Dopamine D₂ receptors mediate amylin’s acute satiety effect

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Received 10 August 2000; accepted in final form 15 December 2000

Lutz, T. A., S. Tschudy, A. Mollet, N. Geary, and E. Scharrer. Dopamine D₂ receptors mediate amylin’s acute satiety effect. Am J Physiol Regulatory Integrative Comp Physiol 280: R1697–R1703, 2001.—The anorectic effect of the pancreatic peptide amylin has been established in numerous studies. Here, we investigated the influence of a pretreatment with dopamine (DA) D₁- and D₂-receptor antagonists on the anorectic effect of intraperitoneally injected amylin in food-deprived rats a medium-fat (18% fat) diet. In 24-h food intake studies, pretreatment with the DA D₂-receptor antagonist raclopride (100 μg/kg [0.2 μmol/kg] ip) significantly attenuated amylin’s (5 μg/kg) anorectic effect, whereas raclopride alone had no effect on food intake (i.e., food intakes 1 h after injection were (n = 12): NaCl/NaCl 7.3 ± 0.5 g; NaCl/amylin 3.9 ± 0.6; raclopride/NaCl 7.7 ± 0.7; raclopride/amylin 5.6 ± 0.7). Pretreatment with another DA D₂ receptor antagonist, sulpiride [50 mg/kg (154 μmol/kg) ip], similarly reduced amylin’s satiety effect, whereas pretreatment with the DA D₁-receptor antagonist SCH-23390 [10 μg/kg (0.03 μmol/kg) ip] did not influence amylin’s effect. SCH-23390, however, completely blocked the anorexia induced by α-amphetamine (0.3 mg/kg ip). These results suggest that, under the present feeding conditions, the dopaminergic system mediates part of amylin’s inhibitory effect on food intake in rats when administered intraperitoneally. This seems to involve DA D₂ receptors but not D₁ receptors.

Food intake; rat; amphetamine; raclopride; sulpiride; SCH-23390

AMYLIN (or islet amyloid polypeptide; see Ref. 48), which is structurally and functionally related to calcitonin gene-related peptide and calcitonin (13, 27, 32, 46, 49), is a 37-amino acid polypeptide that is coreleased with insulin from pancreatic β-cells in response to stimuli such as an elevation of blood glucose concentration and food intake (8, 13). Acute peripheral or central administration of amylin reduces food intake in rats (10, 19, 20, 23, 31, 36, 38), and chronic peripheral administration of amylin leads to a sustained decrease in food intake and a reduction in body weight (2). Several lines of evidence indicate that amylin’s acute anorectic effect is a specific satiety effect (4, 9, 20, 23).

Peripherally administered amylin appears to inhibit feeding by acting in the brain via a humoral mode of action (19, 26). Amylin receptors are present in various brain areas involved in the control of food intake, including areas lacking the blood-brain barrier, such as the area postrema (AP; see Refs. 5 and 42). The AP/nucleus of the solitary tract (NTS) region seems to mediate amylin’s anorectic effect after intraperitoneal injection (26), whereas hypothalamic neurons (47, 42) may mediate its anorectic effect after central administration (25, 28).

We previously reported (22) that the satiety effect of intraperitoneal amylin in rats is abolished by pretreatment with the histamine H₃-receptor agonists R-α-methylhistamine or imetit dihydrobromide, which act at presynaptic autoreceptors to inhibit the release of endogenous histamine (3) and which have been shown to abolish the anorectic action of bombesin (29). Because this effect of histamine H₃ agonism is probably brought about by blocking the release of histamine within the central nervous system, amylin is likely to decrease food intake by increasing the release of histamine in the central nervous system (22).

