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Behavioral Effects of AR-R 15849, a Highly Selective CCK-A Agonist

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SIMMONS, R. D., F. C. KAISER AND T. J. HUDZIK. *Behavioral effects of AR-R 15849, a highly selective CCK-A agonist.* PHARMACOL BIOCHEM BEHAV **62**(3) 549–557, 1999.—The behavioral effects of AR-R 15849, a novel cholecystokinin agonist with high affinity and selectivity for the CCK-A receptor subtype, were examined. Initially, using an operant feeding paradigm to test for anorectic activity and specificity, acute administration of AR-R 15849 was found to alter the intake and pattern of feeding in a manner similar to prefeeding. Further, AR-R 15849 did not induce compensatory feeding as did CCK-8, and did not affect performance on running rates of responding, or motor activity on a running wheel, as did fenfluramine. In tests for subchronic anorectic activity, daily intraperitoneal injections of AR-R 15849 significantly reduced food intake in fasted rats over a 9-day test period with greater efficacy compared to its nonselective predecessor AR-R 14294 (formerly FPL 14294). The sustained decrease in food intake with AR-R 15849 treatment resulted in a significant reduction in body weight gain over 9 days. Finally, an experiment designed to determine the effect of caloric deprivation and subchronic drug exposure on the overall efficacy of AR-R 15849 indicated that pharmacological tolerance does not develop following subchronic treatment. © 1999 Elsevier Science Inc.

AR-R 15849 Fenfluramine Cholecystokinin Food intake Weight loss Tolerance AR-R 14294 CCK-A receptor Motor activity Running wheel Subchronic treatment Operant feeding paradigm

CHOLECYSTOKININ (CCK) and its active fragment CCK-8, have been reported to be involved in a number of mammalian gastrointestinal and CNS functions (9,13,16,25,27). 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 (3,12,17,23,25,31). 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 in a number of animals including humans (18,19,22, 24,28).

Satiety induction or appetite suppression leading to a reduction in caloric intake has proven to be an effective method for the treatment of obesity (4,16). However, the clinical use of such agents has had a number of insufficiencies including; limited efficacy, tolerance, undesired behavioral effects, and abuse liability (7,14,20,21,26). Ideally, a selective anorectic agent should decrease food intake and maintain weight loss without altering other behaviors.

We have previously reported the characterization of AR-R 14294 (formerly FPL 14294) and a new hexapeptide CCK analogue, AR-R 15849 (formerly ARL 15849), that possesses equivalent affinity but greatly enhanced selectivity for the CCK-A vs. the CCK-B receptor compared to AR-R 14294 (29,30). In this study, AR-R 15849 was examined for its efficacy to reduce food intake and maintain body weight loss in comparison with its predecessor AR-R 14294. Corresponding indices of motor activity were examined to ascertain the selectivity of CNS effects of AR-R 15849 on feeding behavior relative to other anorectic agents, in particular, fenfluramine and CCK-8 itself. Along with anorectic efficacy and selectivity, the possiblity of AR-R 15849, relative to AR-R 14294, to induce pharmacological tolerance was also examined.

METHOD

The procedures involving animals and their care were conducted in conformity with the institutional guidelines that comply with the Guide for Care and Use of Laboratory Animals (NIH Publication, No. 85-23, 1995).

Animals

The subjects used in the acute study were sets of six male Long–Evans rats weighing 230–340 g that were housed and

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tested in experimental chambers (see below) with free access to water. The chambers resided in a room that was maintained under a 12-h light:dark cycle (0500 h on, 1700 h off). The subjects used in the subchronic anorectic and drug tolerance studies were sets of 10 male Sprague–Dawley rats that were individually housed and tested in standard wire cages maintained under a reverse-phase 12-h light:dark cycle.

Acute Feeding and Motor Performance Studies in Food-Deprived Rats

The housing/testing chambers were constructed from standard shoebox cages (Nalgene) that were fitted with a running wheel, stimulus lamp, pellet dispenser, and operant lever. The chambers were interfaced with microprocessor equipment that programmed and recorded events in the cages. Between 1700 and 2300 h each day, a small stimulus lamp mounted on the pellet dispenser was turned on, the room lights turned off, and food was available under an FR10 schedule of reinforcement (each 10 lever presses resulted in delivery of two 45-mg food pellets).

Vehicle (physiological saline) or drug, CCK-8 (0.3–10 mg/kg), AR-R 15849 (0.1–30 μg/kg) and fenfluramine (0.3–10 mg/kg), was administered by intraperitoneal (IP) injection at a volume of 0.1 ml/100 g of body weight, 15 min (CCK-8, AR-R 15849) and 30 min (fenfluramine) prior to food availability periods. In the prefeeding experiments, 2, 5, and 10 g of Purina Rat Chow was placed into the chambers 1 h prior to food availability periods.

Subchronic Feeding Studies in Fasted Rats

The subchronic anorectic effects of the drugs were measured by a modification of the method described by Cox and Maickel (7). Male Sprague–Dawley rats were trained to eat powdered Purina Rat Chow during the first 3 h of the dark cycle. A total of 40 rats were divided into groups of 10 based on 3-h food consumption values obtained over a 3-day period preceding the experiment. Groups were formed so that the mean food intake for each group and the mean body weight of each group were comparable. The four groups were composed of the following: 1) 0.9% saline (control group); 2) AR-R 14294—0.4 μg/kg (reference standard group); 3) AR-R 15849—0.3 μg/kg; and 4) AR-R 15849—0.1 μg/kg. Dose levels were based on the calculated ED_{50} and ED_{30} for 3-h food consumption. Vehicle or test compounds were administered daily approximately 10 min preceding food presentation in a volume of 0.5 ml. Daily, 1-, 2-, and 3-h food consumption, as well as body weight values were recorded for each group. The change in body weight for each group was calculated from differences in weight for each rat compared to their day-1 values.

