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FPL 14294: A Novel CCK-8 Agonist With Potent Intranasal Anorectic Activity in the Rat

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SIMMONS, R. D., J. C. BLOSSER AND J. R. ROSAMOND. *FPL 14294: A novel CCK-8 agonist with potent intranasal anorectic activity in the rat.* PHARMACOL BIOCHEM BEHAV 47(3) 701-708, 1994.—Cholecystokinin octapeptide (CCK-8) induces satiety in many species including man. However, its therapeutic utility is restricted due to its short biological half-life and poor bioavailability. FPL 14294 [4-(sulfoxy)-phenylacetyl(MePhe6)CCK-6] is a CCK analog with enhanced metabolic stability that was comparable to CCK-8 in potency to contract isolated gallbladder and in affinity at the CCK-A and CCK-B receptor. However, FPL 14294 was more than 200 times more potent than CCK-8 in inhibiting 3-h feeding in 21-h fasted rats. FPL 14294 also possessed intranasal anorectic activity at 5 μg/kg, while CCK-8 was inactive at doses up to 500 μg/kg. Anorectic activity was inhibited by pretreatment with a CCK-A antagonist (MK-329) but not by a CCK-B antagonist (L365,260). The anorectic effects of CCK-8 and FPL 14294 were the result of a direct effect on feeding and not caused indirectly by effects on water intake. These results indicate that FPL 14294 is a potent, intranasally active, anorectic agent whose enhanced in vivo potency over that of CCK-8 may reflect differences in stability, bioavailability, or receptor kinetics.

Cholecystokinin Octapeptide FPL 14294 Anorectic activity Intranasal CCK-A receptor CCK-B receptor Agonist Antagonist

CHOLECYSTOKININ (CCK), a hormone released from the intestine after eating, was originally isolated from the small intestine as a 33-amino acid polypeptide (39). Other molecular forms including CCK-8, the smallest naturally occurring segment that retains full hormonal activity, have since been identified in intestine, brain, and plasma of various animal species (4,15,16,40). CCK, initially identified by its ability to stimulate gallbladder contraction (19) and pancreatic exocrine secretion (18), has since been shown to act as a neuromodulator in the CNS and enteric nervous system (1,2), contract smooth muscle in intestinal and respiratory tissues (45), regulate gastric emptying and intestinal motility (33-38), and stimulate pepsin secretion in certain species (9). Receptors for CCK have been subclassified pharmacologically as CCK-A, for alimentary, and CCK-B, for brain, although receptor distribution is not limited to these areas. CCK-A receptors require a sulfated tyrosine and the C-terminal heptapeptide for full activity, while the C-terminal tetrapeptide of CCK is the minimally active agonist at CCK-B sites (20,32,35).

Since the original observation by Gibbs et al. (17) that intraperitoneally administered CCK-8 inhibited feeding and produced classical postprandial behaviors in rats, a substantial body of evidence has been gathered to suggest that CCK acts as a physiological satiety signal to terminate feeding [for reviews, see (1,36)]. Peripherally administered CCK-8 is active in inhibiting feeding in a number of species, including man (2,22,42,43,46). In the latter, continuous intravenous infusion decreased meal size in lean or obese subjects at doses below those which produced nausea or other undesirable side effects (22,31,34,36,42,43,46).

Supporting studies with animals suggested that at lower doses of CCK-8 the anorectic effects were not due to taste aversion (31,34) or separate inhibitory effects on water intake (17,37). In rats, using selective receptor antagonists and structural CCK analogs, it has been shown that CCK-A, and not CCK-B receptors mediate the anorectic effects of peripherally administered CCK-8 (1,13,17,21,25,29).

The potential therapeutic effects of exogenous CCK-8 to

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induce satiety and consequent weight loss in man have been restricted due to its relatively short biological half-life (2.5 min) and poor bioavailability (47). FPL 14294 [Hpa(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH_2NH_3] is a chemically stabilized hexapeptide analog of CCK-8. The following studies were done to assess the stability, bioavailability, pharmacological selectivity, anorectic activity, and possible mechanism of action of this novel peptide relative to CCK-8.

