Effects of amylin and salmon calcitonin on feeding and drinking behavior in pygmy goats

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Abstract

In the present study, the effects of peripherally administered amylin and of the amylin-related peptide salmon calcitonin (sCT) on food and water intake was tested for the first time in pygmy goats. In the first series of experiments, the effect of amylin on food (0.5, 1.0 and 2.0 μg/kg b.wt.) and water (2.0 μg/kg) intake was tested. In the second series of experiments, the effect of sCT on food intake (1.0 μg/kg) was tested under ad libitum feeding conditions or after 14 h food deprivation. The relationship of dose on the effect of sCT (0.1, 0.5 and 1.0 μg/kg) on food and water intake was also tested. Finally, the effect of a low dose (0.1 sCT μg/kg) on water intake was also investigated during food withdrawal. We showed for the first time an anorexigenic effect of the satiety peptide amylin (2.0 μg/kg) in ruminants, which was characterized by a reduction in meal size. In pygmy goats, the administration of the three doses of sCT induced an anorexigenic effect, which was larger and of longer duration when compared with amylin, although the anorexigenic effect of the lowest dose never reached significance. This effect was not dose dependent and was partly due to a reduction in meal size and partly to a prolongation of the interval between meals. The anorexigenic effect of sCT was accompanied by a reduced water intake, probably due to reduced prandial drinking. Furthermore, the low dose of sCT (0.1 μg/kg) was dipsogenic during food withdrawal.© 2002 Published by Elsevier Science Inc.

Keywords: Amylin; Salmon calcitonin; Anorexigenic effect; Dipsogenic effect; Pygmy goats

1. Introduction

In recent years, the role of the pancreatic beta-cell hormone amylin being coreleased with insulin as a satiety peptide in rodents has been recognized [1–4]. Amylin reduces food intake in rats and mice after peripheral and central administration [1,2,4–7]. Numerous studies have established the central nervous system as the site of the short-term satiety action of peripherally administered amylin [8,9]. Amylin seems to act directly on neurons in the area postrema (AP) to bring about its anorexigenic effect [8,9].

The anorexigenic effect of salmon calcitonin (sCT), a peptide that is structurally and functionally related with amylin [10,11] and whose effect on food intake in rats, monkeys and humans has been described previously [12,13], seems to be brought about by an interaction with amylin binding sites [14]. Due to its irreversible binding to amylin receptors [15], sCT’s anorexigenic effect lasts much longer than that of amylin [14].

In ruminants, many mechanisms of food intake regulation are parallel to those in rats. A peptidergic control of food intake has been demonstrated in sheep [16,17]. In sheep, intracerebroventricularly, but not intravenously, administered sCT has been shown to depress food intake [18]. In the present study, the effects of peripherally administered amylin and sCT on food intake in another ruminant model, the pygmy goat, was investigated for the first time.

In recent studies, it has been demonstrated that both amylin [19] and calcitonin [20] induce drinking behaviour in rats probably via excitatory effects on angiotensin II (ANGII)-sensitive neurons in the subfornical organ (SFO). It has also been proposed that stimulation of the SFO by nutrient-stimulated amylin secretion may stimulate prandial drinking [19]. Furthermore, sCT has been shown to cause excitation of the amylin- and rat CT-sensitive neurons in the SFO [21]. Therefore, in the present study, a possible effect...
of amylin and the amylin-related peptide sCT on drinking behavior in pygmy goats was investigated together with their effect on feeding. Experiments were performed in the presence and absence of food.

2. Materials and methods

2.1. Animals and maintenance

Eleven to twelve adult female nonlactating and nonpregnant African pygmy goats (age: 2–10 years) that weighed 20–41 kg were used. The goats were individually housed on wood shavings in pens (1.25 × 1.35 m) located in a room with an artificial 12-h light–dark cycle. Lights were on at 09:00 h. Temperature was held constant at 21 ± 2 °C. Goats were fed ad libitum a complete pelleted diet with 86% dry matter (Hypona 888, Volg Winterthur, Switzerland). Water was always available. Goats were fed from spill-resistant feed containers that were fixed on scales (Mettler PE, PM, PG, Greifensee, Switzerland). A similar system was used to record water intake [22].

