Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers

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Lutz, Thomas A., Janine Althaus, Rinaldo Rossi, and Erwin Scharrer. Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1777–R1782, 1998.—Abdominal vagal and splanchnic afferents play an important role in the control of food intake in that they transmit various satiety signals to the central nervous system. Inasmuch as previous studies have shown that the anorectic effect of intraperitoneally injected amylin was not abolished by subdiaphragmatic vagotomy, the aim of the present study was to elucidate the role of splanchnic afferents in mediating amylin’s anorectic effect after intraperitoneal injection. Rats were pretreated intraperitoneally with the neurotoxin capsaicin, which destroys primary sensory (vagal and splanchnic) afferents. Sham-treated rats served as control. Capsaicin-pretreatment had no influence on the anorectic effects of amylin (5 µg/kg) and the related peptide, calcitonin gene-related peptide (CGRP; 5 µg/kg), in 24-h food-deprived rats. Abolition of cholecystokinin’s (3 µg/kg) anorectic effect agrees with previous studies and confirmed the effectiveness of the capsaicin pretreatment. In conclusion, the anorectic effects of intraperitoneally injected amylin and CGRP are not mediated by capsaicin-sensitive primary sensory neurons. Both anorectic peptides are, therefore, most likely to act within the central nervous system. Previous studies suggest that the relevant receptors might be located in neurons of the area postrema-nucleus of the solitary tract region.

calcitonin gene-related peptide; cholecystokinin

AMYLIN (or islet amyloid polypeptide), a recently discovered 37-amino acid pancreatic polypeptide, is cosecreted with insulin in response to food intake (5) and has been regarded as a novel satiety peptide (17). Numerous studies have shown that amylin reduces food intake in rats and mice after peripheral and central administration (4, 7, 14, 15, 17, 22, 23) and that chronic amylin administration reduces food intake and body weight in rats (1). Amylin’s effect seems to be specific in that it does not affect water intake (14) or induce a conditioned taste aversion after peripheral or central administration (6, 17). Hence its anorectic effect does not seem to be secondary to some aversive or toxic effect.

Because the pancreatic β-cells are the main production site for amylin (9), amylin released in response to food intake is physiologically delivered via the portal vein into the systemic circulation. The liver, being the first organ to be reached by amylin, however, does not extract amylin from the blood (24), and the liver or the hepatoportal area does not seem to be involved in the mediation of amylin’s anorectic effect, because hepatic branch vagotomy did not affect amylin’s anorectic effect after intraperitoneal administration (14). The same holds true for other abdominal vagal fibers, because total subdiaphragmatic vagotomy did not influence amylin’s anorectic effect either (15, 23). Amylin was, therefore, supposed to act via central nervous system receptors or peripheral receptors on splanchnic afferents (15). It could, however, not be excluded that, similar to bombesin (30), amylin exerts its anorectic action by an activation of receptors located on both vagal and splanchnic afferents.

Recently, we substantiated the important role of the area postrema-nucleus of the solitary tract (AP-NTS) region in the hindbrain in mediating amylin’s anorectic effect after intraperitoneal injection, because this effect was markedly attenuated in AP-NTS-lesioned rats (19). It is not clear, however, whether the attenuation of amylin’s anorectic effect in AP-NTS-lesioned rats is due to the destruction of central amylin binding sites within the AP-NTS region (2), which is devoid of a blood-brain barrier (33), or whether this attenuation in AP-NTS-lesioned rats is due to the AP-NTS region being an important projection site for abdominal splanchnic and vagal afferents (10, 28). The question, therefore, whether central receptors or peripheral receptors on splanchnic (and vagal) afferents mediate amylin’s anorectic effect after peripheral administration, remained open.

It was, therefore, the aim of the present study to elucidate the role of visceral afferents in mediating amylin’s anorectic effect after peripheral amylin administration. We used rats pretreated with the potent neurotoxin capsaicin, which has been shown to cause substantial damage to unmyelinated primary sensory neurons (25). If capsaicin-sensitive afferents were involved in mediating amylin’s anorectic effect after intraperitoneal administration, this effect would be expected to be attenuated in capsaicin-pretreated rats. Previous studies have shown that the satiety effect of peripherally administered cholecystokinin (CCK; 26) is markedly reduced in rats after capsaicin pretreatment. We therefore also investigated if the anorectic effect of CCK was influenced in capsaicin-pretreated rats under our experimental conditions. Finally, we tested whether the anorectic effect of calcitonin gene-related peptide (CGRP; 18) is reduced by capsaicin pretreatment of rats. CGRP is structurally and functionally related to amylin (9), and extensive crossreaction of amylin and CGRP at their respective receptor sites has been shown (9). Furthermore, the anorectic effect of CGRP shows many similarities to that of amylin in that CGRP is similarly potent in reducing food intake in rats and mice after peripheral administration (18, 21), and CGRP’s anorectic effect is also reduced in rats lesioned in the AP-NTS region (19).
MATERIALS AND METHODS

