] and a significant interaction between MK-329 and time [$F(66,1111) = 5.05$, $P < 0.00001$] and a significant main effect of MK-329 on resting [$F(6,101) = 2.8$, $P < 0.02$].

Merck Sharp & Dohme Research Laboratories, Neuroscience Research Center, Terlings Park, Harlow, Essex CM20 2QR, England.

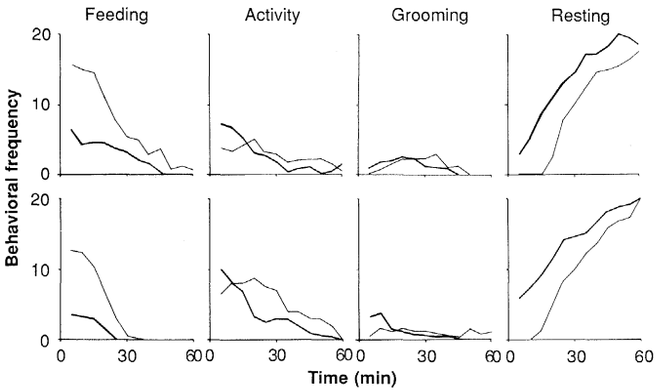

Fig. 2. Comparative responses in the satiety sequence of (lower panel) MK-329–treated rats (thin line) and vehicle-treated rats (dark line), both deprived of food for 17 hours and then given a 40-min prefeeding period, and of (upper panel) vehicle-treated rats, also deprived of food for 17 hours, and then given a 40-min (dark line) or a 20-min (thin line) prefeeding period. The data are the mean of at least 12 rats per group. The effect of MK-329 (10 μg/kg) on the satiety sequence closely resembles the effect of a 20-min decrease in prefeeding time.

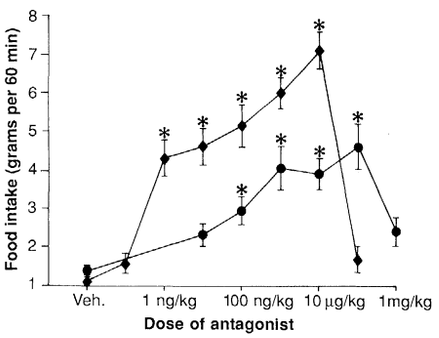

Fig. 4. Comparative potencies of the CCK-A antagonist MK-329 (●) and the CCK-B antagonist L-365,260 (◆) in increasing food intake during a 60-min test in rats deprived of food for 17 hours and then given a 40-min prefeeding period. Both drugs significantly increased food intake during the 60-min test. MK-329 [$F(6,101) = 14.5$, $P < 0.00001$] had a maximal effect at 100 μg/kg, and L-365,260 [$F(7,118) = 46.08$, $P < 0.000001$] had a maximal effect at 10 μg/kg. The data are the mean (±SEM) of at least 12 rats per group. Significant differences are indicated by asterisks ($*P < 0.01$) and were determined by Tukey test after a significant ANOVA.

period of prefeeding in vehicle-treated rats (Fig. 2). There is a striking similarity, both quantitatively and qualitatively, in the patterns of the two graphs. This finding suggests that, in food-deprived rats, MK-329 produces an effect that is equivalent to 20 min less access to food during the prefeeding period.

Our data demonstrate that endogenous CCK plays a role in satiety (*13*) and support findings that exogenous CCK induces satiety (*5*). MK-329 is a selective CCK-A receptor antagonist (*6, 10*). To determine if the effects we observed in the satiety sequence were due to blockade of CCK-A or CCK-B receptors, we compared the potency of MK-329 with that of the selective CCK-B antagonist L-365,260 (*6*) in this test. Doses of L-365,260 [(3*R*)-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N*^{1}-(3-methylphenyl)urea] of 100 pg, 1 ng, 10 ng, 100 ng, 1 μg, 10 μg, or 100 μg per kilogram of body weight or an equal volume of 0.5% carboxymethylcellulose vehicle were injected subcutaneously under the same conditions as used for MK-329. L-365,260 (like MK-329) significantly increased the frequency of feeding and delayed the onset of resting (*14*). The dose-response curves of both antagonists for preventing satiety were bell-shaped, but the explanation for this is unclear at present (*15*). L-365,260 was approximately 100 times more potent than MK-329 in increasing feeding and prolonging the latency to onset of the satiety sequence (Fig. 3). Similarly, both drugs increased the amount of food consumed during the 60-min test, with L-365,260 being more potent and more efficacious (Fig. 4). This difference in potency is in good agreement with the relative potencies of L-365,260 and MK-329 in displacing ^{125}I-labeled CCK binding to brain membranes and in antagonizing the excitatory effect of CCK on VMH neurons (*16*). In contrast, there is no significant linear relation between the potency of MK-329 and L-365,260 in preventing satiety and their relative affinity for CCK-A receptors in pancreas (*17*).

