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Anorectic Effects of Amylin in Rats Over the Life Span

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MORLEY, J. E., P. M. K. MORLEY and J. F. FLOOD. *Anorectic effects of a mylin in rats over the life span.* PHARMACOL BIOCHEM BEHAV 44(3) 577–580, 1993.—Amylin is a pancreatic peptide hormone that has been demonstrated to antagonize a number of the effects of insulin. This study demonstrated that amylin, when administered IP, decreased food intake in 4-month-old rats at doses of 50, 75, and 100 μg/kg. Amylin was slightly more potent at suppressing food intake at 13 months of age and less potent at decreasing food intake in 21- and 25-month-old rats, but the difference was not significant. These studies show that amylin is another peripheral anorectic peptide. They do not implicate amylin in the pathogenesis of the anorexia of aging.

Amylin Anorexia Aging CGRP Food intake Diabetes Mellitus

AMYLIN is a 37 amino acid polypeptide originally isolated from the pancreas of patients with Type II diabetes mellitus and also from insulinoma tissue (7). Amylin has also been demonstrated to be present in the islets of Langerhans of normal rats (25). Amylin is coreleased with insulin (13), inhibits insulin secretion (24), and inhibits insulin-induced glycogen synthesis in muscle (18). Amylin is structurally similar to calcitonin gene related peptide (CGRP) with approximately a 40% homology (7).

A number of gastrointestinal and pancreatic peptide hormones have been demonstrated to modulate feeding when administered IP (19,27). Previously, we reported that IP administration of amylin decreased feeding in normal mice and in streptozocin-induced diabetes mellitus mice (20). In the same study, we found that peripherally administered amylin was more potent than intracerebroventricularly administered amylin at decreasing food intake. CGRP was previously reported to decrease food intake when administered intracerebroventricularly but not when administered peripherally (15,16). Chance and co-workers (1) studied the anorectic effects of amylin in rats. They demonstrated that intrahypothalamic amylin was anorectic and more potent than CGRP. In a second study, they demonstrated that intrahypothalamic amylin inhibited neuropeptide Y induced feeding in rats (6). For these reasons, we decided to investigate whether amylin would decrease food intake in rats when administered peripherally.

It is now well accepted that with advancing age many animal species tend to develop a decrease in food intake, which has been designated as the anorexia of the elderly (22). In part, this syndrome is indirectly related to a decrease in the ability of endogenous opioids to stimulate feeding (12,17). Silver, Flood, and Morley (26) demonstrated that with advancing age the gastrointestinal peptide hormone, cholecystokinin, has a more potent anorectic effect in mice.

Amylin has been postulated to play a role in the pathogenesis of Type II diabetes mellitus (7), a disease that occurs with increasing frequency with advancing age (21). It, therefore, seemed reasonable to study the effect of amylin on food intake over the life span.

METHODS

Fisher 344/Brown Norway FI male rats 4, 13, 21, and 25 months of age were obtained through the NIA from Harlan Industries. This new strain has been validated as a suitable model for aging (5). We received 10 rats in each group but one of the 25-month- old rats was completely hairless and was not utilized for any of the studies. Rats were accustomed to the laboratory for 10 days and were previously used in an experiment examining the effect of angiotensin II on fluid intake. Seven days after the angiotensin II experiment ended, the effect of amylin was studied. Rats were maintained on a light schedule with lights on at 0600 h and off at 1800 h. Rats

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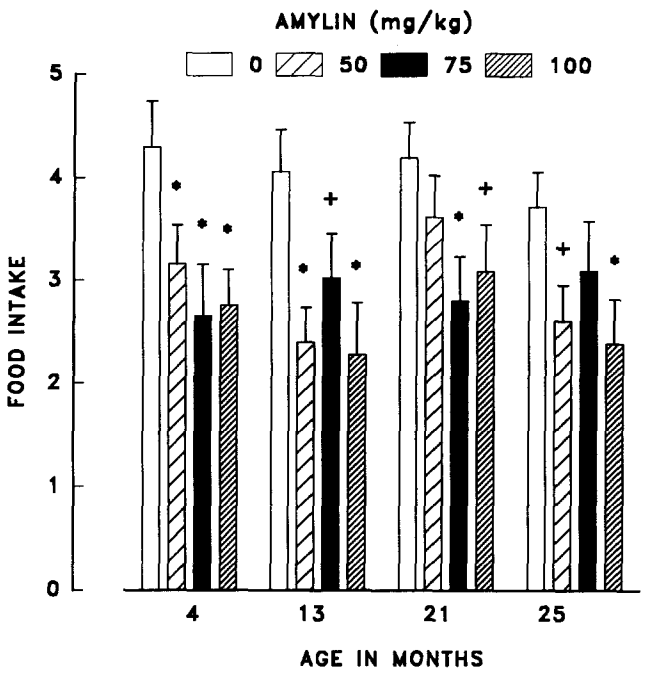

FIG. 1. Effect of amylin on food intake 0 to 30 min postinjection. Error bar represents the standard of the mean.
+ = means differing from the mean of the control groups (0 μg) at $p < 0.05$ or at $*p < 0.01$.

