Pharmacology Biochemistry and Behavior, Vol. 59, No. 2, pp. 439–444, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00446-2

ARL 15849: A Selective CCK-A Agonist With Anorectic Activity in the Rat and Dog

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Received 24 January 1997; Revised 4 June 1997; Accepted 19 June 1997

SIMMONS, R. D., F. C. KAISER, M. E. PIERSON AND J. R. ROSAMOND. *ARL 15849: A selective CCK-A agonist with anorectic activity in the rat and dog.* PHARMACOL BIOCHEM BEHAV **59**(2) 439–444, 1998.—Cholecystokinin octapeptide (CCK-8) and the peptide analog ARL 14294, formerly FPL 14294, [Hpa(SO_3H)-Met-Gly-Trp-Met-Asp-N(Me)Phe-NH_2], have been reported to induce satiety by interaction with the CCK-A receptor subtype. ARL 15849 [Hpa(SO_3H)-Nle-Gly-Trp-Nle-N(Me)-Asp-Phe-NH_2] is an improved ARL 14294 analog with enhanced CCK-A receptor selectivity, greater stability, and a longer duration of action. The affinity of ARL 15849 for the CCK-A receptor (K_i = 0.034 nM) is 6,600 fold greater than for the CCK-B receptor (K_i = 224 nM), whereas CCK-8 and ARL 14294 are nonselective. Although comparable in potency to contract isolated gallbladder and induce pancreatic phosphatidylinositol hydrolysis, ARL 15849 is 3- and 100-fold more potent than ARL 14294 and CCK-8, respectively, to inhibit 3-h feeding in rats. The duration of feeding inhibition was significantly longer for ARL 15849 (>5 h), compared to equipotent doses of ARL 14294 (3 h), and CCK-8 (1 h). Intranasal administration of ARL 15849 inhibits feeding in beagle dogs with a greater separation between doses that induce emesis and those that inhibit feeding. Therefore, ARL 15849 is a potent, selective, intranasally active anorectic agent which may be useful in the treatment of eating disorders. © 1998 Elsevier Science Inc.

Cholecystokinin ARL15849 Anorectic activity Intranasal CCK-A receptor CCK-B receptor Agonist Gallbladder contraction Phosphoinositide hydrolysis

CHOLECYSTOKININ (CCK) and its active fragment, CCK-8, have been reported to be involved in a number of mammalian gastrointestinal and CNS functions (9). These actions are brought about through the interaction of CCK-8 with either of two receptor subtypes, CCK-A, for alimentary and CCK-B, for brain, although receptor distribution is not limited to these areas (23). Characterization of these receptors has revealed a high degree of homology across species (26), which may partially explain why CCK agonists have similar pharmacological profiles in a variety of animals including humans. In particular, a substantial body of evidence has been gathered to suggest that CCK acts as a physiological satiety signal to terminate feeding by stimulation of the CCK-A receptor subtype. Stimulation of the CCK-A receptors located in the brain stem and higher feeding centers, or on the vagal afferents, are thought to be directly involved in the induction of satiety (19,25,26). Furthermore, selective stimulation of CCK-B receptors in the periphery has been associated with the release of hydrochloric acid from gastric parietal cells, pepsinogen, and electrolyte secretion, stimulation of gastric motility, and function as a growth factor for the gastric mucosa (11,18,22,23).

We have previously reported the development of a novel, chemically stabilized, CCK-8 based peptide analog (ARL 14294) with high affinity for CCK receptors and potent activity as a CCK-A agonist to contract gallbladder and inhibit feeding (24). The following studies were done to assess the pharmacological selectivity, potency, anorectic activity, intranasal bioavailability and duration of action of ARL 15849, an improved analog of ARL 14294.

METHOD

CCK Receptor Affinity

Affinity for CCK-A receptors in rat pancreatic membranes was measured according to the methods described by Chang et al. (5). A pancreas from a Sprague–Dawley rat was homogenized for 20 s with a Brinkman Polytron (setting 5) in 50 vol of a 50 mM Tris buffer, pH 8.0 and 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 vol 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 ba-

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citracin. Affinity for CCK-A receptors was measured using either ^{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 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 (7). 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 (6). Tissue was homogenized in 50 vol (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 vol 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 125I-CCK-8 (DuPont) in a final volume of 0.5 ml was incubated at 25°C for 2 h. Membrane-bound ^{125}I 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 (17) and Feldmen (10).

