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Comparison of the Satiating Potencies of Cholecystokinin-33 and Cholecystokinin-8

L. D. MELVILLE, G. P. SMITH[1] AND J. GIBBS

Department of Psychiatry, Cornell University Medical College, and the Edward W. Bourne Behavioral Research Laboratory, Westchester Division, The New York Hospital-Cornell Medical Center, White Plains, NY 10605

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MELVILLE, L. D., G. P. SMITH AND J. GIBBS. *Comparison of the satiating potencies of cholecystokinin-33 and cholecystokinin-8.* PHARMACOL BIOCHEM BEHAV **45**(1) 85–87, 1993. – We investigated the satiating potency of CCK-33 and of CCK-8 administered IP to rats prior to a 30-min food intake test using a high-carbohydrate liquid diet. CCK-33 and CCK-8 produced dose-related inhibitions of intake. The ID_{50}s and the slopes of the dose-response functions of the two peptides were not significantly different. We conclude that CCK-33 is as potent as CCK-8 for inhibiting food intake in the rat.

Food intake Satiety Peptides

IT was initially reported that cholecystokinin-33 (CCK-33) was substantially less potent than the octapeptide (CCK-8) in several visceral test systems, including pancreatic enzyme secretion and gall bladder contraction (6,7). Recently, however, Solomon et al. demonstrated that these results probably reflected problems in the stability and delivery of CCK-33 because the potency of CCK-33 in these tests was equal to or greater than that of CCK-8 when a small quantity of albumin was added to the peptide solution to minimize adsorption and denaturation of the peptide (5).

There has been only one report of a comparison of the potencies of CCK-33 and CCK-8 to reduce food intake. In this study, two doses of CCK-33 and synthetic CCK-8 were administered IP to male rats 30 min before a 1-h intake test with powdered chow after 24.5 h of food deprivation. CCK-8 was more potent than CCK-33, but the difference was not statistically significant (2). To investigate this problem further, we measured the inhibitory effect of porcine CCK-33 and synthetic CCK-8 on 30-min intake of a high-carbohydrate liquid diet in rats after 18 h of food deprivation. A preliminary report of this data has been presented (3).

METHOD

Subjects

Male Sprague-Dawley rats (n = 7, weighing 300–350 g at the start of the experiment) were housed individually and maintained on a 12 L : 12 D schedule. Purina Rat Chow was available between 1130 and 1600 h. Water was available at all times.

CCK Preparation

All doses of powdered CCK-33 and certain doses (see *Testing*) of powdered CCK-8 were dissolved in 0.1% rat serum albumin. Aliquots of 4 μg/0.1 ml were then stored at −80°C until they were dissolved in 0.15 M NaCl just prior to injection. Most doses of CCK-8 were dissolved in 0.15 M NaCl without 0.1% rat serum albumin just prior to injection. Porcine CCK-33 was purchased from Peninsula Laboratories (Belmont, CA) and synthetic CCK-8 was a gift of Bristol-Myers Squibb (Princeton, NJ).

Testing

At 5 min prior to food presentation, animals were given IP injections of either saline vehicle, CCK-33 (0.5, 1, 2, 4, 8, 16, or 32 μg/kg) or CCK-8 (0.5, 1, 2, 4, 8, or 16 μg/kg). This range of doses is equivalent to 0.1–14 nmol/kg. At 1000 h, animals were presented with a high-carbohydrate liquid diet (40% BioServ, Frenchtown, NJ) in graduated drinking tubes, and intakes were measured at 5-min intervals for 30 min. Maintenance diet was returned 30 min after removing the test diet. Peptide doses were given in descending order; tests with CCK-33 were completed before tests with CCK-8 began except all rats received 32 μg/kg of CCK-33 as their final dose. At least one saline baseline was obtained before each peptide treatment. Between one and three peptide treatments were administered in a given week.

Statistics

Total intakes for the 30-min tests were analyzed because inspection of the 5-min interval data did not reveal differences

[1] To whom requests for reprints should be addressed.

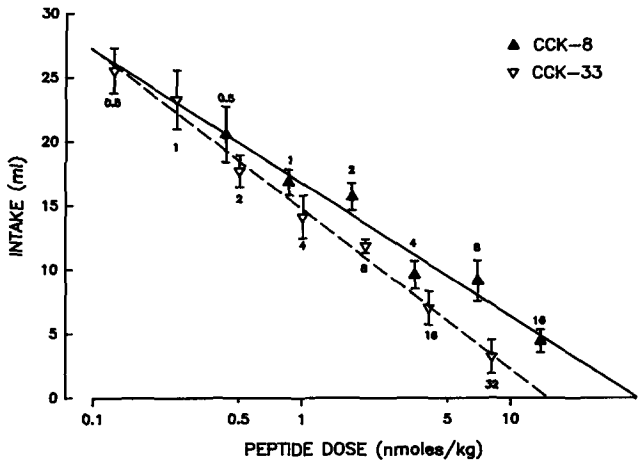

FIG. 1. Linear regressions for inhibitions of food intake produced by range of doses of CCK-8 and CCK-33. Doses of peptide are plotted in nanomoles/kg, and equivalent μg/kg dose appears next to the appropriate point.

in the pattern of the inhibition produced by the peptides. To determine if intakes after vehicle injection could be pooled across dose and treatment, we used a two-way, repeated-measures analysis of variance (ANOVA). There was no significant difference, $F(5) = 1.44$, $p = 0.24$. Therefore, we analyzed intake values for each peptide with a one-way repeated-measures ANOVA followed by Duncan's multiple-range test, using mean intake after saline treatment as the zero dose, to determine the threshold dose for inhibition by each peptide. To determine if there were differences in satiating potencies between CCK-33 and CCK-8, a two-way repeated-measures ANOVA of intake with dose and peptide as factors was performed.