Subchronic Tolerance Study

The design of this study is as described above with the following differences. Three groups of 10 animals were assigned to the following treatments: 1) 0.9% saline (control group); 2) AR-R 15849—0.3 μg/kg; and 3) AR-R 14294—0.4 μg/kg. Dose levels were based on previously determined ED_{50} values (acute doses that reduce 3-h food consumption by 50%). All groups received vehicle or test compounds 10 min prior to food presentation on days 1 and 9 and immediately following the 3-h feeding period on days 2–8. The drugs were given after feeding on days 2–8 to prevent a reduction in food intake leading to caloric deprivation while maintaining daily drug exposure.

Statistical Analysis

For the acute studies, lever presses and food pellet deliveries were summed and recorded for each of 24 successive 15-min segments of the 6-h session. Food intake (g) was recorded by multiplying the number of food pellet deliveries by the weight of the pellets delivered. The average rate of responding that occurred within each FR10 unit (the running rate of responding) was also calculated. The rate of responding was calculated as responses emitted/s, after factoring out periods in which no responding occurred. In other terms, the running rate is the calculated rate of lever pressing after factoring out the periods in which no responding occurred, and can be used as an index of motor performance. For example, an appetite suppressant can cause the animal to respond for fewer food pellets (complete fewer fixed ratios), but the rate with which the fixed ratios are completed may either decrease (indicating a performance effect of the drug) or remain unchanged (indicating a specific anorectic effect). Additionally, turns in the running wheel were summed and recorded hourly and expressed as turns occurring during food availability periods that provide a second index of performance not directly related to feeding behavior. Water intake was recorded daily. Statistical comparisons were made by repeated-measures analysis of variance (ANOVA), and comparisons between control points and drug points by Dunnett's post hoc comparison.

For the subchronic studies, mean and standard error (SEM) for each treatment group were calculated for food consumption and body weight data each day, and a two-way repeated-measures ANOVA over the 9-day period was used to analyze these calculated values across treatments both within days and between days where appropriate. Post hoc comparisons between individual treatment groups and vehicle were performed using a Newman–Keuls multiple comparisons range test. To analyze individual daily differences, one-way ANOVA analyses on the food intakes vs. treatment for each day followed by a post hoc comparison of individual means using a Newman–Keuls multiple comparisons range test were used.

Drugs

The following drugs were used: AR-R 15849 and AR-R 14294 (synthesized on site), cholecystokinin-octapeptide and fenfluramine (Sigma Chemical Co., St. Louis, MO). All drugs were dissolved in physiological saline (one drop of 0.1 N NaOH was added if necessary) and given by intraperitoneal injection.

RESULTS

Food Intake and Activity Following Acute Treatment

The effects of each of the treatments are summarized in Table 1 and Fig. 1. Shown in the left-hand side of the table is food intake (±SEM) across the entire 6-h food availability period. The running rate of responding and wheel turns made during food availability are shown on the right-hand side of the table.

Under control conditions, animals consumed, on the average, from 22 to 28 g of food and 31 to 42 ml of water during a typical 6-h session. Control running rates and wheel turns varied from group to group, largely due to programming changes that were made during the course of the studies in order to compensate for the slow speed of the computer used. The programming changes were only made prior to testing a given compound (i.e., not during a dose–effect curve).

TABLE 1

EFFECTS OF CCK-8, AR-R 15849, FENFLURAMINE, AND PREFEEDING ON FOOD INTAKE, RUNNING RESPONSE RATE, AND ACTIVITY ON THE RUNNING WHEEL IN RATS FASTED FOR 18 H

Treatment	Food Intake Total Session	Running Response Rate	Wheel Turns During Feeding
AR-R15849 (μg/kg)	(g)		
Vehicle	22.1 (0.8)	0.78 (0.1)	905 (206)
0.3	20.9 (2.5)	0.57 (0.18)	394 (105)
3	19.1 (2.7)	0.69 (0.20)	419 (117)
10	20.9 (2.5)	0.38 (0.14)	1219 (427)
30	16.1 (3.9)	0.40 (0.15)	806 (478)
CCK-8 (μg/kg)			
Vehicle	27.1 (1.6)	1.1 (0.15)	262 (64)
1	24.6 (1.7)	1.0 (0.13)	337 (57)
3	24.3 (1.2)	1.0 (0.15)	265 (60)
10	25.3 (1.1)	1.0 (0.18)	301 (68)
Fenfluramine (mg/kg)			
Vehicle	23.8 (0.87)	1.36 (0.10)	853 (206)
1	24.3 (0.90)	1.55 (0.20)	188 (76)*
3	15.3 (3.13)*	0.85 (0.21)*	158 (82)*
10	3.8 (2.15)*	0.15 (0.07)*	44.8 (30.3)*
Prefeeding (g)			
Vehicle	27.6 (1.5)	2.2 (0.21)	960 (384)
2	24.4 (2.2)	2.6 (0.34)	2405 (932)
5	25.8 (1.2)	2.4 (0.21)	1202 (449)
10	18.1 (1.0)	2.4 (0.33)	1192 (715)

Each drug was administered IP at 15 min (CCK-8, AR-R 15849) or 30 min (fenfluramine) before food availability. The vehicle for each group was given at the corresponding time. Prefeeding was done 1 h before the start of the session. Values are expressed as mean (SEM).

*$p < 0.05$, Dunnett's ANOVA post hoc comparison.