The role of neurotransmitters other than histamine in intraperitoneal amylin’s satiety effect is currently unknown. In the present study, we investigated the role of dopamine (DA) in amylin-induced satiety because DA appears to play an important role in several aspects of the control of feeding and because DA also appears to mediate some other effects of amylin (12). Activation of DA D₂ receptors in the nucleus accumbens appears to contribute to the stimulation of feeding by positive feedback from rewarding flavors (e.g., see Ref. 43), and activation of DA D₁ and D₂ receptors in the lateral hypothalamus may contribute to the inhibition of feeding (14, 17, 18). DA binding sites, however, have also been described in the AP/NTS region (34, 45). NTS DA receptors are mainly DA D₂ receptors, with few DA D₁ receptors (34). These receptors may mediate part of the anorectic action of CCK because they are codistributed with CCK-binding sites in this brain area (34) and because CCK, the anorectic effect of which can be blocked by the DA-receptor antagonist cis-flupentixol (6), induces DA release in the NTS (7). These interactions between NTS CCK and DA in the control of food intake are interesting with respect to the present study because amylin mediates part of CCK’s anorectic action (21, 24). Furthermore, the AP/NTS region contains amylin-binding sites.
(5, 42), and amylin excites neurons in the AP (35). Finally, the AP/NTS region is necessary for the anorectic effect of amylin after intraperitoneal injection (19, 26).

To investigate the role of the dopaminergic system in mediating intraperitoneal amylin’s satiating effect, we tested whether it is affected by pretreatment with the DA D2-receptor antagonists raclopride and sulpiride or the DA D1-receptor antagonist SCH-23390. Because the anorectic effect of D-amphetamine (AMP) depends on DA D1 receptors (14), we also performed a control experiment with AMP to test the effectiveness of SCH-23390. Under our conditions, DA D2, but not DA D1, receptor antagonism reduced the satiating potency of amylin.

MATERIALS AND METHODS

Animals and housing conditions. Adult male Sprague-Dawley rats (OFA; BRL, Fullinsdorf, CH) were used. In experiments 1a, 2, and 4, rats weighing 300–450 g were housed individually in specially designed, wire-bottomed Plexiglas cages in which a small tunnel (length: 15 cm; diameter: 6.5 cm) provided access to spillage-resistant feeding cups (23). In experiments 1b, 3, and 5, rats weighing 210–240 g were housed in conventional wire cages. Previous studies indicate that amylin has a similar anorectic potency in rats of different age (20, 23). Rats had ad libitum access to water and food except as described below. Rats were fed a finely ground medium-fat diet (MF) containing (wt/wt) 13% protein, 46% corn starch, and 18% fat (energy density 3.6 kJ/g; Kliba Mühlern; see Ref. 20). Animal rooms were under an artificial 12:12-h light-dark cycle at a room temperature of 21 ± 1°C. Rats were adapted to the housing conditions for at least 2 wk before tests.

Drugs and experimental design. Rat amylin was obtained from Peninsula Laboratories (Belmont, CA). The DA D1-receptor antagonist SCH-23390 [R(+)-SCH-23390 hydrochloride] and the DA D2-receptor antagonists raclopride [S(+)-raclopride t-tartrate] and sulpiride [S(-)-sulpiride] were obtained from RBI. Sterile AMP (1 mg/ml) was prepared by Kantonssapotheke Zurich. All drugs except sulpiride were dissolved or diluted in 0.9% NaCl and injected intraperitoneally in volumes of 1 or 2 ml/kg. Sulpiride was dissolved in hydrochloric acid and brought to an isotonic NaCl solution (pH 7.4) with NaOH. Control injections were equal volumes (1 ml/kg) of 0.9% NaCl.

The experiments were done at dark onset after 24 h food deprivation. SCH-23390 [10–50 μg/kg (0.03–0.15 μmol/kg)] and raclopride [100 μg/kg (0.2 μmol/kg)] were injected −30 min before dark onset, and sulpiride [50 mg/kg (154 μmol/kg)] was injected −75 min before dark onset. Amylin (5 μg/kg) or AMP (0.3 mg/kg) were injected 10–15 min before dark onset. The MF diet was presented at dark onset. The doses of amylin and AMP were chosen based on previous experiments (14, 19). The doses of the DA antagonists were chosen based on demonstrations of an antagonism of DA-mediated effects, such as the reinforcing effect of sucrose solution and the anorectic effect of AMP (14, 41, 43).

On the day before the experiments, food intake was measured during the first 4 h of the dark phase, and rats were divided into experimental and control groups with similar food intakes during this period and similar body weights.

In experiments 1a, 2, and 4, spillage-resistant feeding cups were fixed on electronic precision balances in which outputs were continuously monitored to compute cumulative food intake and meal patterns. Meals were defined using a minimum meal size criterion of 0.3 g, a minimum meal duration criterion of 1 min, and a minimum intermeal interval (IMI) criterion of 15 min, measured from the last measured intake of the meal (23). With the use of these criteria, ~95% of total food intake was resolved into meals. In experiments 1b, 3, and 5, cumulative food intake was assessed manually by weighing the feeding cups. In the latter experiments, meal patterns could not be calculated. Spillage was collected and measured.