METHOD

Peptide Degradation Rate in Dog Kidney Cortex Homogenate

Degradation of peptide by dog kidney cortex homogenate was carried out by a modification of the methods of Lonovics et al. (28). A 10% homogenate of kidney cortex in saline was obtained by applying a Potter homogenizer for 5–10 s in pulses, as needed. To measure peptide degradation, 1.0 mg of peptide and 2.0 ml of 0.01 M ammonium acetate buffer (pH 7.4) containing carbobenzoxy tyrosine (z-tyrosine) as an internal standard were added to 3 dram sample vials and the solutions were checked for clarity. The vials were incubated at 37°C for 5 min and a 100 μl aliquot of kidney homogenate was then added to each vial. Aliquots (200 μl) were removed from the vials at time 0, 2, 5, 10, 30, 60, and 120 min and the enzymatic degradation was quenched by immersion of the aliquot containing tubes into boiling water for 4 min.

Aliquots were allowed to come to room temperature, filtered with a Millipore HV 0.45 μm filter and 100 μl was injected onto HPLC-UV for analysis using a Zorbax ODS column (25 cm 4.6 mm). A gradient mobile phase consisting of MeOH 0.25 M ammonium acetate buffer, pH 4.1 at 50°C was run at a flow of 1 ml/min with UV detection set at 254 nm using carbobenzoxy tyrosine as an internal standard.

A ratio of peptide peak area (using maximum absorbance) to the internal standard peak was calculated for both carbobenzoxy tyrosine and the peptide being assayed. Percent degradation was calculated by comparison of these ratios to those at time zero. Half-life values were determined by a least squares fit of the log of the experimentally determined substrate concentrations vs. time in minutes, using a minimum of four points, values were determined in at least three separate assays. Rates of degradation were readily quantified by a procedure involving comparisons of HPLC peak area vs. time.

CCK-A Receptor Affinity

Affinity for CCK-A receptors in rat pancreatic membranes was measured according to the methods described by Chang et al. (6). A pancreas from a Sprague-Dawley rat was homogenized for 20 s with a Brinkmann Polytron (setting 5) in 50 volumes of a 50 mM Tris buffer, pH 8.0 at 4°C. The homogenate was centrifuged at 50,000 × *g* for 10 min and then the pellet was washed twice, recentrifuged, and resuspended in 2000 volumes of cold 5 mM Tris buffer (pH 7.4, at 37°C) containing 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mg/ml BSA, and 0.14 mg/ml bacitracin. Affinity for CCK-A receptors was measured using either Bolton-Hunter-^{125}I-CCK-8 (DuPont, 2000 Ci/mmol) or [^{3}H]MK 329 (DuPont, Ci/mmol). One milliliter (0.5 mg original wet weight of tissue) of membrane suspension, and either 0.2 nM of [^{3}H]MK 329 or 15 pM of ^{125}I-CCK-8 was incubated for 40 min (^{125}I-CCK-8) or 60 min ([^{3}H]MK 329) at 37°C. Bound ligand was collected onto Whatman GF/B filters and filters were washed twice with 8 ml of ice-cold 50 mM Tris buffer, pH 7.4 and analyzed using a Beckman liquid scintillation counter. Binding constants were determined using ALLFIT, an iterative logistic curve fitting program and apparent K_i values were derived from the formulation described by Cheng and Prusoff (8). The calculated K_d values for each ligand were 0.12 nM for ^{125}I-CCK-8, and 0.22 nM for [^{3}H]MK 329.

Affinity for CCK-B receptors was measured in rat cerebral cortex (7). Tissue was homogenized in 50 volumes (vol/g of wet weight of tissue) of 50 mM Tris (pH 7.5 at 25°C). The homogenate was centrifuged at 50,000 × *g* for 10 min and the resulting pellet was resuspended and recentrifuged. The final pellet was resuspended in 80 volumes of assay buffer (10 mM Hepes, 5 mM $MgCl_2$, 1 mM EGTA, bacitracin (0.25 mg/ml), and 130 mM NaCl, pH 6.5). Then, 0.45 ml of resuspended membranes, unlabeled compound, and 1 nM of ^{125}I-CCK-8 (DuPont) in a final volume of 0.5 ml was incubated at 25°C for 2 h. Membrane bound ^{125}I-CCK-8 was collected by vacuum filtration on Whatman GF/B filters presoaked in 50 mM Tris buffer, pH 7.7, containing 1 mg/ml bovine serum albumin (Sigma). Radioactivity was determined with a Beckman gamma counter (efficiency of 45%). IC_{50} and K_i values were determined using the Lundon AccuFit Competition nonlinear curve fitting software program which is based in part on models described by Linden (27) and Feldman (14).

