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Differential Effects of CCK-JMV-180 on Food Intake in Rats and Mice

K. E. ASIN[1] AND L. BEDNARZ

Neuroscience Research Division,
Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, IL 60064

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ASIN, K. E. AND L. BEDNARZ. *Differential effects of CCK-JMV-180 on food intake in rats and mice.* PHARMACOL BIOCHEM BEHAV **42**(2) 291–295, 1992. – Boc-Tyr(SO3)-Nle-Gly-Trp-Nle-Asp-2-phenylether ester (CCK-JMV-180) has been reported to be a CCK-based heptipeptide with novel in vitro properties. Based on studies conducted in rat and mouse pancreatic acini, it has been proposed that the compound acts as an agonist at the high-affinity site and an antagonist at the low-affinity site in the rat, but as an agonist at both sites in the mouse. In the present study, we examined the effects of CCK-JMV-180 on locomotor activity in the rat and on intake of a liquid diet in the rat and mouse. Although CCK-JMV-180 slightly reduced activity on its own in the rat, it completely reversed the suppression produced by coadministration of CCK-8. In rat feeding studies, CCK-JMV-180 failed to suppress intakes of a liquid diet, but was able to antagonize the anorectic effects of CCK-8. In contrast, in the mouse CCK-JMV-180 potently suppressed intakes on its own, and this effect was blocked by pretreatment with the selective CCK-A receptor antagonist, A-70104. The results of these studies suggest that similar receptor mechanisms are involved in CCK's ability to inhibit food intake in vivo and its effects on pancreatic function in vitro.

CCK-JMV-180 CCK-8 Food intake Locomotor activity Species comparisons

BOC-Tyr(SO3)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester (CCK-JMV-180) represents a novel, CCK-based chemical entity with in vitro properties that suggest it may discriminate between high- and low-affinity CCK receptors: Although both CCK-8 and CCK-JMV-180 produce similar increases in amylase release in isolated rat pancreatic acinar cells at relatively low concentrations, supramaximal concentrations of CCK-8 but not JMV-180 lead to inhibition of this same response. Furthermore, CCK-JMV-180 is able to block the inhibitory effects on amylase secretion produced by high concentrations of caerulein, CCK-7, and CCK-8 (3,6,8,9,13). A similar lack of high-dose inhibition has been reported for the effects of CCK-JMV-180 on the incorporation of [^{3}H]leucine into protein in rat acinar cells (6). Since high-dose inhibition is believed to reflect activation of low-affinity receptors, it has been proposed that CCK-JMV-180 may have agonist properties at high-affinity CCK receptor sites and antagonist properties at low-affinity sites in the rat (3,13). In the mouse, however, CCK-JMV-180's in vitro profile more closely resembles that of CCK-8. Thus, unlike the monophasic response seen in the rat, the effect of CCK-JMV-180 on amylase release and [^{3}H]leucine incorporation in mouse pancreatic acini is biphasic (6), suggesting that CCK-JMV-180 may be devoid of antagonist properties in mouse.

Although the effects of CCK-JMV-180 in vitro have been well characterized, relatively little work has been done on its effect in vivo. It has been reported that in the rat microgram doses of CCK-JMV-180 have no effect on food intake or on brain levels of dopamine or serotonin or their metabolites following systemic administration. Application of the compound into the lateral ventricle produces a small but statistically reliable suppression of intakes 3–4 h postinjection (7).

Since CCK-JMV-180 appears to have different in vitro profiles in the rat and mouse, it would be of interest to correlate these profiles with its in vivo properties. Therefore, we investigated the behavioral effects of CCK-JMV-180 in tests sensitive to CCK-8 administration. Our results suggest a correspondence between the receptor mechanisms underlying CCK-8's anorectic properties and those involved in the inhibition of amylase release and protein synthesis in pancreatic acini by high concentrations of CCK-8.

EXPERIMENT 1: EFFECTS OF CCK-JMV-180 IN THE RAT

METHOD

Animals

Subjects were adult, male Sprague-Dawley rats weighing approximately 220–420 g at the time of testing. Animals were individually housed for feeding experiments (see below) and

[1] Requests for reprints should be addressed to K. E. Asin, Neuroscience Research Division, Pharmaceutical Discovery, Abbott Laboratories, Dept. 47H, Bldg. AP10, Abbott Park, IL 60064.

were group housed for tests involving locomotor activity. Rats in the feeding studies were allowed access to chow for 19 h/day; food was freely available for rats in the locomotor activity tests. Water was always available ad lib.