Experiment 1a tested the influence of the DA D2-receptor antagonist raclopride [100 μg/kg (0.2 μmol/kg)] on the anorectic effect of amylin (5 μg/kg). Four groups (control, amylin, raclopride, amylin + raclopride) of 12 rats each were used. The procedure in experiment 1b was the same as in experiment 1a except the DA D2-receptor antagonist sulpiride [50 mg/kg (154 μmol/kg)] was used instead of raclopride, and only cumulative food intake was determined. Four groups [control (n = 15), amylin (n = 16), sulpiride (n = 15), amylin + sulpiride (n = 16)] of rats were used.

Experiment 2 compared the effects of the DA D2-receptor antagonist SCH-23390 [50 μg/kg (0.15 μmol/kg)] and the DA D2-receptor antagonist raclopride [100 μg/kg (0.2 μmol/kg)] on the anorectic effect of amylin (5 μg/kg). Twenty-two rats were tested in each of four conditions (control, amylin, amylin + SCH-23390, amylin + raclopride). Rats received the treatments in random order using a cross-over design with 4 days of ad libitum feeding between trials.

Experiment 3 investigated whether SCH-23390 [50 μg/kg (0.15 μmol/kg)] alone affects food intake. The effect of raclopride [100 μg/kg (0.2 μmol/kg)] was retested to allow a comparison with experiment 1a. Three groups (control, SCH-23390, raclopride) of eight rats each were used.

Because in experiments 2 and 3 SCH-23390 [50 μg/kg (0.15 μmol/kg)] per se reduced food intake, experiment 4 tested the effects of a reduced dose of 10 μg/kg (0.03 μmol/kg) SCH-23390 on amylin’s (5 μg/kg) anorectic effect. Twenty-four rats were tested in each of four conditions (control, amylin, amylin + SCH-23390, amylin + raclopride). Rats received the treatments in random order using a cross-over design with 4 days of ad libitum feeding between trials.

Experiment 5 tested whether SCH-23390 [10 μg/kg (0.03 μmol/kg)] antagonizes the anorectic effect of AMP (0.3 mg/kg). Because the anorectic effect of AMP is mediated by DA D1 receptors (14), this test served as a control for the effectiveness of this dose of SCH-23390 under our conditions. Thirty-nine rats were used (10 control, 10 AMP, 10 SCH-23390, and 9 AMP + SCH-23390).

Statistics. Results are presented as means ± SE. Results were evaluated by ordinary (experiments 1a, 1b, 3, and 5) or repeated-measures (experiments 2 and 4) ANOVA and the Student-Newman-Keuls post hoc test. In all cases, a value of P < 0.05 was considered significant.

RESULTS

Experiment 1a: influence of the DA-receptor antagonist raclopride [D2 antagonist; 100 μg/kg (0.2 μmol/ kg)] on the anorectic effect of amylin (5 μg/kg) in 24-h food-deprived rats. Amylin significantly reduced cumulative food intake throughout the 2-h observation period (Fig. 1A), mainly by reducing the size and duration of the first meal after injection. Average feeding rate during this meal (data not shown), latency to eat, and the first IMI remained unaffected (Table 1). Subsequent meals also remained unaffected by amylin (results not shown). Raclopride alone had no effect on food intake but significantly attenuated amylin’s anorectic
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Observation period, mainly due to a reduction in the size and duration of the first meal after injection (Table 2). The apparent discrepancy between 30 min food intake and the size and duration of the first meal after injection is due to the fact that, although most rats started eating immediately after injection and food presentation, some did not.

The average feeding rate during the first meal, the latency to eat, the first IMI, and subsequent meals were unchanged (results not shown). Raclopride significantly attenuated amylin’s effect on the size of the first meal after injection, whereas SCH-23390 did not (Table 2). Raclopride tended to attenuate amylin’s action on cumulative food intake, although this was not significant (P < 0.1 at 1, 2, and 4 h after injection; Table 2). In contrast, animals tended to eat somewhat less after treatment with SCH-23390 plus amylin than after amylin alone (Table 2).

