Histamine H₃ receptors mediate the anorectic action of the pancreatic hormone amylin

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Mollet, A., T. A. Lutz, S. Meier, T. Riediger, P. A. Rushing, and E. Scharrer. Histamine H₃ receptors mediate the anorectic action of the pancreatic hormone amylin. Am J Physiol Regulatory Integrative Comp Physiol 281: R1442–R1448, 2001.—We investigated the role of histamine H₃ receptors in mediating the anorectic effect of intraperitoneally injected amylin (5 and 20 μg/kg), the amylin agonist salmon calcitonin (sCT; 10 μg/kg), leptin (1.3 mg/kg), and cholecystokinin (CCK; 20 μg/kg). The experiments were performed with mice lacking functional H₃ receptors (H₃Rko) and wild-type (WT) controls. The mice were also injected with the H₃ antagonist thioperamide (20 mg/kg), which reduces feeding by enhancing the release of endogenous histamine through presynaptic H₃ receptors. The feeding-suppressive effect of thioperamide was abolished in H₃Rko mice. The anorectic effects of amylin and sCT were significantly reduced in 12-h food-deprived H₃Rko mice compared with WT mice [1-h food intake: WT-NaCl 0.51 ± 0.05 g vs. WT-amylin (5 μg/kg) 0.30 ± 0.06 g (P < 0.01); H₃Rko-NaCl 0.45 ± 0.05 g vs. H₃Rko-amylin 0.40 ± 0.04 g; WT-NaCl 0.40 ± 0.09 g vs. WT-sCT (10 μg/kg) 0.14 ± 0.10 g (P < 0.05); H₃Rko-NaCl 0.44 ± 0.08 g vs. H₃Rko-sCT 0.50 ± 0.06 g]. The anorectic effect of leptin was absent in ad libitum-fed H₃Rko mice, whereas CCK equally reduced feeding in WT and H₃Rko animals. This suggests that the histaminergic system is involved in mediating the anorectic effects of peripheral amylin and sCT via histamine H₃ receptors. The same applies to leptin but not to CCK. H₃Rko mice showed significantly increased body weight gain compared with WT mice, supporting the role of endogenous histamine in the regulation of feeding and body weight.

food intake; histamine H₃ receptor knockout mice; thioperamide; salmon calcitonin; leptin; cholecystokinin

AMYLIN, a 37-AMINO ACID POLYPEPTIDE that is secreted from pancreatic β-cells in response to food intake, has been shown to suppress feeding in rats and mice mainly by decreasing meal size (9, 12, 26). Its satiating action is specific, since amylin does not induce a conditioned taste aversion (3, 12). Peripheral amylin has a central role in mediating the anorectic action of the pancreatic hormone amylin. Am J Physiol Regulatory Integrative Comp Physiol 281: R1442–R1448, 2001.

Although the primary site of action for the satiating effect of peripheral amylin is clear, knowledge about the neurotransmitters and signaling pathways that are involved in mediating amylin’s satiating effect in the central nervous system (CNS) is limited. Previous studies have shown that amylin’s satiating effect is partially mediated by the central dopaminergic, but not serotoninergic (11), system because blockade of dopamine D₂ receptors attenuated amylin’s anorectic effect (14). Furthermore, because injection of the H₃ agonists imetit and R-α-methylhistamine, which block the release of endogenous histamine via presynaptic histamine H₃ receptors, reduced amylin’s inhibitory effect on feeding, the histaminergic system also seems to play an important role in mediating amylin’s satiating effect (11).

Because within the CNS, histamine’s inhibitory effect on feeding mainly seems to involve histamine H₁ receptors (17, 27, 28), the aim of the present study was to investigate whether histamine mediates amylin’s satiating effect via this receptor subtype. Histamine H₁ receptors have also been found in the AP/nucleus of the solitary tract (AP/NTS) region, which is involved in mediating the anorectic effect of amylin (13), and in hypothalamic areas (27, 28). To investigate the importance of histamine H₁ receptors in the signaling transmission of the anorectic effect of amylin, we treated histamine H₁ receptor knockout (H₁Rko) mice with amylin and compared the anorectic effect in these mice to that in respective wild-type (WT) controls. We also tested the effect of salmon calcitonin (sCT) on food intake in these animals because sCT has been shown to reduce food intake via amylin binding sites (15). To confirm the lack of H₁ receptors in the H₁Rko mice, they were first injected with thioperamide, an H₂ receptor antagonist that decreases food intake by enhancing the release of endogenous histamine (16, 27). Leptin, the ob gene product that is secreted from white adipose tissue and is involved in the regulation of food intake and body weight by acting on hypothalamic