Drugs

CCK-JMV-180 was purchased from Research Plus, Inc. (Bayonne, NJ) and CCK-8 was purchased from Cambridge Research Biochemicals, Ltd. (Cambridge, England). The vehicle for CCK-8 was sterile, distilled water and all injections were administered IP. Since the higher doses of CCK-JMV-180 were relatively insoluble, the vehicle for all rat tests was 50% DMSO. Injection volumes in rat were 1 ml/kg, except for 10 mg/kg CCK-JMV-180, where the volume for drug and vehicle injection was 2 ml/kg.

Locomotor Activity

Horizontal locomotor activity was measured in Plexiglas cages measuring 49 × 24 × 30 cm and equipped with a photocell beam that bisected the length of the activity boxes. Each box was housed in a sound-attenuating chamber equipped with a fan and a 15-W light bulb centered over the boxes. Using a factorial design (CCK-JMV-180 × CCK-8), 40 animals were randomly assigned to one of four treatment groups. Animals were injected with CCK-JMV-180 (0 or 1.25 mg/kg) 10 min prior to injection with CCK-8 (0 or 10 nmol/kg). Ten minutes later, animals were individually placed into activity boxes and activity was recorded every min for 15 min. (In pilot studies, we had determined that by this time the activity of vehicle-injected animals was negligible.)

Food Intake

Animals were trained to consume a liquid diet (Ensure™, Ross Labs, Columbus, OH) that was presented to them in graduated drinking tubes for 60 min per day. Rats were deprived of their regular chow 4 h prior to presentation of the diet, as well as during the 60-min access period. Intakes were recorded at 30 and 60 min, after which time Ensure was removed and chow was returned. Intakes typically stabilized 10–12 days after initiation of training.

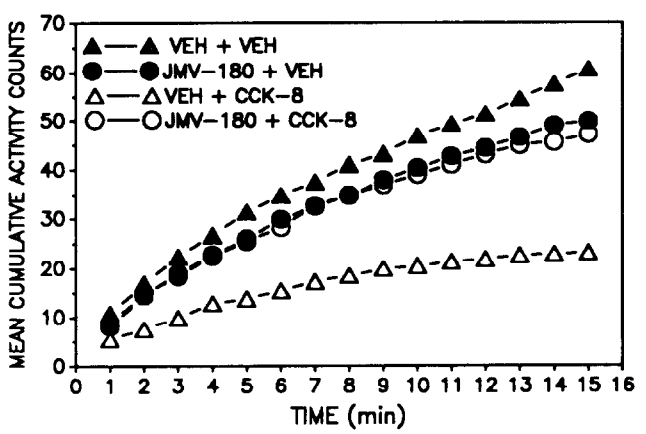

FIG. 1. Mean, cumulative locomotor activity in rats (n = 10/group) treated with either vehicle (VEH) or CCK-JMV-180 [JMV-180 (1.25 mg/kg)] prior to administration of vehicle or CCK-8 (10 nmol/kg). CCK-JMV-180 completely reversed the suppressant effects of CCK-8 on activity (see the text for details).

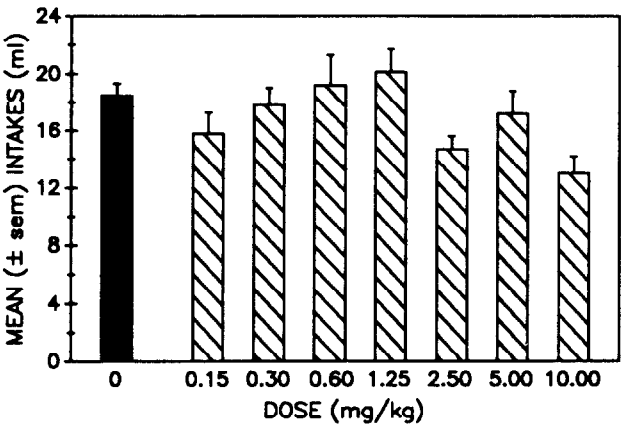

FIG. 2. Effects of various doses of CCK-JMV-180 on intakes of a liquid diet in rats. No dose significantly reduced intakes compared to vehicle (n = 9–26/group).