Agonist Potency in Isolated Guinea Pig Gallbladder

Contraction of guinea pig gallbladder was measured by a modification of the methods described by Rubin et al. (41). The isolated gallbladder (Hartly guinea pig, 300–400 g) was divided longitudinally into two strips and mounted in 10 ml tissue baths containing Krebs solution at 37°C and bubbled with 95% O_2–5% CO_2. Strips were allowed to preequilibrate for 2 to 3 h and a final resting tension of 1 g was established. Isometric responses were recorded using a Grass FT.03 force transducer and a Gould polygraph. Preliminary studies indicated that similar potencies for CCK-8 were obtained whether a dose washout sequence or a cumulative dose–response procedure was used. The latter was found to be necessary for FPL 14294 because of the extremely slow washout period. Regardless of which procedure was used, contractile responses to a peptide were allowed to reach asymptote before the next higher dose was applied. To compensate for tissue to tissue variability in sensitivity to CCK-8, successive dose–response curves were performed with CCK-8 and the test compound and a potency ratio was determined for each strip. Control studies in which similar successive dose–response curves to CCK-8 were performed indicated that there was no change in tissue sensitivity using this procedure. Dose–response curves were constructed using five to eight concentrations of peptide and EC_{50} values were determined using ALLFIT.

Food Intake

Food intake was measured by a modification of the method described by Cox and Maickel (10). Male Sprague-Dawley rats (300–400 g) housed singly on a 12-h reverse dark : light cycle were trained to eat powdered Purina rat chow during a 3-h period following a 21-h fast. To assess anorectic potency, a range of peptide doses was administered intraperitoneally (in 0.5 ml of water, pH 7.0–8.0) or, in some experiments, intranasally (in 50 μl of 0.9% NaCl with 20 mM sodium phosphate, pH 7.0). Food intake was then measured after 30 min or 3 h of feeding. In the intranasal studies, it was found that brief anesthesia with CO_2, accomplished by placing the animal in a CO_2 chamber for 20 to 30 s, facilitated the intranasal adminis-

tration procedure and decreased the within-group variance of food intake in the drug-treated rats without affecting overall food intake (data not shown). There were 10 rats per treatment group and each group received all treatments by a randomized crossover schedule with a 1-week washout period between dosing. Both 0.5- and 3-h time points were determined in the same experiment. A repeated measures ANOVA followed by a Newman-Keuls post hoc analysis were used to determine significant differences of food intake between groups. The dose of compound producing 50% inhibition of food intake (RD_{50}) was estimated using ALLFIT. The effect of the CCK-A antagonist, MK329 (Merck), or CCK-B receptor antagonist, L365,260 (Merck), on the anorectic activity of peptides was evaluated by administering the antagonist IP in a vehicle consisting of 2% methyl cellulose (clear gel) 30 min before the feeding bout (15 min before agonist administration). In the antagonist experiments the peptides were also suspended in 2% clear gel. All treatment effects were analyzed by a two-way ANOVA followed by a Newman-Keuls post hoc analysis.

Water Intake

Water consumption was measured following drug treatment by weighing tared water bottles after the 3-h feeding period. Additionally, the relationship between food intake and water consumption in 21-h fasted rats was characterized by using a paired feeding strategy. Different groups of rats (n = 8–10/group) received either 5, 10, 15, 20, or 25 g of powdered chow for the 3-h feeding period and mean water consumption of each group was calculated and analyzed by linear regression. Comparison of the effects of equipotent doses (RD_{30} dose, dose that inhibits food intake by 30%) of CCK-8 or FPL 14294 on water intake with those of pair feeding was made using the 95% confidence limits of the regression curve.

RESULTS

Representative HPLC elution profiles for the degradation of FPL 14294 in dog kidney homogenate are presented in Fig. 1. The half-life was determined to be 4.3 ± 1.0 min for CCK-8 and 19.7 ± 2.4 min for FPL 14294 indicating that structural replacement of amino acids at the major cleavage sites (12,23,30,31,44) extended biological half-life of the peptide (Table 1). Substitution of the Asp-Tyr(SO_3H) moiety by 4-(sulfoxy)phenylacetyl (abbreviated Hpa(SO_3H) where Hpa is 4-hydroxyphenylacetyl) protected against 1–2 cleavage and the replacement of Phe with *N*-methyl phenylalanine (i.e., MePhe) protected against 7–8 cleavage.