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arccuate nucleus neurons (2, 31), was used as a positive control because its anorectic effect has been reported to be absent in H1Rko mice (18). Cholecystokinin (CCK) was also included in the study because the anorectic effect of CCK does not seem to depend on the histaminergic system (33).

**MATERIALS AND METHODS**

**Animals and Housing Conditions**

The experiments were performed with knockout mice lacking functional histamine H1 receptors (H1Rko; strain C57BL) and respective WT controls (strain C57BL). The H1Rko mice were generated by homologous recombination (7) and appear phenotypically normal but show increased ambulation during the light period, decreased locomotor activity in the dark period, and reduced exploratory behavior in a new environment (7). ([3H]pyrilamine and doxepin binding studies were used to verify the lack of H1 receptors (7).

Adult male H1Rko and WT mice, respectively, were used. The average age was ~3.5 mo when they were used for the first time in the feeding experiments. The animals were housed in standard wire cages (24 x 24 x 11 cm) at a room temperature of 22 ± 1°C and with an artificial inverted 12:12-h light-dark cycle (lights on at 2100). The mice were adopted to the housing conditions for at least 3–4 wk before the experiments. Mice had ad libitum access to water and food (powdered medium-fat diet containing 13% protein, 46% carbohydrate, 8% fat, and an average energy density of 16.5 kcal/g; Kliba Mühlten, Kaiseraugst, Switzerland) except during food deprivation just before the experiments (see below). Body weight was registered at weekly intervals.

**Drugs and Experimental Design**

Rat amylin, sCT, and CCK octapeptide (CCK-8) were obtained from Peninsula Laboratories (Belmont, CA). Mouse recombinant leptin and the H3 antagonist thioperamide were obtained from RBI Products/Sigma (St. Louis, MO). All peptides were freshly dissolved in 0.9% NaCl just before the experiments. The H3 antagonist thioperamide was dissolved in sterile 0.5% sodium carboxymethylcellulose (CMC) solution. Amylin was injected at doses of 5 and 20 μg/kg and the amylin receptor agonist sCT at doses of 1 and 10 μg/kg. Leptin (1.3 mg/kg) was used as a positive control (18). CCK (20 μg/kg) was included in the study because its anorectic effect is reported not to depend on the histaminergic system (33). To confirm the lack of functional histamine H1 receptors, mice were also injected with thioperamide (20 mg/kg), a selective H3 receptor antagonist (1) that, by enhancing the release of endogenous histamine, has been shown to reduce food intake in rats (16, 27).

For each experiment, H1Rko and WT mice were randomly divided into two groups, each with similar body weight. The order of treatment was randomized. When a crossover design was used (see below), at least 5 days were allowed for recovery between tests. All solutions were injected intraperitoneally with an injection volume of 10 μl/kg. Injection of the same volume of the appropriate solvent served as control. Food was presented just after the injections. All experiments were performed at dark onset (0900). Food intake was assessed by measuring the weight of the food containers (±0.1 g) after correcting for food spillage and removing feces and urine. To prevent disturbance of the mice by light during the dark period, the measurement was performed under red light.

**Experiment 1.** H1Rko (n = 15; 30.3 ± 0.6 g) and WT mice (n = 16; 27.8 ± 0.5 g) were food deprived for 12 h during the light period (2100–0900). Thioperamide (20 mg/kg) or vehicle (0.5% sodium-CMC solution) were injected at dark onset (0900). The experiment was performed using a crossover design so that each animal served as its own control.

