Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior

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Baldo, Brian A., and Ann E. Kelley. Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior. Am J Physiol Regulatory Integrative Comp Physiol 281: R1232–R1242, 2001.—Amylin, a calcitonin gene-related peptide-like peptide coreleased with insulin, exerts anorexic effects on central administration. Because previous studies revealed dense amylin binding in the nucleus accumbens (Acb), we investigated the behavioral effects of amylin infusions (10, 30, and 100 ng/side) into Acb subregions. Intra-Acb shell amylin infusions decreased ambulation, rearing, feeding, and drinking in either food-deprived rats or water-deprived rats; motor activity was affected more potently than ingestive behavior. Moreover, intra-Acb shell amylin reduced motor activity in nondeprived rats tested in the absence of food or water, indicating that the expression of amylin’s effects is independent of drive or proximal incentives. Intra-Acb core amylin infusions in water-deprived rats also decreased ambulation and water intake, although anterior Acb placements were associated with smaller motor effects, regardless of Acb subregion. In contrast to amylin’s effects, intra-Acb shell infusions of orexin-A (50, 100, and 500 ng/side) had no effects on motor activity, feeding, or drinking. Hence the Acb may be a target for behavioral regulation by satiety-related peptides like amylin.

IN RECENT YEARS, there have been remarkable advances in the identification of new peptides that regulate feeding behavior via actions within the central nervous system. One peptide that has attracted considerable interest as a satiety factor is amylin. Amylin (also called islet amyloid polypeptide), a 37-amino acid peptide with considerable homology to calcitonin gene-related peptide (CGRP) (39), is colocalized and cosecreted with insulin from pancreatic beta cells in the islets of Langerhans (1, 28). Originally isolated from diabetic pancreas (16) and an insulin-producing tumor (41), amylin exerts opposite effects compared with insulin on metabolic measures such as glycogen synthesis and glucose uptake in muscle (15, 24, 27).

In addition to its role in peripheral metabolic processes, amylin has also been shown to possess anorexic effects that are, at least in part, centrally mediated. Amylin has been shown to cross the blood-brain barrier (6), and infusion of amylin into the lateral ventricle (8, 25, 29), third ventricle (33), or hypothalamus (11) reduces feeding in rats. In accordance with amylin’s anorexic effects, amylin binding has been detected in feeding-related brain centers such as the dorsomedial and arcuate hypothalamic nuclei, the parabrachial area, and the nucleus of the solitary tract (35, 40).

Another potentially important site for the mediation of amylin’s feeding-related effects is the nucleus accumbens (Acb). The Acb contains among the highest levels of amylin binding in the entire brain (39); binding sites are particularly dense in the Acb shell. Recent studies have shown that the Acb shell has a specialized role in regulating feeding behavior. Stimulation of GABA receptors (36) or blockade of glutamate receptors (26) in the Acb shell elicits marked hyperphagia; this effect is not seen with similar pharmacological manipulations of the Acb core or neighboring striatal regions (7, 20). The effects of GABA stimulation or glutamate blockade are specific to feeding behavior; drinking or gnawing behavior is not affected by these manipulations in the Acb shell (7, 37). Hence, feeding responses are rapidly and specifically activated in the Acb shell, rendering this site a potentially efficient central target for the meal-terminating effects of satiety-related peptides.

Given the anorexic effects of amylin, the dense concentration of amylin binding sites in the Acb shell, and the important role of this site in the central regulation of feeding behavior, we aimed to investigate the behavioral effects of amylin infusion into the Acb shell. Our initial hypothesis was that amylin would exert anorexic effects when infused into the Acb shell. To ascertain the behavioral specificity of amylin’s effects, experiments were carried out under different motivational states (i.e., food deprivation or water deprivation), and spontaneous motor activity was measured concomitantly with ingestive behavior as an index of overall performance capacity. In addition, infusions were made into the Acb core to determine the anatomic specificity of amylin’s effects. As a general control for potential

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nonspecific effects of intra-Acb peptide infusion, we also tested the effects of the feeding- and arousal-related peptide orexin-A \(^{17, 34}\).

**METHODS**

**Subjects**

Subjects in all experiments were male Sprague-Dawley rats, obtained from Harlan (Indianapolis, IN), weighing 260–280 g on arrival at the laboratory. The rats were housed in clear polycarbonate cages (9.5-in. width \(\times\) 17-in. length \(\times\) 8-in. height), with cob bedding, in a light- and temperature-controlled vivarium. Animals were maintained under a 12:12-h light-dark cycle (lights on at 7:00 AM). Food and water were available ad libitum, except during the drug testing phase of the experiment. Animals were handled daily to reduce stress. All facilities and procedures were in accordance with the guidelines regarding animal use and care put forth by the National Institutes of Health and were supervised and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