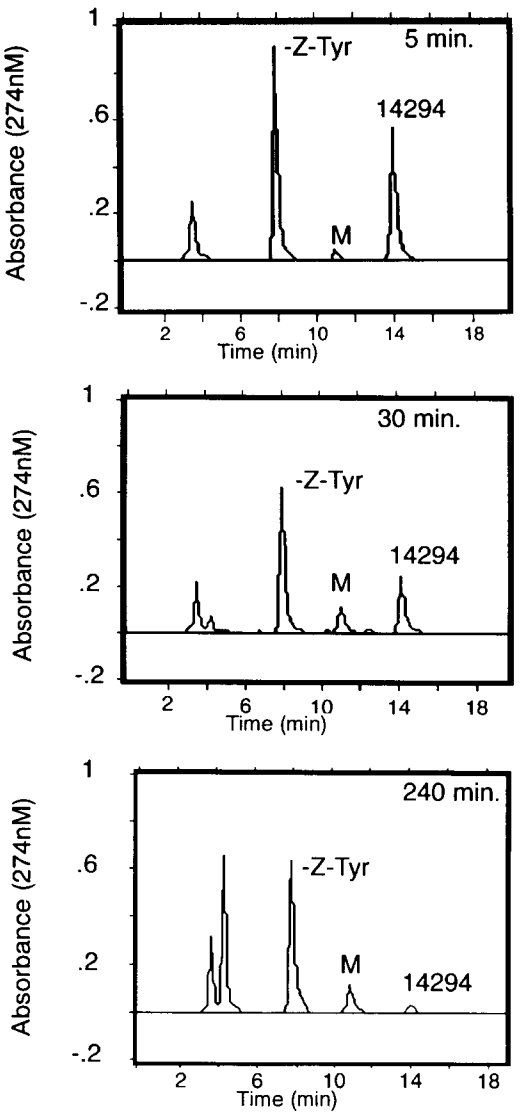

FIG. 1. A representative HPLC elution profile for the degradation of FPL 14294 in dog kidney homogenate. Note the disappearance of the parent compound and increasing accumulation of the major tetrapeptide metabolite (M) over 5, 30, and 240 min of incubation.

TABLE 1

DOG KIDNEY CORTEX HOMOGENATE DEGRADATION OF CCK-8 AND FPL 14294

Compound	Cleavage Sites	T½ (min)
	1 2 3 4 5 6 7 8	
CCK-8	H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH	4.3 + 1.0
	↑ ↑ ↑	
FPL 14294	Hpa(SO_3H)-Met-Gly-Trp-Met-Asp-MePhe-NH	19.7 + 2.4*
	↑	

n = three experiments per group.
Values are expressed as mean ± SE.
↑Indicates major cleavage site.
*Indicates significantly different from CCK-8 ($p < 0.05$, Student's t-test).

TABLE 2

AFFINITY OF FPL 14294 FOR RAT PANCREATIC CCK-A RECEPTORS AND RAT BRAIN CORTICAL CCK-B RECEPTORS IN VITRO

	CCK-A		^{125}I-CCK-8		CCK-B	
Compound	^{3}H-MK-329 K_i (nM)	n	K_i (nM)	n	^{125}I-CCK-8 K_i (nM)	n
CCK-8	7.6 ± 1.3	0.6	0.34 ± .03	1.2	1.0 ± .30	0.6
FPL 14294	6.1 ± 1.0	0.5	0.33 ± .03	1.2	0.33 ± 0.08	0.9

Values are expressed as mean K_i ± SE.
n = Hill coefficient.
Number of replications per measure is ≥3.

To determine whether replacement of the amino acids at the 1-2 and 7-8 cleavage sites may have hindered the binding of FPL 14294 to the CCK-A and/or the CCK-B receptor, the affinity for these receptors was assayed. As described in Table 2 the affinities of FPL 14294 for CCK-A and CCK-B receptors, measured by standard procedures, were similar to those of CCK-8 indicating a relatively good structural fit to these receptors. When measuring the affinity of FPL 14294 for CCK-A receptors on pancreatic membranes through displacement of an antagonist, MK329, FPL 14294 had an apparent K_i of 6.1 nM with a Hill coefficient of 0.5, which was essentially identical to that of CCK-8. When measuring the affinity of FPL 14294 through displacement of a labelled agonist ^{125}I-BH-CCK-8, FPL 14294 also resembled CCK-8 in affinity with a Hill coefficient near unity. FPL 14294 did not exhibit selective affinity for CCK-A or CCK-B receptors, the K_i values for pancreatic and rat brain cortical receptors being virtually identical (Table 2).