**Experiment 3: influence of the DA-receptor antagonists SCH-23390 [D_1 antagonist; 50 μg/kg (0.5 μmol/kg)] and raclopride [D_2 antagonist; 100 μg/kg (0.2 μmol/kg)] on food intake in 24-h food-deprived rats.** This test was designed to determine whether the apparent increase in amylin’s anorectic effect after pretreatment with SCH-23390 observed in experiment 2 was due to a specific interaction between amylin and SCH-23390 or an effect of SCH-23390 alone on food intake. The results support the latter possibility [that SCH-23390 at 50 μg/kg (0.13 μmol/kg) significantly reduced cumulative food intake compared with control animals (Fig. 2)]. As in experiment 1a, raclopride [100 μg/kg (0.2 μmol/kg)] had no effect on food intake when given alone. In light of these results, in experiment 4, we reduced the dose of SCH-23390 from 50 to 10 μg/kg.

**Experiment 4: influence of the DA-receptor antagonist SCH-23390 [D_1 antagonist; 10 μg/kg (0.03 μmol/kg)] on the anorectic effect of amylin (5 μg/kg) in 24-h food-deprived rats.** SCH-23390 [10 μg/kg (0.03 μmol/kg)] did not affect feeding when given alone (Fig. 3) and significantly attenuated amylin’s effect on the size of the first meal after injection, whereas SCH-23390 did not (Table 2). Raclopride tended to attenuate amylin’s action on cumulative food intake, although this was not significant (P < 0.1 at 1, 2, and 4 h after injection; Table 2). In contrast, animals tended to eat somewhat less after treatment with SCH-23390 plus amylin than after amylin alone (Table 2).

**Experiment 5: influence of the DA-receptor antagonist SCH-23390 [D_1 antagonist; 10 μg/kg (0.03 μmol/kg)] on the anorectic effect of AMP (0.3 mg/kg) in 24-h food-deprived rats.** This test was designed to determine whether the apparent increase in amylin’s anorectic effect after pretreatment with SCH-23390 observed in experiment 2 was due to a specific interaction between amylin and SCH-23390 or an effect of SCH-23390 alone on food intake. The results support the latter possibility [that SCH-23390 at 50 μg/kg (0.13 μmol/kg) significantly reduced cumulative food intake compared with control animals (Fig. 2)]. As in experiment 1a, raclopride [100 μg/kg (0.2 μmol/kg)] had no effect on food intake when given alone. In light of these results, in experiment 4, we reduced the dose of SCH-23390 from 50 to 10 μg/kg.

**Fig. 1. A: influence of the dopamine (DA) D_2-receptor antagonist raclopride [100 μg/kg (0.2 μmol/kg)] on the anorectic effect of amylin (5 μg/kg) in 24-h food-deprived rats injected at dark onset. Values with different superscript letters differ significantly (P < 0.05; n = 12 experiments for all groups, ANOVA with the Student-Newman-Keuls post hoc test). B: influence of the DA D_2-receptor antagonist sulpiride [50 mg/kg (154 μmol/kg)] on the anorectic effect of amylin (5 μg/kg) in 24-h food-deprived rats injected at dark onset. **P < 0.01 and ***P < 0.001, significant difference between groups. Values with different letters at respective time points differ significantly (n = 15–16; ANOVA with the Student-Newman-Keuls post hoc test).**

<table>
<thead>
<tr>
<th>Meal Pattern</th>
<th>Size, g</th>
<th>Duration, min</th>
<th>Latency to Eat, min</th>
<th>First IMI, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNCl</td>
<td>7.4 ± 0.7*</td>
<td>42 ± 3*</td>
<td>3 ± 0</td>
<td>97 ± 15</td>
</tr>
<tr>
<td>Amylin</td>
<td>4.1 ± 0.1*</td>
<td>26 ± 5*</td>
<td>4 ± 1</td>
<td>84 ± 14</td>
</tr>
<tr>
<td>Raclopride</td>
<td>8.0 ± 0.9*</td>
<td>44 ± 5*</td>
<td>3 ± 2</td>
<td>76 ± 13</td>
</tr>
<tr>
<td>Amylin + raclopride</td>
<td>5.8 ± 0.8‡</td>
<td>36 ± 4*</td>
<td>3 ± 1</td>
<td>91 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE. DA, dopamine; IMI, intermeal interval. *P values determined by ANOVA. Values with different symbols differ significantly (P < 0.05; n = 12 for all groups, ANOVA with the Student-Newman-Keuls post hoc test).
food-deprived rats. SCH-23390 (10 μg/kg (0.03 μmol/kg)) completely blocked the anorectic effect of AMP (0.3 mg/kg; Fig. 4). As in experiment 4, SCH-23390 alone did not affect food intake.

