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RAPID COMMUNICATION

The CCK-A Receptor Antagonist, Devazepide, Blocks the Anorectic Action of CCK but Not Peripheral Serotonin in Rats

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EBERLE-WANG, K. AND K. J. SIMANSKY. *The CCK-A receptor antagonist, devazepide, blocks the anorectic action of CCK but not peripheral serotonin in rats.* PHARMACOL BIOCHEM BEHAV 43(3), 943–947, 1992. – A role has been proposed for cholecystokinin (CCK)-A-type receptors in mediating the anorectic action produced by serotonergic stimulation in rats. We examined the effect of pretreatment with the CCK-A antagonist devazepide (DVZ) on anorexia produced by peripheral administration of serotonin [5-hydroxytryptamine (5-HT)] or CCK-8 in 3-h food-deprived rats consuming a 30-min test meal of sweetened mash. The anorectic effect of CCK-8 (4.0 nmol/kg, IP) was antagonized in a dose-dependent manner by DVZ (0.03, 0.10, and 0.30 μmol/kg, IP), with even the lowest dose producing a significant reversal. Under identical testing conditions, a supramaximal dose of DVZ (.75 μmol/kg) did not attenuate the reductions in food intake produced by either a moderate (4.0 μmol/kg) or a high dose (10.0 μmol/kg) of 5-HT. These data confirm established findings that the anorectic action of peripheral CCK depends upon CCK-A receptors. However, peripherally administered 5-HT reduces food intake independently of CCKergic function.

Serotonin Peripheral serotonin Cholecystokinin Devazepide CCK-A receptors Feeding Anorexia

SYSTEMIC injection of the indoleamine serotonin [5-hydroxytryptamine (5-HT)] reduces food intake and elicits satiety-associated behaviors in rats (9,14). Beyond the particular serotonergic receptors involved (15), the anorectic effect of 5-HT may depend also upon the activity of other endogenous satiety factors. Interactions between monoamines and peptides have been suggested to mediate controls of food intake (2,5). The peptide cholecystokinin (CCK) elicits satiety in rats (1), and this action is blocked by the nonpeptide CCK-A antagonist devazepide (DVZ, also MK-329, or L-364,718) (6,11-12). Recently, Cooper et al. (3) demonstrated that devazepide also blocked the anorectic effect of the indirectly acting serotonergic agonist *d*-fenfluramine. These data implicated endogenous CCK in mediating anorexia produced by serotonergic stimulation. However, the specific locus for this peptide-monoamine interaction remains to be addressed. It has been proposed that activating 5-HT receptors in the periphery recruits CCKergic mechanisms to reduce food intake (2,5). The present study, therefore, tested whether devazepide would inhibit the anorectic effect of peripherally administered 5-HT in rats. We report that blocking CCK-A receptors fails to reverse the peripherally induced action of 5-HT to decrease food intake.

METHOD

Subjects and Diet

Male Sprague-Dawley rats (AAI, Inc., Boyertown, PA), weighing 475–550 g at the time of the experiments, were housed individually in suspended wire mesh cages (20.3-cm wide × 22.9-cm deep × 17.8-cm high) maintained at an ambient temperature of 22 ± 1°C with a 12-h light photoperiod beginning at 0600 h. Rats had ad lib access to tapwater from glass bottles with stainless steel sipper tubes (Ancare, Inc, Manahasset, NY) attached to the front of each cage. They were maintained under the following feeding schedule: lab chow pellets from 1230 to 0900 h, no food from 0900 to 12.00 h, and a sweetened mash mixture from 1200 to 1230 h. Mash intakes were measured during this 30-min test period (1200–1230 h). The mash was a mixture of 800 g powder made from

A portion of these results were given in a preliminary report (8) at the annual meeting for the Society for Neuroscience (New Orleans, 1991).

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pelleted chow (Prolab, Agway Inc., Syracuse, NY) and 1,000 ml 10% (w/v) sucrose in distilled water (dH_20). The mash was presented in glazed clay bowls (9-cm diameter × 5-cm high) and measurements (g/30 min) were taken by weighing each bowl before and after the 30-min test period using a top-loading balance (Port-O-Gram Scale Model C501, Ohaus, Florham Park, NY). Intakes were corrected for spillage. Testing began when stable baseline intakes were attained.