In initial studies, we examined the ability of CCK-JMV-180 to suppress intakes across a wide dose range. The CCK-JMV-180 was injected (IP) approximately 10 min prior to the presentation of Ensure and intakes were recorded 30 and 60 min thereafter. In subsequent studies, the effects of CCK-JMV-180 on the suppression of food intake produced by CCK-8 were examined. On the test day, rats were divided into four treatment groups (N = 10–12/group) using a factorial design, matched for baseline intakes. Animals were injected with CCK-JMV-180 (0–5 mg/kg) 10 min prior to receiving CCK-8 (0 or 10 nmol/kg, IP); the liquid diet was presented 10 min later. Each group of rats was used to test only one dose of CCK-JMV-180.

Both 30- and 60-min intakes following presentation of food were recorded. However, since we (1) and others [e.g., (14)] have found that the period of maximal intake suppression produced by CCK-8 is within 30 min, only these data are presented. Results obtained at 60 min were essentially the same as those obtained at 30 min.

RESULTS

Locomotor Activity

Activity data were analyzed using a three-way (CCK-8 × JMV-180 × time) analysis of variance (ANOVA) with repeated measures on the time factor. The results of the analysis indicated significant effects of CCK-8, $F(1, 36) = 33.30$, $p < 0.001$, and JMV-180, $F(1, 36) = 4.80$, $p < 0.04$, and a significant CCK × JMV-180 interaction, $F(1, 36) = 24.66$, $p < 0.001$. As expected, there was a significant time effect, $F(14, 504) = 30.60$, $p < 0.001$ (reflecting habituation to the testing environment in all groups), but no interaction between time and either of the other two factors [$F(14, 504) < 1.60$, $p > 0.07$ for all comparisons]. Post hoc contrast analysis on the data collapsed across the time factor indicated that the activity of CCK-8-injected rats pretreated with CCK-JMV-180 was significantly greater than those pretreated with vehicle, $F(1, 36) = 25.62$, $p < 0.001$. There was a trend for CCK-JMV-180 to reduce activity on its own compared to vehicle, $F(1, 36) = 3.85$, $p < 0.06$. Cumulative activity scores are

shown in Fig. 1. As may be seen, although CCK-JMV-180 slightly reduced activity on its own, it completely blocked the suppression of activity produced by CCK-8.

Food Intake

Thirty-minute food intakes following various doses of CCK-JMV-180 are shown in Fig. 2. A one-way ANOVA with planned comparisons (where all treatments were compared with vehicle) indicated no significant effect of treatment, $F(1, 84) = 2.52, p < 0.116$. Thus, CCK-JMV-180 failed to affect intakes in any group compared to vehicle injections.

Data from the CCK-JMV-180 × CCK-8 interaction studies were analyzed using 2 × 2 factorial ANOVAs (Fig. 3); post hoc analysis of significant interaction terms were conducted using the method of contrasts. For all studies, the CCK-8 treatment effect was statistically reliable ($p < 0.001$) (Fig. 2). The CCK-JMV-180 × CCK-8 interaction term, which would reflect the attenuation of CCK-8's effects by CCK-JMV-180, was statistically reliable ($p < 0.04$) at all but the lowest dose (0.04 mg/kg) of CCK-JMV-180 ($p > 0.30$). Post hoc analysis indicated that the anorectic effects of CCK-8 were significantly attenuated by pretreatment with CCK-JMV-180 at doses of 5.0, 1.25, and 0.31 mg/kg [$F(1, 40) = 7.47, p < 0.009$, $F(1, 40) = 8.96, p < 0.005$, $F(1, 36) = 4.76, p < 0.04$, respectively].

EXPERIMENT 2: EFFECTS OF CCK-JMV-180 ON FOOD INTAKE IN THE MOUSE

METHOD

Animals

Subjects were adult, male mice (CD1 strain) weighing approximately 30–35 g at the start of the experiment. Animals were allowed access to a 60% Ensure solution for 60 min in the morning and 90 min approximately 6 h later. (In pilot studies, we determined that diet dilution was necessary to generate sufficient baseline intakes against which reductions could be measured.) All tests were conducted on intakes during the morning feeding period after stable baseline intakes had been achieved.