**DISCUSSION**

The present study shows that the acute satiating effect of amylin is significantly attenuated by doses of the DA D2-receptor antagonists raclopride and sulpiride that alone have no effect on feeding. In contrast, amylin’s anorectic action was not affected by a dose of the DA D1-receptor antagonist SCH-23390 that alone had no effect on feeding but completely blocked the anorectic action of 0.3 mg/kg AMP. Hence, we show for the first time that the amylin-induced reduction in food intake is partly mediated by DA via DA D2 receptors and does not require DA D1 receptors, at least under the present experimental conditions. Raclopride’s antagonistic effect was especially clear, both in experiments 1 and 2, in the measurement of the size of the first meal after injection. This is important because amylin’s satiating action on meal size seems to be mainly responsible for its anorectic effect after acute administration both in food-deprived and in ad libitum-fed animals (23).

There was some discrepancy between experiment 1a and experiment 2 regarding the influence of raclopride on amylin’s inhibitory effect on cumulative food intake. Although this influence was significant in experiment 1a, it failed to reach the level of significance in experiment 2. In the latter experiment, a clear tendency of raclopride to attenuate amylin’s anorectic effect was, however, evident, and raclopride did significantly attenuate amylin’s effect regarding the diminution of meal size in both experiments. Therefore, the conclusion of an important role of DA in mediating peripheral amylin’s anorectic effect via DA D2 receptors appears to be justified. This conclusion is clearly corroborated by the result of experiment 1b showing that sulpiride, another DA D2 antagonist, significantly reduced amylin’s anorectic action regarding amylin’s inhibitory effect on cumulative food intake.

Our conclusion that the dopaminergic system mediates amylin’s anorectic effect via DA D2 receptors fits with the wealth of data supporting the importance of various brain dopaminergic systems in the control of feeding (17, 34, 43). It has been clearly established that

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**Table 2. Influence of the DA receptor antagonists SCH-23390 [D1 antagonist; 50 μg/kg (0.15 μmol/kg)] and raclopride [D2 antagonist; 100 μg/kg (0.2 μmol/kg)] on the anorectic effect of amylin (5 μg/kg) in 24-h food-deprived rats injected at dark onset**

<table>
<thead>
<tr>
<th>Time after injection:</th>
<th>Cumulative Food Intake, g</th>
<th>First Meal</th>
<th>Duration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.4 ± 0.3 a</td>
<td>7.1 ± 0.4 a</td>
<td>9.1 ± 0.5 a</td>
</tr>
<tr>
<td>Amylin</td>
<td>2.8 ± 0.3 b</td>
<td>4.2 ± 0.4 c</td>
<td>5.6 ± 0.6 d</td>
</tr>
<tr>
<td>Amylin + SCH-23390</td>
<td>2.0 ± 0.3 f</td>
<td>3.3 ± 0.5 g</td>
<td>6.1 ± 0.7 h</td>
</tr>
<tr>
<td>Amylin + raclopride</td>
<td>3.2 ± 0.3 h</td>
<td>5.4 ± 0.7 i</td>
<td>7.3 ± 0.6 j</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. P values determined by ANOVA. Values with different symbols at respective time points differ significantly (n = 22 for all groups; repeated-measures ANOVA with the Student-Newman-Keuls post hoc test).

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**Fig. 2. Influence of the DA-receptor antagonists SCH-23390 [D1 antagonist; 50 μg/kg (0.15 μmol/kg)] and raclopride [D2 antagonist; 100 μg/kg (0.2 μmol/kg)] on cumulative food intake in 24-h food-deprived rats. *P < 0.05 and **P < 0.01, significant difference between groups. Values with different letters differ significantly (P < 0.05; n = 8 for all groups, ANOVA with the Student-Newman-Keuls post hoc test).**

**Fig. 3. Influence of the DA D1-receptor antagonist SCH-23390 [10 μg/kg (0.03 μmol/kg)] on the anorectic effect of amylin (5 μg/kg) in 24-h food-deprived rats. *P < 0.01, significant difference between groups. Values with different letters differ significantly (P < 0.05; n = 24 for all groups, repeated-measures ANOVA with the Student-Newman-Keuls post hoc test).**
peripherally administered amylin elicits its satiety effect through a central mechanism (19, 26, 31). Furthermore, the DA D2 antagonists used in this study have been shown to act at central sites after systemic administration (17, 43).

