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The Effect of Calcitonin Gene-Related Peptide on Food Intake Involves Aversive Mechanisms

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KRAHN, D. D., B. A. GOSNELL, A. S. LEVINE AND J. E. MORLEY. *The effect of calcitonin gene-related peptide on food intake involves aversive mechanisms.* PHARMACOL BIOCHEM BEHAV **24**(1) 5–7, 1986.—Calcitonin gene-related peptide (CGRP), the product of the calcitonin gene produced primarily in the central nervous system, has been shown to decrease food intake when administered intracerebroventricularly (ICV). Testing of CGRP (ICV) in both single bottle conditioned-aversion and differential starvation paradigms was done. In both paradigms, results using CGRP were consistent with those predicted for aversive agents. Therefore, CGRP apparently decreases feeding via aversive mechanisms.

Calcitonin gene-related peptide Feeding Aversion Satiety

RECENTLY, calcitonin gene-related peptide (CGRP), a product of alternative processing of RNA transcripts from the calcitonin gene, has been characterized [1]. mRNA encoding CGRP is found in several brain nuclei important in ingestive behavior [8]. Though CGRP was suggested as the primary gene product in the brain, recently CGRP was detected by radioimmunoassay in the thyroid as well [3]. We recently reported that CGRP administered intracerebroventricularly (ICV) resulted in decreased food intake in both rats feeding spontaneously at night and rats feeding after 24 hours of starvation [5]. Central administration of CGRP was markedly more potent than peripheral CGRP administration [5]. Calcitonin, another product of the calcitonin gene, likewise decreases food intake in rats as well as other species [4, 6, 7, 9].

The mechanisms by which CGRP causes this decrease in food intake is unknown. This effect could be explained as either a natural satiating effect of CGRP or a behaviorally disruptive, aversive effect of the peptide [5]. In previous experiments, however, behavioral ratings done each minute for 2 hours after injection of CGRP failed to demonstrate any behaviors that precluded food intake. In order to clarify the mechanism, we tested CGRP in two experimental models proposed to differentiate aversive from satiating chemicals. We used a single choice taste aversion method in which injections of CGRP were paired with a novel-tasting liquid as well as the differential starvation method proposed by Billington *et al.* [2]. The former test relies on the rationale that an animal will learn to decrease subsequent intake of a substance if after the initial ingestion of that substance the animal is treated with a sickness- or malaise-inducing agent. It has been reported that calcitonin does not cause such a conditioned taste aversion [4]. The latter test depends primarily on the following rationale: A satiety factor should inhibit feeding less in an animal starved for 48 hours than one starved 12 hours, while an agent that acts through aversive mechanisms should be relatively unaffected by the degree of hunger in the experimental subjects. This method was used to demonstrate that cholecystokinin behaves as predicted for a satiety factor and that lithium chloride behaves as predicted for an aversive factor [2].

METHOD

Experiment 1: Differential Starvation Method

The experimental subjects were 46 male Sprague-Dawley rats weighing 250–350 grams. Each animal was used twice with five to seven days between trials. Animals had resumed normal 24 hour consumption by the time of re-testing. The animals were housed singly in a light-controlled room (lights on 0700–1900). Injections and testing were done in the home cages.

Under Nembutal anesthesia, stainless steel guide cannulae were stereotactically implanted into the right lateral ventricle. Coordinates for the cannula tip were 0.5 mm posterior and 1.5 mm lateral to bregma, and 3.5–3.75 mm ventral to the surface of the skull. The incisor bar was 1.5 mm below the inter-aural line. Cannulae were anchored to skull screws with dental acrylic, and at least 5 days recovery were allowed before testing.