To compare the inhibitory potency of the molecular quantities of peptide administered, doses in μg/kg were converted to nmol/kg and the results were plotted against intake. For each peptide, a regression line was calculated and the slope was determined. An approximate ID_{50} for each peptide was calculated using nonlinear regression. All analyses were performed with the Statistical Analysis System (SAS, Cary, NC) except calculation of the ID_{50}, which was performed with InStat and InPlot (GraphPad, Inc.).

RESULTS

Both CCK-8 and CCK-33 produced dose-related inhibition of 30-min intake (see Table 1). The threshold dose for CCK-8 was 0.5 μg/kg, $F(6, 6) = 38.83$, $p < 0.05$] and for CCK-33 1 μg/kg, $F(6, 7) = 30.83$, $p < 0.05$. The largest doses of both peptides produced a nearly complete suppression of intake. There was no evidence of a decline in satiating potency with the largest doses tested.

When intakes were plotted against peptide dose in nanomoles, CCK-33 was at least as potent and efficacious as CCK-8 (Fig. 1). Regression analysis revealed no difference between the slopes (CCK-8 $m = -3.60 \pm 0.26$, CCK-33 $m = -3.64 \pm 0.23$). The estimated ID_{50}s for CCK-8 (≈ 1.2 nmol/kg) and for CCK-33 (≈ 0.9 nmol/kg) were not significantly different, $t(12) = 0.74$, $p = 0.47$.

To ensure that the satiating potency of CCK-8 was not affected by the absence of albumin in the test preparation, the same animals were tested with 2 and 4 μg/kg CCK-8 prepared in the same manner as CCK-33, that is, using 0.1% rat serum albumin. The addition of albumin had no effect on the potency of CCK-8. The percent inhibition of CCK-8 without albumin was 46 ± 4 for 2 μg/kg and 65 ± 4 for 4 μg/kg; the percent inhibition of CCK-8 with albumin was 39 ± 5 for 2 μg/kg and 55 ± 4 for 4 μg/kg. These effects were not significantly different (correlated $t = -1.62$, $p = 0.2$ for 2 μg/kg; correlated $t = -1.89$, $p = 0.12$ for 4 μg/kg respectively).

DISCUSSION

The equivalent satiating potency of CCK-33 and CCK-8 observed in our experiments confirms the brief report by Hagiwara et al. (2) and extends it by using a complete range of doses that permitted a quantitative and statistical comparison of the potency and efficacy of the peptides.

The relative satiating potency of CCK-33 to CCK-8 was 1.3:1 based upon the estimated ID_{50}s. This is similar to the relative potency of these peptides in rat visceral systems in studies that employed albumin to prevent adsorption of CCK-33. Three studies have reported the potency of CCK-33 and CCK-8 to be equivalent for stimulation of rat pancreatic secretion in vitro (2,4,5). Two of these studies used synthetic human CCK-33 and porcine CCK-33, which were also equipo-

TABLE 1

INTAKES OF LIQUID FOOD AFTER IP INJECTION OF CCK-33 OR CCK-8

	Dose (μg/kg)							
	0	0.5	1	2	4	8	16	32
CCK-33	28 ± 1^{a}	26 ± 2^{ab}	23 ± 2^{b}	18 ± 1^{c}	14 ± 2^{cd}	12 ± 1^{d}	7 ± 1^{e}	3 ± 1^{e}
CCK-8	28 ± 1^{a}	21 ± 2^{b}	17 ± 1^{c}	16 ± 1^{c}	10 ± 1^{d}	9 ± 2^{d}	4 ± 1^{e}	

Intakes are expressed as mean ml (± SEM) of liquid food consumed during a 30-min test period. Values for 0 dose are the pooled intakes after vehicle injection for that peptide. Intakes with the different superscript letters are significantly different by Duncan's multiple-range test, $p < 0.05$. $n = 7$ for all doses except 32 μg/kg CCK-33, where $n = 5$.

tent (2,4). Two reports examined pancreatic secretion in the anesthetized rat: One found no significant difference between porcine CCK-33, human CCK-33, and synthetic CCK-8 (1) and one found CCK-33 to be somewhat more potent than CCK-8, with a ratio of 5.4 : 1 (5). The reason for this discrepancy is unclear because both studies utilized albumin to protect CCK-33.

We conclude that the satiating potency of CCK-33 is equivalent to that of CCK-8 in the rat when both peptides are administered IP. Further work is required to determine the relative satiating potencies of these peptides when administered intraportally or intravenously.

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