Drugs

For these studies, 5-HT creatinine sulfate (MW = 387) was purchased from Sigma Chemical Co. (St. Louis, MO). CCK-8 (sulfated cholecystokinin octapeptide, MW = 1143), was purchased from Bachem, Inc. (Torrance, CA). Devazepide (formerly MK-329 and L-364,718, MW = 408) was donated generously by Dr. R.S.L. Chang of Merck Sharp and Dohme Research Laboratories (West Point, PA). The vehicle for devazepide was 0.5% (v/v) dimethyl sulfoxide (DMSO) in 1.0% (w/v) sodium carboxymethylcellulose (Sigma). Both 5-HT and CCK-8 were dissolved in distilled water. All drugs were injected in a volume of 2 ml/kg. CCK-8 was dissolved in aliquots and stored at −70°C to be thawed as needed. 5-HT and devazepide were prepared on the day of the experiment. Devazepide (or vehicle) was injected 30 min prior to presentation of the mash and 24 min prior to injection with either 5-HT, CCK-8, or vehicle.

Experimental Design

A dose-response curve for CCK-8 was generated by injecting rats with either 0 (vehicle), 1.0, 2.0, 4.0, or 8.0 nmol/kg IP CCK-8. Doses were administered in a random order using a repeated-measures design such that each rat (n = 8/group) received vehicle and each of the four doses of CCK-8 over the course of the experiment. There were at least 2 nontest days intervening between each of the 5 test days. CCK-8 or vehicle was injected 6 min prior to presentation of the mash, and 30-min mash intakes were measured.

To establish an appropriate probe dose of DVZ to test against 5-HT, we determined a dose-response curve for the ability of DVZ to antagonize the anorectic effect of CCK-8. First, baseline intakes of mash were measured for 27 rats after injection of the vehicles for DVZ and CCK-8. On the next day, four groups of these rats (n = 6–7/group) were injected IP with 4.0 nmol/kg CCK-8 after IP pretreatment with either 0 (vehicle), 0.03, 0.10, or 0.30 μmol/kg DVZ. Within each group, intakes after injection of CCK-8 on the test day were compared to the respective baselines. In addition, each rat's intake was expressed as a percentage of its own baseline for analyzing the dose-response function for DVZ to reverse the action of CCK-8.

The antagonism by DVZ of CCK-induced anorexia was confirmed in a separate group of animals that were used also for testing the effects of DVZ on the action of 5-HT. In the first experiment, we determined mash intakes in four groups of rats (n = 8–9/group) that received injections of either: a) both vehicles; b) 0.75 μmol/kg DVZ followed by the vehicle for CCK-8; c) the vehicle for DVZ plus 4.0 nmol/kg CCK-8; or d) DVZ plus CCK-8.

In subsequent experiments, we used similar designs for testing whether the same dose of DVZ would block the effects of either 4.0 or 10.0 μmol/kg 5-HT. These doses were chosen on the basis of a dose-response study that determined intakes after injecting vehicle, 1.6, 4.0, or 10.0 μmol/kg 5-HT (n = 9/group).

Statistics

The data were analyzed by analysis of variance (ANOVA) followed by a Newman-Keuls posthoc test for comparing individual means. The data for the devazepide dose-response experiment were analyzed also by comparing intakes on the test day after CCK-8 to baselines using orthogonal t-tests for related measures. The threshold for significance was $\alpha = p < 0.05$.

RESULTS

Systemic administration of CCK-8 produced a dose-related reduction of intake in 3-h food-deprived rats consuming a sweetened mash diet, $F(4, 28) = 21.4$, $p < 0.01$. The four doses of CCK-8 (1.0, 2.0, 4.0, and 8.0 nmol/kg, IP) decreased mash intakes by 38, 60, 78, and 72%, respectively, compared to intakes after treatment with vehicle (veh = 10.5 ± 1.1 g; $p < 0.01$ vs. vehicle for all doses).