**Surgery and Microinjection Procedures**

Rats (weighing 330–370 g at the time of surgery) were anesthetized with a xylazine/ketamine mixture (intraperitoneal injection of 13 mg/kg xylazine, 87 mg/kg ketamine; Research Biochemicals International, Natick, MA) and then secured in a Kopf stereotaxic frame. Bilateral stainless steel cannulas (10-mm long, 23 gauge) were implanted according to standard stereotaxic surgical procedures. Cannulas were aimed at the Acb core [final target coordinates were AP, 3.1 mm anterior to bregma; mediolateral (ML), \(\pm 2.0\) mm; dorsoventral (DV), \(-7.0\) mm from skull surface, toothbar at \(-3.3\) mm below interaural zero] or the Acb shell [final target coordinates were AP, \(3.1\) mm anterior to bregma; ML, \(\pm 1.0\) mm; DV, \(-7.7\) mm from skull surface, toothbar at \(5\) mm above interaural zero] and fixed in place 2.5 or 3 mm above the target site with dental acrylic (New Truliner, Skokie, IL) and anchoring skull screws (Plastics One, Roanoke, VA). Acb shell experiments were also conducted with cannulas angled at 20° from the vertical to avoid the lateral ventricles (final target coordinates were AP, \(+3.0\) mm anterior to bregma; ML, \(\pm 3.4\) mm; DV, \(-7.8\) mm from skull surface at a 20° angle). Wire stylets (10-mm long, 30 gauge) were placed in the cannulas to prevent blockage. Animals were given an intramuscular injection of penicillin (0.3 ml of a 300,000 U/ml suspension; Phoenix Pharmaceuticals, St. Joseph, MO), placed in a warm recovery cage, returned to their home cages on awakening, and given a recovery period of no less than 5 days (with daily health checks) before behavioral testing commenced.

\(^1\)The hypocretin/orexin peptides were discovered almost simultaneously by two different groups (17, 34). Each group chose a different name for this peptide family; consequently, issues of nomenclature have yet to be finalized. Presently, the convention is to refer to the peptides as hypocretins/orexins. Nevertheless, because some of the commercially available hypocretins lack part of the hypocretin/orexin amino acid sequence that contains amidation sites, these nonamidated peptides are likely biologically less active than commercially available amidated orexin peptides (see Ref. 21 for a discussion of this issue). Therefore, for purposes of clarity, we refer to hypocretin 1/orexin-A as “orexin-A” to emphasize that the amidated peptide was used in these experiments.

Bilateral intracerebral microinfusions were made through stainless steel injectors (30 gauge) attached with polyethylene tubing (PE-10, Becton Dickinson, Sparks, MD) to 10-μl capacity glass Hamilton syringes (Hamilton, Reno, NV) mounted on a Harvard microdrive pump. The rate of infusion was 0.32 μl/min; the total infusate volume for all experiments was 0.5 μl/side. Injectors were left in place for 1 min after the infusion to allow for injectate diffusion into the tissue.

**Behavioral Testing**

**Observational studies.** Observational studies were carried out in clear polycarbonate testing cages (9.5-in. width \(\times\) 17-in. length \(\times\) 8-in. height) with wire grid floors. A pre-weighed quantity of food (standard rat chow pellets) was placed on the cage floors, and water was available from an overhead graduated burette. A sheet of paper was placed underneath each testing cage to collect food spillage.

Before testing, rats were habituated to the intracerebral injection procedures with a sham infusion (injectors lowered into the brain site but no infusion given) and a saline infusion given on consecutive days. On the saline infusion day, animals were habituated to the testing cages for 30 min.

On testing days, animals were moved from the vivarium into the testing room, given an intracerebral microinfusion, returned to their home cages for 5 min (orexin-A experiment) or 15 min (amylin experiments), and then placed into the testing cages. The following behaviors were rated for 30 min by an experimenter blind to treatment: 1) crossovers, defined as ambulation across the center of the cage; 2) rears, 3) feeding, and 4) drinking. These parameters were recorded using a switch box connected to a microprocessor (Paul Fray, Cambridge, UK). Using this system, the experimenter monitored the frequency and duration for all behaviors except for crossovers, for which only frequency was recorded. From the frequency and duration data, the mean duration of each rearing, feeding, or drinking bout was calculated. Data for the 30-min testing session were recorded in three 10-min time bins. At the conclusion of the session, uneaten food, food spillage, and water intake were recorded. Testing occurred between 2:00 and 5:30 PM.

**Photocell cages.** In one experiment, the effect of intra-Acb shell amylin on spontaneous motor activity was measured with an automated photocell system. Motor activity was tested in clear polycarbonate testing cages (8-in. width \(\times\) 19-in. length \(\times\) 10-in. height) with four evenly spaced photocells along the bottom length and eight photocells along the top width of the cages (San Diego Instruments, San Diego, CA). The cages had wire grid cage floors with aspen chips beneath the wire grid. Neither food nor water was present in the testing cages. The photocells were interfaced with a computer; total beam breaks were detected and recorded for 90 min in 10-min time bins. On testing days, animals were moved from the vivarium to a testing room, given an intracerebral microinfusion, returned to their home cages for 15 min, and then placed into the photocell testing cages for 90 min.