Each rat was tested twice during the experiment: once after 12 hours of starvation and once after 48 hours of star-

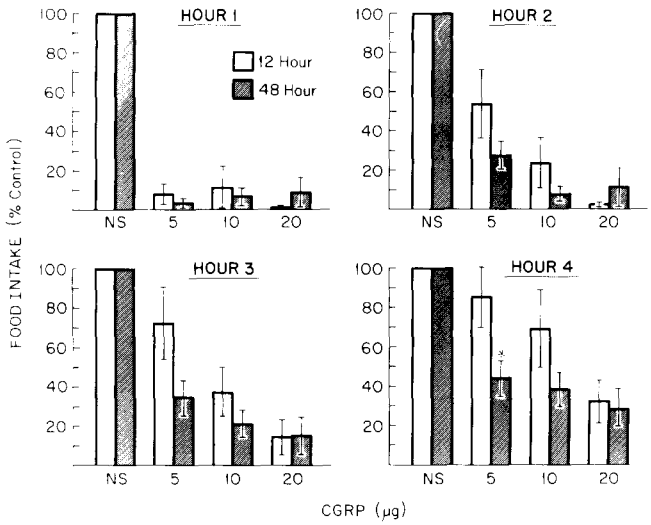

FIG. 1. The results of the differential starvation test are shown. Food intake is expressed in percentage of control intake. *Indicates a significant increase in food intake expressed as a percentage of control in the 12 hour starved rats vs. the 48 hour starved rats.

vation, with equal numbers of animals experiencing each treatment (i.e., 48 hour starvation or 12 hour starvation) first. Starvation periods ended at 1230–1330 hours, at which time the rats were given intracerebroventricular injections of 0, 5, 10, or 20 μg of CGRP in a 10 μl volume of normal saline. Each animal received a different dose of CGRP at each testing. The doses received were otherwise randomly assigned. These doses were chosen on the basis of their effectiveness at suppressing food intake, as reported in previous experiments [5]. After injection, two preweighed Purina rat pellets were placed on the floor of the cage. Each hour, for 4 hours, the remaining pellets and the spillage were collected and weighed with the difference of this measure from the original weight equaling the food eaten.

Data from the two trials were pooled and analyzed with a two-way analysis of variance (ANOVA) (starvation × dose) at each time point.

Experiment 2: Conditioned Taste Aversion

Twenty-four male Sprague-Dawley rats housed in the same quarters as those described above were fitted with chronic indwelling ventricular cannulae. For four days following surgery rats were given ad lib food and water. Then, rats were trained to reliably drink water in a defined period by restricting access to water to only 1200–1300 hours each day for 5 days. The next day rats were given a preweighed amount of 0.15% saccharin solution in their usual water bottles from 1230–1300 hours. Immediately after the 30 minutes of exposure to saccharin, a 10 μl ICV injection containing 10 μg of CGRP (a dose shown to be effective in reducing food intake) or normal saline was administered or a peripheral injection of one ml of NS or one ml of 0.65 molar LiCl was given. To quantify fluid intake, the bottles containing the saccharin solution were reweighed and the amount drunk was calculated. After a one day return to the one hour/day access to water regimen (during which water intake was measured), the animals were re-exposed to saccharin in

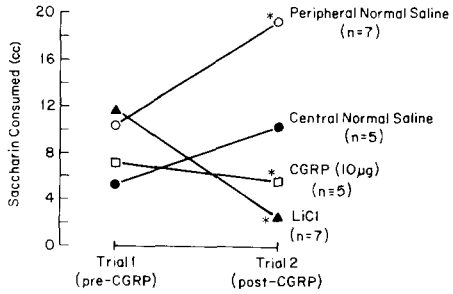

FIG. 2. The results of the single bottle taste aversion test are shown. *Indicates that the saccharin intake on trial 2 was significantly different ($p<0.05$) from the intake on trial 1 for the same group.

the same manner as above and the amount drunk on this test day was measured.

t-Tests comparing post-exposure intake to pre-exposure intake in a repeated measures design for each of the four treatment groups were carried out.