**Experiment 2.** For amylin at 5 μg/kg, WT mice had an average body weight of 28.2 ± 0.5 g (n = 16), and H1Rko mice had an average body weight of 29.8 ± 0.8 g (n = 15). For amylin at 20 μg/kg, WT mice had an average body weight of 26.8 ± 0.6 g (n = 16), and H1Rko mice had an average body weight of 29.2 ± 0.7 g (n = 16). The animals were food deprived for 12 h during the light phase (2100–0900). Amylin (5 or 20 μg/kg) or vehicle was injected intraperitoneally immediately before dark onset (0900). Each experiment was performed using a crossover design.

**Experiment 3.** CCK at 10 μg/kg, WT mice had an average body weight of 26.6 ± 0.4 g (n = 16), and H1Rko mice had an average body weight of 28.1 ± 0.6 g (n = 15). For sCT at 10 μg/kg, WT mice had an average body weight of 30.8 ± 0.8 g (n = 15), and H1Rko mice had an average body weight of 34.1 ± 0.6 g (n = 16). WT and H1Rko mice were injected intraperitoneally with sCT (1 or 10 μg/kg) or vehicle immediately before dark onset (0900) after 12-h food deprivation.

**Experiment 4.** The experimental procedure was similar to that in the study by Morimoto et al. (18). Ad libitum-fed WT (n = 16; 27.7 ± 0.4 g) and H1Rko mice (n = 15; 29.6 ± 0.6 g) received an intraperitoneal injection of leptin at a dose of 1.3 mg/kg or vehicle at dark onset (0900). The experiment was performed using a crossover design.

**Experiment 5.** Twelve-hour food-deprived H1Rko (n = 16; 31.1 ± 0.6 g) and WT mice (n = 16; 28.7 ± 0.8 g) were injected with CCK-8 (20 μg/kg) or vehicle at dark onset (0900).

**Statistics**

Results are presented as means ± SE. The treatment groups were compared using one-way or, when a crossover design was used, repeated-measures ANOVA with the Student-Newman-Keuls post hoc test or paired Student’s t-test. The influence of the absence of functional histamine H1 receptors in the H1Rko mice on the effects of the tested substances on food intake was investigated by two-factor ANOVA. Body weight, which was assessed at weekly intervals, was compared using the unpaired Student’s t-test.

In all cases, P < 0.05 was considered significant.

**RESULTS**

**Experiment 1.** Thioperamide (20 mg/kg) significantly reduced 1-h cumulative food intake in WT mice (paired Student’s t-test) by ~40% [food intake 1 h after injection: WT-vehicle (n = 16) 0.49 ± 0.05 g vs. WT-thioperamide (n = 15) 0.29 ± 0.07 g; P < 0.05]. In H1Rko mice, thioperamide did not reduce feeding [food intake 1 h after injection: H1Rko-vehicle (n = 15) 0.36 ± 0.04 g vs. H1Rko-thioperamide (n = 15) 0.43 ± 0.06 g]. Because thioperamide’s anorectic effect was completely abolished in the H1Rko mice (2-factor ANOVA: 1 h, P < 0.05), this experiment confirms the abolition of functional H1 receptors in these animals.

**Experiment 2.** In experiment 2, amylin was injected at 5 or 20 μg/kg. In WT mice, amylin (5 μg/kg) significantly (paired
Students’ t-test) reduced cumulative food intake throughout the 4-h observation period by 20–40%. Amylin’s anorectic effect was completely abolished in H_1Rko mice (Fig. 1A) (2-factor ANOVA: 4 h, P < 0.05).

At a dose of 20 μg/kg, amylin significantly (repeated-measures ANOVA) reduced food intake throughout the 4-h observation period in WT mice. In H_1Rko mice, amylin only slightly reduced feeding, without reaching the level of significance (Fig. 1B).

**Experiment 3**

In experiment 3, sCT was injected at 1 or 10 μg/kg. sCT (10 μg/kg) significantly (1-way ANOVA) reduced feeding in WT mice by 40–65% but had no effect on cumulative food intake in H_1Rko mice (Fig. 2A) (2-factor ANOVA, interaction between the lack of H_1 receptors and the effect of treatment: 1 h, P < 0.05; 2 h, P < 0.01; 4 h, P < 0.01; and 8 h, P < 0.05).