In the mice feeding studies, CCK-JMV-180 (0–0.10 mg/kg, IP) was injected in a 1% DMSO/sterile water solution, in a volume of 10 ml/kg, 10–12 min prior to presentation of the liquid diet. In pharmacologic specificity studies, the CCK-A receptor antagonist A-70104, the dicyclohexylammonium salt of A-65186 (4), was administered (300 nmol/kg, IP) in a 5% DMSO/sterile water solution approximately 10 min prior to CCK-JMV-180. CCK-JMV-180 was administered 10–12 min prior to presentation of the liquid diet.

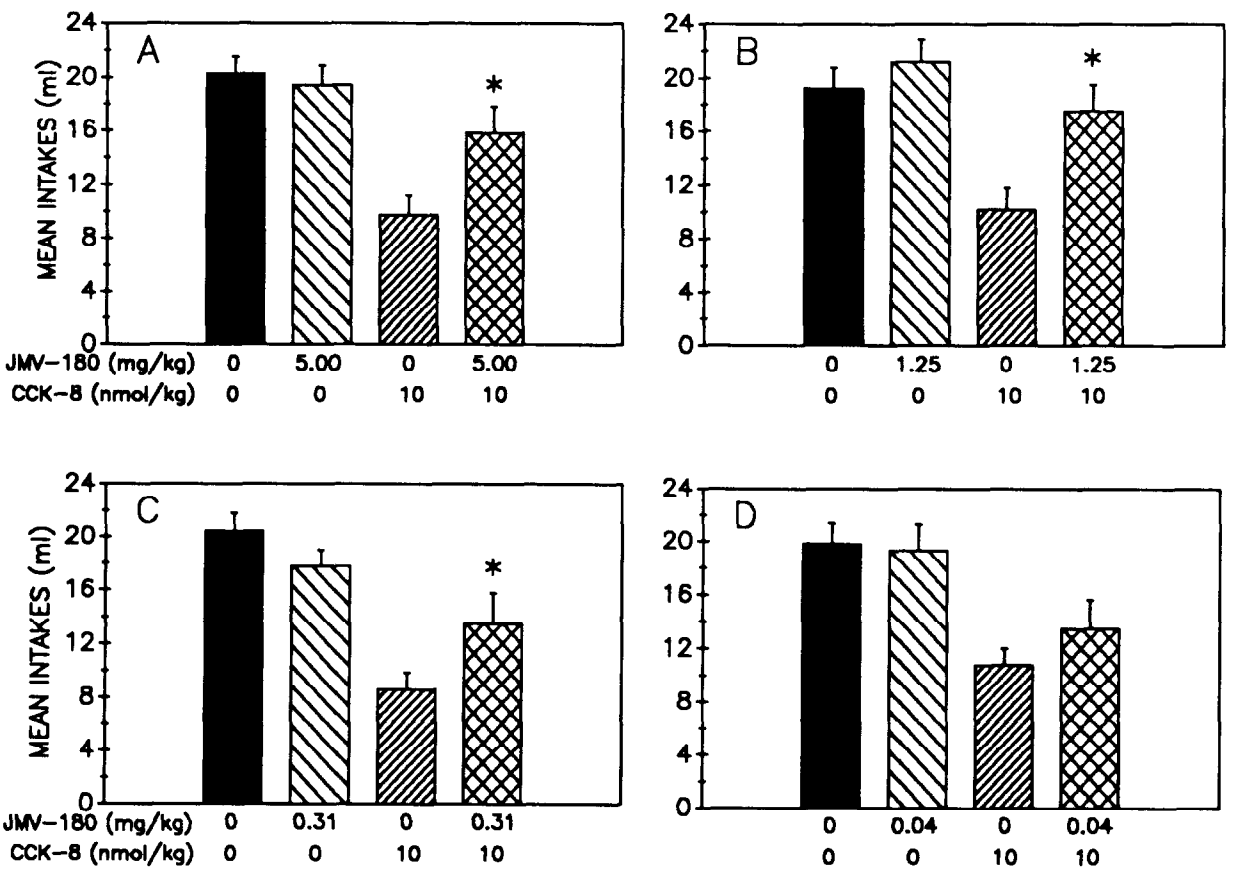

FIG. 3. Treatment of rats with CCK-JMV-180 (JMV-180) (0.31–5.00 mg/kg) blocks the effects of CCK-8 on feeding [$*p < 0.05$ compared to JMV-180 (0 mg/kg) + CCK-8 (10 nmol/kg)] (n = 10–12/group).