**Peptides**

Rat amylin was obtained from Bachem Bioscience (Torrance, CA). The peptide was dissolved in sterile 0.9% saline at a concentration of 1 μg/μl and stored at \(-20°C\) in 50-μl aliquots. Orexin-A (human, mouse, rat) was obtained from Phoenix Pharmaceuticals (Mountain View, CA), dissolved in sterile 0.9% saline at 1 μg/μl, and stored at \(-20°C\) in 10-μl aliquots.
Experimental Design

Separate groups of rats were used for all experiments in this study (i.e., rats were not used in multiple experiments).

Effect of amylin in food-deprived rats. Animals (n = 8) were subjected to bilateral intra-Acb shell infusions of amylin (0, 10, 30, and 100 ng/side) administered in a counterbalanced order according to a within-subjects Latin square design. After amylin infusions, spontaneous motor activity, eating, and drinking were monitored in the behavior observation cages as described in Behavioral Testing. Each dose was tested on a separate day; testing days were separated from each other by one treatment-free day. Each rat received all amylin doses.

Beginning 3 days before behavioral testing, and lasting for the duration of the experiment, rats were placed on a food-restriction regimen in which food and water were available for 6 h/day (between 2:00 and 8:00 PM). On testing days, animals were not fed before testing but received food for 2 h after the testing session (6:00 to 8:00 PM) in addition to any food eaten during testing. In their home cages, rats had free access to water at all times.

Effect of amylin in water-deprived rats. Rats with cannulas aimed at the Acb core (n = 8), the Acb shell with a straight placement (n = 8), or the Acb shell with an angled placement (20° to the vertical) to avoid the lateral ventricles (n = 8) were subjected to bilateral infusions of amylin (0, 10, 30, and 100 ng/side), and their spontaneous motor activity, eating, and drinking were monitored in the behavior observation cages. The general design for these experiments was identical to the experiment testing the effect of amylin in hungry rats (see Effect of amylin in food-deprived rats), except that animals were maintained under water restriction rather than food restriction. The water-restriction regimen was carried out according to the same time schedule as the food-restriction regimen. Water-restricted rats tended to eat less in their home cages, leading to small increases in feeding behavior in addition to the expected large increases in drinking during testing.

Effect of amylin on general motor activity. Animals (n = 7, nondeprived) were subjected to bilateral intra-Acb shell infusions of amylin (0, 10, 30, and 100 ng/side) administered in a counterbalanced order according to a within-subjects Latin square design. Each rat received all amylin doses. After amylin infusions, spontaneous motor activity was monitored for 90 min in photocell testing cages with neither food nor water present (see Photocell cages).

To reduce neophobia, all animals were habituated to the photocell cages for 90 min 3 days before testing commenced. To minimize between-session habituation to the photocell cages, testing days were separated from one another by 3 days. Food and water were freely available to the rats in their home cages.

Effect of orexin-A in nondeprived rats. Animals (n = 10) were subjected to bilateral intra-Acb shell infusions of orexin-A (0, 50, 100, and 500 ng/side) administered in a counterbalanced order according to a within-subjects Latin square design. After orexin-A infusions, spontaneous motor activity, eating, and drinking were monitored in the behavior observation cages (see Behavioral Testing). Each dose was tested on a separate day; testing days were separated from each other by one treatment-free day. Each rat received all orexin-A doses. Food and water were freely available to the rats in their home cages.

Histology

Rats were deeply anesthetized with pentobarbital sodium and perfused transcardially with a 0.9% saline solution followed by a solution of 10% formalin in phosphate buffer (Sigma Diagnostics, St. Louis, MO). Brains were removed and stored in 10% formalin. The brains were then cut on a cryostat microscope; 60-μm sections were taken through the injection site. Brain slices were mounted on slides, stained with cresyl violet, and subsequently reviewed to verify correct placement of the intracerebral injections. Images of representative sections from each experiment were captured using Scion Image software on a computer interfaced with a microscope-mounted Hitachi HV-C20 charge-coupled device camera.

Statistical Analyses

For the behavioral tests, data scores were summed across the three 10-min time bins of the 30-min testing sessions and subjected to one-factor (dose) repeated-measures ANOVAs. Preplanned contrasts were used for specific comparisons between vehicle-associated means and the means for each individual peptide dose.

For the photocell activity study, data from the entire 90-min session (divided into 10-min time bins) were analyzed with a two-factor ANOVA (dose × time). After significant dose × time interactions, data were further analyzed for simple effects with two-factor ANOVAs (dose × time). In addition, data scores summed across the first 30 min of the session (i.e., the first 3 time bins) were analyzed with a one-factor (dose) repeated-measures ANOVA with preplanned contrasts (see above).

For all experiments, the level of statistical significance was set at P < 0.05.

