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BRIEF COMMUNICATION

Devazepide Antagonizes the Inhibitory Effect of Cholecystokinin on Intake in Sham-Feeding Rats

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MELVILLE, L. D., G. P. SMITH AND J. GIBBS. *Devazepide antagonizes the inhibitory effect of cholecystokinin on intake in sham-feeding rats.* PHARMACOL BIOCHEM BEHAV **43**(3) 975–977, 1992. – 3*S*(-)-*N*-(2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide (devazepide), a potent and selective cholecystokinin$_A$ (CCK_A) antagonist, has been shown to reverse the inhibitory effect of exogenously administered CCK-8 on food intake. In all tests, however, the inhibition of food intake could have been due not only to the CCK-8 administered but also to synergistic interactions between administered CCK-8 and endogenous satiety signals, such as glucagon or CCK released from the small intestine, elicited by the postingestive effects of the test diet. To eliminate these possible interactions, we investigated the effect of devazepide on the inhibitory effect of CCK-8 on the intake of a milk diet during 30 min of sham feeding, a procedure that minimizes or eliminates the postingestive satiating effect of food. Under these conditions, devazepide was a potent antagonist of the inhibitory effect of CCK-8 (16 μmol/kg, IP): The approximate ED_{50} was 625 ng/kg (1.3 nmol/kg) and the threshold dose was between 62.5 and 625 ng/kg.

Cholecystokinin antagonists Food intake CCK_A receptors Satiety Gut peptides

3*S*(-)-*N*-(2,3-Dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-yl)-1*H*-indole-2-carboxamide [devazepide (DV)], a selective and potent cholecystokinin$_A$ (CCK_A) antagonist (1), has been shown to block the inhibitory effect of peripherally administered CCK-8 on food intake in a variety of test conditions (3,9,11,12,16). In all tests, however, the inhibition of food intake could have been due not only to the CCK-8 administered but also to additive or synergistic interactions between administered CCK-8 and endogenous satiety signals elicited by the postingestive effects of the test diet, such as pancreatic glucagon (6) or endogenous CCK released from the small intestine (14,17).

To eliminate these possible interactions, we investigated the effect of DV on the inhibitory effect of CCK-8 on intake during sham feeding in the chronic gastric fistula rat (18). In this preparation, sham feeding is accomplished by letting ingested liquid diet drain out of the open gastric cannula during an intake test. Because food does not accumulate in the stomach or enter the small intestine, satiety signals elicited by the postingestive effects on food are minimized (13) or eliminated (5). For example, it is relevant to the purposes of this experiment that sham feeding does not increase the concentration of bioactive CCK in the peripheral plasma (15).

We report here that DV was a potent antagonist of the inhibitory effect of CCK-8 on intake during sham feeding. A preliminary communication of this work has appeared (7).

METHOD

Subjects

Five male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 350–450 g at the time of surgery, were housed individually in Plexiglas cages adapted for sham feed-

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ing and maintained on a 12 L : 12 D schedule. Maintenance diet was pelleted chow (Purina Rat Chow) and ad lib tapwater.

Surgery

Under surgical anesthesia produced by a mixture of chloral hydrate and pentobarbital (3 ml/kg, IP), rats were implanted with a chronic gastric cannula according to the procedure previously described (10). They were allowed 1 week to recover before being adapted to the test conditions.

Drug Preparation

Devazepide, a gift of Merck, Sharpe & Dohme (West Point, PA), was mixed into stock suspensions of 1,000, 50, and 12.5 μg/ml 0.5% carboxymethylcellulose and kept refrigerated. Other doses were made by diluting one of these stock suspensions after it had been sonicated for 2–5 min. All suspensions were vortexed prior to filling syringes for injection. Solutions of CCK-8 were made by diluting frozen (−80°C) CCK-8 with 0.15 M NaCl immediately prior to testing.

Test Conditions

After overnight food deprivation (approximately 18 h), DV (0.0625–1,000 μg/kg) or vehicle (0.5% carboxymethylcellulose) was given intraperitoneally 30 min (−30) prior to food presentation. A rat was then prepared for sham feeding by opening the gastric cannula and lavaging the interior of the stomach with warm water or 0.15 M NaCl until gastric drainage was clear of food particles and debris. A drainage tube (18) was attached to the cannula and the rat was returned to its home cage. CCK-8 (16 μg/kg) or saline was administered intraperitoneally at 5 min (−5) before access to the test diet [2 : 1 deionized water and sweetened condensed milk (Bordens)]. All tests began at 1030 h and intake was measured at 5-min intervals for 30 min.

Criteria for complete gastric drainage (5) were: Ingested food must begin to drain from the collection tube within 15 s after feeding began and the volume of gastric drainage collected in plastic pans under the cage must be equal to or larger than the volume of milk ingested. When these criteria are met, gastric drainage is complete or nearly so. Data from tests that failed to meet these criteria were discarded.

At the end of a test, the drainage tube was removed, the gastric cannula was closed, and the rat was returned to its cage, where it had access to chow pellets and water until the deprivation period began.

The sequence of tests for each dose of DV was vehicle (−30) + saline (−5), vehicle (−30) + CCK-8 (−5), vehicle (−30) + saline (−5), and DV (−30) + CCK-8 (−5). At least two tests with vehicle (−30) + saline (−5) were given before beginning the sequence of tests using the next dose of DV. Doses of DV were given in a quasi random order.