AR-R 15849 (10 and 30 μg/kg) decreased food intake in the first hour following its administration. The highest dose (30 μg/kg) tended, although not significantly, to decrease intake for the entire 6-h session. AR-R 15849 did not significantly alter turning in the wheel or running rates of responding across the dose range studied, and did not alter water intake (data not shown).

All doses of CCK-8 decreased feeding in the first hour after its administration and did so without significantly altering the other behavioral measures. No dose of CCK-8 decreased intake across the entire food availability period. Like AR-R 15849, CCK-8 administration did not alter running rates of responding or wheel turns during the food availability period. Water intake was unaltered by CCK-8.

Prefeeding with each of the three amounts of food resulted in decreases in intake in the first hour of the session in an amount-dependent manner. None of the prefeeding treatments resulted in changes in the running rate of responding or in the number of wheel turns. Prefeeding did not alter water intake.

Fenfluramine at 3 mg/kg decreased food intake in the first hour after its administration, but this dose also decreased running in the wheel, which indicates some behavioral impairment. Fenfluramine at doses of 10 mg/kg decreased food intake across the entire 6-h food availability period, but also decreased the running and overall rate of responding as well as wheel-running behavior, indicating a lack of anorectic specificity. Because of its long duration of action, water intake was

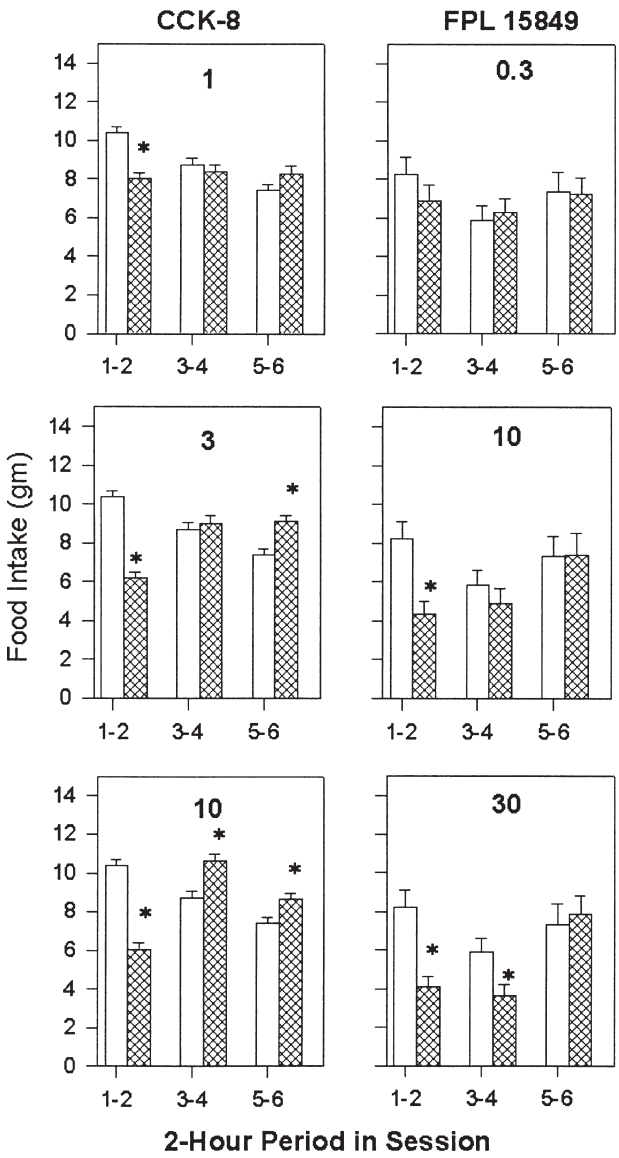

FIG. 1. Local food intake (g) during the 6-h food availability period shown in successive 2-h blocks (thirds of the session). Filled bars show the effects of the drug treatments at the specified doses after IP administration 15 min before food availability. The food intake at 1 to 2 h was significantly reduced at all doses except 0.3 μg/kg of AR-R 15849, and was significantly decreased at the 3- to 4-h session following 30.0 μg of AR-R 15849. Intake significantly increased at the 3- to 4-h session with 10.0 μg/kg of CCK-8 and at the 5- to 6-h interval for the 3.0 and 10.0 μg/kg CCK-8–treated groups ($p < 0.05$).

significantly decreased after the highest dose of fenfluramine, to 16 ± 3.7 ml ($p < 0.05$). The effects of drug treatment on local food intake compared to control values are shown in Figs. 1 and 2. CCK-8 decreased intake of food rather specifically within the early parts of the session, consistent with its relatively short duration of action (Fig. 1, left side). However, compensatory feeding later in the session was evident following 3 μg/kg in the final 2 h of the session and following 10 μg/kg in the final 2/3 of the session. AR-R 15849 administration (Fig. 1, right side) decreased feeding selectively in the first 1/3

of the session after 10 μg/kg and in the first 2/3 of the session after 30 μg/kg. However, unlike CCK-8, AR-R 15849 did not result in compensatory feeding later in the session over the dose range studied. This compensatory feeding observed with CCK could be the result of its shorter duration of action compared to AR-R 15849, most probably due to a shorter half-life and a faster on–off rate at the CCK-A receptor (30). Prefeeding the animals with 5 g of food resulted in compensatory feeding in the final third of the session (Fig. 2, left side). Like AR-R 15849, 10 g of food decreased food intake in the first 2/3 of the session without causing compensatory eating later in the session. Fenfluramine administration did not result in compensatory feeding, likely due to its longer duration of action (Fig. 2).