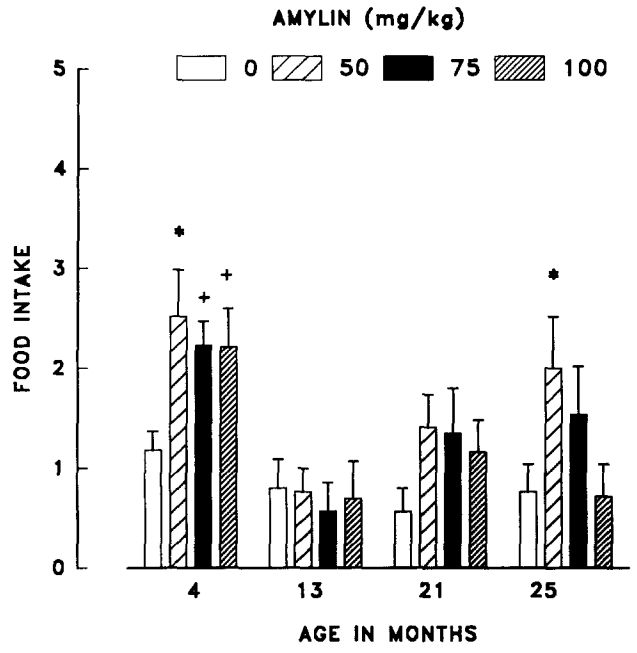

FIG. 2. Effect of amylin on food intake 31 to 60 min postinjection. The error bar represents the standard of the mean. The + = means differing from the mean of the control groups (0 μg) at $p < 0.05$ or at $*p < 0.01$.

were fed Purina 5001 rodent chow ad lib except for the evening before the experiments when they were food deprived at 1500 h. All experiments were begun between 0700 and 0800 h the next morning.

To measure food intake, the Purina food pellets were preweighted and placed in the food hopper. At 30 and 60 min after receiving an injection of amylin or saline, the food pellets were reweighed to determine the amount of food consumed. This strain of rat does not powder their food and therefore the spillage was negligible. Amylin was prepared by dissolving it in saline to a concentration that permitted 0.01 ml/g body weight to be injected.

Rats were food deprived on four occasions, with at least 3 days between subsequent deprivation periods. Rats received amylin (Peninsula Laboratories, Belmont, CA) IP in doses of 0, 50, 75, and 100 μg/kg utilizing a randomized block design. All rats received all doses with at least 3 days between drug testing. Food was made available immediately after the injection and food intake was measured at 30 min and 60 min. The grams of food consumed were analyzed by two separate two-way analysis of variance (ANOVA) (age by dose of amylin); one was for 0 to 30 min eating and the other for 31 to 60 min eating. Tukey's *t*-test was used to determine if treatment group means differed significantly from the control mean within each age group (14).

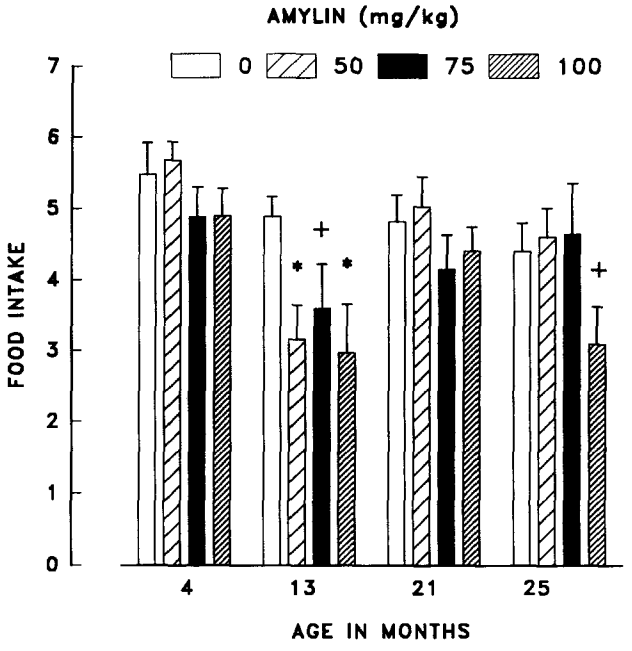

FIG. 3. Effect of amylin on food intake 0 to 60 min postinjection. The apparent lack of effect in all but the 13-month-old rats was due to rebound eating during the second 30-min test period while the poor effects in 21- and 25-month-old rats was due to a combination of reduced effectiveness of amylin during the first 30 min and a tendency toward rebound eating during the second 30 min. This suggests overall that 13-month-old mice are the most sensitive to the anorectic effect of amylin. The error bar represents the standard of the mean. The + = means differing from the mean of the control groups (0 μg) at $p < 0.05$ or at $*p < 0.01$.