Adult male Sprague-Dawley rats (ZUR:SD; Institut für Laboratoriumskauf, University of Zürich, Switzerland) were used for the experiments. Rats were kept in a colony room at a constant room temperature of 21 ± 1°C and under an artificial 12:12-h light-dark cycle (lights off at 2000). The rats were housed individually in wire cages. They were adapted to the housing conditions for at least 2 wk before the capsaicin treatment. The rats were fed a medium-fat diet with a fat content (wt/wt) of 18% (14). Food was available ad libitum, except during food deprivation just before the experiments (see below). Water was always available ad libitum.

Capsaicin pretreatment of rats. On the day of the capsaicin pretreatment, the mean body weight of rats was ~270 g. Rats were randomly allocated into two groups. The first group was to be pretreated with capsaicin, the second group served as sham-treated control group injected with the vehicle.

Systemic capsaicin pretreatment was performed by a procedure similar to that described in previous reports (8, 26, 27). Briefly, after 12 h food deprivation, rats were premedicated with atropine (2 mg/kg ip) and theophylline (5 mg/kg ip) ~30 min before induction of general anesthesia (27). Rats were kept under ketamine (80 mg/kg)-xylazine (4 mg/kg) anesthesia during capsaicin injection and during the postinjection period. The total dose of capsaicin (117.5 mg/kg; Sigma) was administered as a series of three intraperitoneal injections of 12.5, 30, and 75 mg/kg within a 24-h period (injections at 0, 18, and 24 h). Capsaicin was dissolved in a vehicle consisting of ethanol (10%), Tween 80 (10% Sigma), and 0.9% saline solution (80%). The concentration of the capsaicin solution was 25 mg/ml for all injections. Sham-treated rats received three intraperitoneal injections of equivalent volumes of the vehicle. After capsaicin or vehicle injections, all rats received analgetic treatment with buprenorphine (0.5 mg/kg).

Rats treated with capsaicin showed marked cutaneous hyperemia and acral hyperthermia. All rats treated with capsaicin exhibited prolonged respiratory arrest for various durations (5–45 min). During respiratory arrest, rats received artificial positive pressure ventilation via a respiratory mask until they resumed spontaneous respiration. In most rats, respiratory arrest was observed after all three capsaicin injections, but it was generally of shorter duration after the second and third capsaicin injection. The survival rate after the first capsaicin injection was ~65%, all rats survived the subsequent capsaicin injections. After the capsaicin treatment, about one-half of the rats developed skin lesions on their head due to self-mutilation during grooming subsequent to the destruction of sensory (pain) fibers. These lesions were minor in most rats, except in four rats, where the skin lesions comprised more than one-third of the skin on the head. Skin lesions were treated with astringent zinc ointment, and infections of the lesions did not occur. Despite these lesions, the general condition of all capsaicin-pretreated rats was good. Sham-treated rats did not show any sign of discomfort, respiratory arrest, or skin lesions.

Functional tests for verification of the neurotoxic effect of capsaicin. All rats were subjected to two functional tests to verify the destruction of primary sensory afferents (11) by the capsaicin pretreatment (12, 26, 27). First, an eye-wiping test was carried out ~1 wk after capsaicin or vehicle pretreatment, i.e., before the feeding experiments were performed (12). After the application of an irritating solution to the cornea, rats normally respond by vigorously wiping their eyes, whereas eye wiping is markedly reduced subsequent to the capsaicin pretreatment. For the test, one drop of 1% NaOH solution was applied to both eyes by a Pasteur pipette, and the number of eye wipes during a 10-s period after application was recorded. All rats fulfilled the criteria for successful capsaicin treatment (<4 eye wipes in 10 s), because capsaicin-pretreated rats (n = 16) showed significantly less eye wiping than vehicle-treated controls (n = 12; left eye: vehicle-treated rats 13 ± 1 vs. capsaicin-pretreated rats 1 ± 0 eye wipes/10 s (P < 0.05); right eye: 13 ± 1 vs. 1 ± 0 (P < 0.05)).