Agonist Potency in Isolated Guinea Pig Gallbladder

Contraction of guinea pig gallbladder was measured by a modification of the methods described by Bishop et al. (2). The isolated gallbladder (Hartly guinea pig, 300–400 g) was divided longitudinally into 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 equilibrate 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 ARL 15849 because of the extremely slow wash out period. Regardless of which procedure was used, contractile responses to a peptide were allowed to reach asymptote before the next higher dose was applied. Dose–response curves were constructed using five to eight concentrations of peptide, and ED_{50} values were determined using ALLFIT.

Agonist Potency in Guinea Pig Pancreatic Tissue

The CCK induced stimulation of phosphoinositide (PI) hydrolysis in guinea pig pancreatic fragments was measured by the method of Lin et al. (15). The isolated pancreas was washed, cut into fragments (10–15 mg each), and placed into 20-ml glass scintillation vials containing 0.3 M [^{3}H] myo-inositol in 2 ml of HEPES buffer, pH 7.4. The vials were bubbled with O_2 for 10 s, capped, and incubated at 37°C for 2 h in a Dubnoff metabolic shaker (150 oscillations/min). After incubation, the fragments were rinsed five times with 20 ml of warm buffer and placed individually in test tubes with 2 ml of buffer containing BSA (0.2%), bestatin (0.1 mM), phosphoramidon (3.0 mM), LiCl (10 mM), and the test peptide compounds at various concentrations. Each tube was then bubbled with O_2, capped, incubated, and shaken for 30 min at 37°C as above. The incubation was terminated by adding 3 ml methanol-chloroform (1:2), and each sample was homogenized for 10 s with a Brinkman polytron (setting 6). The samples were centrifuged for 10 min at 1000 × *g* at 4°C and 2 ml of the aqueous layer were removed and diluted with 2 ml of water. Then, 0.5 ml aliquots from the total aqueous volume were removed and counted with a Beckman 1100 scintillation counter in 10 ml of Ecolume scintillation fluid. The remaining 3.5 ml was added to 1 ml AG1-X8 anion exchange column, 100–200 mesh (Bio-Rad) and the total inositol phosphates ([^{3}H] PI), were eluted with 2 × 1.5 ml aliquots of 1.0 M ammonium formate: 0.1 M formic acid solution and counted in 15 ml of Ecolume. The percentage of PI formation is expressed as the ratio between the sum of the radioactive fractions collected in the ammonium formate/formic acid elution and the total amount of water soluble [^{3}H] inositol containing species. EC_{50} values were determined as described above.

Food Intake

Food intake was measured by a modification of the method described by Cox and Maickel (8). Male Sprague–Dawley rats (300–400 g) housed singly under a 12 D: 12 L reverse 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) 10 min prior to food availability, and food intake was then measured after 30 min and 3 h of feeding. 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. Dose–response curves were constructed using multiple doses of peptide and ED_{50} values were determined using ALLFIT.

In experiments where the duration of action of test compounds was evaluated, rats received treatment at various time intervals (1, 2, 3, 4, and 5 h) before food was presented, and 1 h food intake was determined and compared to vehicle-injected control animals. The number of animals per group, dosing schedules, and statistical analysis were performed as described above.

Food intake in male beagle dogs (n = 16) was evaluated according to the following protocol. After a 12-h fast, each

TABLE 1

AFFINITY OF ARL 14294 AND ARL 15849 FOR RAT PANCREATIC CCK-A RECEPTORS AND RAT BRAIN CORTICAL CCK-B RECEPTORS IN VITRO

Compound	CCK-A ^{3}H-MK-329 K_i (nM)	^{125}I-CCK-8 K_i (nM)	CCK-B ^{125}I-CCK-8 K_i (nM)	Selectivity* CCK-B K_i / CCK-A K_i
CCK-8	4.1 ± 0.2	0.087 ± 0.009	0.41 ± 0.06	5
ARL 14294	4.2 ± 0.8	0.069 ± 0.017	1.28 ± 0.03	18
ARL 15849	1.8 ± 0.2	0.034 ± 0.005	224 ± 19†	6590

Values expressed as mean ± SEM, n = 3 experiments.
*Ratio of CCK-B affinity over CCK-A affinity (vs. agonist).
†Significantly different from all other groups, $p < 0.05$.