Pretreatment with DVZ (0, 0.03, 0.1, and 0.3 μmol/kg, IP) reversed the anorexia produced by 4.0 nmol/kg CCK-8 (Fig. 1A), $F(3, 23) = 9.6$, $p < 0.01$. The lowest dose of DVZ attenuated partially the reduction in food intake by CCK-8, whereas the 10-fold higher dose completely reversed the anorectic effect of the peptide. These results were confirmed in a separate experiment (Fig. 1B) using a dose of DVZ (0.75 μmol/kg) that was 25-fold greater than the lowest dose (0.03 μmol/kg) that inhibited CCK's action.

As with CCK-8, 5-HT produced a dose-related anorexia, $F(3, 24) = 28.9$, $p < 0.01$, with doses of 1.6, 4.0, and 10.0 μmol/kg of the amine decreasing mash intakes by 24, 54, and 75%, respectively, when compared to the intake after vehicle treatment (veh = 7.0 ± 0.7 g; $p < 0.01$ vs. vehicle for the two highest doses). Unlike CCK-8, however, the same dose of DVZ (0.75 μmol/kg) did not alter the anorectic effects of either the EC_{50} (4.0 μmol/kg) or the EC_{75} (10.0 μmol/kg) doses of 5-HT (Fig. 2).

DISCUSSION

Peripheral administration of 5-HT produced an anorectic effect that was insensitive to blockade by the CCK-A antagonist devazepide. A dose of approximately 300 μg/kg devazepide did not alter the action of either a large or a moderately anorectic dose of the amine. This dose of devazepide was 25 times greater than the lowest dose that inhibited the decrease in food intake produced by CCK. Thus, the failure of devazepide to block 5-HT apparently was not due to an insufficient dose of the antagonist. In other experiments, we have found (unpublished data) that devazepide did not reverse 5-HT when the vehicle used was 50% DMSO [as in (12)] or when the antagonist was administered subcutaneously (8). Overall, the results dissociate the mechanisms for the anorectic action of exogenous 5-HT and CCK in rats.

The inability of DVZ to antagonize 5-HT is consistent with other evidence apparently separating the peripheral mechanisms for CCK- and 5-HT-induced anorexia. Specifically, abdominal vagotomy attenuates or blocks this action of CCK (16–18) but not that of 5-HT (7,10). The present data with 5-HT contrast, however, with the reported involvement of CCK-A receptors in the anorectic action of the serotonergic agonist fenfluramine (3). It is possible that fenfluramine recruits central CCKergic mechanisms because peripherally ad-

