0013-7227/00/$03.00/0
Endocrinology

Vol. 141, No. 2
Printed in U.S.A.

Amylin: A Novel Action in the Brain to Reduce Body Weight*

PAUL A. RUSHING, MARY M. HAGAN, RANDY J. SEELEY, THOMAS A. LUTZ, AND STEPHEN C. WOODS

Department of Psychiatry (P.A.R., M.M.H., R.J.S., S.C.W.), College of Medicine, University of Cincinnati, Cincinnati, OH 45267-0559, USA; and Institute of Veterinary Physiology (T.A.L.), University of Zürich, Winterthurerstrasse 260, CH 8057 Zürich, Switzerland

ABSTRACT

Amylin is a 37-amino acid peptide hormone that is co-secreted with insulin by pancreatic B cells in response to a nutrient stimulus (e.g., during meals). To test the hypothesis that amylin acts within the brain to reduce long-term food intake and body weight, we examined the effects of acute and chronic 3rd-ventricular (i3vt) infusion of low doses of amylin on food intake and body weight in rats.

In one experiment, separate groups of *ad lib*-fed male Long Evans rats were given one i3vt infusion (3 μl over 30 s) of synthetic cerebrospinal fluid vehicle or 1 to 100 pmol amylin, and food intake and body weight were monitored for 7 days. Amylin potently and dose-dependently reduced 1-h food intake, with all doses producing significant reductions. The largest dose (100 pmol) significantly reduced 24-h intake by over 30%. The effect was persistent in that both 7-day cumulative food intake and body weight change were significantly decreased over the 7 days following a single injection of 100 pmol of amylin.

Other groups of rats received continuous i3vt infusion (0.5 μl/h volume) of saline or 2.0 pmol/h amylin via osmotic minipumps over 10 days. Food intake over the 10-day infusion was significantly suppressed in amylin-treated rats as compared to that of controls. Consequently, by the 4th day of infusion, amylin rats weighed significantly less than baseline relative to saline controls, and this difference persisted throughout the remainder of the infusion period. At sacrifice (Day 10), the percent of body weight from retroperitoneal fat depots was significantly lower in the amylin-treated rats, indicative of a reduction of total body adiposity.

In summary, the results support the hypothesis that amylin acts as a signal to the brain contributing to the maintenance of long-term energy balance.

AMYLIN IS A 37-amino acid peptide hormone that is co-secreted with insulin by pancreatic B cells in response to a nutrient stimulus (1). Until recently, amylin has been considered to be mainly a controller of various peripheral metabolic functions including the control of blood glucose and the rate of stomach emptying (2). However, analogously to what has been discovered for many other digestion-related peptides, amylin has also become implicated in the normal control of food intake. Several groups have reported that when amylin is administered to rats or mice prior to being given a meal, food intake is reduced (3).

The vast majority of data relating amylin to the control of food intake has centered on the short-term control of meal size. This is because amylin is secreted in response to meals (4) and when administered acutely into the periphery, its effect on food intake is relatively transient (5, 6), much like that observed for other putative satiety signals such as gastrin-releasing peptide and cholecystokinin (7). Central administration of amylin also reduces short-term food intake (8, 9, 10). However, the potential long-term effects of central amylin administration have not been pursued, perhaps due to the prevailing mindset that amylin impacts food intake purely as a satiety factor that limits meal size.

There is evidence, however, that amylin also affects food intake and body weight over periods of days. For example, continuous subcutaneous infusion of amylin via osmotic minipumps has been reported to reduce long-term food intake and body weight in rats (11). In addition, mice with a targeted deletion of the amylin gene, resulting in amylin deficiency, weigh more than wild-type controls (12). Thus, amylin may contribute to the maintenance of long-term energy balance.

In fact, in addition to its anorexic action, amylin shares many other properties with established adiposity signals such as insulin and leptin (13). Amylin, like insulin and leptin, is synthesized peripherally and is rapidly and efficiently transported across the blood-brain barrier into discrete brain regions (14, 15), including the hypothalamus, where populations of amylin binding sites are located (16, 17, 18). Importantly, the circulating levels of all three peptide hormones are directly correlated with body adiposity (13, 19).

We therefore hypothesized that amylin's role in the control of food intake is not limited to that of purely a satiety signal that brings individual bouts of ingestion to an end, but also serves as an adiposity signal acting within the brain to regulate long-term food intake, body weight and adiposity. Here, we present data consistent with this hypothesis.