RESULTS

A one-way ANOVA indicated a significant effect of treatment, $F(4, 35) = 42.94$, $p < 0.001$, and post hoc analysis indicated that the intakes of all drug-treated groups were significantly suppressed compared to the intakes of controls ($p < 0.002$ for all comparisons) (see Fig. 4A).

A two-way (A-70104 × CCK-JMV-180) factorial ANOVA conducted on 30-min data generated in the pretreatment study indicated a significant effect of A-70104, $F(1, 36) = 18.75$, $p < 0.001$, JVM-180, $F(1, 36) = 18.37$, $p < 0.001$, and a significant interaction, $F(1, 36) = 11.70, p < 0.002$. Post hoc contrasts indicated that pretreatment with A-70104 completely reversed the effects of JMV-180 on feeding, $F(1, 36) = 30.03$, $p < 0.001$ (Fig. 4B).

GENERAL DISCUSSION

The results of the present study indicate that the behavioral effects of CCK-JMV-180 are complex and species dependent. In the rat, we found that CCK-JMV-180 produced a small reduction in locomotor activity and failed to suppress intake of a liquid diet, although when the compound was administered prior to CCK-8 administration it was able to block the suppressant effects of CCK-8 on both locomotor activity and food intake. In contrast to the results obtained in the rat, CCK-JMV-180 potently suppressed food intakes in mice and this effect was shown to be mediated via stimulation of the CCK-A receptor subtype.

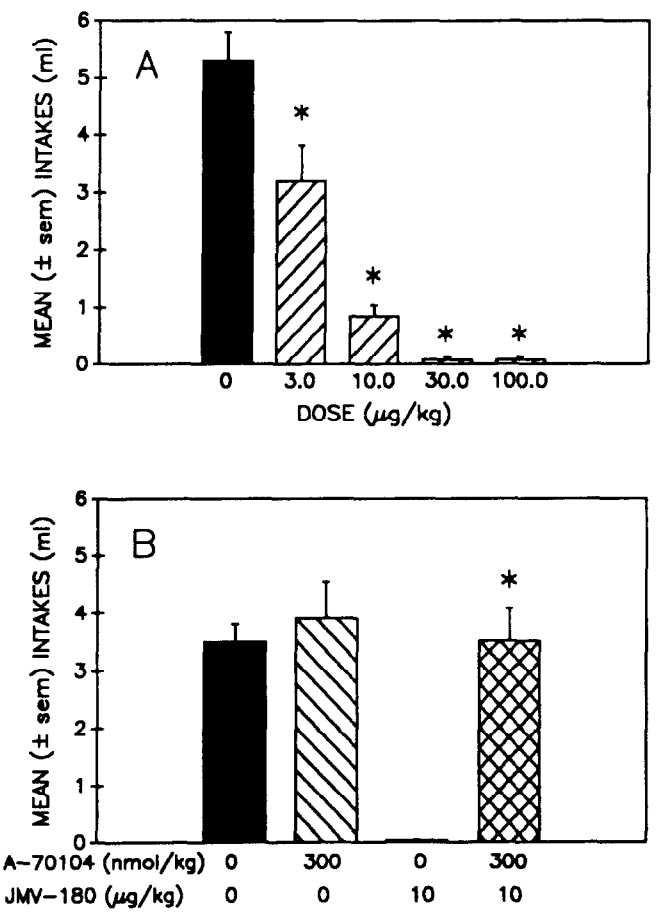

FIG. 4. Effects of CCK-JMV-180 on food intakes in mice. (A) All doses of CCK-JMV-180 tested significantly suppressed intakes compared to vehicle ($n = 8$/group) (*$p < 0.05$ compared to vehicle). (B) Anorectic effects of CCK-JMV-180 are reversed by pretreatment with the selective CCK-A receptor antagonist, A-70104 (*$p < 0.05$ compared to A-70104) (0 nmol/kg) + JMV-180 (10 μg/kg) ($n = 10$/group).