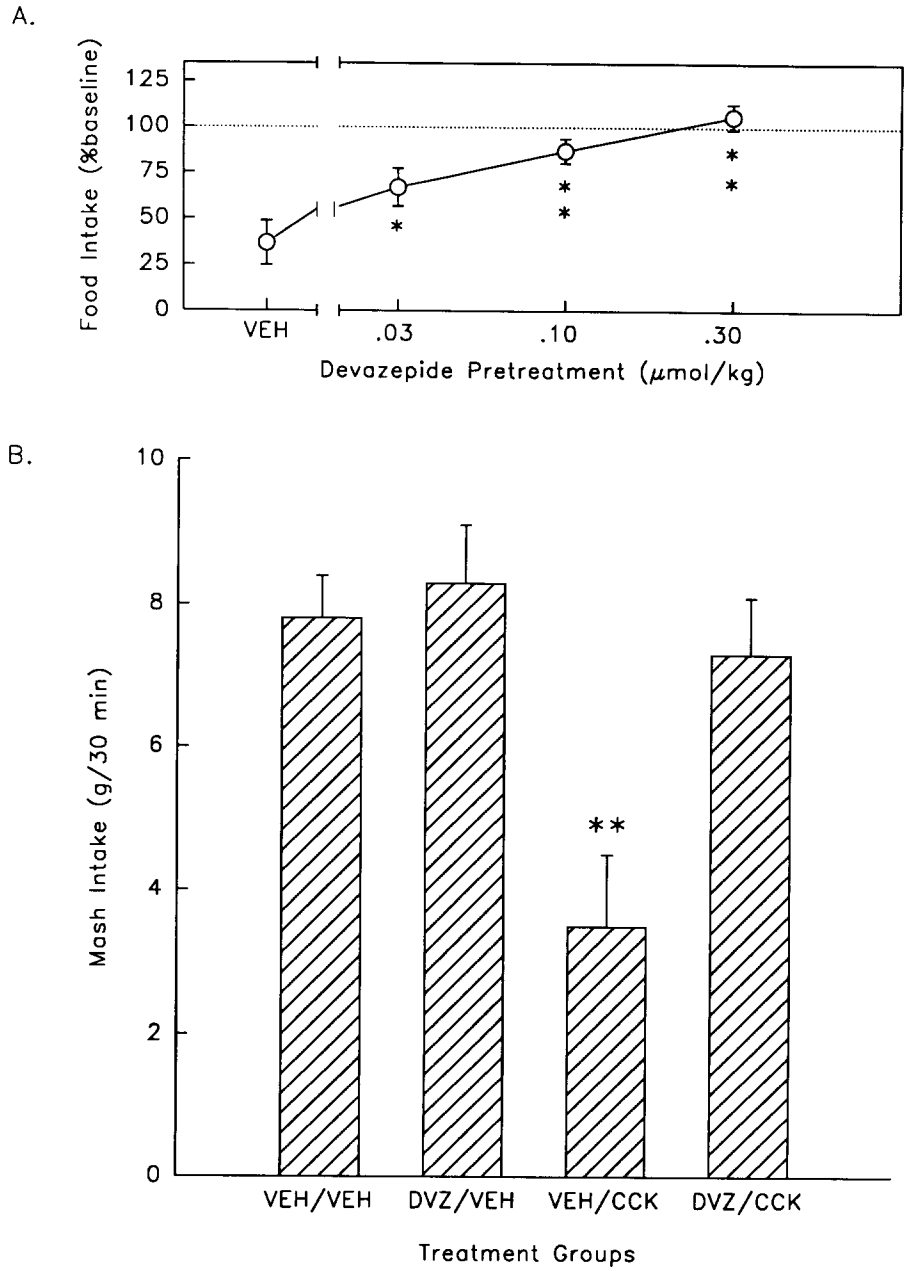

FIG. 1. (A). Devazepide (DVZ) produced a dose-dependent reversal of the anorectic effect of cholecystokinin (CCK)-8. Four groups of seven rats each received DVZ (0, 0.03, 0.10, and 0.30 μmol/kg, IP) followed 24 min later by CCK-8 (4.0 nmol/kg, IP) 6 min before mash was presented. Mash intakes on the test day were expressed as a percentage of each animal's baseline intake on the previous day. Pretreatment with each of the doses of DVZ increased mash intake compared to rats injected with vehicle (0 dose of DVZ) before CCK-8. $*p < 0.05$; $**p < 0.01$, Newman-Keuls test after ANOVA. Actual intakes on the test day were also compared to baselines on the previous day by two-tailed t-tests for related measures. The Veh + CCK-8 group ate 64% less than its baseline (5.8 ± 0.4 g, $p < 0.01$). Rats given the lowest dose of DVZ also ate less than their baseline ($p < 0.05$), but those given the two highest doses of DVZ did not differ from their control means. Baselines did not vary significantly among the four groups (overall baseline = 6.0 ± 0.5 g, $n = 27$). (B). In a separate group of rats, DVZ (0.75 μmol/kg, IP) reversed anorexia produced by 4.0 nmol/kg CCK-8. Four treatment groups ($n = 8$ except $n = 9$ for the Veh/Veh group) were injected with either DVZ or its vehicle followed 24 min later by either CCK-8 or its vehicle. CCK-8 reduced mash intake by 55% compared to Veh/Veh controls. **Mean for Veh/CCK-8 group differed from means for all other groups, $p < 0.01$, Newman-Keuls test.