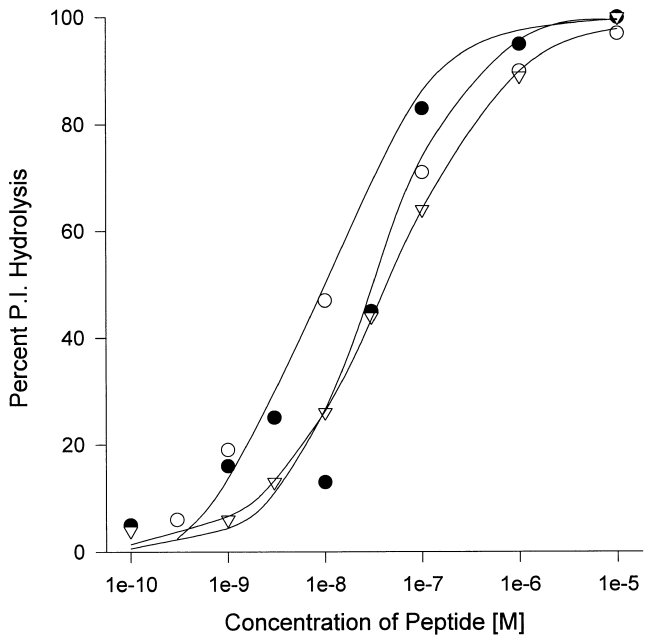

FIG. 1. The dose response of ARL 15849 (●), EC_{50} = 31.4 ± 9.4 nM, ARL 14294 (▽), EC_{50} = 43.8 ± 2.3 nM, and CCK-8 (○), EC_{50} = 12.2 ± 1.8 nM to stimulate phosphatidylinositol hydrolysis in guinea pig pancreatic fragments.

dog was given 100 g of food and allowed to eat for 15 min; 30 min later, 400 g of food was presented to each dog for an additional 15-min period. Food consumption was calculated on the amount of the 400 g of food eaten. Vehicle or drug was administered intranasally in a volume of 50 μl per nostril per 10 kg body weight, 10 min before the second feeding period. Values for food intake, expressed as (g/kg b.wt.) and the incidence of emesis (%) were analyzed by (ANOVA) followed by Newman–Keuls multiple range tests and *Z*-test for comparing proportions, respectively. Dose–response curves were constructed using multiple doses of peptide and ED_{50} values were determined using ALLFIT.

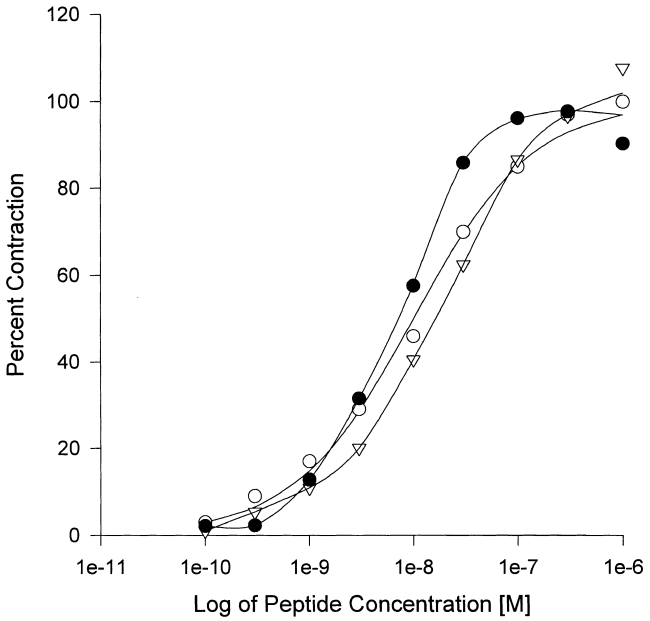

FIG. 2. The dose response of ARL 15849 (●), EC_{50} = 6.63 ± 0.67 nM, ARL 14294 (▽), EC_{50} = 7.84 ± 0.21 nM, and CCK-8 (○), EC_{50} = 10.1 ± 0.8 nM to contract isolated guinea pig gallbladder.

RESULTS

To determine whether replacement of the Met in ARL 14294 to increase stability (Met can be oxidized in solution) may have hindered receptor binding and if movement of the *N*-methyl group from Phe to Asp imparted the selectivity for CCK-A receptors, the affinity of ARL 15849 for both CCK-A and CCK-B receptors was assayed. As described in Table 1, the affinity of ARL 15849 for the CCK-A receptor as measured by standard procedures was similar to those of CCK-8 and ARL 14294, indicating a relatively good structural fit to this receptor. When measuring the affinity of ARL 15849 for CCK-A receptors on pancreatic membranes through displacement of an antagonist, MK-329, ARL 15849 had an apparent K_i of 1.8 nM, which is approximately equivalent to that of CCK-8 and ARL 14294. When measuring the affinity of ARL 15849 through displacement of a labeled agonist ^{125}I-CCK-8, ARL 15849 also resembled CCK-8 and ARL 14294. However, ARL 15849 did exhibit a high degree of selectivity for CCK-A receptors compared to CCK-B receptors (6,590-fold) unlike CCK-8 or ARL 14294, which showed little or no significant selectivity.