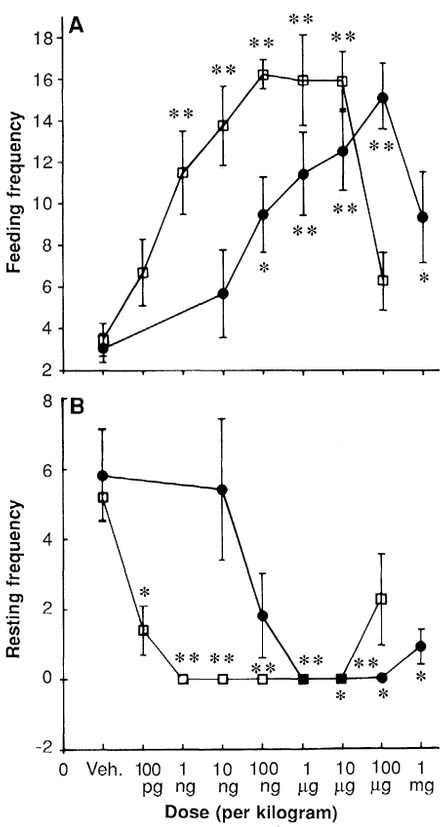

Fig. 3. Comparative potencies of the CCK-A antagonist MK-329 (●) and the CCK-B antagonist L-365,260 (□) in (**A**) increasing the frequency of feeding and (**B**) postponing the onset of resting in the satiety sequence. The graph illustrates effects of the antagonists during the first 5 min of the test (time of maximal effect of both drugs on these two behaviors). The data are the mean (±SEM) of at least 12 rats per group. Significant differences from vehicle are indicated by asterisks ($*P < 0.05$; $**P < 0.01$) and were determined by Tukey test after a significant ANOVA.

It has been suggested that CCK decreases food intake and produces satiety by slowing gastric motility (*1*). Indeed, blockade of CCK-A receptors by MK-329 increases gastric emptying of a meal in cats (*18*) and prevents the delayed gastric emptying induced by exogenous CCK in rodents (*19*). Our data, which show that the selective CCK-B antagonist L-365,260 is considerably more potent than MK-329, argue against the gastric motility hypothesis as a complete explanation for CCK-induced satiety because L-365,260 is much less effective than MK-329 in antagonizing CCK-induced inhibition of gastric emptying (*20*). This is in agreement with suggestions that effects on gastric motility can only partially account for the effects of exogenous CCK on feeding (*21*).

Our results argue in favor of an important role for CCK-B receptors in the mediation of CCK-induced satiety. The location of this CCK-B receptor population is uncertain, but both the PVN and VMH contain high densities of CCK-B receptors (*22*). Injection of CCK and cerulein into the hypothalamus has been reported to decrease feeding in rats (*1, 8*), whereas lesions of the PVN and VMH are claimed to cause overeating and obesity and to abolish the effects of CCK on feeding (*9*) [although not in all studies (*1, 9*)]. Whatever the precise neuroanatomical location involved, our findings support the concept that CCK-B receptor agonists may be clinically effective appetite-suppressant drugs with minimal peripheral side effects.

Furthermore, CCK receptor antagonists may have therapeutic use in the treatment of various forms of anorexia.