RESULTS

Behavioral Effects of Amylin Infusions into the Acb Shell in Food-Deprived Rats

Food intake [F(3, 21) = 16.45, P < 0.0002] and feeding duration [F(3, 21) = 3.93, P < 0.03] were both decreased by intra-Acb shell amylin in food-deprived rats (see Table 1). Preplanned contrasts indicated that the effect of the 100-ng dose differed significantly from vehicle control for both measures; in addition, the effect of the 30-ng dose differed significantly from control for the food intake measure (for α values, see legend for Table 1). Amylin treatment tended to increase the latency to feed, although this effect did not achieve statistical significance [F(3, 21) = 2.61, not significant (NS)]. Latency to drink was decreased by 10 ng amylin and increased by 100 ng amylin [F(3, 21) = 3.14, P < 0.05, Table 2].

Intra-Acb shell amylin infusions in hungry rats also significantly decreased locomotor activity [F(3, 21) = 7.92, P < 0.002, Fig. 1] and rearing [F(3, 21) = 8.19, P < 0.0009, Fig. 1]. For both measures, the means associated with the 30-ng dose and the 100-ng dose differed significantly from vehicle-associated means (for α values, see legend for Fig. 1).
### Table 1. Effects of intra-Acb amylin on feeding behavior

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Food Intake, g</th>
<th>Feeding Duration, s</th>
<th>Feeding Bouts</th>
<th>Mean Bout Duration, s</th>
<th>Latency to Feed, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acb shell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylin dose, ng</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.71 ± 0.4</td>
<td>1,066.3 ± 50.9</td>
<td>24.0 ± 2.6</td>
<td>45.5 ± 5.2</td>
<td>14.7 ± 4.4</td>
</tr>
<tr>
<td>10</td>
<td>6.48 ± 0.4</td>
<td>1,081.3 ± 56.6</td>
<td>19.8 ± 2.1</td>
<td>56.7 ± 9.4</td>
<td>14.6 ± 4.9</td>
</tr>
<tr>
<td>30</td>
<td>5.59 ± 0.7</td>
<td>892.1 ± 97.0</td>
<td>20.5 ± 2.2</td>
<td>45.2 ± 5.3</td>
<td>19.4 ± 4.7</td>
</tr>
<tr>
<td>100</td>
<td>3.73 ± 0.8‡</td>
<td>736.5 ± 156.4*</td>
<td>16.5 ± 2.1</td>
<td>43.0 ± 9.3</td>
<td>74.8 ± 37.6</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Acb, nucleus accumbens. *P < 0.05, †P < 0.01, ‡P < 0.001, different from dose of 0 ng/side.

### Table 2. Effects of intra-Acb amylin on drinking behavior

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Water Intake, ml</th>
<th>Drinking Duration, s</th>
<th>Drinking Bouts</th>
<th>Mean Bout Duration, s</th>
<th>Latency to Drink, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acb shell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylin dose, ng</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.4 ± 0.9</td>
<td>120.8 ± 29.4</td>
<td>7.4 ± 1.5</td>
<td>14.6 ± 3.3</td>
<td>875.5 ± 209.8</td>
</tr>
<tr>
<td>10</td>
<td>3.6 ± 0.7</td>
<td>142.9 ± 19.6</td>
<td>8.5 ± 1.6</td>
<td>20.8 ± 5.6</td>
<td>514.7 ± 105.0</td>
</tr>
<tr>
<td>30</td>
<td>4.4 ± 1.0</td>
<td>137.8 ± 37.7</td>
<td>5.6 ± 1.4</td>
<td>20.5 ± 6.3</td>
<td>695.4 ± 183.7</td>
</tr>
<tr>
<td>100</td>
<td>2.8 ± 0.9</td>
<td>94.0 ± 44.2</td>
<td>5.3 ± 2.6</td>
<td>8.9 ± 4.0</td>
<td>1,208.0 ± 281.2</td>
</tr>
</tbody>
</table>

Values represent means ± SE. *P < 0.05, †P < 0.01, ‡P < 0.001, different from dose of 0 ng/side.

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Behavioral Effects of Amylin Infusions into Acb Subregions in Water-Deprived Rats

The effects of amylin were tested in water-deprived rats to determine the extent to which the effects of intra-Acb amylin were selective for feeding behavior or for a specific deprivation state. To examine the anatomic specificity of amylin's effects, the peptide was injected into the Acb shell (with either a straight placement or an angled placement to eliminate possible spread into the ventricles) or into the Acb core.

Acb shell (straight placement). Intra-Acb shell amylin infusions in water-deprived rats depressed water intake [$F(3,21) = 5.95, P < 0.005$, Table 2], total drinking duration [$F(3,21) = 6.63, P < 0.003$, Table 2], and the number of drinking bouts [$F(3,21) = 4.69, P < 0.002$, Table 2]. As well, food intake [$F(3,21) = 4.82, P < 0.02$, Table 1], feeding duration [$F(3,21) = 4.41, P < 0.02$, Table 1], and the number of feeding bouts [$F(3,21) = 4.27, P < 0.02$, Table 1] were decreased. For all of these drinking and feeding measures, the effects of the highest amylin dose (100 ng) differed significantly from vehicle control values (for $\alpha$ values, see legends for Tables 1 and 2). Amylin also decreased mean feeding bout duration, although this effect did not achieve statistical significance [$F(3,21) = 2.72, P < 0.07$]. There were no consistent effects on mean drinking bout duration or latency to begin drinking ($F$ values $= 0.74–1.02$, NS).