The experiment was also performed with chow-fed mice at a dose of 1 μg/kg sCT. Under these conditions, sCT significantly (1-way ANOVA) reduced cumulative food intake in WT mice by ~20–55% but not in H_1Rko mice (Fig. 2B) (2-factor ANOVA, interaction between the lack of H_1 receptors and the effect of treatment: 1 h, P < 0.05; 2 h, P < 0.01; 4 h, P < 0.01; and 8 h, P < 0.05).

**Experiment 4**

Leptin (1.3 mg/kg) significantly (paired Student’s t-test) reduced cumulative food intake in WT mice by ~10–30%, whereas in H_1Rko mice, its anorectic effect was completely abolished (Fig. 3) (2-factor ANOVA: 6 h, P < 0.05; 12 h, P = 0.06).
Experiment 5

In experiment 5, CCK-8 was injected at 20 μg/kg. Cumulative food intake was significantly (1-way ANOVA) reduced by CCK-8 throughout the 2-h observation period in both WT and H1Rko mice (Fig. 4). The two-factor ANOVA showed that the absence of the histamine H1 receptor in H1Rko mice did not influence the anorectic effect of CCK-8. In another experiment, CCK-8 was used at a dose of 5 μg/kg. In this case, CCK-8 tended to reduce feeding in WT and H1Rko mice, but the effects were not significant [e.g., food intake 1 h after injection (body weight of: WT 27.7 ± 0.6 g, n = 16; H1Rko 28.5 ± 0.7 g, n = 16); WT-NaCl 0.48 ± 0.04 g vs. WT-CCK 0.38 ± 0.08 g; H1Rko-NaCl 0.52 ± 0.05 g vs. H1Rko-CCK 0.38 ± 0.05 g].

All experiments (except with CCK-8) were repeated using another group of H1Rko and WT mice. In all cases, the outcome was as described before, i.e., the anorectic effects of thioperamide, amylin, sCT, and leptin, which were significant in WT mice, were abolished in H1Rko animals (results not shown).

We observed a significantly increased body weight gain in H1Rko mice compared with WT animals (Fig. 5). In H1Rko mice, basal food intake tended to be higher by ~5% than in WT control mice, but this effect was not significant (data not shown).

DISCUSSION

In this study, we have shown that functional histamine H1 receptors are essential for the anorectic effects of intraperitoneally administered amylin and sCT. In contrast to WT animals, in which amylin markedly reduced feeding, the anorectic action of amylin was absent in H1Rko mice when a dose of 5 μg/kg was used. At the higher amylin dose (20 μg/kg), only a slight nonsignificant reduction in feeding was observed in H1Rko mice. Interestingly, the inhibitory effect of sCT on feeding was also completely abolished in H1Rko mice at both doses of sCT (1 and 10 μg/kg). sCT is structurally and functionally related to amylin (5) and seems to reduce feeding by interaction with amylin receptors (15). In previous studies, a stronger and prolonged effect of sCT compared with amylin was observed (15). This can be explained by the irreversible binding of sCT to amylin receptors (21), causing a persistent neuronal activation (24).

To verify the ablation of histamine H1 receptors in the H1Rko mice, we injected these mice with the H3 antagonist thioperamide, which reduces food intake in rats (16, 27) by enhancing the synthesis and release of...
endogenous histamine (1). It has been shown that histamine reduces feeding mainly via histamine H₁ receptors (17, 28). The lack of an anorectic effect of thioperamide in H₁Rko mice compared with WT animals confirms the absence of functional histamine H₁ receptors in H₁Rko mice.

The findings of the present experiments complement our previous study (11) demonstrating a mediation of amylin’s anorectic effect by the histaminergic system. The pretreatment with the H₃ receptor agonists imetit and R-α-methylhistamine, which are able to penetrate the blood-brain barrier and block the release of endogenous histamine via presynaptic histamine H₃ receptors, reduced amylin’s inhibitory effect on feeding in rats (11). We now show that histamine mediates amylin’s satiating effect through H₁ receptors.