Feeding Inhibition and Weight Loss Following Subchronic Treatment

Rats used in this study were maintained on the 21-h food deprivation regimen and were assigned to treatment groups and dosed as described in the Method section.

Analysis of mean body weight changes recorded during the 9-day treatment period indicated a significant effect of days, $F(8,24) = 20.18$, $p < 0.0001$, and a significant treatment × days interaction, $F(8, 288) = 4.54$, $p < 0.0001$. A significant difference related to treatment alone was not reached because the day 1 body weights were included in the overall analysis, and as expected, differences in weights did not occur this early in the treatment period. Individual analyses on body weight vs. treatment for each day from days 2–9, revealed the following significant ($p < 0.05$) differences (Fig. 3). The changes in body weight were different from the corresponding control values for all treatment groups through day 9, excluding the AR-R 14294 and the 0.1 μg/kg AR-R 15849 groups on days 7 and 9. AR-R 15849 at the ED_{50} dose showed the highest potency and efficacy compared to the two other treatments by producing a sustained weight loss from days 3–9, which was significantly greater than the other treatments ($p < 0.05$).

Similar analysis of one hour food intakes across the 9-day treatment period indicated a significant effect of treatment, $F(3, 36) = 210.16$, $p < 0.0001$, and days, $F(8, 24) = 17.46$, $p < 0.0001$, and a significant treatment × days interaction, $F(8, 288) = 5.93$, $p < 0.0001$. Individual analyses on the food intakes vs. treatment for each day, revealed the following significant ($p < 0.05$) treatment effects (Fig. 4). All drug treatment values were significantly different from control intake values for the entire 9-day period, indicating a sustained anorectic effect during the first hour of feeding. Although the lower dose of AR-R 15849 produced an equipotent effect with the ED_{50} doses of AR-R 15849 and AR-R 14294 for the first 2 days, efficacy was significantly lower during days 3–9, suggesting a dose-related decrease in duration of effect with subchronic treatment.

The analysis of 2-h food intakes across 9 days indicates a significant effect of treatment, $F(3, 36) = 97.69$, $p < 0.0001$, and days, $F(8, 24) = 42.06$, $p < 0.0001$, and a significant treatment × days interaction, $F(8, 288) = 6.58$, $p < 0.0001$. Individual analysis of food intake following treatment for each day indicated that only the ED_{50} dose treatment with AR-R 15849 was significantly different across all 9 days. The equipotent dose of AR-R 14294 and the lower dose of AR-R 15849 did not reach significance on day 9. Similarly, all treatments were equipotent up until day 4, after which, the ED_{50} dose of AR-R 15849 produced a significantly greater reduction in feeding through out the remainder of the treatment period with the exception of day 8. This suggests that the duration of anorectic effect observed over 9 days of treatment is compound specific, as well as dose related.

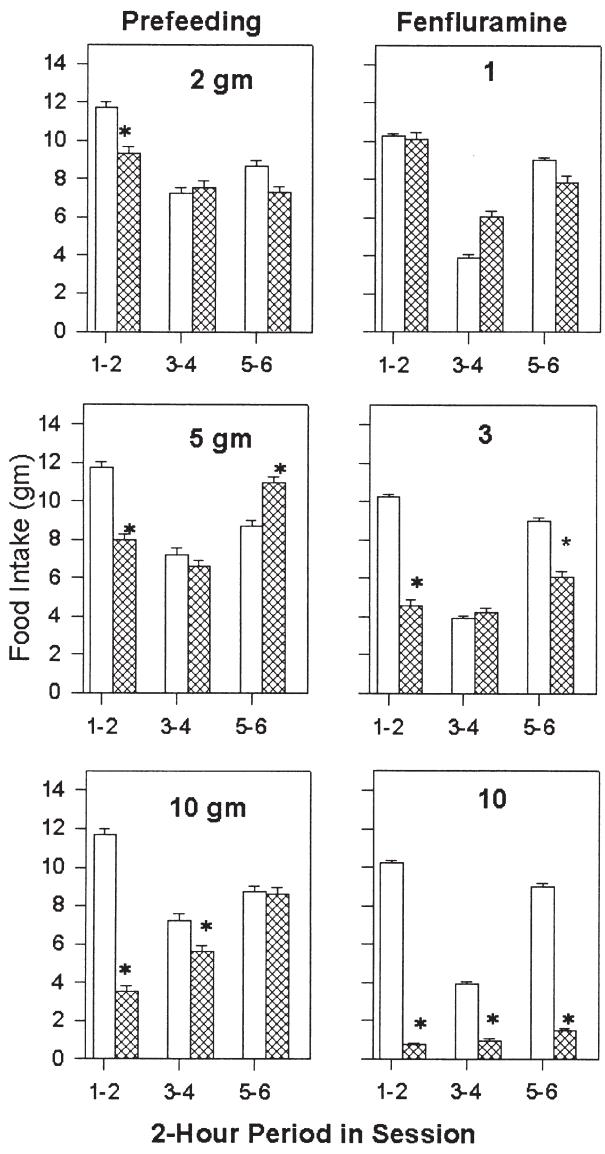

FIG. 2. Local food intake (g) during the 6-h food availability period shown in successive 2-h blocks (thirds of the session). Filled bars show the effects of the drug treatment at the specified doses after IP administration 30 min before food availability. In the prefeeding experiments, food was placed into the chambers 1 h prior to food availability. The food intake at all hourly intervals was significantly ($p < 0.05$) reduced in the 3- and 10-mg/kg fenfluramine treatment group except at 3 to 4 h in the 3-mg/kg group. Prefeeding with each amount significantly reduced intakes at the 1- to 2-h period and for the 10 g prefeeding at the 3- to 4-h period. Intake was significantly increased at 5 to 6 h following prefeeding with 5 g of food ($p < 0.05$).