REFERENCES AND NOTES

1. G. P. Smith, in *Eating and Its Disorders*, A. J. Stunkard and E. Stellar, Eds. (Raven, New York, 1984), pp. 67–75; J. E. Morley, *Life Sci.* **30**, 479 (1982); H. R. Kissileff, F. X. Pi-Sunyer, J. Thorton, G. P. Smith, *Am. J. Clin. Nutr.* **34**, 154 (1981).
2. J. Gibbs, R. C. Young, G. P. Smith, *J. Comp. Physiol. Psychol.* **84**, 488 (1973).
3. ———, *Nature* **245**, 323 (1973).
4. J. D. Falasco, G. P. Smith, J. Gibbs, *Physiol. Behav.* **23**, 887 (1979).
5. J. Antin, J. Gibbs, J. Holt, R. C. Young, G. P. Smith, *J. Comp. Physiol. Psychol.* **89**, 784 (1975).
6. B. E. Evans *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4918 (1986); R. S. L. Chang and V. J. Lotti, *ibid.*, p. 4923; M. G. Bock *et al.*, *J. Med. Chem.* **32**, 13 (1989); V. J. Lotti and R. S. L. Chang, *Eur. J. Pharmacol.* **162**, 273 (1989).
7. J. N. Crawley and J. S. Schwaber, *Brain Res.* **295**, 289 (1984); C. E. Fulwiler and C. B. Saper, *Neurosci. Lett.* **53**, 289 (1985); L. Zaborszky, M. C. Beinfeld, M. Palkovits, L. Heimer, *Brain Res.* **303**, 225 (1984).
8. M. L. McCaleb and R. D. Myers, *Peptides* **1**, 47 (1980); M. A. Della-Fera and C. A. Baile, *Science* **206**, 471 (1979); R. R. Schick, T. L. Yaksh, V. L. W. Go, *Regul. Pept.* **14**, 277 (1986).
9. J. N. Crawley and J. Z. Kiss, *Peptides* **6**, 927 (1985); J. N. Crawley and J. S. Schwaber, *ibid.* **4**, 743 (1983); P. J. Kulkosky and C. Breckenridge, *Behav. Biol.* **18**, 227 (1976).
10. R. B. Innis and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6917 (1980); T. H. Moran, P. Robinson, M. S. Goldrich, P. McHugh, *Brain Res.* **362**, 175 (1986); D. R. Hill, N. J. Campbell, T. M. Shaw, G. N. Woodruff, *J. Neurosci.* **7**, 2967 (1987).
11. Individually housed adult male Sprague-Dawley rats (250 to 300 g) were deprived of food for 17 hours before the experiments. Groups of animals were given access to food (standard pellets, Beekay Foods, Hull) for various times (0, 10, 20, 30, or 40 min) before a 60-min test. During the test, rats were observed 20 times every 5 min and their behavior was recorded (with a BBC microcomputer) in one of four mutually exclusive categories: feeding, active, grooming, or resting. At the end of the 60-min test the computer generated scores for each behavior during each 5-min period (maximum score was 20). Food consumed was also recorded by weighing the amount of food presented before the test and then the amount of food not consumed by the end of the test.
12. Injection of exogenous CCK produces the opposite effect (*5*).
13. Increased food intake after injection of MK-329 and the weak CCK antagonist proglumide in rats has been observed [G. Hewson, G. E. Leighton, R. G. Hill, J. Hughes, *Br. J. Pharmacol.* **93**, 79 (1988); C. T. Dourish, J. Coughlan, D. Hawley, M. L. Clark, S. D. Iversen, in *Cholecystokinin Antagonists*, R. Y. Wang and R. Schoenfeld, Eds. (Liss, New York, 1988), pp. 307–325; G. Schillabeer and J. S. Davison, *Regul. Pept.* **8**, 171 (1984); R. D. Reidelberger, M. F. O'Rourke, E. Solomon, *Soc. Neurosci. Abstr.* **14**, 1196 (1988)].
14. Analysis of variance revealed a significant main effect of L-365,260 on feeding frequency [$F(7,118) = 16.3$, $P < 0.0001$] and a significant interaction between L-365,260 and time [$F(77,1298) = 4.23$, $P < 0.00001$]. Similarly, there was a significant main effect of L-365,260 on resting [$F(7,118) = 3.76$, $P < 0.001$].
15. A bell-shaped dose-response curve has also been observed with MK-329, L-365,260 and proglumide in analgesia tests [C. T. Dourish, D. Hawley, S. D. Iversen, *Eur. J. Pharmacol.* **147**, 469 (1988); C. T. Dourish, D. Hawley, M. F. O'Neill, S. D. Iversen, *Br. J. Pharmacol.*, in press; L. R. Watkins, I. B. Kinscheck, D. J. Mayer, *Science* **224**, 395 (1984)].
16. The concentrations of L-365,260 and MK-329 producing 50% inhibition (IC_{50}) of ^{125}I-labeled CCK binding to guinea pig brain homogenates were 2.0 and 250 n*M*, respectively (*11*); D. R. Hill and G. N. Woodruff, *Br. J. Pharmacol.* **98**, 629P (1989). The pA_2 values for L-365,260 and MK-329 as antagonists of CCK-induced excitation of VMH neurons (a response that is mediated by CCK-B receptors) were 7.5 and 6.6, respectively. J. A. Kemp, G. Marshall, G. N. Woodruff, *Br. J. Pharmacol.* **98**, 630P (1989).
17. The IC_{50} of L-365,260 and MK-329 for displacing ^{125}I-labeled CCK binding to guinea pig pancreas homogenates were 280 and 0.08 n*M*, respectively (*11*).
18. R. J. Gould, P. G. Cook, C. Fioravanti, H. F. Solomon, *Clin. Res.* **35**, 590A (1987).
19. V. J. Lotti *et al.*, *J. Pharmacol. Exp. Ther.* **241**, 103 (1987).
20. The dose producing 50% inhibition (ED_{50}) of L-365,260 and MK-329 for antagonizing CCK-induced inhibition of gastric emptying in mice were greater than 30.0 and 0.04 mg/kg, respectively (*11*).
21. T. H. Moran and P. R. McHugh, *Am. J. Physiol.* **254**, R628 (1988); K. L. Conover, S. M. Collins, H. P. Weingarten, *ibid.* **256**, R56 (1989).
22. M. A. Zarbin, R. B. Innis, J. K. Walmsley, S. H. Snyder, M. J. Kuhar, *J. Neurosci.* **3**, 877 (1983); T. R. Akesson, P. W. Mantyh, C. R. Mantyh, D. W. Matt, P. E. Micevych, *Neuroendocrinology* **45**, 257 (1987).

