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RAPID COMMUNICATION

Type-A CCK Receptors Mediate the Inhibition of Food Intake and Activity by CCK-8 in 9- to 12-Day-Old Rat Pups

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SMITH, G. P., A. TYRKA AND J. GIBBS. *Type-A CCK receptors mediate the inhibition of food intake and activity by CCK-8 in 9- to 12-day-old rat pups.* PHARMACOL BIOCHEM BEHAV **38**(1) 207–210, 1991.—To determine the type of cholecystokinin (CCK) receptor that mediates the inhibitory effects of peripherally administered CCK-8 on food intake and activity in 9- to 12-day-old rat pups, we gave injections of a type-A CCK receptor antagonist, MK-329, or of the type-B CCK receptor antagonist, L-365,260, prior to CCK-8 (IP). MK-329 reversed the inhibitory effects of CCK-8, but L-365,260 did not. This demonstrates that the inhibitory effects of CCK-8 (IP) are mediated by type-A, but not type-B, CCK receptors in pups of this age.

Ontogeny of ingestion CCK antagonists L-365,260 MK-329

IN 1973, we proposed that one of the actions of endogenous cholecystokinin (CCK) released from the small intestine by ingested food was to terminate eating (7). On the basis of subsequent work by us and others with exogenous CCK-8 in adult rats, we refined the hypothesis by stipulating three additional details: 1) the satiating effect of CCK was mediated by CCK interacting with type-A CCK receptors in the abdomen (20); 2) the satiating effect was produced through a paracrine mode of action in the case of CCK-8 (8), but not necessarily for larger forms of CCK, e.g., CCK-22 or CCK-33; 3) the effect of CCK receptor activation was carried to the brain over vagal afferent nerves (23).

The strongest evidence for this hypothesis comes from experiments in adult rats in which an antagonist of CCK, administered prior to a food-intake test, increased food intake due to blockade of the satiating effect of CCK released from the small intestine (1, 5, 6, 10, 11, 16, 17, 21, 22, 25–27).

To test the hypothesis further, we recently investigated the satiating effect of CCK released from the small intestine by soybean trypsin inhibitor (28), the most potent releasing stimulus for CCK in the rat (14). We did this experiment in 9- to 12-day-old rat pups eating independently away from the dam for the first time in order to eliminate any conditioned controls on meal size that may be operating in adult rats. The results supported the hypothesis—pretreatment with trypsin inhibitor decreased the test meal significantly and this inhibition was reversed by MK-329, a potent and specific antagonist of type-A CCK receptors (2).

One of the assumptions underlying our interpretation of that experiment was that MK-329 would antagonize the satiating effect of CCK in the 9- to 12-day-old pup as well as it does in the adult. Another assumption was that the dose of MK-329 (1 mg/kg, IP) was selective for type-A CCK receptors and did not produce its effect through an action on type-B CCK receptors (4, 12, 13). Since neither of these assumptions has been demonstrated in preweanling pups, we tested the effect of MK-329 and of L-365,260, an antagonist of type-B CCK receptors (15), on the inhibition of food intake produced by exogenous CCK-8 in 9- to 12-day-old pups eating independently (9) under the same conditions that we used in the experiment with trypsin inhibitor.

METHOD

Subjects were the offspring of timed-pregnancy Sprague-Dawley rats (Taconic Farms, Germantown, NY). Pregnant females were obtained 1 week before delivery and housed individually in Plexiglas cages on corn cob bedding with water and Purina 5012 Formulab chow available ad lib. Ambient temperature was maintained at 23 ± 2°C and the light phase occurred between 0600