2.2. Feed and water intake measurement

The actual weight of the food and water containers was automatically monitored by a computerized system. Meals were defined as feed removals exceeding 5 g that were separated by at least 15 min of nonfeeding. With this meal definition, the recorded meals accounted for 95% of the cumulative food intake during ad libitum feeding [22]. Parameters recorded were cumulative feed intake and meal pattern, including latency to eat (min), meal size (g), intermeal interval (IMI, min), meal frequency and eating rate (g/min).

Every meal, which began before 21:00 h (beginning of dark phase), was defined to belong to the meals ingested during the light phase, regardless of a possible continuation of a meal in the dark phase (Tables 1–4). However, the time frame for the cumulative food intake was exactly 0–10 h postinjection (Figs. 1–4).

2.3. Experiments

Depending on the experiment, 11 or 12 goats were subdivided in two or four treatment groups with similar weight.

On test days, in order to coordinate the beginning of feeding between the goats, food was withdrawn during the first 2 h of the light phase. The goats were injected intraperitoneally (ip) in the paralumbal fossa 2 h after the onset of the light phase, and cumulative food and/or water intake was recorded for the following 22 h. Control animals received an equivalent volume of saline.

All experiments (exception: effect of 1.0 μg/kg b.wt. amylin on food intake) were performed in counterbalanced

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Meal pattern from 11:00 to 21:00 h after injection of amylin (2.0 μg/kg b.wt.) in pygmy goats at 11:00 h</th>
<th>Saline</th>
<th>Amylin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to eat (min)</td>
<td>10.4 ± 7.1</td>
<td>3.6 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Average meal size (g)</td>
<td>54.8 ± 6.2</td>
<td>35.7 ± 3.9*</td>
<td></td>
</tr>
<tr>
<td>Meal frequency</td>
<td>7.1 ± 0.5</td>
<td>9.5 ± 1.0*</td>
<td></td>
</tr>
<tr>
<td>Average IMI* (min)</td>
<td>67.0 ± 5.5</td>
<td>57.0 ± 7.3</td>
<td></td>
</tr>
<tr>
<td>Eating rate (g/min)</td>
<td>3.3 ± 0.5</td>
<td>2.7 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. n = 11 for each group.
* IMI: intermeal interval.
* Significantly (P < .05, paired Student’s t test) different from saline value.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Meal pattern from 11:00 to 21:00 h after injection of sCT (1.0 μg/kg b.wt.) in pygmy goats at 11:00 h</th>
<th>Saline</th>
<th>sCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to eat (min)</td>
<td>28.5 ± 15.8</td>
<td>7.9 ± 4.2*</td>
<td></td>
</tr>
<tr>
<td>Average meal size (g)</td>
<td>57.8 ± 9.7</td>
<td>37.7 ± 3.5*</td>
<td></td>
</tr>
<tr>
<td>Meal frequency</td>
<td>7.6 ± 0.8</td>
<td>7.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Average IMI* (min)</td>
<td>72.6 ± 8.8</td>
<td>91.2 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Eating rate (g/min)</td>
<td>3.4 ± 0.4</td>
<td>2.5 ± 0.3*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. n = 12 for each group.
* IMI: intermeal interval.
* Significantly (P < .05, paired Student’s t test) different from saline value.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Meal pattern from 11:00 to 21:00 h after injection of sCT (1.0 μg/kg b.wt.) in pygmy goats at 11:00 h, after 14 h of food deprivation</th>
<th>Saline</th>
<th>sCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to eat (min)</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Average meal size (g)</td>
<td>61.3 ± 8.7</td>
<td>43.1 ± 5.7*</td>
<td></td>
</tr>
<tr>
<td>Meal frequency</td>
<td>8.5 ± 0.5</td>
<td>7.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Average IMI* (min)</td>
<td>67.1 ± 7.5</td>
<td>87.3 ± 11.1</td>
<td></td>
</tr>
<tr>
<td>Eating rate (g/min)</td>
<td>2.8 ± 0.2</td>
<td>3.0 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. n = 12 for each group.
* IMI: intermeal interval.
* Significantly (P < .05, paired Student’s t test) different from saline value.
order to have each goat to act as its own control. The period between treatments was 1 week.

In the first series of experiments, the effect of rat amylin (molecular weight 3921, Peninsula Laboratories, Belmont CA, USA) on food (0.5, 1.0 and 2.0 µg/kg b.wt.) and water intake (2.0 µg/kg b.wt.) was tested. The effect of amylin (1.0 µg/kg b.wt.) on water intake during food withdrawal was also tested.