The analysis of three hour food intakes across 9 days indicates a significant effect of treatment, $F(3, 36) = 25.74$, $p < 0.0001$, and days, $F(8, 24) = 36.64$, $p < 0.0001$, and a significant treatment × days interaction, $F(8, 288) = 7.63$, $p < 0.0001$. The analyses of daily food intake between groups showed that all treatments were equipotent in reducing feed-

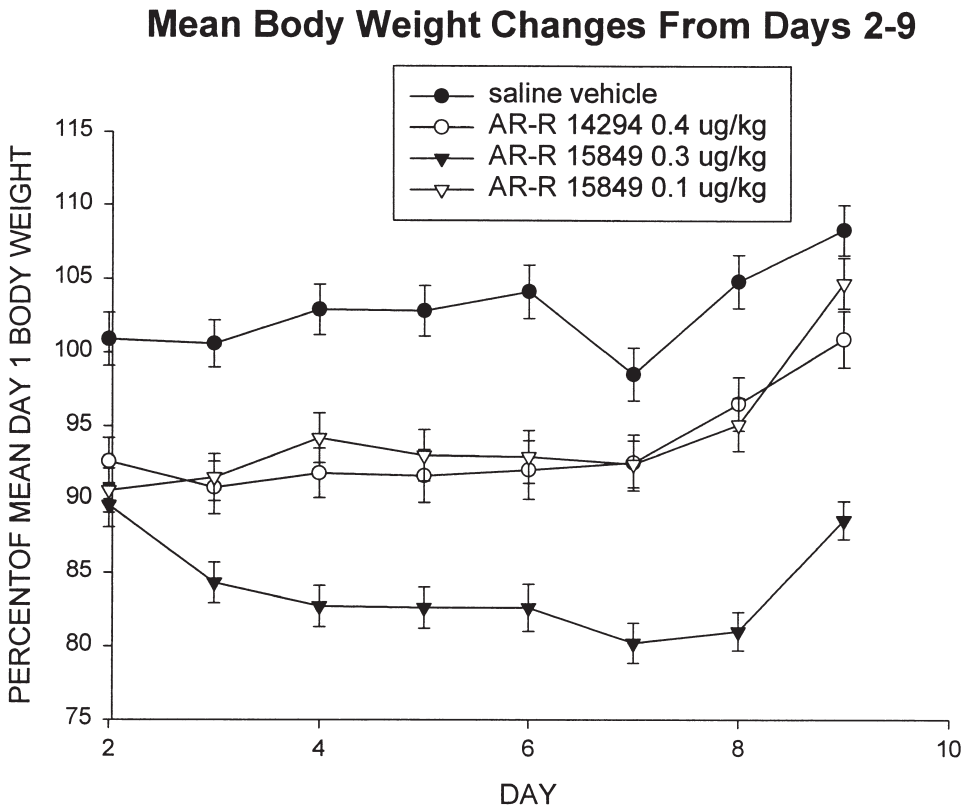

FIG. 3. Effects of daily administration of AR-R 15849 and AR-R 14294 over 9 days on weight gain in male Sprague–Dawley rats trained to eat during daily 3-h sessions. Both drugs were administered by intraperitoneal injection 15 to 20 min prior to the start of the feeding period. Values are expressed as percent of mean body weight ± SEM, taken on day 1 for each group. The number of rats for each group is 10. All values were significantly different from controls for all treatment groups through day 9 ($p < 0.05$), except for the AR-R 14294 and the AR-R 15849 (0.3 μg/kg) groups on days 7 and 9. The AR-R 15849 (0.4 μg/kg) group was significantly different ($p < 0.05$) from all other groups from day 3 to day 9.

ing on day 1 only. From day 2 through day 9 the ED_{50} AR-R 15849 dose produced a greater inhibition than the lower dose and there was a trend for greater anorectic potency compared to AR-R 14294, although significance was not reached on days 3, 5, and 8. All treatments were effective in reducing intake until day 5 (except the low dose AR-R 15849 on day 4) and only the ED_{50} dose of AR-R 15849 continued to produce a reduction until day 8 when all treatment effects were not found to be significant.

In summary, analysis of the 1, 2, and 3-h food intake data over 9 days of treatment indicates both a dose- and compound-dependent duration of effect over the daily 3-h feeding period as well as over the 9-day treatment period with the ED_{50} dose of AR-R 15849 being most efficacious. The results also show a trend toward an increase in food intake over time, specifically in the cumulative 2 and 3-h time periods, which suggests the possibility of tolerance.

Tolerance Study

To address the possibility of pharmacological tolerance development to the anorectic effects of the selected treatments, a subchronic study was performed comparing treatment without a reduction in food intake and therefore without caloric deprivation on days 2–8, as described in the Method section.

Figure 5 presents the effects on the 1, 2, and 3-h, food intakes of vehicle, AR-R 14294, and AR-R 15849 on days 1 through 9 when administered to rats daily for 9 days. Each group was administered drug or vehicle before food presentation on day 1 and day 9 and after feeding on days 2–8. Cumulative food intakes on day 1 and day 9 of the treatment period were analyzed using a two-way ANOVA. When the day 1 and day 9 food intake values were compared to control values for each drug treatment group there was a significant difference at all time intervals for the AR-R 15849 group but not for the AR-R 14294 group. The AR-R 14294 group was not different from control at 3 h on day 1 and at 2 or 3 h on day 9. Comparison of food intake values between AR-R 15849–treated animals on day 1 and day 9 revealed no significant differences. These findings suggest that AR-R 15849 retains its anorectic potency during the 3-h feeding period over 9 days of treatment when the animals are not concurrently calorically deprived. However, some lessening of effect at 2 h postdosing was observed with AR-R 14294 on day 9, which is probably related to its comparatively shorter duration of action.