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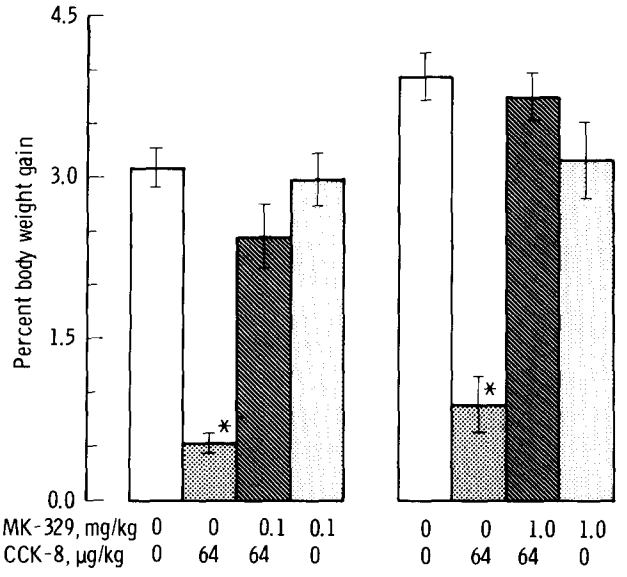

FIG. 1. Data are means ± SE percent body weight gain (%BWG) during the 30-minute intake test. %BWG was significantly smaller after CCK-8 than after vehicle-saline treatment (*$p<0.05$, Tukey's HSD test). Both doses of MK-329 reversed the inhibitory effect of CCK-8 so that %BWG after 0.1 and 1.0 mg/kg MK-329 was not significantly different from the respective vehicle-saline group.

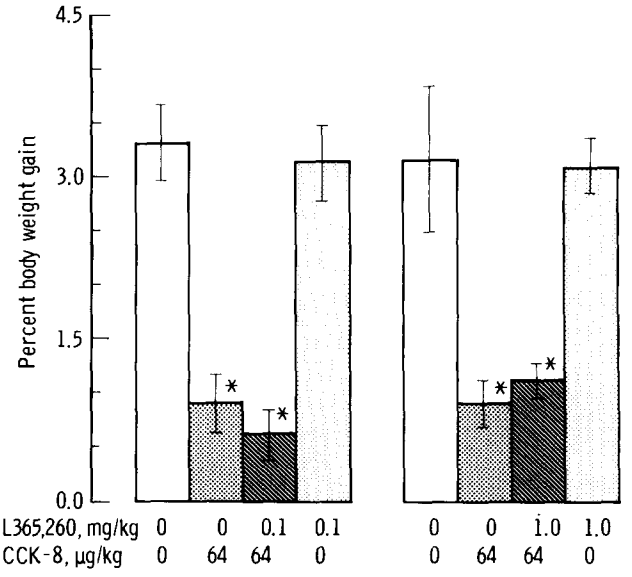

FIG. 2. Data are means ± SE percent body weight gain (%BWG) during the 30-minute intake test. %BWG was significantly smaller after CCK-8 than after vehicle-saline treatment (*$p<0.05$, Tukey's HSD test). Neither dose of L-365,260 changed the inhibitory effect of CCK-8.

and 1800 hours. Litters were culled at 24–48 hours after birth to a maximum of 10 pups. Animals were not handled again until the time of testing except during weekly maintenance. Pups were tested at 9 to 12 days of age; no test condition included more than one animal drawn from a single litter. Each animal was tested once.

Pups were removed from the home cages in the morning, 4 hours prior to the start of the test, and placed in individual containers into a humid, 32°C incubator. After 3.5 hours, the animals received an intraperitoneal injection of MK-329, L-365,260 or vehicle. Fifteen minutes later, pups were removed from the incubator to room temperature. Urination and defecation were induced by manual stroking of the anogenital region with a cotton swab and the urethral meatus was occluded with cyanoacrylate glue. An intraperitoneal injection of CCK-8 (provided by Squibb Medical Research Institute) or its vehicle (0.15 M sodium chloride) was administered 5 minutes before the start of the intake test. Animals were then weighed and placed individually in 1-liter, Nalgene beakers in a humid test chamber maintained at 36°C to 38°C. Tissue paper (Kimwipes, Kimberly-Clark) that had been soaked with 4 milliliters of commercially available Half and Half (Crowley Foods, Inc.) at 37°C covered the floor of each beaker; 1 additional ml was added at 18 to 20 minutes after the test began. The test conditions were identical to those used in our recent report (28).