Having improved selectivity, while retaining the binding affinity of ARL 15849 to be comparable to that of CCK-8 and ARL 14294, the question arises as to its functional potency relative to these two compounds. Therefore, all three compounds were profiled in the guinea pig pancreatic phosphatidylinositol (PI) hydrolysis assay (Fig. 1) and in the isolated gallbladder assay (Fig. 2). ARL 15849, ARL 14294, and CCK-8 were approximately equipotent in both the PI turnover (EC_{50} = 31, 44 and 12 nM, respectively) and the gallbladder contraction assay (EC_{50} = 6.6, 7.8 and 10.1 nM, respectively), which would be predicted from the receptor binding data. However, the time to peak contraction (Table 2) with ARL 14924 (10.2 min) and ARL 15849 (16.9 min) was significantly longer ($p = 0.0175$, $F = 7.63$) than with CCK-8 (8.6 min). Similarly, contractions produced by ARL 15849 and ARL 14294 could not be readily reversed by washing out the peptide with repeated exchanges (three or greater) of the tissue bath media. In contrast, CCK-8–induced contractions were readily reversed by one exchange of bath media.

Intraperitoneally administered ARL 15849 inhibited the food intake of fasted rats during the first 30 min of feeding in

TABLE 2

EFFECT OF ARL 14294 AND ARL 15849 ON GALLBLADDER CONTRACTION IN VITRO

Compound	*n* (No. of Strips)	Time to Peak Conraction at 1.0 nM (min)
CCK-8	4	8.60 ± 0.82
ARL 15849	3	16.93 ± 1.92*
ARL 14924	3	10.22 ± 2.16

Values are expressed as mean ± SEM.
*Significantly different from both other groups, $p < 0.05$.

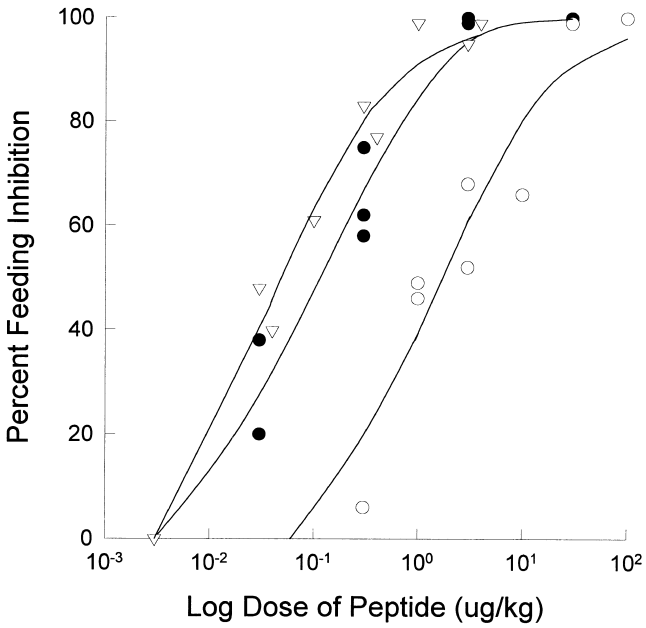

FIG. 3. The dose response of ARL 15849 (●), ED_{50} = 0.16 ± 0.02 μg/kg, ARL 14294 (▽), ED_{50} = 0.07 ± 0.008 μg/kg, and CCK-8 (○), ED_{50} = 3.0 ± 0.11 μg/kg to inhibit 30-min food intake after IP administration in fasted rats.

a dose dependent manner (Fig. 3) with a potency that was 19-fold greater than CCK 8 (ED_{50} = 0.16 vs. 3.0 μg/kg) and slightly less potent than ARL 14294 (ED_{50} = 0.07 μg/kg). When the feeding period was extended to 3 h the dose to inhibit feeding by 50% increased approximately 10- and 35-fold for ARL 14924 (ED_{50} = 0.80 μ/kg) and CCK-8 (100 μg/kg), respectively (Fig. 4). However, the ED_{50} dose of ARL 15849 to inhibit feeding at 3 h was only slightly higher (0.26 vs. 0.16 μg/kg) than the 30-min value, suggesting that ARL 15849 may have a longer duration of action than ARL 14294 and CCK-8.