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Factors That Predict Individual Vulnerability to Amphetamine Self-Administration

PIER VINCENZO PIAZZA, JEAN-MARIE DEMINIÈRE, MICHEL LE MOAL, HERVÉ SIMON

Clinical observations show that there is considerable individual variability in the response to the addictive properties of drugs. This individual variability needs to be taken into account in animal models of addiction. Like humans, only some rats readily self-administer low doses of psychostimulants. The individual animals at risk can be identified on the basis of their response to environmental or pharmacological challenges. This predisposition to develop self-administration can be induced by repeated treatment with amphetamine. These results may help elucidate the neurobiological basis of addiction liability observed in both rats and humans.

THE ETIOLOGY OF DRUG ADDICTION is largely unknown, although an individual's vulnerability to addiction is one of the main factors that influences the prognosis. As pointed out by O'Brien *et al.* (*1*, p. 329), "Some addicts go for months or years using heroin or cocaine only on weekends before becoming a daily (addicted) user. Others report that they had such an intense positive response that they became addicted with the first dose. . . ." Although the importance of individual differences in humans is well accepted in clinical practice, it has often been neglected in animal studies. Intravenous self-administration (SA), a useful method for analysis of drug-taking behavior in animals, is typically studied after the behavior is well established after prolonged training with relatively high doses of drugs. Although this procedure has provided useful information on the neurobiological substrate of SA (*2*), it has obscured individual differences in vulnerability to the drug. However, during the acquisition of SA, individual differences among rats can be seen if low doses of drug are used. We addressed two questions in this study: (i) Could individual differences in the development of amphetamine SA in rats be predicted by a particular set of traits? (ii) Could these individual differences be modified?

In the first experiment, 30 male Sprague-Dawley rats (280 to 300 g body weight) were separately tested for individual reactivity (locomotor response) in a novel environment (*3*) and after an intraperitoneal injection of *d*-amphetamine sulfate. Novelty-induced locomotor activity was measured every 10 min for 2 hours. Two subgroups of animals were selected on the basis of their level of activity (either below or above the median of the group). Half the animals were classified as low responders to novelty (LRs; $n = 15$) and the other half, with a slower habituation response, were classified as high responders (HRs; $n = 15$) (Fig. 1A) (*4*). The two groups did not differ in other variables such as body weight or health. On the day after exposure to the novel environment, the animals were placed in the same apparatus for a habituation period of 3 hours and then were injected with amphetamine (1.5 mg per kilogram of body weight, intraperitoneally), after which their locomotor activity was recorded for three more hours. Analysis of variance (ANOVA) indicated that the responses of LR and HR

Psychobiologie des Comportements Adaptatifs, INSERM U.259, Université de Bordeaux II, Domaine de Carreire, rue Camille Saint-Saëns, 33077 Bordeaux, France.