In the second series of experiments, we first tested the effect of 1.0 µg/kg b.wt. sCT (molecular weight 3396.9, Peninsula Laboratories) on food intake. The effect of 1.0 µg/kg b.wt. sCT on food intake was also tested after 14 h of

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**Fig. 1.** Effect of amylin (2.0 µg/kg b.wt. ip) on the cumulative food intake in pygmy goats. Values are means ± S.E.M., n = 11 each group. * P < .05, significantly different from saline (paired Student’s t test).

**Fig. 2.** Effect of sCT (1.0 µg/kg b.wt. ip) on the cumulative food intake in pygmy goats. Values are means ± S.E.M., n = 12 each group. * P < .05, ** P < .01, *** P < .001, significantly different from saline (paired Student’s t test).
food deprivation. Further, the effect of three doses of sCT (0.1, 0.5 and 1.0 μg/kg b.wt) on food and water intake was investigated. Finally, the effect of 0.1 μg/kg b.wt. sCT on water intake during food withdrawal was tested.

2.4. Statistical analysis

The values in the tables and figures represent means ± S.E.M. The differences between the two treatment groups
were statistically evaluated using the unpaired (comparison of two groups of goats) or paired (comparison of two groups of goats injected in counterbalanced order) Student’s t test. The differences among the four treatment groups were statistically evaluated with a repeated-measures ANOVA, since the experiments were performed in counterbalanced order with the Bonferroni post hoc test.

Fig. 5. Effect of sCT (0.1, 0.5 and 1.0 μg/kg b.wt. ip) on the cumulative water intake in pygmy goats. Values are means ± S.E.M., n = 11 each group. At 8 and 10 h after injection: significant difference between groups (P < .05, repeated-measures ANOVA). * P < .05, significantly different from saline (Bonferroni post hoc test).

Fig. 6. Effect of sCT (0.1 μg/kg b.wt. ip) on the cumulative water intake in pygmy goats during food withdrawal. Values are means ± S.E.M., n = 11 each group. At 8 and 10 h after injection: significant difference between groups (P < .05, repeated-measures ANOVA). ** P < .01, significantly different from saline (paired Student’s t test).

3. Results

The intraperitoneal injection of amylin (2.0 μg/kg, Fig. 1) reduced the cumulative food intake, which was significant 2 h after injection. This reduction in food intake was characterized by a significantly reduced average meal size 0–10 h postinjection, which was associated with a signific-
ant increase in meal frequency (Table 1). The eating rate was not affected (Table 1). The intraperitoneal injection of 0.5 and 1.0 μg/kg amylin (results not shown) did not produce such a short-term inhibition of feeding. Water intake of pygmy goats was not affected by the injection of amylin (results not shown).

The injection of 1.0 μg/kg sCT produced a long-lasting reduction in the cumulative food intake both under ad libitum feeding conditions (significant from 3 to 22 h, Fig. 2) and after 14 h of food deprivation (significant from 7 to 10 h, Fig. 3). The reduction in food intake was characterized by a significantly reduced average meal size 0–10 h postinjection, and a trend towards an increased average IMI (Tables 2 and 3) was observed. Interestingly, the latency to eat was significantly shorter after injection of 1.0 μg/kg sCT under ad libitum feeding conditions (Table 2). The eating rate was significantly reduced under ad libitum feeding conditions (Table 2).

In the dose–response experiment, the injection of 0.5 and 1.0 μg/kg sCT produced a reduction in the cumulative food intake, which was significant from 4 to 10 h after injection (Fig. 4). The eating rate was significantly reduced only after injection of 0.5 μg/kg sCT (Table 4). Although the lowest dose of sCT (0.1 μg/kg) also produced a reduction in the cumulative food intake of comparable magnitude, this effect was not significant (Fig. 6). Both average meal size and meal frequency seemed to be diminished following sCT injection, but these effects did not reach statistical significant (Table 4). Furthermore, the latency to eat after injection of 0.5 and 1.0 μg/kg sCT was shorter when compared to saline-injected goats (Table 4). This effect, however, was not significant.

The anorexigenic effect of sCT (Fig. 4) was accompanied by a reduction in the cumulative water intake, which was significant at 8 h after injection of 0.5 and 1.0 μg/kg (Fig. 5). The lowest dose of sCT (0.1 μg/kg) also produced a reduction in the cumulative water intake of comparable magnitude. However, this effect was not significant (Fig. 5). Interestingly, during food withdrawal, sCT was dipsogenic. As shown in Fig. 6, the injection of 0.1 μg/kg sCT produced a delayed increase in water intake, which was significant 8 h after injection.