Having found the binding affinity of FPL 14294 to be comparable to that of CCK-8, the question arises as to its functional potency relative to that of CCK-8. Therefore, both compounds were profiled in the isolated guinea pig gallbladder assay. CCK-8 potently contracted isolated gallbladder strips with an EC_{50} of 1.05 nM in good agreement with values reported by others (16,24,41). The effects of CCK-8 were completely blocked by pretreatment with the CCK-A receptor antagonist MK329 (data not shown). FPL 14294 appeared to be equal to CCK-8 in efficacy and exhibited similar potency (Table 3). However, the time to peak contraction with FPL 14294 was much longer (10 min at 1 nM), than with CCK-8 (approximately 1 min). Similarly, contractions produced by FPL 14294 could not be readily reversed by washing out the peptide with repeated exchanges of the tissue bath media. In contrast, CCK-8 induced contractions were readily reversed by one exchange of the bath media.

Intraperitoneally administered FPL 14294 inhibited food intake during the first 30 min of feeding in a dose-dependent manner (Fig. 2) with a potency which was over 50-fold greater than that of CCK-8 (RD_{50} = 0.04 vs. 2.6 μg/kg). When the feeding period was extended to 3 h, the RD_{50} increased approximately tenfold for FPL 14294 (RD_{50} = 0.56 μg/kg, Fig. 3). However, it was necessary to increase the dose of CCK-8 in this and other studies by 40-fold or more, to inhibit feeding by 50% for 3 h (RD_{50} ≥ 100 μg/kg) suggesting that FPL 14294 may have a longer duration of action than CCK-8.

FPL 14294 demonstrated anorectic activity when administered intranasally but was much less potent than when delivered intraperitoneally. Inhibition of feeding for the 30-min feeding period by 50% required approximately 30 μg/kg, over 700 times the intraperitoneal dose (Fig. 4). The RD_{50} for 3 h of feeding was approximately tenfold higher (250-300 μg/kg) than that for 30 min, similar to the incremental increase observed with intraperitoneal administration. FPL 14294 was active intranasally at doses as low as 5 μg/kg (30% inhibition) for the 30-min feeding period and 15 μg/kg (20% inhibition) for the 3-h period (Fig. 4). In contrast, CCK-8 was inactive when delivered intranasally at doses as high as 500 μg/kg (data not shown).

The reduction in food intake as compared to controls by both FPL 14294 (F = 13.13, $p < 0.0000$) and CCK-8 (F = 11.14, $p < 0.0000$) when administered IP were reversed by pretreatment with 100 μg/kg IP of the CCK-A antagonist, MK 329 (F = 20.49, $p < 0.0000$), but not by 300 μg/kg of the CCK-B antagonist, L365,260 (F = 29.0, $p < 0.0000$), as shown in Table 4. The effect of intranasally administered FPL 14294 to inhibit feeding (Table 5) was also blocked by IP pretreatment with MK329 (F = 3.5, $p < 0.0445$). Taken together these results indicate that, despite the nonselectivity in affinity for CCK-A and B receptors, peripherally administered FPL 14294 acts only through CCK-A receptors to inhibit feeding in rats.

Because of the close quantitative link between feeding and

TABLE 3

EFFECT OF FPL 14294 ON GUINEA PIG GALLBLADDER CONTRACTION IN VITRO

Compound	N (No. of strips)	EC_{50}* (nM)	EC_{50} Ratio† (FPL 14294/CCK-8)	Time to Peak Contraction at 1.0 nM (s)
CCK-8	4	1.05 ± .12		85 ± 15
FPL 14294	4	1.09 ± .05	1.1	585 ± 30

*Values expressed as geometric mean ± SE.
†The potency ratio is the mean of the individual ratios for each tissue strip.
EC_{50} was determined by ALLFIT.