Materials and Methods

Animals and Housing

Adult male Long Evans rats (Harlan, Indianapolis, IN) were individually housed in standard plastic tub cages with corn-cob bedding. The rats were maintained on a 12/12-h light-dark cycle (lights off, 1300 h) in a temperature-controlled vivarium. Pelleted laboratory chow (Harlan Teklad LM 485 Mouse/Rat Diet) and tap water were continuously available ex-

Address all correspondence and requests for reprints to: Paul A. Rushing, Ph.D., Department of Psychiatry, University of Cincinnati, P. O. Box 670559, Cincinnati, OH 45267-0559, USA.

* This work was supported by NIH grants DK17844 and DK54080.

Received 10/07/99

cept as otherwise indicated. This work complied with guidelines of the *National Institutes of Health Guide for Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee (IACUC).

Stereotaxic Surgery

Rats were anesthetized with intraperitoneal injection of 60 mg/kg ketamine (Ketaset, Fort Dodge, Iowa) and 8 mg/kg xylazine (Rompun, Mobay, Shawnee, KS). A 21-gauge stainless-steel guide cannula (Plastics One, Roanoke, VA) was implanted in the skull of each rat with its tip aimed at the 3rd-cerebral ventricle (i3vt). With bregma and lambda at the same vertical coordinate, the sagittal venous sinus was carefully displaced laterally with a metal probe. The guide cannula was then lowered directly on the midline, 2.2 mm posterior to bregma, to a point 7.4 mm ventral to dura, and was fixed to the skull with anchor screws and dental acrylic. Cannulas were fitted with removable obturators that extended 0.5 mm beyond the tip. One week after surgery, cannula placement was confirmed by infusion of 10 ng angiotensin II in 1 µl of saline while the rats were water replete. Rats that did not drink at least 5 ml of water in 60 min were not used.

Acute 3rd-Ventricular Amylin Infusion

To investigate the hypothesis that amylin is a signal to the brain that reduces long-term food intake and body weight, we first examined the effects of bolus i3vt infusion of amylin. Immediately prior to lights out, separate groups of *ad lib*-fed rats were given one i3vt infusion (3 µl volume over 30 s) of synthetic cerebrospinal fluid vehicle (n = 8) or 1 (n = 8), 10 (n = 9) or 100 (n = 8) pmol amylin (American Peptides, Sunnyvale, CA, 1 pmol amylin = 0.004 µg). Food intake and body weight were monitored daily for the next 7 days.

Chronic 3rd-Ventricular Amylin Infusion

In order to produce sustained central elevations of amylin, other groups of rats were continuously infused with amylin (2.0 pmol/h) or saline vehicle into the 3rd cerebral ventricle via osmotic minipumps. After rats received i3vt cannulas, and placement had been confirmed by the angiotensin drinking test, rats were weight-matched into two groups of 6 rats each. Each rat was anesthetized with the same ketamine/xylazine mixture used in the i3vt cannula surgery, and an osmotic minipump (Alzet, Palo Alto, CA, Model 2002; 14-day capacity at 0.5 µl/h infusion rate) containing the appropriate solution (sterile 0.9% saline or amylin at the concentration necessary to deliver 2.0 pmol/h) was implanted subcutaneously into the intrascapular pocket. The pump was then attached via polyethylene tubing to the i3vt cannula. After implantation, food intake and body weight were monitored daily for 10 days. The rats were then sacrificed, and retroperitoneal (RP) fat pads were dissected and weighed.

Analysis of Data

For the dose-response data from the acute i3vt amylin tests, repeated-measures analyses of variance (ANOVAs) were employed, followed by Newman-Keuls tests for multiple comparisons ($p < 0.05$, two-tailed). For the data from the chronic amylin experiment, independent t-tests ($p < 0.05$, two-tailed) were used to compare the amylin and vehicle conditions.

Results

Acute 3rd-Ventricular Amylin Infusion

As can be seen in Figure 1A, amylin potently and dose-dependently reduced 1-h food intake, $F(3, 29) = 22.58$, $p < 0.001$, with all doses producing significant reductions. A significant reduction of 24-h intake by amylin was also apparent, $F(3, 29) = 6.87$, $p = 0.001$, with a significant suppression of over 30 % observed after the 100 pmol dose (Fig. 1B).