Nevertheless, our data are unable to allow any definite conclusion about the site of DA receptors involved in mediating amylin’s satiating effect. However, we think that there is reason to believe that it may be AP/NTS DA that is involved in mediating amylin’s satiety effect. First, the AP/NTS region contains both DA (45) and amylin (42) binding sites, and amylin excites neurons in the AP (35). Furthermore, the AP/NTS region is necessary for the anorectic effect of amylin after intraperitoneal injection (26). Second, this region contains mainly DA D2 but only few D1 receptors (34). Third, NTS DA D2 receptors may be involved in the control of food intake by CCK (34). These receptors, which codistribute with CCK-binding sites in the NTS (34), seem to mediate at least part of the anorectic effect of CCK because the DA-receptor antagonist cis-flupentixol reduced CCK’s anorectic effect (6) and CCK increases DA levels in NTS tissue probes (7). These findings are especially interesting in relation to our previous finding that CCK’s anorectic effect, which shows some parallels to that of amylin, is partly mediated by amylin (21, 24). Finally, recent preliminary experiments showed that an infusion of raclopride (10 $\mu$g/rat in 0.5 $\mu$l) in the AP/NTS region blocked the anorectic action of peripherally injected amylin (5 $\mu$g/kg; unpublished observation).

To summarize, the hypothesis that AP/NTS DA is involved in mediating amylin’s satiety effect awaits definite proof. Nevertheless, the data provided here are consistent with the following model for the interaction between amylin and DA in the control of food intake. Peripheral amylin reaches amylin receptors in the AP that directly or indirectly activate dopaminergic neurons in the AP (15) projecting to the NTS. The DA released by these neurons acts on postsynaptic DA D2 receptors that provide a necessary input for part, but not all, of the amylin satiety signaling pathway. Furthermore, these same NTS neurons may integrate the effects of various satiety signals, such as amylin and CCK, so that, for example, CCK’s effect depends in part on coactivation of the amylin pathway.

Apart from the control of food intake by the AP/NTS dopaminergic system, both DA D1 and D2 receptors in the hypothalamus mediate an important inhibitory control of feeding (14, 17, 18, 33). Whether these receptors may also play a role in amylin’s feeding effects is unknown. However, it is of interest in this context that peripheral injection of a DA agonist (apomorphine) suppressed intake not only in intact rats but also in chronic decerebrate rats, suggesting that the forebrain was not necessary for this DA receptor-mediated effect (16).

DA also plays a role in the mediation of food reward, mainly via mesolimbic dopaminergic projections to both DA D1 and DA D2 receptors in the nucleus accumbens (for review, see Ref. 43). For example, in rats sham feeding with open gastric cannulas, DA D1 and D2 receptor antagonists reduce the intake of a sucrose solution similar to the effect of reducing the concentration of sucrose (41, 43). Several considerations, however, suggest that this role of DA in the control of feeding does not account for amylin’s satiating effect. First, this effect of DA increases rather than decreases feeding. Second, Asarian et al. (4) showed that, although amylin reduced sham feeding in rats, the initial rate of licking was not affected, as would be the case if amylin reduced food reward (4). Third, in the present study only DA D2 but not D1 receptor antagonism attenuated amylin’s anorectic effect, whereas both receptor subtypes appear to be involved in dopaminergic food reward (43). Finally, if amylin lowered food intake through an antagonism of food reward, i.e., through a decrease in dopaminergic transmission in the nucleus accumbens, coadministration of amylin plus raclopride or sulpiride should result in an enhancement of amylin’s anorectic effect rather than its attenuation.

The dose of raclopride used in the present study was chosen on the basis of previous experiments demonstrating an antagonistic action of DA-mediated effects, such as the reinforcing effect of sucrose (41). In that study (41), raclopride alone produced some suppression of sucrose intake while in the present study raclopride left basal food intake unaltered. This apparent discrepancy may well be due to differences in the experimental design between both studies, such as measuring the intake of sucrose-containing fluids (41) vs. solid food and use of a sham-feeding (41) vs. a real-feeding design. If higher doses of raclopride had been used in the present study, it is possible that some suppression of basal food intake would have been observed.