As in food-deprived rats, intra-Acb shell infusions of amylin in water-deprived rats markedly depressed locomotor activity [$F(3,21) = 24.65, P < 0.0002$, Fig. 2] and rearing [$F(3,21) = 16.25, P < 0.0002$, Fig. 2]. Preplanned contrasts revealed that for both behavioral measures, the effect of all three amylin doses differed significantly from vehicle control (for $\alpha$ values, see legend for Fig. 2).

Acb shell (angled placement). Intra-Acb shell infusions of amylin using angled guide cannulas (20° to the vertical) to avoid the lateral ventricles produced similar inhibitory effects on locomotor activity [$F(3,21) = 6.51, P < 0.003$] and rearing [$F(3,21) = 3.19, P < 0.05$] as observed with straight placements (see Fig. 2). For both of these activity measures, the effects of the 30-ng dose and the 100-ng dose differed significantly from vehicle control values (for $\alpha$ values, see legend for Fig. 2). There was a tendency for amylin to reduce drinking duration, water intake, and the number of drinking bouts, although these effects did not reach statistical significance ($F$ values $= 1.60–2.31$, NS, see Table 2).

There were no consistent effects of amylin in this experiment on feeding duration, food intake, feeding bouts, mean feeding or drinking bout duration, or latency to begin feeding ($F$ values $= 0.17–1.40$, NS, Table 1). There was a nonsignificant tendency for the 30-ng amylin dose to increase the latency to drink [$F(3,21) = 2.22$, NS], although this effect was due to a very large increase in latency in only one rat of eight, as reflected in the large SE for that dose (see Table 2).

Acb core. As shown in Fig. 2, intra-Acb core infusions of amylin significantly depressed locomotor activity [$F(3,21) = 6.53, P < 0.003$] but not rearing [$F(3,21) = 1.92$, NS]. For the locomotor activity measure, preplanned contrasts indicated that the effects of the 30-ng dose and the 100-ng dose differed significantly from the vehicle control value (for $\alpha$ values, see legend for Table 2).
for Fig. 2). Intra-Acb core amylin infusions also produced a small but statistically significant decrease in water intake \(F(3,21) = 4.57, P < 0.02\); preplanned contrasts revealed that the means associated with all three amylin doses differed significantly from the vehicle-associated mean (for \(\alpha\) values, see legend for Table 2). The other measures of ingestive behavior were unaffected by amylin treatment in this experiment (\(F\) values = 0.18–1.80, NS, Tables 1 and 2).

Effects of Amylin Infusions into the Acb Shell on General Motor Activity

Amylin infusion into the Acb shell significantly decreased spontaneous motor activity in nondeprived rats tested in photocell cages in the absence of food or water [dose \times time interaction: \(F(24,144) = 3.12, P < 0.0002\), Fig. 3]. Two-factor ANOVAs for simple effects revealed significant dose \times time interactions for the comparisons between the effect of vehicle and the effects of the two highest amylin doses [vehicle vs. 30 ng: \(F(8,144) = 2.72, P < 0.05\); vehicle vs. 100 ng: \(F(8,144) = 6.17, P < 0.001\)]. Analysis of activity counts summed over the first 30 min of the testing session also revealed an effect of amylin treatment [\(F(3,18) = 3.71, P < 0.04\)]; preplanned contrasts indicated that the effect of the 100-ng dose differed significantly from vehicle control (for \(\alpha\) value, see legend for Fig. 3).

Effect of Orexin-A in Nondeprived Rats

As a pharmacological control, the effects of intra-Acb shell infusion of the feeding- and arousal-related peptide, orexin-A, were tested. As with amylin, there is evidence for the presence of orexin-A receptors in the Acb shell (38). The two peptides were tested in overlapping molar dose ranges (amylin dose range, 2.5–25 pmol/side; orexin A dose range, 14–140 pmol/side). Because orexin-A was expected to increase feeding, rats were not food deprived in this experiment.

In contrast to the effects of amylin, infusion of orexin-A into the Acb shell did not influence any of the measures of motor activity or ingestive behavior in this study (\(F\) values = 0.04–1.13, NS, Fig. 4, Table 3).

Histological Analyses

As shown in Fig. 5, there was no excessive or unexpected tissue damage noted in any of the experiments. Figure 6 shows chartings of injector tip placements for the rats in all experiments except the orexin-A experiment. Shaded symbols indicate percent locomotor suppression [calculated as 100 – [(100-ng dose effect)/(0-ng dose effect)] \times 100] for the horizontal activity measures associated with each respective placement. As can be seen in Fig. 6, placements in the middle-to-posterior Acb were associated with the largest motor effects, regardless of whether the placement was in the Acb shell or Acb core. In the anterior sectors of the Acb, amylin-induced locomotor suppression was less consistent and often of a smaller magnitude than the effects observed with more posterior placements.