A two-site model has been proposed for the CCK pancreatic receptor: a high-affinity site, stimulation of which promotes (in part) amylase release, protein synthesis, and inositol phosphate$_3$ (IP_3) formation, and a low-affinity site, stimulation of which promotes inhibition of these responses. Considering the functional responses evoked by CCK-JMV-180 in the rat in vitro, it has been suggested that CCK-JMV-180 may act as an agonist at the high-affinity site and as an antagonist at the low-affinity site (which would explain the compound's ability to block the inhibitory effects of supramaximal concentrations of CCK-8 on these same responses) (10,11,13,15). Although other explanations have been offered for JMV-180's unique in vitro properties in the rat [i.e., (6)], it is clear that there are distinct differences between the functional responses elicited by CCK-JMV-180 in the rat and mouse.

The pharmacologic profile obtained with CCK-JMV-180 in the current study parallels that reported from in vitro studies. In the rat, CCK-JMV-180 stimulates amylase secretion from pancreatic acinar cells in the absence of inhibition at supramaximal concentrations. Furthermore, CCK-JMV-180 blocks the inhibition of amylase release produced by supramaximal concentrations of CCK-8 but not carbachol (3,12), indicating that the response is mediated via the CCK receptor. A similar monophasic dose-response effect is seen with CCK-JMV-180 with regard to [^{3}H]leucine incorporation into protein in rat acini (6), with antagonism of CCK-8's high-dose inhibitory effects on protein synthesis. CCK-JMV-180 is only minimally effective in promoting production of IP_3 in the rat, although it reduces 1,4,5-IP_3 production by CCK-8 (5).

In contrast to what has been reported in the rat, in mouse pancreatic acini CCK-JMV-180's effects on amylase release and protein synthesis are biphasic, similar to what is seen with CCK-8 (6).

In locomotor activity tests in the rat, CCK-JMV-180 (1.25 mg/kg) produced a small suppression of activity on its own ($p < 0.06$) and effectively blocked the profound suppression produced by CCK-8. These results suggest that the reduction of locomotor activity produced by CCK-8 may have two components: Stimulation of the high-affinity CCK receptor might lead to some, relatively slight, attenuation of activity, whereas stimulation of the low-affinity site may lead to more pronounced effects on activity. CCK-JMV-180, as a proposed agonist at the high-affinity site, would produce a mild suppression of activity on its own, but, as a proposed antagonist at the low-affinity site, would effectively block the actions of exogenous CCK-8 on locomotion. It is notable that a dose of CCK-JMV-180 eight times that used in the activity test failed to affect food intake in rat.

Both the inability of CCK-JMV-180 to suppress food intakes on its own in the rat as well as its reversal of the anorectic actions of CCK-8 suggest that the food suppressant actions of exogenous application of CCK may be mediated via stimulation of the low-affinity site (i.e., the same receptor that promotes high-dose inhibition of amylase release, etc. in the rat). Functional antagonism of this site by CCK-JMV-180, therefore, would not affect intake on its own (or might even promote it), but would be expected to block the actions of exogenous application of CCK-8. In contrast to these effects

in the rat, JMV-180 potently suppressed intakes on its own in the mouse, an observation consistent with reports that in this species CCK-JMV-180 is an agonist at both high- and low-affinity sites in pancreas. The suppression of intakes by CCK-JMV-180 was blocked by the selective CCK-A antagonist A-70104, suggesting that although CCK-JMV-180 has similar affinity for both the CCK-A and CCK-B receptor subtypes (3) its effects on feeding are mediated via stimulation of the CCK-A site. Similar conclusions have been reached for the effects of exogenous application of CCK-8 on food intake (2). Thus, our results are consistent with the notion that in the mouse, the high-dose inhibition of amylase release and protein synthesis seen in vitro with supramaximal concentrations of CCK-8 and CCK-JMV-180 reflect the stimulation of the same receptor subtype and affinity site as that responsible for the suppression of food intake.

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