As a second functional test (experiment 3), the feeding response to CCK (3 µg/kg ip) was compared in capsaicin-pretreated rats and vehicle-pretreated rats because previous studies have shown that the anorectic effect of CCK is markedly attenuated by capsaicin pretreatment (8, 26). This test, which was performed after the amylin and CGRP experiments, confirmed the success of the capsaicin pretreatment [the results of this test are shown in RESULTS (experiment 3)].

Drugs, experimental procedure. Rat amylin, CGRP, and CCK-sulfated octapeptide were obtained from Peninsula Laboratories (Belmont, CA). The peptides were dissolved in 0.9% NaCl and injected intraperitoneally (1 ml/kg). Injection of 0.9% NaCl served as control. Rats were injected at dark onset (2000) after 24-h food deprivation. The doses applied were amylin 5 µg/kg (experiment 1), CGRP 5 µg/kg (experiment 2), and CCK 3 µg/kg (experiment 3).

Cumulative food intake was measured for 2 h (1 h with CCK) after injection by weighing the food containers (~0.1 g). Spillage of food was taken into account. Both capsaicin- and sham-treated rats were divided into two groups based on body weight and food intake (within capsaicin- and sham-treated rats, respectively) during the dark phase preceding the experiment. Experiment 1 was performed in a counterbalanced design, with each animal tested under experimental (amylin, 5 µg/kg) and control (NaCl) conditions. The interval between trials was 4 days.

Statistics. Results are presented as means ± SE. A two-factor ANOVA was used to assess the effects of treatment (anorectic peptide vs. control) and the effect of capsaicin pretreatment. The results were analyzed separately at each individual time point after injection. For the assessment of the effect of treatment within the capsaicin-treated and the sham-treated group, respectively, the unpaired or the paired Student’s t-test (corrected for multiple comparisons) was used where appropriate. A value of P < 0.05 was considered significant.

RESULTS

Development of food intake and body weight in capsaicin-pretreated rats. Before capsaicin or sham pretreatment, body weight was ~270 g in all rats. Basal food intake in capsaicin-pretreated rats was initially higher than in sham-treated controls [e.g., dark-phase food intake ~1 wk after pretreatment: sham-treated rats 16.4 ± 1.1 g (n = 12) vs. capsaicin-pretreated rats 20.0 ± 0.8 g (n = 16); P < 0.05]. This difference in basal food intake had disappeared by ~3 wk after pretreatment (results not shown). The transient increase in basal food intake was paralleled by a higher body weight gain [e.g., body weight 2 wk after pretreatment: 314 ± 9 vs. 341 ± 7 g (P < 0.05)]. This difference was maintained until the end of the experiments [6 wk after pretreatment: 443 ± 13 vs. 471 ± 9 g (P ~ 0.08)].
Experiment 1. Influence of amylin on food intake in 24-h food-deprived capsaicin- and sham-treated rats. In experiment 1 we tested the influence of the destruction of primary sensory afferents by capsaicin on the anorectic effect of intraperitoneally injected amylin (5 µg/kg) in 24-h food-deprived rats. Amylin significantly reduced cumulative food intake in sham-treated rats compared with its respective NaCl control group (30 min and 1 h after injection: *P < 0.05; Fig. 1). Basically the same was observed in capsaicin-treated rats (Fig. 1). Two-factor ANOVA revealed a significant main effect of (amylin) treatment (30 min and 1 h after injection: *P < 0.001), but no main effect of capsaicin pretreatment or interaction between treatment × capsaicin pretreatment.

Experiment 2. Influence of CGRP on food intake in 24-h food-deprived capsaicin- and sham-treated rats. Under the same experimental conditions, similar observations were made with CGRP. CGRP (5 µg/kg) significantly reduced cumulative food intake in sham-treated rats and in capsaicin-treated rats for 1 h after injection (Fig. 2). There was no difference in the anorectic potency of CGRP in capsaicin-treated versus sham-treated rats (2-factor ANOVA).