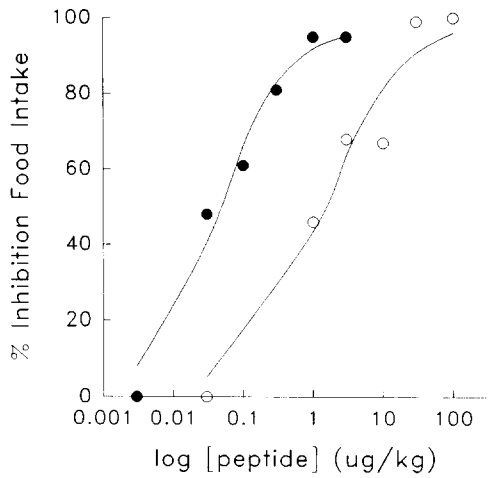

FIG. 2. The dose response of FPL 14294 (●) and CCK8 (○) to inhibit 30-min food intake after IP administration to fasted rats.

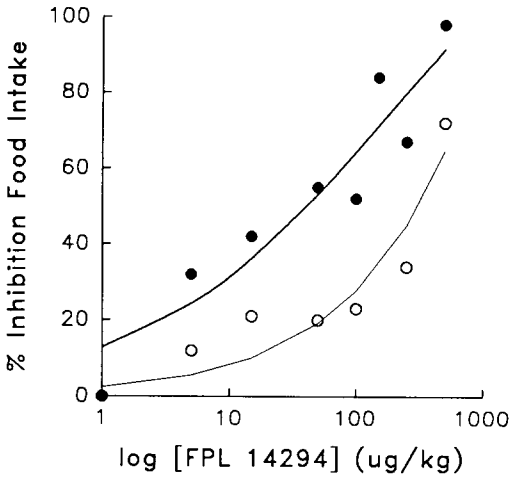

FIG. 4. The dose response to inhibit 30-min (●) and 3-h (○) food intake following intranasal administration of FPL 14294 to fasted rats.

drinking in rats, measurement of possible inhibitory effects of FPL 14294 on drinking were made by comparing water intake with that of control rats fed different quantities of food. As seen in Fig. 5, there was a linear relationship between meal size and water intake in the pair-fed rats during the 3-h feeding period ($r = 0.98$). Intraperitoneal treatment with 30 μg/kg of CCK-8 or 0.4 μg/kg of FPL 14294 decreased water and food intake by approximately 30% over the 3-h feeding period. However, these effects on food and water intake fell within the 95% confidence limits of the linear regression curve for the pair-fed animals, indicating that FPL 14294 and CCK-8 have no inhibitory effect on drinking beyond that associated with decreased meal size.

DISCUSSION

Because peptides are inherently unstable biological molecules and in vivo potency and enzymatic stability are often intimately related, a more efficacious CCK-8 related peptide was designed by stabilizing the weak links in the peptide backbone. By radioimmunoassay, Thompson et al. (47) found the circulation half-life of CCK-33, a precursor to CCK-8, to be 2.4 min in man and 2.6 min in dog. In dog and several other species, the kidney cortex is one of the major CCK-8 degrad-

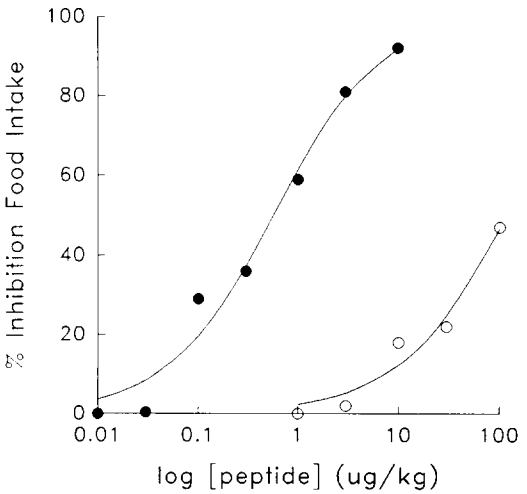

FIG. 3. The dose response of FPL 14294 (●) and CCK-8 (○) to inhibit 3-h food intake after IP administration in fasted rats.