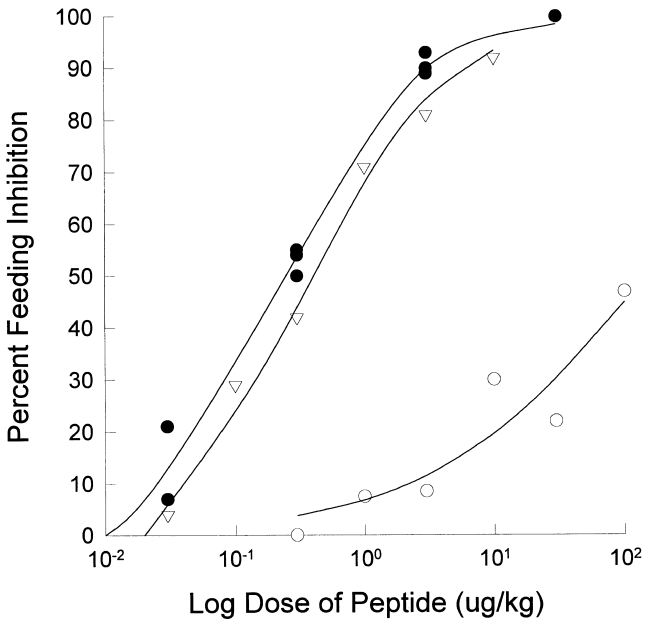

FIG. 4. The dose response of ARL 15849 (●), ED_{50} = 0.26 ± 0.01 nM, ARL 14294 (▽), ED_{50} = 0.80 ± 0.12 nM, and CCK-8 (○) ED_{50} = 100 ± 3.8 nM to inhibit 3-h food intake after IP administration in fasted rats.

The duration of action of ARL 15849 relative to CCK-8 and ARL 14294 was directly addressed by administering the compounds at selected intervals (1, 2, 3, or 4 h) before food presentation (Fig. 5). ARL 15849 maintained a significant reduction (21%) in 1-h food intake for a period of 5 h after IP dose administration in fasted rats. Equipotent doses of CCK-8 and ARL 14294 did not maintain a significant reduction in food intake beyond 2 h after dosing. This finding supports the suggestion of a significantly longer in vivo duration of action of ARL 15849 to inhibit feeding.

Both ARL 14294 and ARL 15849 demonstrated anorectic activity when administered intranasally in the beagle dog (Fig. 6). Analysis of food intake and the incidence of emesis (i.e., number of dogs responding per group) revealed that ARL 15849 (ED_{50} = 5.0 μg/kg) and ARL 14294 (ED_{50} = 3.2 μg/kg) are equipotent to inhibit feeding but ARL 15849 has a significantly greater threshold to induce emesis (p = 0.0018, Z-test for comparing proportions). ARL 15849 was without effect on emesis at 1.0, 4.0, 10.0, and 20.0 μg/kg. At 40.0 μg/kg a modest effect was noted (12%). At 60 μg/kg less than half of the dogs showed emesis, whereas with ARL 14294, no emesis was evident at 1.0 and 4.0 μg/kg, but dose-dependent increases in the incidence of emesis were noted at the three higher dose levels (10, 20, and 40 μg/kg). The highest dose level given for ARL 14294 (40 μg/kg) produced a substantially greater incidence of emesis than the corresponding dose level of ARL 15849 (50 vs. 12%, respectively).