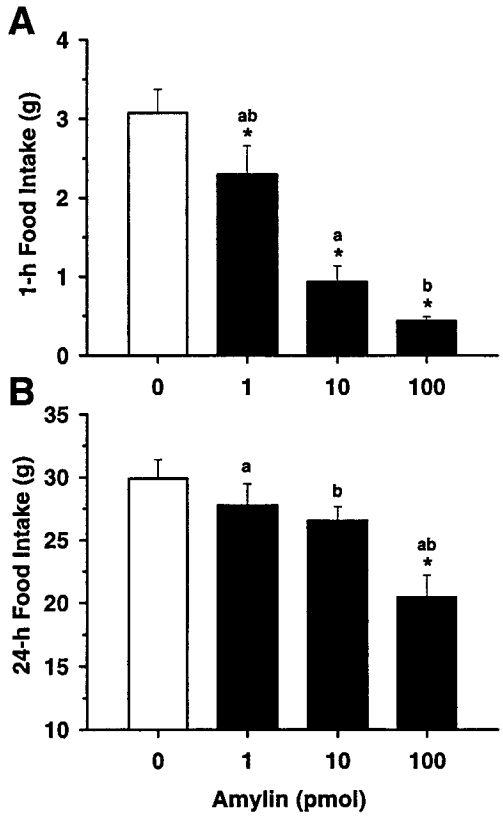

FIG. 1. A. Mean 1-h food intake after a single bolus infusion of synthetic CSF vehicle (0 pmol) or 1, 10, or 100 pmol amylin into the 3rd cerebral ventricle (i3vt) in rats. B. Mean 24-h food intake after vehicle or i3vt amylin. * Significantly different from 0 pmol, $p < 0.05$, *error bars* as ± SEM. Like letters indicate significant differences between conditions.

After the initial suppression of 24-h food intake, no significant reduction of daily, 24-h intake was observed for the remainder of the week. However, no apparent compensation for the initial amylin-induced reduction was observed. Hence, 7-day cumulative food intake was significantly decreased by a single i3vt injection of 100 pmol of amylin (vehicle = 209 ± 8 g; amylin = 177 ± 11 g, $p < 0.05$). Consequently, body weight change over the 7 days post-infusion was significantly reduc-

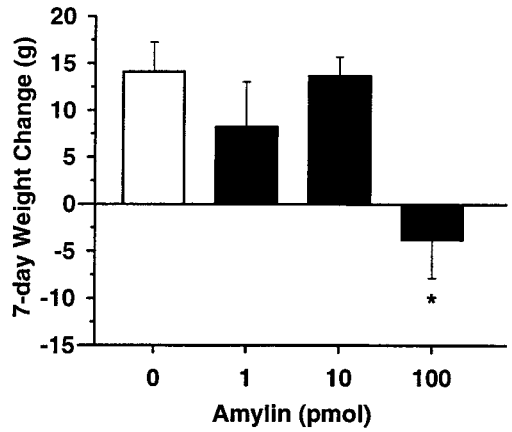

FIG. 2. Mean change of body weight over the 7 days after bolus i3vt infusion of CSF vehicle (0 pmol) or 1, 10, or 100 pmol amylin. * Significantly different from 0 pmol, $p < 0.05$, *error bars* as ± SEM.

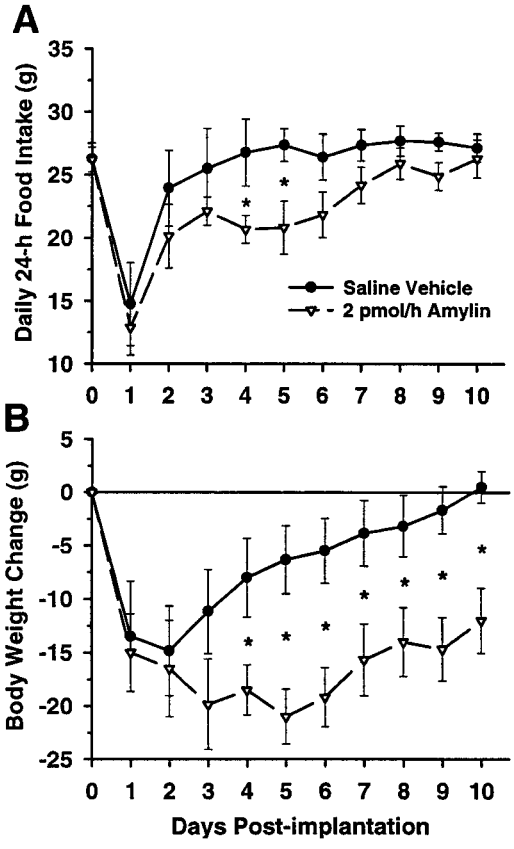

FIG. 3. A. Mean daily, 24-h food intake immediately preceding and for 10 days after implantation of osmotic minipumps prepared to continuously deliver (i3vt) 2 pmol/h (0.05 μl/h volume) amylin ($n = 6$) or saline vehicle ($n = 6$) in rats. B. Mean change of body weight for saline and amylin-treated rats over the 10-day infusion period. * Significantly dif-ferent from saline vehicle, $p < 0.05$, *error bars* as ± SEM.