TABLE 4

EFFECT OF PRETREATMENT WITH MK329 OR L365,260 (CCK-A AND CCK-B RECEPTOR ANTAGONIST, RESPECTIVELY) ON THE ANORECTIC EFFECT OF INTRAPERITONEALLY ADMINISTERED CCK-8 OR FPL 14294 IN 21 H FASTED RATS

Treatment	Dose, IP (μg/kg)	½ h Food Intake (g/Animal)	Percent Inhibition
Experiment 1			
Vehicle		8.6 ± .45	
CCK-8	10	4.0 ± 0.92*	53*
MK329	100	8.9 ± 0.50	0
CCK-8/MK329	10/100	6.8 ± 0.72	21
Experiment 2			
Vehicle		8.9 ± 0.35	
FPL 14294	0.4	4.4 ± 1.0*	50*
MK329	100	9.1 ± 0.35	0
FPL 14294/MK329	0.4/100	8.3 ± 0.5	7
Experiment 3			
Vehicle		8.8 ± 0.53	
CCK-8	10	5.0 ± 1.4*	43*
L365,260	10/300	8.6 ± 0.30	2
CCK-8/L365,260	10/300	1.7 ± 0.67†	81†
Experiment 4			
Vehicle		7.7 ± 0.34	
FPL 14294	0.4	2.1 ± 0.62‡	73‡
FPL 14294/L365,260	0.4/300	3.1 ± 0.65‡	60†

Values are expressed as mean ± SE. (Two-way ANOVA followed by Newman-Keuls analysis: $p < 0.05$).

*Indicates significant difference compared with all other treatment groups $p < 0.05$.

†$p < 0.05$, compared with vehicle and L365,620 treated groups.

‡$p < 0.05$, compared with vehicle treated group $n = 10$ per group.

TABLE 5

ANTAGONISM BY MK329 OF THE ANORECTIC EFFECT OF INTRANASALLY ADMINISTERED FPL 14294 IN 21 H FASTED RATS

Treatment	Dose (μg/kg)	Route	½ h Food Intake (q/Animal)	Percent Inhibition
Vehicle			9.4 ± 0.5	
FPL 14294	50	i.n.	7.5* ± 0.6	20*
FPL 14294/MK329	50/100	i.n./i.p.	9.0 ± 0.5	4

Values are expressed as mean ± SE.

*Indicates significant differences from vehicle-treated and FPL 14294 + MK329 treated groups, $p < 0.05$ (two-way ANOVA followed by Newman-Keuls analysis), $n = 10$ per group.

ing organs that contains enkephalinases, thiol protease, and aminopeptidases that cleave the peptide bonds mainly at the Asp-Tyr, Asp-Phe and, to a lesser extent, at the Gly Trp bond (referred to as 1-2, 7-8, and 4-5 cleavage, respectively) (12, 23,30,32,44). Substitution of the Asp-Tyr (SO_3H) moiety by Hpa (SO_3H) blocked 1-2 enzymatic cleavage and replacement of Phe with N-MePhe blocked 7-8 cleavage leading to a chemically stabilized hexapeptide with a fivefold longer half-life than CCK-8 which was determined by using in vitro dog kidney homogenate.

Alteration of the chemical structure of CCK-8 did not, however, inhibit its ability to bind to the CCK-8 receptors or elicit agonist activity. Both functional and receptor binding assessments have indicated that FPL 14294 is a nonselective CCK agonist with potency and affinity for CCK-A receptors comparable to that of CCK-8. However, the relatively long time to peak contraction and slow reversibility of FPL 14294-induced gallbladder contractions suggest that the kinetics of interaction with the CCK-A receptor are different than those of CCK-8.

Despite having similar binding affinities and gallbladder contraction potency in vitro, FPL 14294 was some 60 times more potent than CCK-8 to inhibit feeding for 30 min in rats when administered intraperitoneally. The greater potency cannot be explained by some non-CCK receptor-mediated effect because the CCK-A antagonist, MK329, completely blocked the anorectic activity of FPL 14294. Increased metabolic stability and prolonged resident time at the receptor could both contribute to an apparently high in vivo anorectic potency. If the catabolic rate of an agent is rapid relative to the period of time its effect is measured, apparent potency could vary as a function of half-life even though absolute affinity remains constant. Pharmacokinetic studies in humans and pigs (3,11) indicate that the half-life of IV administered CCK-8 is very short (0.5 to 2 min) compared to the relatively longer experimental feeding period in the current rat studies. Our in vitro findings with kidney homogenates indicate that the stability of FPL 14294 is nearly fivefold greater than that of CCK-8. In regards to the possibility of a longer residence time at the receptor, results from in vitro gallbladder studies suggest that FPL 14294 may dissociate from the CCK-A receptor more slowly than CCK-8. This was evidenced by a longer onset and duration of contraction, thus prolonging its overall agonist effect. An alternate explanation that cannot be ruled out at this time, is the possibility that a yet unidentified subtype of CCK-A receptor predominately mediates satiety and that FPL 14294 has high affinity and efficacy for this putative receptor subtype.