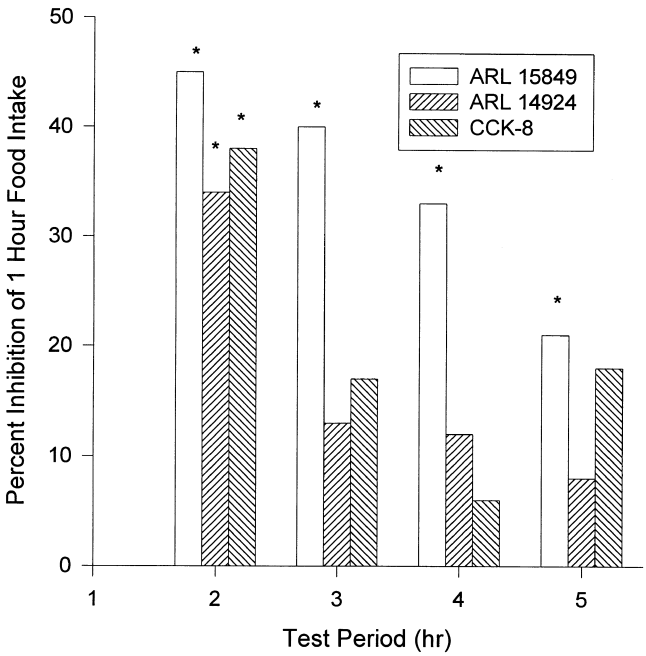

FIG. 5. Duration of feeding inhibition activity of ARL 15849 (0.16 μg/kg), ARL 14294 (0.07 μg/kg), and CCK-8 (3.0 μg/kg) after IP administration in fasted rats. *p < 0.05 when compared to vehicle controls (one-way ANOVA followed by Newman–Keuls analysis). n = 10 animals per group. Test period is defined as feeding delay (from IP dosing to food presentation) plus the feeding period (1 h).

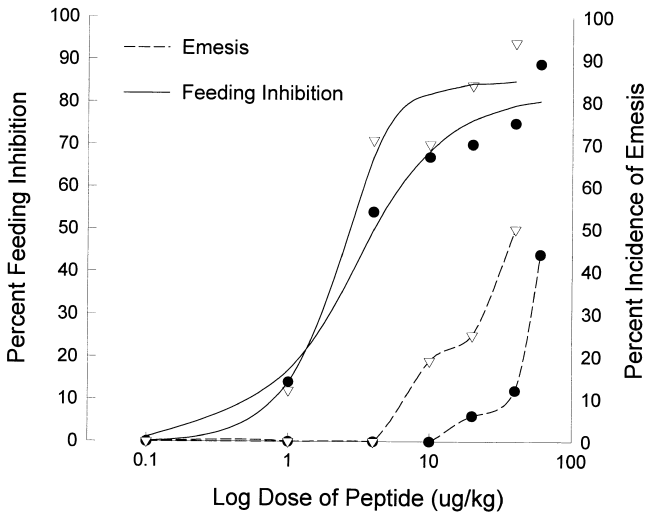

FIG. 6. The dose response of ARL 15849 (●), ED_{50} = 5.0 ± 1.5 μg/kg and ARL 14294 (▽), ED_{50} = 3.2 ± 0.8 μg/kg, to inhibit feeding and cause emesis after intranasal administration in dogs.

DISCUSSION

As stated previously, agonist stimulation of CCK-A receptors induces satiety, whereas stimulation of CCK-B/gastrin receptors causes gastric acid and electrolyte secretion, gastric motility, and functions as a growth factor for the gastric mucosa (23). Therefore, a selective CCK-A agonist may be useful in inhibiting food intake in various eating disorders without interfering with the normal CCK-B physiological processes. Transferring the *N*-methyl group from the Phe on ARL 14294 to the Asp has lead to a highly CCK-A receptor-selective compound while retaining potent full CCK-8 agonist activity. Receptor binding assessments have indicated that ARL 15849 is about 6000-fold more selective for CCK-A vs. CCK-B receptors with CCK-A agonist affinity that is comparable to that of CCK-8 and ARL 14294.

Replacement of the Met groups in ARL 14294 with Nle lead to a more stable compound because Met can be oxidized in solution. This structural change together with the transfer of the *N*-methyl group did not significantly alter the in vitro agonist potency and efficacy as measured in the gallbladder contraction and PI turnover assay, indicating a good structural fit to the receptor. The ability to stimulate gallbladder contraction was measured because it has been found to be a highly selective and sensitive indicator of CCK-A potency as well as a functional correlate and predictor of feeding inhibition potency. However, gallbladder contraction is not regarded as a reliable measure of CCK-A efficacy because of the large quantity of spare receptors located on this tissue. Therefore, the pancreatic PI hydrolysis assay was performed to determine the efficacy of ARL 15849 and to characterize the compound as a partial or full agonist relative to CCK-8. Although all the results of these assays revealed equivalent potency in vitro, ARL 15849 was approximately 20 times more potent than CCK-8 and equipotent with ARL 14294 to inhibit feeding for 30 min in rats when administered intraperitoneally. Further, ARL 15849 was threefold more potent than ARL 14924 to inhibit feeding at 3 h in rats. Additional experiments showed that the duration of action to inhibit feeding in rats was significantly longer for ARL 15849 than either ARL 14294 or CCK-8. Improved bioavailability, greater stability, and a prolonged resident time at the receptor could contribute to an apparently high in vivo potency and duration of effect for ARL 15849. Evidence to support a longer resident time at the receptor is apparent in the guinea pig isolated gallbladder model where the time-to-peak contraction is significantly longer for ARL 15849 compared to CCK-8 and ARL 14294.