Experiment 3. Influence of CCK on food intake in 24-h food-deprived capsaicin- and sham-treated rats. The results of this experiment, which served as a functional test for successful capsaicin pretreatment (see MATERIALS AND METHODS; Ref. 26), are shown in Fig. 3. In the sham-treated group, CCK significantly (P < 0.01) reduced cumulative food intake during the first 30 min after injection compared with the NaCl control group. In contrast, CCK had no effect on food intake in the rats pretreated with capsaicin (Fig. 3). Two-factor ANOVA revealed a significant (P < 0.05) main effect of treatment (CCK vs. NaCl) but no main effect of capsaicin. The attenuation of CCK's anorectic effect by capsaicin pretreatment was significant (interaction treatment × capsaicin pretreatment: *P < 0.01) 30 min after injection. One hour after injection, there was no difference in food intake between CCK- or NaCl-treated rats in either the capsaicin group or the sham-treated group (Fig. 3).

This functional test, showing an abolition of the anorectic effect of CCK in capsaicin-pretreated rats, corroborated the results of the eye-wiping test (see MATERIALS AND METHODS) and indicated a successful destruction of primary sensitive afferents.

DISCUSSION

In the present study, we have shown for the first time that a destruction of primary sensory afferents by capsaicin pretreatment did not attenuate the anorectic effect of peripherally administered amylin in rats. The anorectic effect of CCK, however, which is transmitted by vagal afferents (20, 26), was abolished in capsaicin-pretreated rats. Visceral afferents, therefore, do not seem to be involved in mediating the anorectic effect of peripherally administered amylin. This study comple-
ments previous studies by our group and others (14, 15, 23) providing evidence for a central site of amylin action after intraperitoneal injection.

In previous studies, we have shown that the anorectic effect of peripherally injected amylin was not abolished by hepatic branch (14) or subdiaphragmatic vagotomy (15). The absence of an effect of subdiaphragmatic vagotomy confirmed findings by Morley et al. (23). It was, therefore, suggested that amylin exerts its anorectic effect after intraperitoneal injection via receptors located within the central nervous system or via receptors located on afferents of the splanchnic nerves (15). A cooperative effect mediated by receptors on vagal and splanchnic afferents, however, which seems to underlie bombesin's anorectic action after peripheral injection (30), could not be excluded. Further studies substantiated the important role of the AP-NTS region in the hindbrain in the anorectic effect of intraperitoneally injected amylin, because an AP-NTS lesion attenuated this effect after intraperitoneal injection of the same amylin dose (5 µg/kg; Ref. 19) and because a subcutaneous injection led to c-Fos activation in the AP-NTS region (29). The question, however, whether amylin receptors within the AP-NTS region (2) or receptors on splanchnic and vagal afferents projecting to the AP-NTS region (10, 28) mediate amylin's effect remained open (19).

With the present study showing that the anorectic effect of intraperitoneally injected amylin does not depend on intact primary sensory afferents, we now provide evidence for receptors within the central nervous system being responsible for amylin's anorectic effect. Subsequent to previous studies, it appears very likely that these receptors are located within the AP-NTS region for the following reasons: 1) the AP-NTS region has a high density of amylin binding sites (2), 2) the AP-NTS region, lacking a blood-brain barrier (33), can easily be reached by circulating amylin, and 3) lesion of the AP-NTS region markedly attenuated amylin's (5 µg/kg ip) anorectic effect (19). However, it cannot yet be excluded that amylin acts at least partially via capsaicin-insensitive; Ref. 25) neurons within the spinal cord, which relay the pertinent information from visceral afferents to the AP-NTS region. To our knowledge, binding sites for amylin in the spinal cord have not been described. However, binding sites for CGRP are widely distributed in the spinal cord (31), and a mediation of amylin's anorectic effects via these receptors cannot be excluded.

In previous studies, we and others have reported that peripherally administered CGRP, which is structurally and functionally related to amylin and belongs to the same superfamily of peptides (9), elicits an anorectic effect in rats and mice similar to amylin (18, 19, 21, 22). This similarity in the anorectic effects of the two peptides was confirmed in the present study because the anorectic effect of CGRP, which was of comparable magnitude to that of amylin, was not influenced by capsaicin pretreatment. It is, therefore, likely that CGRP also elicits its anorectic action via central receptors located in the AP-NTS region (19). However, the exact nature of the receptors mediating the anorectic effects of amylin and CGRP is not known yet, because receptors for both amylin (2) and CGRP (32) have been found in the AP-NTS region, and amylin and CGRP show extensive crossreaction at their respective binding sites (9). As mentioned earlier, a mediation of amylin's and CGRP's anorectic effects via binding sites for CGRP in the spinal cord (31) cannot be totally excluded.