At 4-minute intervals throughout the test, the behavior of a pup was observed for 5 seconds and was scored according to the method of Robinson et al. (19). The sum of these scores was expressed as total activity. In addition to these observations, the latency (seconds) to initiate mouthing of the tissue paper soaked with milk was measured. If a pup did not initiate mouthing in less than 300 seconds, the latency was considered 300 seconds for data analysis. At the end of the test, the pups were dried, weighed to 0.01 g (XT Top Loading Balance, Fisher Scientific) and intake was measured as percent body weight gain (%BWG).

The first intraperitoneal injection, administered 30 minutes before the intake test, was 0.05 ml of either MK-329 (0.1 or 1.0 mg/kg), L-365,260 (0.1 or 1.0 mg/kg), or the vehicle 0.5% carboxymethylcellulose in distilled water (w/v). The antagonist solutions were prepared at one time and refrigerated between uses. The second intraperitoneal injection, administered 5 min before the test, was 0.05 ml of CCK 8 (64 μg/kg) in 0.15 M sodium chloride or 0.05 ml of 0.15 M sodium chloride. The CCK solution was mixed fresh just before each test. We used a pharmacological dose of CCK-8 that was much larger than doses that have been reported to decrease food intake in rats of this age or younger (19) in order to decrease activity (24) and to increase the possibility that CCK-8 would have access to type-B CCK receptors in the brain. There were 4 treatment conditions consisting of the following pairs of injections at −30 and −5 min in each experiment: 1) vehicle-saline, 2) vehicle-CCK, 3) antagonist-CCK, 4) antagonist-saline. Four experiments were performed. Each experiment tested CCK-8 and one dose of either MK-329 or L-365,260.

Statistical Procedures

Intake was measured as %BWG. The number of animals per treatment condition was 8 to 9 in the two experiments that tested 1.0 mg/kg of the antagonists and 5 to 6 in the two experiments with 0.1 mg/kg of the antagonists. Each experiment—one dose of CCK antagonist and its comparison groups—was analyzed as a separate data set by one-way analysis of variance followed by Tukey's HSD test.

RESULTS

CCK-8 decreased %BWG significantly in each of the 4 experiments (all $F's>9.42$, $p's<0.001$, Figs. 1 and 2), and this decrease in %BWG was completely reversed by both 0.1 and 1.0 mg/kg MK-329 (Fig. 1). In contrast, neither dose of L-365,260 had an effect on the decreased intake produced by CCK-8 (Fig. 2). Finally, both MK-329 and L-365,260 failed to increase %BWG when given alone (Figs. 1 and 2).

The antagonists had the same differential effect on the effects

TABLE 1

EFFECT OF CCK-8 ANTAGONISTS ON ACTIVITY AND LATENCY TO INGESTION

Condition	Latency to Ingestion	Total Activity
	MK-329 0.1 mg/kg	
Vehicle/Saline	208 ± 41 (6)	4.7 ± 0.7 (4)
Vehicle/CCK-8	300 ± 0 (6)	2.3 ± 0.4 (4)*
Antagonist/CCK-8	257 ± 28 (5)	4.8 ± 0.7 (5)
Antagonist/Saline	178 ± 51 (6)	5.5 ± 0.5 (6)
	MK-329 1.0 mg/kg	
Vehicle/Saline	126 ± 31 (6)	8.9 ± 0.7 (4)
Vehicle/CCK-8	300 ± 0 (6)*	3.0 ± 0.8 (4)*
Antagonist/CCK-8	161 ± 25 (5)	7.2 ± 0.5 (5)
Antagonist/Saline	169 ± 33 (9)	6.8 ± 0.7 (9)
	L-365,260 0.1 mg/kg	
Vehicle/Saline	92 ± 30 (6)	7.1 ± 1.4 (4)
Vehicle/CCK-8	300 ± 0 (6)*	1.6 ± 0.6 (4)*
Antagonist/CCK-8	300 ± 0 (5)*	2.7 ± 0.5 (5)*
Antagonist/Saline	133 ± 38 (6)	7.5 ± 0.6 (4)
	L-365,260 1.0 mg/kg	
Vehicle/Saline	154 ± 38 (6)	5.6 ± 0.9 (4)
Vehicle/CCK-8	300 ± 0 (6)*	2.4 ± 0.5 (4)*
Antagonist/CCK-8	282 ± 15 (5)*	2.6 ± 0.7 (5)*
Antagonist/Saline	166 ± 36 (8)	7.2 ± 1.4 (8)