Our observation that 10 $\mu$g/kg (0.03 $\mu$mol/kg) of SCH-23390, which specifically blocks DA D1 receptors, did not attenuate amylin’s anorectic effect suggest that DA D1 receptors are not crucially involved in amylin's...
satiety effect. This was unlikely to be due to the low dose of SCH-23390 used because we also found that this dose completely blocked AMP-induced anorexia, which is mediated by hypthalamic DA D1 receptors (14). That the dose of 50 μg/kg (0.15 μmol/kg) SCH-23390 alone reduced food intake in animals agrees with previous reports (14). That raclopride and sulpiride, but not SCH-23390, attenuated the anorectic action of amylin is consistent with our conclusion that DA receptors in the NTS mediate at least part of amylin’s anorectic effect because the NTS appears to contain mainly DA D2 but only few DA D1 receptors (34).

The experiments described here were performed using the same procedures (e.g., experimental design, MF diet, etc.) as a previous study in which we showed that antagonism of the release of endogenous histamine by stimulation of histamine H3-autoreceptors attenuated the anorectic effect of amylin (22). These data indicate that the histaminergic system mediates part of amylin’s anorectic effect (22); we believed that this was due to histamine release in the central nervous system (22). Activation of presynaptic H3 receptors not only reduces the release of histamine, however, but via receptors on nonhistaminergic neurons also reduces the release of serotonin (39), norepinephrine (40), and DA (11). Therefore, it is possible that the attenuation of amylin’s anorectic effect by histamine H3 agonists (22) was due to an inhibition of DA release rather than of histamine release. Further work is required to clarify the interaction of the dopaminergic and the histaminergic systems in amylin’s anorectic effect.

In summary, we have shown that the DA D2-receptor antagonists raclopride and sulpiride, but not the DA D1-receptor antagonist SCH-23390, significantly attenuated the anorectic effect of intraperitoneally injected amylin. Therefore, DA D2 receptors seem to partially mediate amylin’s anorectic effect under the present experimental conditions.

Perspectives

The findings of this and a previous (22) study implicate both brain histamine and DA in amylin’s anorectic effect. It is not yet known to what extent, how, and where histamine and DA interact to bring about amylin’s anorectic action. One possibility is that, in the AP/NTS region, amylin activates dopaminergic neurons that act locally on D2 receptors of other neurons that then increase hypothalamic histamine release. Alternatively, amylin may reduce feeding by activating dopaminergic AP/NTS neurons in which DA release may be under histaminergic control. These neurons then inhibit feeding by acting on D2 receptors in the AP/NTS region or elsewhere.

Clarifying the neurotransmitters mediating amylin’s anorectic effect may also reveal possible points of contact between the control of feeding by amylin and by other short-term (direct; see Ref. 44) and long-term (indirect; see Ref. 44) satiety signals. This may include an interaction between amylin, CCK, and DA in the AP/NTS region because both of their anorectic effects are partly mediated by DA and because CCK’s effect depends partly on amylin (21, 24). Another example relates to leptin. Like amylin (22), leptin’s anorectic action is partly mediated by histamine (30, 50). Furthermore, recent studies suggest that lasting elevations in amylin levels tonically decrease feeding (37).

What remains unclear is whether amylin’s persistent anorectic effect during chronic administration (2) is mediated by the same neurotransmitters as the short-term anorectic effect induced by a single injection. Interestingly, a recent study showed that salmon calcitonin, which is structurally and functionally related to amylin (13, 32, 46, 49) and which reduces feeding via amylin-binding sites (27), activates tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of DA (and other catecholamines), in brain cell cultures (1). Therefore, it is possible that amylin, in addition to enhancing DA release from dopaminergic neurons, also increases tyrosine hydroxylase activity and thus DA synthesis in these neurons. This mechanism could ensure that amylin’s anorectic effect induced by chronic administration is mediated by these neurons for an extended period of time.

The critical input of T. Riediger (Institute of Veterinary Physiology, University of Zurich) and P. A. Rushing (Department of Psychiatry, University of Cincinnati) is gratefully acknowledged.

This work was supported by Swiss National Research Foundation Grant 3100–045 583.95/1.

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