CCK-8 and related analogs have been reported to inhibit food intake in beagle dogs (1,13,14,20,21). We have found that intravenous administration of 1 μg/kg of CCK over a 15-min. period reduced intake by 40% in our testing paradigm (data not shown). However, intranasal administration of CCK-8 was not effective. Conversely, intranasal administration of ARL 15849 did significantly inhibit feeding in beagle dogs, albeit at higher doses than those required to inhibit feeding activity in the rat after intraperitoneal administration. This finding may be the result of several factors, the nasal administration technique, the kinetics of intranasal absorption, and any possible species differences. First, Asin et al. have reported that the dog appears to be somewhat unique compared to other species because CCK-8 was significantly more potent in suppressing food intakes than A-17623 at the same molar dose, which is contrary to several other species tested including the rat, mouse, and monkey (1). Secondly, nasal sprays have been shown to be more effective than drops in the absorption of peptides (4). In our studies, a 50-μl volume was administered into the dog nasal cavity by a spray. However, much of the dose may be lost due to rapid ingestion following the leakage of drug down through the nasal cavity and into the mouth and esophagus. In contrast with ARL 15849, CCK-8 is inactive in the dog when given intranasally. We speculate that the enhanced availability of ARL 15849 and ARL 14294 compared to CCK-8 may be due to a greater resistance to peptidase activity. The nasal mucosa possess aminopeptidase activity that could metabolically degrade CCK-8 at a faster rate than either analog, thereby allowing less time for CCK-8 to be absorbed. Other important physical properties, including lipophilicity and molecular weight, do not differ markedly between the three peptides.

The enhanced separation between doses that inhibit feeding and induce emesis in the dog could be related to the specificity of ARL 15849 relative to ARL 14294 for the CCK-A receptor. The emesis response could result from both higher dose stimulation of CCK-A and CCK-B receptors that have been shown to be present on the vagal afferents (16), together with a lower dose stimulation of CCK-B receptors in the gastric mucosa. Therefore, a nonselective compound would act through both CCK-A and CCK-B receptor mechanisms to induce emesis at lower doses compared to a selective CCK-A compound that would only act through CCK-A mechanisms. In effect, the increased release of stomach acid and gut motility through CCK-B receptor stimulation may increase the susceptibility of the dog to the emesis inducing effect of CCK-A peptide agonists.

In summary, ARL 15849 appears to be an effective, intranasally biovailable, anorectic agent that inhibits feeding for a longer duration of time and with a lower risk of CCK-B–related side effects than its predecessor, ARL 14294. Such a compound could provide a novel therapy for the treatment of eating disorders resulting in obesity through selective, peripheral stimulation of the CCK-mediated satiety pathway. Several investigators have reported that an effective treatment for the control of appetite is a critical component in the overall management of obesity and its related diseases (3,12).