DISCUSSION

Ideally, an anorectic agent should suppress feeding and maintain weight loss at doses that do not simultaneously alter

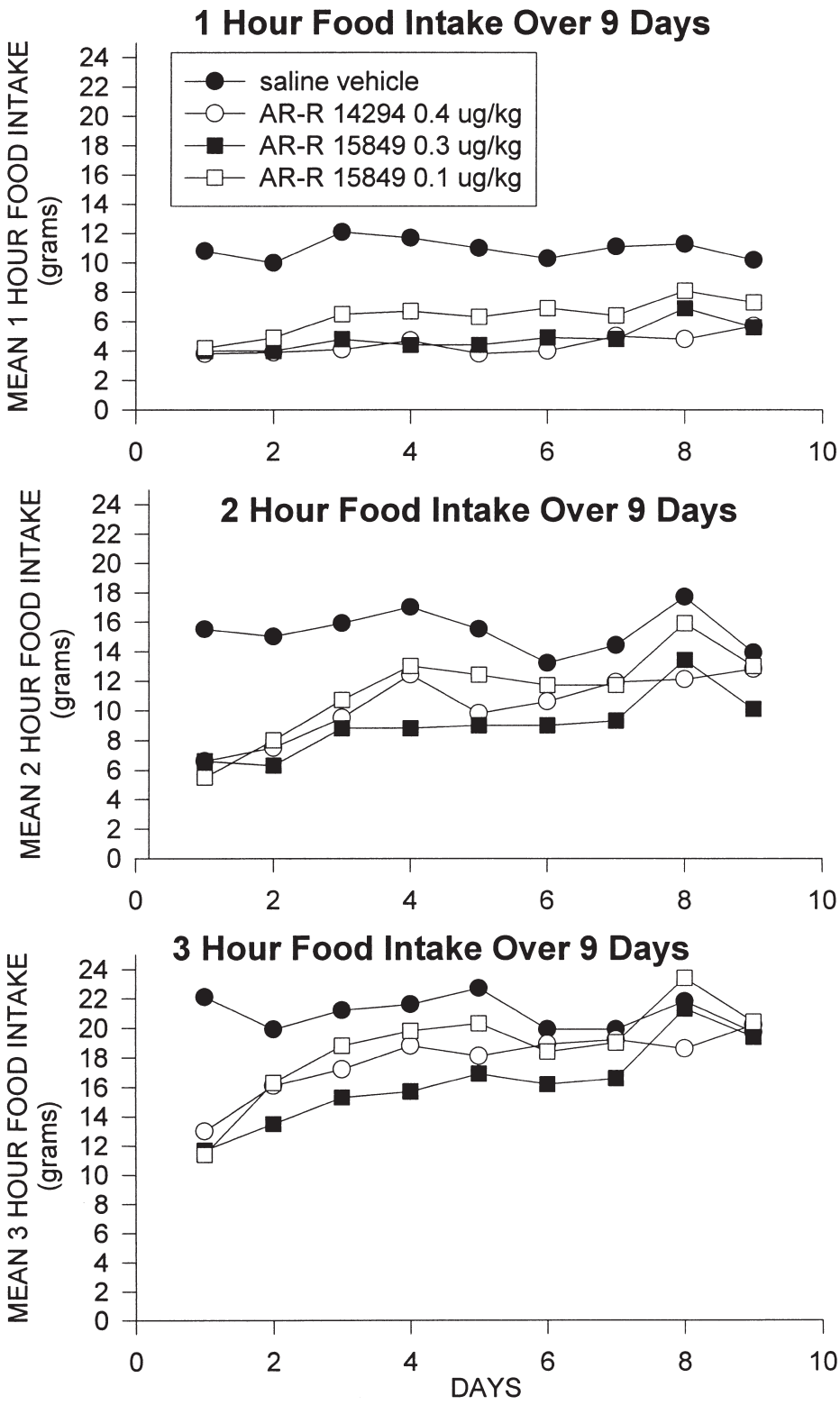

FIG. 4. Effects of daily adminstration of AR-R 15849 and AR-R 14294 over 9 days on 1, 2, and 3-h food intakes in male Sprague–Dawley rats trained to eat during daily 3-h sessions. Both drugs and vehicle were administered by intraperitoneal injection 15 to 20 min prior to the start of the feeding period. Values are expressed as 1, 2, or 3-h mean food intakes for each group. The number of rats in each group is 10. During the 1-h feeding period, all treatment group values were significantly different from controls ($p < 0.05$) for the entire 9 day period. At 2 h, all treatments were equipotent and significantly different from control values ($p < 0.05$) up until day 4; after that, the AR-R 15849 (0.03-mg/kg dose) produced a greater reduction in feeding ($p < 0.05$) compared to the other groups except on day 8. At 3 h, all treatments were effective in reducing intake until day 5 (except the low dose AR-R 15849 on day 4), and only the ED_{50} dose of AR-R 15849 continued to produce a significant reduction ($p < 0.05$) until day 8.