The apparent increase in anorectic potency relative to CCK-8 with longer feeding periods suggests that FPL 14294 has a longer duration of action. For example, a 10- to 15-fold increase in dose was required to extend feeding inhibition by 50% from 30 min to 3 h for FPL 14294. CCK-8 in contrast, required a greater than 40-fold increase in dose to produce a comparable extension. More direct measures of duration of action will be required to substantiate this.

Intranasal administration of FPL 14294 also inhibited feeding in rats. However, relatively high doses were required to inhibit feeding (30 min RD_{50} = 30 μg/kg) as compared to intraperitoneal delivery. This may be more a reflection of the technique of administration than of peptide absorption. Nasal sprays have been shown to be more effective than drops in the absorption of other peptides as well (5). In our studies, a 50 μl volume was administered into a small volume of rat nasopharynx (estimated to be approximately 400 μl) by a drop procedure. In addition, experiments using intranasal administration of dyes confirmed that a large portion of the delivered volume was rapidly ingested by rats. Thus, it is difficult to realistically estimate intranasal bioavailability based solely on apparent intranasal vs. intraperitoneal anorectic potency in rat. In contrast with FPL 14294, CCK-8 was inactive intranasally in rat. We speculate that the enhanced intranasal bio-

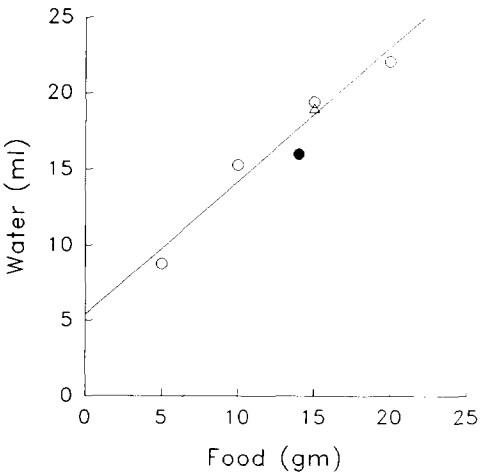

FIG. 5. Relationship between meal size and water intake over a 3-h period in rats fasted for 21 h. Meal-fed controls (○). CCK-8 △, 30 μg/kg, IP) FPL 14294 (●, 0.4 μg/kg, IP).

availability of FPL 14294 as compared to CCK-8 may be due to its greater resistance to peptidase activity. The nasal mucosa possesses aminopeptidase activity approaching that of the small intestine and could metabolically degrade CCK-8 at a greater rate than FPL 14294, thereby allowing less time for intact CCK-8 to be absorbed. Other important physical properties, including lipophilicity and molecular weight, do not differ markedly between the two peptides.

Decreasing food intake by treatment with FPL 14294 or by providing small meals also decreased water intake, and the magnitude of this decrease appeared to be related to meal size. However, the suppression of water intake by FPL 14294 was no greater than that observed in animals given a similar quantity of chow. Therefore, FPL 14294, like CCK-8, only appears to indirectly affect drinking as a consequence of reducing food intake.

In summary, although CCK-8 is a naturally occurring hormone that produces satiety in many species including man, the therapeutic application of CCK-8 as an anorectic is limited because of its poor bioavailability, short duration of action, and multiple physiological effects in addition to feeding inhibition. FPL 14294 is a chemically stabilized peptide analog of CCK-8 that is nasally bioavailable, has a longer duration of action, and is 50–100 times more potent to inhibit food consumption relative to other CCK-8-mediated actions such as gallbladder contraction and binding to pancreas and cortex. Additionally, the anorectic effects of FPL 14294 appear to be selectively mediated through peripheral CCK-A and not by CCK-B receptor interactions. These pharmacological properties of FPL 14294 suggest that it would be a good candidate for the therapeutic treatment of eating disorders. Further study to determine the potential therapeutic index of FPL 14294 based on the differences in dose that induce satiety relative to doses that stimulate other CCK-8 driven physiological processes would be essential for evaluation of FPL 14924 as a therapeutic agent.

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