RESULTS

Differential Starvation Method

The results support two conclusions. First, the fact that those animals starved for 48 hours and treated with NaCl did eat much more than those saline-treated rats starved for 12 hours (9.16±1.62 g in 4 hr after 48 hr starvation vs. 4.3±0.66 g after 12 hr starvation) suggests that one of the premises of this test is fulfilled. Second, the data show that the doses of CGRP used did decrease food intake, as an analysis of variance showed a significant dose effect at 1, 2, 3 and 4 hours. To compare the magnitude of suppression of food intake across starvation conditions, data were expressed as percentages of their respective control intakes and re-analyzed (excluding control values) by ANOVA (Fig. 1). No significant starvation, dose or interaction effect was noted at 1, 2 or 3 hours. At four hours there was a significant effect of starvation, $F(1,64)=5.71$, $p<0.02$, and of CGRP dose, $F(2,64)=3.64$, $p<0.05$. However, examination of Fig. 1 reveals that the group starved for 48 hours actually ate less on a percentage basis than did those starved for 12 hours at each dose of CGRP. Thus, the only point at which there was a significant starvation effect was at 4 hours and it was in the opposite direction from that predicted for a satiety agent.

One-Bottle Conditioned Aversion Method

Results of this testing are shown in Fig. 2. It shows that those rats treated with normal saline both increased their intake on successive trials while those treated with LiCl and CGRP decreased their mean intake. This treatment did not affect water intake on the intervening day, however, when CGRP-treated rats drank more than the other three groups (CGRP=38.4±12.1 cc; LiCl=22.5±9.7; control normal saline=17.5±4.8; peripheral normal saline=30.9±3.9). This was supported by the results of a two-way ANOVA which revealed a significant drug effect, $F(3,20)=6.54$, $p<0.003$. *t*-Tests were then used to compare the intake of saccharin of each group on Trial 2 vs. Trial 1 (LiCl, $t(6)=8.53$, $p<0.05$; CGRP, $t(4)=3.78$, $p<0.05$; central normal saline, $t(4)=1.52$,

NS; peripheral normal saline, $t(6)=7.31$, $p<0.05$; all t-tests 2-tailed). Thus, LiCl and CGRP administered after a novel food both resulted in decrements in subsequent intake of that substance. The decrement due to CGRP was smaller than that after LiCl, however. Saline administration (ICV) after saccharin did not have a statistically significant effect on subsequent intake, while those animals treated with peripheral normal saline showed a significant increase.

DISCUSSION

Any substance that decreases feeding should be subjected to scrutiny regarding its aversive and/or satiating qualities. While it is unclear whether aversion and satiety are mutually exclusive categories, results on certain testing paradigms have been used to distinguish operationally substances in each group. Despite the lack of overt toxicity or behavioral disruption on previous formal behavioral observation, we chose to test CGRP in two of these paradigms. We selected two paradigms, conditioned taste aversion and differential starvation, that depend on different rationales for distinguishing satiety from aversion. Yet in both test situations CGRP behaved as these models predicted an aversive substance would. In the differential starvation method, CGRP decreased food intake on a percentage basis to a greater degree in those rats that were more hungry than in those that were less hungry. Interestingly, but inexplicably, those rats starved 12 hours actually ate more on a percentage basis than those starved for 48 hours when given CGRP but not when given saline. CGRP may interact with other factors affecting feeding in a complex manner that changes with length of starvation. Billington [2] showed that calcitonin also acted as an aversive substance in this paradigm.

In the conditioned taste aversion paradigm, the CGRP-exposed rats decreased their subsequent saccharin intake (although not as much as LiCl treated rats) while those exposed to saline by ICV or peripheral routes did not decrease their intake. Thus, CGRP resulted in a conditioned taste aversion in contrast to the results reported by Freed *et al.* for calcitonin [4]. While a two-bottle taste aversion paradigm may provide a more sensitive test of aversion, the demonstration of aversion on the less sensitive single bottle test is sufficient to identify CGRP as aversive. It is, of course, possible that the reduced intake of saccharin in the taste aversion paradigm resulted from a long-lasting satiety effect of CGRP. However, this is unlikely as the CGRP-treated animals actually drank more water than saline-treated animals on the day between the first and second exposures to saccharin.

Thus, two products of the calcitonin gene decrease feeding [4,5]. While conceptual difficulties remain in categorizing substances as aversive, results in two currently available paradigms define the process by which CGRP decreases food intake as involving an aversive process.

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