DISCUSSION

Infusions of amylin into the Acb shell potently reduced ambulation, rearing, and ingestive behaviors in both food-deprived and water-deprived rats. Motor-depressant effects induced by intra-Acb shell amylin were also noted in nondeprived rats tested in the ab-
The effects of amylin on motor behavior were frequently observed at lower doses than those required to suppress ingestive behaviors. Amylin infusion into the Acb core of water-deprived rats diminished ambulation and water intake, although these effects tended to be of smaller magnitude than the results obtained in the Acb shell. Nevertheless, inspection of injector placements revealed an anteroposterior gradient in which more caudal placements in the Acb produced stronger, more consistent effects on motor activity regardless of Acb subregion. In contrast to amylin, orexin-A infusion into the Acb shell did not influence any of the behavioral parameters measured in this study. Taken together, these results indicate that stimulation of amylin receptors in the Acb reduced the degree to which rats explored their environment, whether or not motivational arousal was heightened by a deprivation state coupled with the presence of a desired goal object (i.e., food or water to a deprived rat). Moreover, the effects of amylin infusions into the Acb were not restricted to feeding behavior; drinking behavior was also potently depressed by intra-Acb amylin. Hence, stimulation of amylin receptors in the Acb might modulate fundamental processes related to general motor output or arousal, leading to decreases in exploratory and ingestive behaviors. It is unlikely that

Table 3. Effects of intra-Acb shell orexin-A on feeding and drinking behavior in nondeprived rats

<table>
<thead>
<tr>
<th>Orexin-A Dose, ng</th>
<th>Food Intake, g</th>
<th>Feeding Duration, s</th>
<th>Feeding Bouts</th>
<th>Mean Bout Duration, s</th>
<th>Latency to Feed, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.36 ± 0.1</td>
<td>24.0 ± 12.3</td>
<td>1.5 ± 0.6</td>
<td>8.2 ± 3.0</td>
<td>1,367.3 ± 162.2</td>
</tr>
<tr>
<td>50</td>
<td>0.44 ± 0.2</td>
<td>25.7 ± 16.6</td>
<td>1.6 ± 0.8</td>
<td>5.3 ± 3.7</td>
<td>1,520.3 ± 149.5</td>
</tr>
<tr>
<td>100</td>
<td>0.41 ± 0.1</td>
<td>14.7 ± 7.9</td>
<td>1.5 ± 0.7</td>
<td>3.9 ± 1.8</td>
<td>1,392.2 ± 188.3</td>
</tr>
<tr>
<td>500</td>
<td>0.6 ± 0.2</td>
<td>49.0 ± 35.5</td>
<td>1.3 ± 0.9</td>
<td>7.4 ± 5.2</td>
<td>1,494.1 ± 204.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Orexin-A Dose, ng</th>
<th>Water Intake, ml</th>
<th>Drinking Duration, s</th>
<th>Drinking Bouts</th>
<th>Mean Bout Duration, s</th>
<th>Latency to Drink, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2 ± 0.6</td>
<td>22.1 ± 14.2</td>
<td>2.6 ± 1.2</td>
<td>6.4 ± 3.4</td>
<td>921.3 ± 224.8</td>
</tr>
<tr>
<td>50</td>
<td>2.6 ± 0.6</td>
<td>27.5 ± 12.6</td>
<td>2.7 ± 0.9</td>
<td>5.1 ± 1.9</td>
<td>972.6 ± 227.3</td>
</tr>
<tr>
<td>100</td>
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<td>72.8 ± 41.8</td>
<td>3.4 ± 2.1</td>
<td>6.3 ± 2.3</td>
<td>1,147.6 ± 192.1</td>
</tr>
<tr>
<td>500</td>
<td>2.6 ± 0.6</td>
<td>49.8 ± 22.5</td>
<td>4.8 ± 1.8</td>
<td>6.7 ± 2.0</td>
<td>785.3 ± 183.7</td>
</tr>
</tbody>
</table>

Values represent means ± SE.

Fig. 5. Photomicrographs depicting representative injector placements. A–C: placements in the nucleus accumbens shell for the amylin experiments with food-deprived, water-deprived, and nondeprived rats, respectively. D: representative placement in the nucleus accumbens core. E and F: intranucleus accumbens shell placements angled 20° from the vertical for the experiments with amylin in food-deprived rats and orexin-A in nondeprived rats, respectively.
the observed motor-depressant effect of amylin was due to malaise, because in several instances, amylin doses that reduced ambulation and rearing did not influence ingestive behaviors. Moreover, previous studies failed to demonstrate a conditioned taste aversion induced by intraperitoneal (30) or intrahypothalamic (9) amylin infusions. In the present study, amylin doses that suppressed ambulation and rearing often did not influence drinking behavior, even though drinking from the overhead bottles was a motorically demanding task. Moreover, previous studies failed to demonstrate a conditioned taste aversion induced by intraperitoneal (30) or intrahypothalamic (9) amylin infusions.