4. Discussion

A major finding of the present study consists of the fact that the satiety peptide amylin [1–4] reduced food intake in a ruminant model, the pygmy goat. This is the first time that an anorexigenic effect of amylin has been shown in ruminants. The anorexigenic effect of amylin in pygmy goats was characterized by a significant reduction in the average meal size. In rats, the anorexigenic effect of amylin was also characterized by a reduction in meal size [6]. The observation that the anorexigenic effect of intraperitoneal amylin was due to a reduction in meal size without a change in eating rate supports the hypothesis that in pygmy goats, as observed in rats [6,14], exogenous amylin can reduce meal size by facilitating normal meal-ending satiety processes.

Amylin significantly increased meal frequency, suggesting that the decrease in meal size was partly compensated by an increase in meal frequency.

Compared with amylin, the anorexigenic effect of sCT in pygmy goats was larger and of longer duration. This finding is consistent with previous studies in rats [14,23], which showed that the anorexigenic potency of sCT was higher than that of amylin. Irreversible binding of sCT to amylin receptors may lead to a stronger and prolonged effect in comparison to amylin due to a sustained activation of the binding sites [14].

In rats, the anorexigenic effect of sCT appeared during the hour following injection [14]. Interestingly, in pygmy goats, the anorexigenic effect of intraperitoneally applied sCT was delayed when compared to rats. This could be explained by a centrally mediated inhibitory effect of sCT on reticulum motility [18], which could reduce abomasal filling. The stimulation of the emptying of the abomasum has been shown to lead to an increased food intake [24] and, therefore, immediately after injection of sCT, a stimulatory effect on food intake (in the present study expressed by the reduced latency to eat after sCT application under ad libitum feeding conditions) elicited by diminished abomasal fill could counteract the inhibitory effect on food intake induced by reticulum hypomotility leading to an increase in rumen fill [18,25,26]. It is well established that rumen distension inhibits food intake [27].

The anorexigenic effect of sCT in pygmy goats was not clearly dose related. The three doses tested (0.1, 0.5, 1.0 μg/kg) similarly reduced the cumulative food intake, although the anorexigenic effect of the lowest dose (0.1 μg/kg) never reached significance. This suggests that the central receptors mediating sCT’s anorexigenic effect were already fully activated at the lower doses.

The anorexigenic effect of sCT in pygmy goats was partly due to a reduction in meal size and partly due to a prolongation of the interval between the meals, which is consistent with the results obtained in rats [14]. It is possible that the observed effect of sCT on the IMI in pygmy goats and in rats [14] was due to its long duration of action after a single injection. The anorexigenic effect of sCT under ad libitum feeding conditions, but not after restricted feeding, was associated with a reduced eating rate. This suggests that sCT produces an aversion only under certain conditions. Therefore, its aversive property appears to be weak [28].

Under ad libitum feeding conditions, sCT reduced cumulative water intake. This observation confirms results in rats, which suggest that decrease in water intake by calcitonin may be a consequence of the food intake suppression, i.e. reduced prandial drinking [13,29]. The anti-dipsogenic effect of sCT in pygmy goats can therefore be explained by a reduction in prandial drinking, which in pygmy goats appears to depend on the release of histamine.
Interestingly, in the present study, under conditions of food withdrawal, a low dose of sCT induced drinking in pygmy goats, indicating a possible specific dipsogenic effect of sCT. This observation is consistent with the results of a recent study in rats [21], which showed the ability of sCT to activate neurons in the SFO, a brain region stimulated by amylin-related peptides and believed to be involved in control of drinking via excitatory effects on angiotensin II (ANGII)-sensitive neurons [19,21]. However, the long latency of the effect (>3 h) rather suggests an indirect action of sCT on drinking. An involvement of the known diuretic effect of sCT [31] in the observed increased water consumption after sCT application is possible, since in rats given peripheral injection of sCT, the increased water consumption was accompanied by marked increase in urine volume [13].

In conclusion, the present study showed for the first time an anorexigenic effect of amylin and the related peptide sCT after peripheral administration in a ruminant model, the pygmy goat. Furthermore, a dipsogenic effect of sCT has been observed.

References