ed by amylin, F (3, 29) = 5.34, p = 0.005, with 100 pmol producing a significant decrease compared to vehicle (Fig. 2).

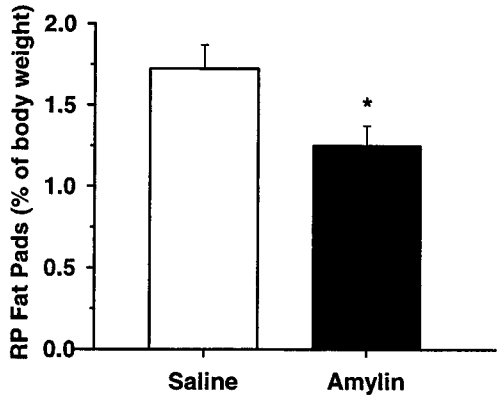

FIG. 4. Mean percentage of total body weight from the retroperitoneal (RP) fat depots after 10 days of continuous i3vt infusion of saline or 2 pmol/h amylin. * Significantly different from saline control, $p < 0.05$, *error bars* as ± SEM.

Chronic 3rd-Ventricular Amylin Infusion

As depicted in Figure 3A, all rats had depressed food intake on the day following surgical implantation of the minipumps. Cumulative intake over the subsequent 9 days of infusion as well as on individual Days 4 and 5 was significantly lower in amylin-treated rats than in controls ($p < 0.05$). By Day 4, amylin rats weighed significantly less than baseline relative to saline controls ($p < 0.05$), and this difference persisted throughout the remainder of the infusion period (Fig. 3B). At sacrifice (Day 10), the percent of body weight from retroperitoneal fat depots was significantly reduced in the amylin-treated rats as compared to controls (Fig. 4).

Discussion

Consistent with the previous observation of reductions of short-term food intake after acute infusion into the lateral ventricle of food-deprived rats (9), amylin potently and dose-dependently reduced short-term intake of non food-deprived rats after bolus i3vt infusion. Significant reductions of 1-h food intake were observed at doses as low as 1 pmol (0.004 μg), a dose level more than 100 times lower than has been observed to be effective peripherally (5, 6).

New to the literature is the documentation of long-term reductions of food intake and body weight by amylin when delivered either acutely or continuously into the brain (i3vt) of rats. The highest acute i3vt dose of amylin (100 pmol) significantly decreased 24-h food intake by over 30 %. Moreover, this effect was persistent in that animals did not compensate over the subsequent week for the initial amylin-induced reduction of intake, resulting in significantly suppressed 7-day cumulative food intake after a single i3vt infusion of 100 pmol (0.4 μg) of amylin. Likewise, body weight was significantly reduced over the 7 days following infusion of 100 pmol of amylin as compared to vehicle.

It should be noted that the animals appeared normal immediately after and throughout the week following acute amylin infusion. Further, amylin has been reported (20) not to pro-

duce a conditioned taste aversion following the intrahypothalamic administration of 1 μg (250 pmol), more than twice the highest acute amylin dose used in the present study.

When amylin (2 pmol/h) was slowly and continuously infused into the brain (i3vt) of rats via osmotic minipumps, significant reductions of both food intake and body weight were observed over the course of 10 days of infusion. Upon sacrifice and dissection of fat pads post-infusion, the percent of body weight from the retroperitoneal fat depots was found to be significantly reduced in the amylin-treated rats, indicative of a reduction of total body adiposity.

Again, as with the acute infusion experiment, the chronic i3vt amylin dose (2 pmol/h) is substantially lower (more than 200 times lower) than that reported to similarly affect long-term food intake and body weight when administered peripherally via a minipump infusion model (11). Clearly, therefore, the reductions of food intake and body weight reported here appear to be centrally-mediated.