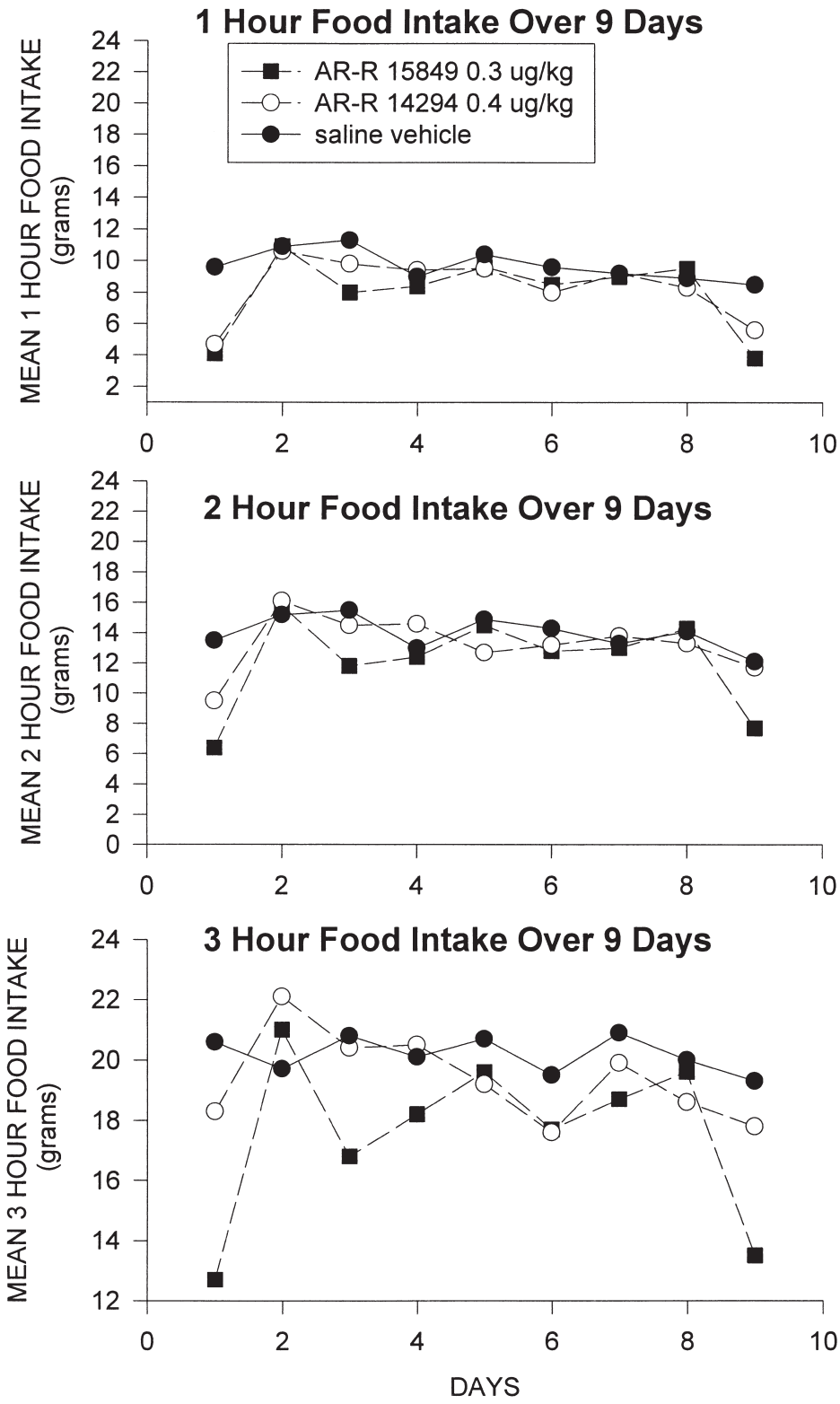

FIG. 5. Effects on the 1, 2, and 3-h food intakes of AR-R 14294 and AR-R 15849 when administered to fasted rats daily for 9 days. Each group was administered drug or vehicle by intraperitoneal injection 15 to 20 min before food presentation on day 1 and day 9 and after feeding on days 2 to 8. Values are expressed as 1, 2, or 3-h mean food intakes for each group. The number of rats in each group is 10. Daily intakes on days 2–8 did not differ between the treatment group and the control values at any time interval of feeding with the exception of day 3 values for the AR-R 15849 treatment group relative to controls ($p < 0.05$). Day 1 and day 9 food intake values for each drug treatment group were significantly different from control values ($p < 0.05$) at all time intervals for the AR-R 15849 group and all except at 3 h on day 1 and at 2 and 3 h on day 9 for the AR-R 14294 group. Comparison of food intake values between AR-R 15849–treated animals on day 1 and day 9 at each hourly interval revealed no significant differences.

other behaviors. An evaluation of the relationship between the appetite-suppressant and behavioral effects of AR-R 15849 in comparison to those of three efficacious standards: CCK-8, fenfluramine (a serotonergic agonist) and prefeeding was performed to characterize AR-R 15849 as a anorectic agent. In the acute treatment experiments, animals responded on levers for food and ran in a wheel during daily 6-h periods. Administration of AR-R 15849, CCK-8, as well as prefeeding prior to food availability each caused decreases in food intake, and did so without altering motor activity, demonstrating good anorectic specificity. Fenfluramine, on the other hand, decreased running in the wheel during the food availability period, indicating behavioral effects within the dose range causing decreases in food intake. These data confirm that AR-R 15849 can offer advantages over other types of appetite suppressants.