Note. Total activity data are mean ± SE of total activity scores (see the Method section). Latency data are mean ± SE seconds to initiate eating. Number in parentheses is the number of rats tested. Significantly different from value after vehicle/saline, *$p<0.05$, Tukey's HSD test.

of CCK-8 on latency and activity (Table 1). CCK-8 produced significantly longer latencies in three of four tests $p<0.05$; the failure to prolong latency in the experiment in which MK-329 (0.1 mg/kg) was used appears to be due to the unusually long latency observed in the vehicle saline-treated pups. MK-329 reversed the prolonged latencies produced by CCK-8, but neither dose of L365,260 had a significant antagonistic effect (Table 1).

CCK-8 also decreased the total activity scores significantly ($p<0.05$, Table 1). Both doses of MK-329 reversed this inhibition, but neither dose of L365,260 did (Table 1).

DISCUSSION

The major result of these experiments is that prior administration of MK-329 reversed the inhibition of food intake and of activity produced by CCK-8, but prior administration of L-365,260 did not. The efficacy of MK-329 confirms the results in adult rats for food intake (3) and for activity (24), and extends them to 9- to 12-day-old pups.

The failure of L365,260 to change the inhibitory effect of CCK-8 on food intake confirms the results in adult rats (3) and extends them to 9- to 12-day-old rats. The failure of L365,260 to change the inhibitory effect of CCK-8 on activity has not been reported before.

The differential effects of the two antagonists demonstrate that even when a large dose of CCK-8 is used, all of its inhibitory effects on food intake and activity are mediated by interaction with type-A CCK receptors. We found no evidence for any contribution of type-B CCK receptors. Previous work has demonstrated the presence of type-A CCK receptors in the gastrointestinal tract of rats at this age (18). The site of the type-A CCK receptors that mediate these effects of CCK-8 is probably in the abdomen, but our experiments do not exclude the possible involvement of central type-A CCK receptors.

The importance of type-A CCK receptors for mediating the inhibition of food intake by the peripheral administration of *exogenous* CCK-8 demonstrated here supports our interpretation of our previous results that used soybean trypsin inhibitor (STI) to release *endogenous* CCK from the small intestine in 9- to 12-day-old pups (28). In that experiment, we reversed the inhibition produced with STI by pretreatment with MK-329 and inferred that endogenous CCK released by STI produced inhibition of food intake by interacting with type-A CCK receptors.

Although MK-329 reversed the inhibition of food intake produced by exogenous CCK-8 in these experiments, neither MK-329 nor L-365,260 increased food intake when given alone. This is evidence that the satiating effect of the mixture of cream and milk ingested by the 9- to 12-day-old pups under these experimental conditions was not mediated through endogenous CCK despite the presence of sufficient type-A receptors to mediate an inhibition of food intake to exogenous CCK-8 and the ability of endogenous CCK to inhibit food intake when released by soybean trypsin inhibitor (28). Further experiments are required to clarify this problem.

In summary, these experiments demonstrate that peripherally administered CCK-8 inhibits food intake and activity in 9- to 12-day-old rat pups through an interaction with type-A, but not type-B, CCK receptors.

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