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Hence, although central administration of amylin depresses exploration-related motor behaviors, it is unlikely that this effect is exclusively due to generalized sensorimotor impairment. Instead, stimulation of amylin receptors may interact with neural processes underlying arousal or exploratory drive to suppress behaviors such as ambulation and rearing. It should be noted, however, that the profound suppression of locomotor and ingestive behaviors produced by the highest amylin dose (100 ng or 25 pmol) in the present study might have been due to generalized sensorimotor impairment. Nevertheless, the results of the present study clearly demonstrate that infusion of a low (10 ng or 2.5 pmol) amylin dose into the Acb is sufficient to reduce locomotion and rearing but not feeding or drinking, suggesting that mechanisms other than sensorimotor impairment are responsible for amylin’s effects at this dose.

The potency of intra-Acb shell amylin to reduce ambulatory activity in the present study was at least 100-fold greater than the previously reported potency of the motor-depressant effects of intraventricular amylin (8, 14), suggesting that the Acb represents an important site for the motor activity-suppressing effects of ventriculally administered amylin. In contrast, the effect of intra-Acb amylin on feeding behavior was less potent than previously reported for intraventricular amylin (25, 33). For example, Lutz et al. (25) reported an EC50 of 7 ng/rat (2 pmol/rat) for acute suppression of feeding behavior by administration of amylin into the lateral ventricle. Moreover, Rushing et al. (33) found that an amylin dose as low as 3.5 ng/rat (1 pmol/rat), infused into the third ventricle (which is immediately adjacent to the hypothalamus), was sufficient to acutely inhibit feeding behavior. Locomotor activity was not measured in these two studies, although in the latter study, the authors commented that rats “appeared normal” immediately after amylin infusions were given, implying a lack of overt motor effects (33). In the present study, the lowest dose at which feeding suppression was observed was 30 ng (60 ng/rat or 15 pmol/rat). These potency differences suggest that the Acb may be a relatively more sensitive substrate for amylin effects on locomotor activity compared with feeding behavior. This hypothesis is not inconsistent with a role for Acb-localized amylin receptors in satiety. Thus it is well-established that postprandial behaviors include an initial brief bout of exploratory activity immediately after feeding, followed by a longer-lasting overall reduction in behavioral output and arousal (e.g., Ref. 3). It may be that amylin receptors in the Acb are preferentially involved in satiety-related effects on arousal and motor output, whereas other structures, such as the hypothalamus, are more closely linked to amylin’s meal-terminating effects. Hence amylin’s role in satiety may require coordinated action at several different brain sites, each mediating a distinct satiety-related behavioral process.

In contrast to amylin, orexin-A produced no behavioral effects when infused into the Acb. Intraventricular or intrahypothalamic infusion of hypocretin/orexin...
peptides produces a modest increase in feeding in rats
(for review, see Ref. 21), and recent studies have shown
that the hypocretin/orexin peptides are also involved in
the central regulation of arousal and of the sleep dis-
order narcolepsy (21). Considering that orexin-2 recep-
tor mRNA has been observed in the Acb shell (38), we
initially predicted that intra-Acb shell infusion of
orexin-A would influence feeding behavior and in-
crease motor activity, the latter resulting indirectly
from orexin-A effects on arousal. However, orexin-A
influenced neither motor activity nor feeding behavior
in this study at doses as high as 140 pmol (500 ng). It
is possible that because orexin-A was tested in awake
rats, motor activity increases resulting from enhanced
arousal were undetectable because of ceiling effects.
This hypothesis is supported by the finding that infu-
sion of a 35-pmol (125 ng) orexin-A dose into the medial
septal area (adjacent to the Acb shell) of sleeping rats
produced alert waking with a consequent increase in
motor activity and feeding behavior (18). Nevertheless,
the lack of orexin-A effects in the present study serves
as a control for possible toxicity associated with peptide
infusion into the Acb, thereby providing further evi-
dence of the specificity of amylin’s effects.

Presently, the neurochemical bases for the behav-
ioral effects of intra-Acb amylin are unknown. One
possible mechanism is that amylin interacts with the
dopamine system, as suggested by the similarities be-
tween the behavioral effects of interfering with dopa-
mine neurotransmission and stimulating amylin re-
ceptors in the Acb. For example, as with amylin, small
6-hydroxydopamine lesions or low-to-medium doses of
dopamine receptor antagonists in the Acb reduce loco-
motor activity while sparing feeding behavior (4, 5, 22).
With larger lesions or higher doses, feeding behavior
is also impaired. As well, intraventricularly adminis-
tered amylin blocks the behavioral effects of the dopa-
mine agonists amphetamine and apomorphine (13, 14),
and systemic injection of amylin decreases striatal
concentrations of the dopamine metabolite 3-methoxy-
tyramine (10). Taken together, these results indicate
that stimulating amylin receptors might produce a
functional inhibition in dopaminergic transmission,
which could account for the potent effects on motor
activity and ingestive behaviors observed in the
present study.