RESULTS

At 30 min, the main effect of the dose of amylin on food intake was significant [$F(3, 135) = 10.98, p < 0.001$], but the main effect of age on food intake was not ($F = 1.41$) nor was the interaction of dose by age ($F = 0.74$). Within the main effect of dose, the overall mean of each dose differed significantly from the mean of the saline treated groups at $p < 0.01$. Figure 1 shows the food consumption by age group resulting from administration of amylin. In 4- and 13-month-old rats, all doses significantly suppressed food intake compared to the saline control. At 21 months of age, only rats receiving 75 and 100 μg/kg showed significant suppression of food intake. For rats 25 months of age, 100 μg/kg suppressed feeding relative to the saline control at $p < 0.01$, 75 μg/kg did not suppress feeding significantly and the mean food intake of the group receiving 50 μg/kg of amylin only differed from the mean of the control group at $p < 0.05$.

A separate ANOVA run on food intake over 31 to 60 min test period, indicated that the main effect of amylin [$F(3, 136) = 4.71, p < 0.005$] and age [$F(3, 136) = 11.18, p < 0.001$] were significant but the interaction was not. During the second half of the test, it is clear that overall the effect of amylin on food suppression was greatly reduced. The significant effect was due to significant rebound eating in 4-month-old rats treated with amylin and a tendency for rebound eating in 21- and 25-month-old rats (Fig. 2). The lack of significant rebound eating, in 13- and to a lesser extent in 21-month-old rats, implies that amylin continued to exert an anorectic effect over this time period but was not detectable because of reduced eating by the control groups. The significant main effect of age was due mostly to rebound eating in 4-month-old rats and its absence in most of the older rats.

A separate ANOVA run on food consumption over the entire 60-min-test period yielded significant main effects for dose of amylin [$F(3, 136) = 4.00, p < 0.01$] and age [$F(3, 136) = 8.78, p < 0.001$]. It is clear from Fig. 3 that the significance of both main effects was primarily due to a significant reduction in food intake of 13-month-old rats treated with amylin.

DISCUSSION

This study demonstrates that, in rats, amylin is a peripherally active anorectic agent. This extends the previous studies of Chance and co-workers (1,6), which reported that intrahypothalamic amylin decreased food intake. Based on average body weight per group, the lowest total amount of amylin to inhibit food intake significantly was 9.9 μg in the 4-month-old rats and 27.5 μg in the 13-month-old rats. While these amounts are higher than the 1 to 2 μg injected intrahypothalamically by Chance et al. (1,6), it seems unlikely, considering the volume of distribution and the small amount of peptide that crosses the blood-brain-barrier (2), that the peripherally injected amylin is producing its effect on feeding by a direct effect on the hypothalamus. Thus, it seems that amylin like cholecystokinin (11) and bombesin (10) can reduce food intake by independent central and peripheral mechanisms.

Based on our experience over a decade of studying the effect of sulfated cholecystokinin octapeptide (CCK-8S) on feeding, utilizing paradigms similar to the one used for amylin in this study, we have found that doses of 2 to 10 μg/kg are necessary to inhibit feeding in rats (23); others have found lower doses to be effective (11). We have found a consistent decrease of food intake using 0.5 μg/kg of CCK-8S in mice (8). The amylin molecule is approximately four times heavier than CCK-8S. Amylin circulates at a concentration of 5.2 pMol after meals (4) while CCK-8S peaks at 3.2 pMol after meals (3). Amylin may be better thought of as a circulating hormone like glucagon since it inhibits feeding in the same dose range as glucagon (9).

These studies suggest that amylin is most effective in fully mature (13- month-old) rats, as this was the only group that failed to show rebound eating during the second half of the feeding study indicating that amylin continued to exert some anorectic effect. This is clearly demonstrated in Fig. 3, where the 1-h food consumption is inhibited. The failure to see suppression in the other age groups over the full 60 min was due to significant rebound eating in the second 30-min period in the 4-month-old rats (Fig. 2). The lack of suppression in the 21- and 25-month-old rats was due to a reduced effect on food intake during the first 30 min and a tendency for rebound eating during the second 30 min. Our previous studies indicated that CCK-8S was more effective in older mice (26) and may play a role in the pathogenesis of the anorexia of aging (22). Our data do not support a role of amylin in the development of the anorexia seen in older animals as no marked change in the dose-response effect was observed.

In conclusion, amylin joins glucagon as a pancreatic hormone that decreases food intake after peripheral administration in rats. Further studies will be necessary to determine the mechanism(s) by which amylin reduces food intake after peripheral administration.

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