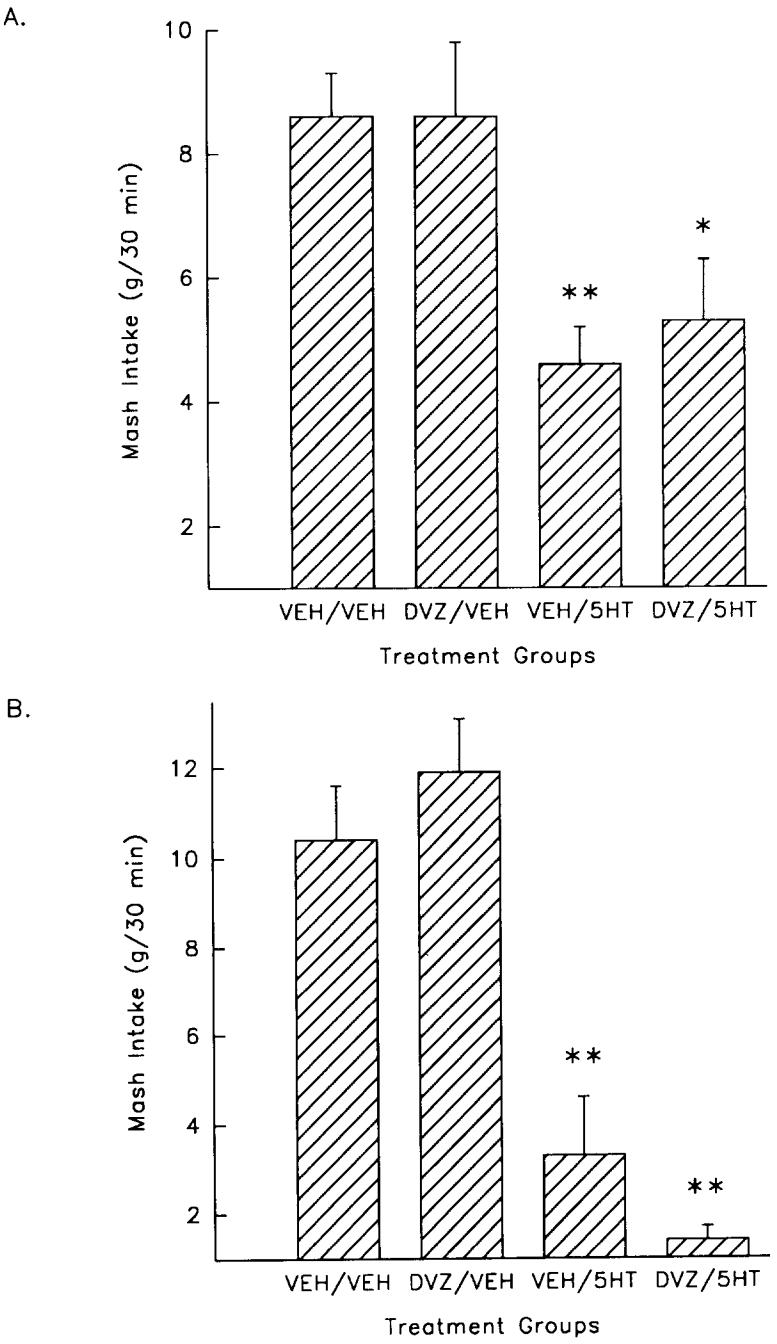

FIG. 2. Rats described in Fig. 1B were injected IP with either 0.75 μmol/kg devazepide (DVZ) or its vehicle followed 24 min later by either 5-HT or its vehicle (n = 8 except n = 9 for the Veh/Veh group). (A). A dose of 4.0 μmol/kg 5-HT reduced mash intake by 47% compared to Veh/Veh controls. (B). A dose of 10.0 μmol/kg 5-HT reduced intake by 68%. Asterisks indicate significant difference from each of the controls (Veh/Veh and DVZ/Veh): *$p < 0.05$, **$p < 0.01$, Newman-Keuls test.

ministered fenfluramine but not 5-HT penetrates into the CNS. It should be noted, however, that although devazepide attenuates fenfluramine's reduction of feeding this CCK antagonist does not alter anorexia produced by the 5-HT uptake inhibitor fluoxetine (4). Thus, the extent to which serotonergic drugs reduce food intake via CCKergic processes requires further clarification. The present results argue strongly that peripherally administered 5-HT reduces food intake independently of CCKergic function.

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