REFERENCES

1. Asin, K. E.; Bendnarz, L.; Nikkel, A. L.; Gore, P. A.; Nadzan, A. M.: A-71623, a selective CCK-A receptor agonist, suppresses food intake in the mouse, dog and monkey. Pharmacol. Biochem. Behav. 42:699–704; 1992.
2. Bishop, L. A.; Gerskowitch, V. P.; Hull, R. A. D.; Shankley, N. P.; Black, J. W.: Combined dose-ratio analysis of cholecystokinin receptor antagonists, devazepide, lorglumide and loxiglumide in the guinea-pig gallbladder. Br. J. Pharmacol. 100:61–66; 1992.
3. Bray, G. A.: Use and abuse of appetite-suppressant drugs in the treatment of obesity. Ann. Intern. Med. 119:707–713; 1993.
4. Campbell, D. E. S.; Richeter, W.: An observational method estimating toxicity and drug actions in mice applied to 68 reference drugs. Acta. Pharmacol. Toxicol. 25:345–363; 1967.
5. Chang, R. S.; Lotti, J.; Chen, T. B.; Kunkel, J. A.: Characterization of the binding of [^{3}H]-(+)L-364,718: A new potent, non-peptide cholecystokinin antagonist radioligand selective for peripheral receptors. J. Pharmacol. Exp. Ther. 30:212–217; 1986.
6. Chang, R. S.; Lotti, V. S.: Biochemical and pharmacological characterization of an extremely potent and selective monopeptide cholecystokinin antagonist. Proc. Natl. Acad. Sci. USA 86:4923–4926; 1986.
7. Cheng, Y. C.; Prusoff, W. H.: Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22:3099–3108; 1973.
8. Cox, R. H.; Maickel, R. P.: Comparison of anorexigenic and behavioral potency of phenylethylamines. J. Pharmacol. Exp. Ther. 181:1–9; 1972.
9. Crawley, J. N.; Corwin, R. L.: Biological actions of cholecystokinin. Peptides. 15:731–755; 1994.
10. Feldman, H. A.: Mathematical theory of complex ligand-binding systems at equilibrium: Some methods of parameter fitting. Anal. Biochem. 48:317–338; 1972.
11. Grider, J. R.: Role of cholecystokinin in the regulation of gastrointestinal motility. J. Nutr. 124(Suppl. 8): 1334S–1339S; 1994.
12. Hill, A. J.; Weaver, C. F.; Blundell, J. E.: Food craving, dietary restraint and mood. Appetite 17:187–197; 1991.
13. Inui, A.; Okita, M.; Inoue, T.; Sakatani, N.; Oya, M.; Morioka, H.; Oimomi, M.; Baba, S.: Effect of cholecystokinin octapeptide analogues on food intake in the dog. Am. J. Physiol. 257:R946–R951; 1989.
14. Kalogeris, T. J.; Reidelberger, R. D.; Mendel, V. E.; Solomon, T. E.: Interaction of cholecystokinin-8 and pancreatic glucagon in control of food intake in dogs. Am. J. Physiol. 260:R688–R692; 1991.
15. Lin, C. W.; Bianchi, B. R.; Grant, D.; Miller, T.; Danaher, E. A.; Tufano, M. D.; Kopecka, H.; Nadzan, A. M.: Cholecystokinin receptors: Relationships among phosphoinositide breakdown, amylase release and receptor affinity in pancreas. J. Pharmacol. Exp. Ther. 236:729–733; 1986.
16. Lin, C. W.; Miller, T. R.: Both CCK-A and CCK-B/gastrin receptors are present on rabbit vagus nerve. Am. J. Physiol. 263:R591–R595; 1992.
17. Linden, J.: Calculating the dissociation constant of an unlabelled compound from the concentration required to displace radiolabel binding by 50%. J. Cyc. Nucleot. Res. 8:162–172; 1982.
18. Qian, J. M.; Rowely, W. H.; Jensen, R. T.: Gastrin and CCK activate phospholipase C and stimulate pepsinogen release by interacting with two distinct receptors. Am. J. Physiol. 264:G718–G272; 1993.
19. Raybould, H. E.; Lloyd, K. C.: Integration of postprandial function in the proximal gastrointestinal tract. Role of CCK and sensory pathways. Ann. NY Acad. Sci. 713:143–156; 1994.
20. Reidelberger, R. D.; Kalogeris, T. J.; Solomon, T. E.: Plasma CCK levels after food intake and infusion of CCK analogues that inhibit feeding in dogs. Am. J. Physiol. 256:R1148–R1154; 1989.
21. Rubin, B.; Engel, S. L.; Drungis, A. M.; Dzelzkalns, M.; Grigas, E. O.; Waugh, M. H.; Yiacas, E.: Cholecystokinin-like activities in guinea pigs and dogs of the C-terminal octapeptide (SQ 19,844) of cholecystokinin. J. Pharm. Sci. 58:955–959; 1969.
22. Sandvik, A. K.; Waldum, H. L.: CCK-B (gastrin) receptor regulates gastric histamine release and acid secretion. Am. J. Physiol. 260:G925–G928; 1991.
23. Silvente-Poirot, S.; Dufresne, M.; Vaysse, N.; Fourmy, D.: The peripheral cholecystokinin receptors. Eur. J. Biochem. 215:513–529; 1993.
24. Simmons, R. D.; Blosser, J. C.; Rosamond, J. R.: ARL 14294: A novel CCK-8 agonist with potent intranasal anorectic activity in the rat. Pharmacol. Biochem. Behav. 47:701–708; 1994.
25. Smith, G. P.; Gibbs, J.: Satiating effect of cholecystokinin. Ann. NY Acad. Sci. 713:236–241; 1994.
26. Wank, S. A.; Pisegna, J. R.; De Weerth, A.: Cholecystokinin receptor family. Ann. NY Acad. Sci. 713:49–66; 1994.