We performed two experiments that constitute established functional tests to demonstrate the successful destruction of capsaicin-sensitive nerve fibers (8, 12, 27). In the first test, we showed that capsaicin-treated rats have a markedly reduced eye-wiping response to the application of an irritating substance to the cornea. This demonstrates a deficit in the chemosensory response due to the destruction of trigeminal afferents originating in the cornea that occurs after intraperitoneal capsaicin treatment (12). In the second test, we have shown that the anorectic effect of CCK, which depends on abdominal vagal afferents (20), was abolished in capsaicin-treated rats. Our findings thus confirm previous reports that CCK-induced satiety is transmitted via capsaicin-sensitive afferents (26). Together, these two functional tests provide evidence for a successful destruction of primary sensory neurons in our capsaicin-treated rats.

In two previous studies, we had reported that part of the anorectic effect of CCK seems to depend on the release of amylin (or CGRP) because coadministration of an amylin and CGRP antagonist reduced the anorec-
tic effect of CCK (16, 18). At first sight, the observation of the present study that capsaicin pretreatment completely abolished the anorectic effect of CCK was surprising, because we would have expected that at least the amylin-dependent component of CCK’s anorectic action was still effective in capsaicin-pretreated rats.

The apparently contradictory findings of the previous (16, 18) and the present studies can be reconciled by the hypothesis that amylin has a neuromodulator function within the AP-NTS region on incoming satiety signals being, e.g., elicited by CCK binding to vagal receptors (20). Interestingly, the primary role of CGRP, whose anorectic effect is comparable to that of amylin (18, 19), seems to be a modulation of sensory neurotransmission in the peripheral and central nervous system (31). To our knowledge, however, it is unknown whether CGRP release is involved in the anorectic effect of CCK.

The hypothesis of a modulating role of amylin on CCK’s satiating action is supported by a recent report (3) showing that amylin markedly potentiated CCK’s anorectic effect. Coadministration of both peptides was up to 30 times more potent than administration of either peptide alone (3). On the presumption of a modulating role as amylin’s primary mode of action, the lack of incoming signals to the AP-NTS region due to the destruction of nerve afferents by capsaicin could explain why CCK’s anorectic effect was completely abolished in capsaicin-treated rats in our study. Amylin released in response to CCK administration would then no longer be sufficient to reduce food intake.

In the present study, we observed transient hyperphagia leading to an increased rate of body weight gain in capsaicin-pretreated rats versus sham-treated controls for the first few weeks after capsaicin pretreatment. These findings agree with previous reports that showed overconsumption of highly palatable diets in systemically capsaicin-treated rats (8, 12). The transient overconsumption of food after capsaicin treatment is probably due to the interruption of feedback signals from the periphery, such as metabolic cues (13) or satiety peptides (12, 26), reaching the brain.

In conclusion, we have shown in the present study that the anorectic effects of peripherally injected amylin and CGRP are not abolished by systemic capsaicin treatment damaging primary sensory neurons in rats. Hence, visceral capsaicin-sensitive afferents do not seem to be involved in mediating amylin’s and CGRP’s anorectic effects. Amylin and CGRP, therefore, appear to have a central site of action, probably in the AP-NTS region.

Perspectives

With the present study, evidence is provided for amylin’s and CGRP’s role as centrally acting satiety peptides. Future studies should be directed at clarifying the exact nature of the receptors mediating these effects. The main role of amylin, which is coreleased with insulin in response to food intake, may be that of a neuromodulator acting during meals within the AP-NTS region on incoming satiety signals elicited, e.g., by CCK. This issue needs to be investigated in future studies. Furthermore, future studies should try to clarify amylin’s role in the long-term regulation of food intake and body weight (1), because the plasma concentrations of insulin and amylin depend on adiposity (9, 24), with the former being considered a lipostatic signal.

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The financial support of the Swiss National Research Foundation, the Hypothesis of a modulating role of amylin on CCK’s satiating action is supported by a recent report (3) showing that amylin markedly potentiated CCK’s anorectic effect. Coadministration of both peptides was up to 30 times more potent than administration of either peptide alone (3). On the presumption of a modulating role as amylin’s primary mode of action, the lack of incoming signals to the AP-NTS region due to the destruction of nerve afferents by capsaicin could explain why CCK’s anorectic effect was completely abolished in capsaicin-treated rats in our study. Amylin released in response to CCK administration would then no longer be sufficient to reduce food intake.

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