These results support the hypothesis that amylin is an important signal to the brain contributing to the maintenance of long-term energy balance. In this regard, amylin may be analogous to other adiposity signals secreted in the periphery such as insulin and leptin. Although considerable work and effort has established insulin and leptin as adiposity signals to the CNS (13), little has been done to explore the hypothesis that amylin also provides such an afferent signal concerning some aspect of adipose stores in the periphery.

Thus, the present data add amylin to the growing list of hormones and neuropeptides that regulate energy homeostasis in the brain. Understanding the interactions of these compounds as they enable the CNS to match caloric intake to energy expenditure is an important goal. Such fundamental information can ultimately be applied to strategies of treatment and prevention of disorders of body weight regulation.

References

1. **Ludvik B, Kautzky-Willer A, Prager R, Whitehouse FW** 1997 Amylin: History and overview. Diabet Med 14(Suppl. 2):S9-S13
2. **Cooper GJS** 1994 Amylin compared with calcitonin gene-related peptide: Structure, biology, and relevance to metabolic disease. Endocr Rev 15:163-201
3. **Young A** 1997 Role of amylin in nutrient intake - animal studies. Diabet Med 14(Suppl. 2):S14-S18
4. **Butler PC, Chou J, Carter WB, Wang YN, Bu BH, Chang D, Chang JK, Rizza RA** 1990 Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. Diabetes 39:752-756
5. **Lutz TA, Del Prete E, Scharrer E** 1994 Reduction of food intake in rats by intraperitoneal injection of low doses of amylin. Physiol Behav 55:891-895
6. **Rushing PA, Seeley RJ, Woods SC** 1998 Intravenous amylin transiently reduces food intake in nondeprived rats. Soc Neurosci Abstr (abstract) 24:1019
7. **Smith GP, Gibbs J,** 1998 The satiating effects of cholecystokinin and bombesin-like peptides. In: Smith GP (ed) Satiation: From Gut to Brain. Oxford University Press, New York, 97-125
8. **Chance WT, Balasubramaniam A, Zhang FS, Wimalawansa SJ, Fischer JE** 1991 Anorexia following the intrahypothalamic administration of amylin. Brain Res 539:352-354
9. **Lutz TA, Rossi R, Althaus J, Del Prete E, Scharrer E** 1998 Amylin reduces food intake more potently than calcitonin gene-related peptide (CGRP) when injected into the lateral brain ventricle in rats. Peptides 19:1533-1540
10. **Morley JE, Flood JF** 1991 Amylin decreases food intake in mice. Peptides 12:865-869
11. **Arnelo U, Permert J, Adrian TE, Larsson J, Westermark P, Reidelberger RD** 1996 Chronic infusion of islet amyloid polypeptide causes anorexia in rats. Am J Physiol 271:R1654-R165
12. **Gebre-Medhin S, Mulder H, Pekny M, Westermark G, Tornell J, Westermark P, Sundler F, Ahren B, Betsholtz C** 1998 Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). Biochem Biophys Res Commun 250:271-277
13. **Woods SC, Seeley RJ, Porte Jr. D, Schwartz MW** 1998 Signals that regulate food intake and energy homeostasis. Science 280:1378-1383
14. **Banks WA, Kastin AJ** 1998 Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. Peptides 19:883-889
15. **Banks WA., Kastin AJ, Maness LM, Huang W, Jaspan JB** 1995 Permeability of the blood-brain barrier to amylin. Life Sci 57:1993-2001
16. **Beaumont K., Kenney MA, Young AA, Rink TJ** 1993 High affinity amylin binding sites in rat brain. Mol Pharmacol 44:493-497
17. **Christopoulos G, Paxinos G, Huang XF, Beaumont K, Toga AW, Sexton PM** 1995 Comparative distribution of receptors for amylin and the related peptides calcitonin gene related peptide and calcitonin in rat and monkey brain. Can J Physiol Pharmacol 73:1037-1041
18. **Sexton PM, Paxinos G, Kenney MA, Wookey PJ, Beaumont K** 1994 In vitro autoradiographic localization of amylin binding sites in rat brain. Neuroscience 62:553-567
19. **Pieber TR, Roitelman J, Lee Y, Luskey KL, Stein DT** 1994 Direct plasma radioimmunoassay for rat amylin-(1-37): Concentrations with acquired and genetic obesity. Am J Physiol 267:E156-E164
20. **Chance WT, Balasubramaniam A, Chen X, Fischer, JE** 1992 Tests of adipsia and conditioned taste aversion following the intrahypothalamic injection of amylin. Peptides 13:961-964