Somewhat surprisingly, we did not observe major
differences between the behavioral effects of amylin
infusions into the Acb shell vs. the Acb core, particu-
larly with regard to ambulation and rearing. Receptor
autoradiography studies have revealed dense concen-
trations of sites in the Acb that bind amylin, salmon
calcitonin, and, with a lesser affinity, CGRP (for re-
views, see Refs. 32 and 39); this pharmacological pro-
file is similar to that obtained in cultured cells when
calcitonin receptors are coexpressed with receptor ac-
tivity-modifying protein (RAMP)-1 (12). The distribu-
tion of these binding sites appears to be denser in the
Acb shell than in the Acb core (35, 40). Previous work
from this laboratory demonstrated that the Acb shell
has a specialized role in generating feeding behavior
(7, 20, 26, 36); therefore, we initially predicted that
amylin’s effects on ingestive behaviors would be stron-
ger in the shell than in the core. The present results,
however, indicate that the behavioral effects of amylin
infusion into the Acb shell vs. the Acb core are similar
but that effects obtained in the more caudal regions of
the Acb on both motor and ingestive behaviors are
stronger and more consistent relative to effects ob-
tained with more anterior placements, regardless of
subregion. The basis for this anteroposterior gradient
is unknown, although amylin binding sites are also
abundant in the fundus striati (also called the intersti-
tial nucleus of the posterior limb of the anterior com-
missure) and the bed nucleus of the stria terminalis
(35), regions that are anatomically and functionally
interrelated to the caudal Acb (see Ref. 2). The more
posteriorly placed infusions in this study might have
gained access to these sites in addition to the Acb, and
the full expression of amylin’s behavioral effects may
require combined action at the Acb together with these
related sites. Detailed mapping studies will be re-
quired to further elucidate the anatomic substrates
supporting amylin’s behavioral effects.

Given that amylin binding sites are densely concen-
trated within the Acb (35, 40) but that amylin mRNA is
not found within the brain (19, 23), the question arises
regarding the identity of the endogenous ligand that
acts at Acb-localized binding sites. One possibility is
that amylin, released from the pancreas, crosses the
blood-brain barrier to gain access to its binding sites in
the Acb. Banks and Kastin (6) found that a significant
portion of intravenously administered radiolabeled
amylin crossed the blood-brain barrier and accumu-
lated in several brain structures, including the stria-
tum. Indeed, these authors (6) determined that pene-
tration of amylin into the brain exceeded that of
insulin. Another candidate is CGRP. CGRP has a high
affinity (although not as high as amylin’s) for Acb-
localized amylin binding sites (40), and CGRP-immu-
noreactive processes have been reported in the Acb (for
review, see Ref. 39). Hence, it is possible that Acb-
localized CGRP, peripherally derived amylin, or both
peptides act at amylin binding sites in the Acb to
suppress motor activity and ingestive behavior. It is
tempting to speculate that feeding-related amylin re-
lease from the pancreas represents a physiological sig-
nal of peripheral origin that coordinates the activity of
the several brain regions involved in the behavioral
satiety sequence. Further studies with intra-Acb infu-
sions of selective antagonists could shed further light
on this issue.

In summary, intra-Acb infusions of amylin potently
reduced motor activity and ingestive behavior. Dose-
related dissociations between amylin-induced suppress-
on of exploratory motor behaviors vs. ingestive be-
haviors suggest that amylin’s effects were not due to
malaise or motor incapacity but rather may have re-
sulted from a diminution of exploratory drive or
arousal. Such an effect could account for the observed
decreases in ambulation, rearing, and ingestive behav-
iors. Considering that peripherally administered amy-
lin crosses the blood-brain barrier and gains access to the striatum (6), amylin may relay a satiety signal from the periphery to the brain and mediate satiety-induced decreases in behavioral output and arousal via actions within the Acb.

**Perspectives**

Presently, the interaction of hormones and circulating factors that modulate satiety and energy balance with brain regions that govern goal-directed behaviors is poorly understood. The present findings suggest the possibility that a peptide of pancreatic origin, amylin, can act at the level of the Acb to diminish investigatory motor responses. The Acb has long been hypothesized to mediate the translation of motivational states, such as hunger, into appropriate, adaptive behaviors, including exploration and appetitive approach behaviors. Hence, this site could represent a substrate for the integration of homeostatic or meal-limiting physiological signals with limbic-mediated motivational arousal associated with drive states.

In addition, the pattern of behavioral changes induced by intra-Acb amylin infusions in the present study resembled the effects commonly observed after dopamine-receptor blockade in the Acb. If receptors for the CGRP/amylin family of peptides exert a negative regulatory influence on Acb-mediated dopaminergic function, these receptors could represent targets at which the effects of the large class of reinforcers known to activate the mesolimbo-dopamine system (i.e., food, sex, and drugs of abuse) could be modulated.

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