In the subchronic studies, AR-R 14294 and AR-R 15849 consistently inhibited cumulative food intake and significantly reduced body weight over a 9-day period when the drug was administered once daily. The effect on daily food intake was dependent on dose , time, and treatment, with AR-R 15849 at the ED_{50} dose, showing the most potent, longest lasting effect across both the 3-h feeding interval and the 9-day treatment period. Previous studies have shown that AR-R 15849 has a longer duration of action compared to AR-R 14294, most probably due to a longer on–off rate at the CCK-A receptor (30). Other pertinent characteristics between the two compounds are similar, such as metabolic stability, potency, and lipophilicity. Although the reduction of daily food intake was significant during the first hour following subchronic drug administration, this effect lessened during the last two feeding hours over the 9-day test period, as has been reported for another CCK-A agonist, A71623 (2). This lessening of effect could be the result of compensatory hunger increases due to sustained, severe, caloric deprivation, changes in the CCK-A receptor sensitivity, or increased metabolic clearance of the compounds. Similar observations have been cited for CCK-8 treatment, with the suggestion that the most probable cause is the rapid acquisition of behaviors that might overcome the anorectic activity of the drug (8). Therefore, the effect of 9-day AR-R 15849 and AR-R 14294 treatment on food consumption was measured in rats, with and without caloric deprivation, to determine whether tolerance, that is, a diminished anorectic response to the drug over time is influenced by the state of caloric intake. Therefore, AR-R 15849 and AR-R 14294 were administered before food presentation on days 1 and 9 and after food was removed on days 2–8. Both drugs were administered after feeding so that the animals receiving drug would maintain a normal food intake with corresponding drug exposure. Thus, any reduction in anorectic efficacy would be related to subchronic drug exposure (pharmacological tolerance) and not an adaptation to insufficient caloric intake. Both AR-R 14294 and AR-R 15849 inhibited food intake on day 1 and day 9. The reduction in food intake caused by both drugs on day 1 was not significantly different from that observed on day 9, which indicates that pharmacological tolerance did not develop when the drugs were given after feeding. Therefore, the trend toward apparent tolerance observed in the earlier studies does not appear to be pharmacological tolerance (reduced biological half-life or efficacy of the drug) but more likely, an adaptation in behavior due to sustained caloric deprivation. The continued reduction of daily caloric intake by administration of AR-R 15849 could lead to an increase in the hunger drive that would lessen its effects on satiety. It has been suggested that because the actions of CCK peptides are mediated by gastric afferents, they may inhibit gastric emptying and intensify a food-dependent distension. Tolerance could then develop by rapid extinction of the conditioned satiety component of the distension (8).

With respect to the effects on body weight, the sustained decrease produced by AR-R 15849 at the 0.3 μg/kg dose level was significantly greater than that induced in the AR-R 14294 or AR-R 15849 (0.1 μg/kg) treatment groups at all time points following day 2. The enhanced efficacy of AR-R 15849 appears to be related to its greater duration of action compared to AR-R 14294, which possesses equivalent receptor affinity and acute potency (30). Duration of action is thought to be an important factor in determining weight-loss efficacy for other related anorectic compounds (6).

Two different feeding paradigms were used in the present studies: operant responding for food and direct feeding studies. AR-R 15849 was much less potent in suppressing operant feeding than it was in suppressing food intake in the direct feeding studies, and a number of factors could have contributed to the potency difference. For example, a different rat strain was used for those studies (largely because the operant procedure had been previously standardized and characterized in-house in the L.E. strain). Additionally, the type of food used was different between the operant studies and the direct feeding studies (pellets vs. powdered chow), as well as the duration of the daily food access periods (6 vs. 3 h). The size of the testing arenas and the fact that rats had access to a running wheel in the operant studies were two other notable differences. Additionally, it is likely that a major contributor to the difference was the fact that in the operant paradigm, feeding is suppressed by disruption of behavior under the control of the operant schedule and associated stimulus conditions. Although these factors may reflect fundamental differences between the two procedures, it is important to remember that the primary purpose of the operant studies in the present series of experiments was to measure anorectic specificity—decreases in food intake and motor activity at the same time in the same animals. This was clearly demonstrated in the present study.

In summary, appetite suppressant agents such as amphetamine and fenfluramine have proven useful in the treatment of obesity (11). These agents are believed to act, at least in part, through central mechanisms to reduce caloric intake leading to weight loss (5,6,15). However, the use of these agents has been compromised because of several insufficiencies such as, limited efficacy, tolerance, undesired behavioral effects, and associated abuse potential. Unlike these agents, CCK-8 and its analogs such as A-71623 (2), Ac-CCK-7 derivatives (10) and the 1,5-benzodiazepines (1) are believed to induce satiety through a peripheral activation of CCK-A receptors on the vagus nerve rather than by a direct central mechanism. We hypothesized that a peripherally acting selective CCK-A agonist, which is without the pharmacodynamic limitations of CCK-8, would be more likely to inhibit feeding without behavioral side effects. The results of these studies indicate that AR-R 15849, a new selective CCK-A agonist, does indeed possess several advantages over its predecessor AR-R 14294 and other anorectic agents such as fenfluramine. AR-R 15849 administration resulted in inhibition of feeding that was quantitatively and qualitatively similar to CCK-8 and prefeeding. AR-R 15849 evoked decreases in food intake without altering running rates of responding or turns in the running wheel, indicating anorectic specificity. Unlike CCK-8, however, AR-R 15849 administration did not result in compensatory feeding later in the session, as evidenced by an increase

in food intake above control values for CCK-8. Fenfluramine, while efficacious in decreasing food intake, was unable to do so without altering running rates of responding or turns in the running wheel, indicating a less favorable side effect liability. These data describe the efficacy and the relative safety of peripherally acting peptides over centrally acting agents. In this regard, AR-R 15849 has profiled as a potent, selective, long-lasting anorectic agent that significantly decreases body weight over 9 days in the rat, without affecting other behaviors such as locomotor activity. Such an agent could prove useful in the treatment of eating disorders leading to obesity and its related illnesses.

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