Statistics

The three test conditions were vehicle + saline, vehicle + CCK-8, and DV + CCK-8. We used a one-way analysis of variance (ANOVA) with repeated measures to analyze these three conditions for each dose. We performed separate ANOVAs on each dose because intakes after vehicle + saline and vehicle + CCK treatments differed significantly across doses of DV. It was possible, however, to collapse the intakes of the vehicle + saline tests that occurred prior to vehicle + CCK and DV + CCK for each dose.

Duncan's multiple-range test was used to analyze differences among the three conditions. Significance was set at $p \leq 0.05$.

TABLE 1

EFFECT OF DEVAZEPIDE ON CCK-INDUCED INHIBITION OF INTAKE IN SHAM-FEEDING RATS

	Intake (ml/30 min)		
DV(μg/kg)	Vehicle + Saline	Vehicle + CCK-8	DV + CCK-8
0.0625	69 ± 3	51 ± 3	45 ± 9
0.625	63 ± 4	31 ± 6	53 ± 5*†
6.25	60 ± 4	21 ± 3	53 ± 6*
12.5	69 ± 5	33 ± 4	48 ± 7*†
25	65 ± 3	27 ± 4	46 ± 6*†
50	60 ± 4	34 ± 2	61 ± 5*
1,000	73 ± 7	43 ± 9	76 ± 7*

Intakes are mean ± SEM from tests in five rats except for the data from 50 μg/kg, which were obtained from four rats because one rat died before receiving this dose. The mean intake ± SEM for vehicle + saline treatment was obtained from pooling the intakes from the vehicle + saline test prior to vehicle + CCK-8 and the vehicle + saline test prior to DV + CCK-8. Intakes after vehicle + CCK-8 were significantly smaller than the intakes after vehicle + saline in all cases ($p < 0.05$).

†All doses of DV ≥ 0.625 μg/kg decreased the inhibitory effect of CCK-8 significantly ($p < 0.05$ significantly larger than vehicle + CCK-8) and the 6.25-, 50-, and 1,000-μg/kg doses completely reversed the inhibitory effect of CCK-8 (†$p < 0.05$, significantly smaller than vehicle + saline).

RESULTS

All doses of DV ≥ 0.625 μg/kg decreased the inhibitory effect of CCK-8 and the 6.25-, 50-, and 1,000-μg/kg doses completely reversed it (Table 1). The minimum dose for decreasing the effect of CCK-8 by 50% or more was 0.625 μg/kg. Thus, the approximate ED_{50} was 1.3 nmol/kg. The threshold dose was between 0.0625 and 0.625 μg/kg. We cannot explain the complete reversal by the 6.25-μg/kg dose when the next two larger doses produced a partial reversal.

The relatively large dose of CCK-8 (16 μg/kg) was used to provide approximately a 50% inhibition over repeated sham-feeding tests because our measurement of the potency of DV depended upon that assumption. This was achieved because all tests with vehicle + CCK-8 produced a significant inhibition ($p < 0.05$) and the mean percent inhibition after vehicle + CCK-8 was 51%.

DISCUSSION

Devazepide is quite potent for antagonizing the inhibitory effect of exogenous CCK-8 on milk intake during sham feeding. The approximate ED_{50} of 1.3 nmol/kg is equal to a concentration of DV in the extracellular fluid of about 6 nM assuming immediate and uniform distribution in the extracellular fluid compartment, calculated as 20% of body weight. This potency is only slightly less than the potency of DV we (12) previously reported during real feeding of milk after overnight food deprivation (minimum effective dose for real feeding = 0.20 μg/kg compared to 0.63 μg/kg for sham feeding; ED_{50} for real feeding = ~0.4 nmol/kg and for sham feeding = ~1.3 nmol/kg). Furthermore, it is a less ambiguous estimate because other postingestive satiety signals that could be

synergistic with administered CCK-8 for the inhibition of intake were minimized or eliminated in the current experiments.

Aside from our previous report (12), DV is more potent for antagonizing the inhibitory action of exogenous CCK-8 on intake in the sham-feeding test than in any other in vivo test involving food intake or visceral measurements (2,3,11). Thus, the sham-feeding rat is a sensitive assay for the measurement of potency of CCK_A antagonists.

When doses of DV (6.25, 50, and 1,000 μg/kg) were administered that abolished the satiating effect of CCK-8, they did not produce a significantly larger intake than after vehicle + saline treatment (Table 1). This suggested that if these doses were given alone they would have no effect on milk intake during sham feeding. We tested this directly in three rats by giving DV (1,000 μg/kg, IP) at −30 min and saline at −5 min. DV had no effect (vehicle + saline = 60 ± 4 ml/30 min, DV + saline = 58 ± 4 ml/30 min). This confirms a prior report of Garlicki et al. (4). Because DV penetrates the blood–brain barrier readily (8), the failure of this large dose of DV to change milk intake is evidence that neuronal CCK acting at CCK_A receptors in the brain is not necessary for the control of sham feeding under these conditions.

In summary, our results demonstrate that DV is a potent antagonist of the inhibitory effect of peripherally administered CCK-8 on milk intake during sham feeding and that sham feeding is a sensitive in vivo assay for evaluating CCK_A antagonists.

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