Hypothalamic histamine is well known to be involved in the regulation of food intake and body weight (16, 17, 27–29). Increased concentrations of central histamine are able to reduce feeding via histamine H₁ receptors (17, 28), whereas a blockade of central histamine H₁ receptors (28) increases food intake. Additional studies showed that the H₁ receptors in the ventromedial (VMH) and paraventricular hypothalamic nuclei, which are the areas richest in hypothalamic histamine and H₁ receptors (29, 30), play the major role in this regard (29). We therefore conclude that the amylin-induced activation of AP neurons that underlies the anorectic effect of peripheral amylin (13, 23, 25) directly or indirectly leads to an increase in hypothalamic histamine release to bring about amylin’s effect by acting on hypothalamic histamine H₁ receptors. This main conclusion of our present study is corroborated by preliminary findings that an infusion of the H₁ receptor antagonist chlorpheniramine into the VMH blocked the anorectic effect of peripheral amylin in rats (unpublished data).

In previous studies, we showed that the dopaminergic system is also involved in mediating amylin’s satiating effect because blockade of D₂ receptors attenuates amylin’s action in rats (14). In another study, a high density of dopamine D₂ receptors has been detected in the AP/NTS region (22). In analogy to a blockade of the anorectic effect of amylin by H₁ antagonists into the VMH, we recently demonstrated that an infusion of the dopamine D₂ antagonist raclopride directly into the AP/NTS region also attenuates the amylin-induced reduction of feeding (unpublished observations). We therefore propose that at least a dopaminergic hypothalamic mechanism (e.g., in the VMH) are involved in the ascending signaling pathway mediating the anorectic effect of amylin.

Because activation of presynaptic H₃ receptors, which attenuates amylin’s satiating effect (11), not only reduces the release of histamine but, via receptors on nonhistaminergic neurons, also the release of dopamine (4), it could theoretically be possible that the attenuation of amylin’s anorectic effect by histamine H₃ agonists (11) was due to an inhibition of dopamine rather than histamine release. However, we showed in the present study that amylin’s anorectic effect was markedly reduced in H₁Rko mice. It is therefore unlikely that the attenuating effect of H₃ agonists on amylin’s satiating action (11) is due to an inhibition of dopamine release. Instead of a direct interaction between the dopaminergic and the histaminergic systems, we suggest the model presented above with dopamine in the AP/NTS region and histamine in the VMH as sequential mediators of amylin’s anorectic effect.

In our study, we observed an increased body weight gain in H₁Rko mice compared with the WT control animals. Higher body weight appeared to be associated with an increased fat pad mass (unpublished observations). This was paralleled by an increase in basal food intake in the H₁Rko mice, although the latter effect was not significant. Over time, however, the higher food intake probably contributes to the observed differences in body weight (and fat mass). These findings underline the overall importance of the histaminergic system and H₁ receptors in the regulation of food intake and body weight (29).

Our results support previous findings that the histaminergic system plays an important role in the feeding-suppressive effect of intraperitoneally administered leptin. Morimoto et al. (18, 19) and Yoshimatsu et al. (35) have shown that the central histaminergic system is involved in leptin’s anorectic effect because the specific histidine decarboxylase inhibitor α-fluoromethylhistidine abolished the anorectic effect of leptin in mice and because leptin’s inhibitory effect on feeding was absent in H₁Rko mice (18, 35). The latter effect was confirmed in the present study. It is possible that leptin is able to reduce feeding in H₁Rko mice when administered at higher doses, indicating that other neurotransmitters are also involved in leptin action (2, 34). A similar conclusion can be drawn for amylin, which, at the high dose used (20 μg/kg), appeared to produce some weak, albeit nonsignificant, reduction in feeding in the H₁Rko mice.

The findings of this and other studies (18, 35) that the anorectic effects of both amylin and leptin are mediated via histamine H₁ receptors raise the question as to whether the central nervous signaling pathways for amylin, which acts through activation of AP neurons (13, 23, 25), and leptin, which acts primarily via arcuate hypothalamic nucleus neurons (31, 34), converge via the histaminergic system in the VMH.

We also injected the mice with CCK, which acts as an important satiating peptide (6). We have shown that CCK reduces food intake with equal potency in both H₁Rko and WT mice compared with saline controls. Consequently, histamine H₁ receptors are not necessary in the transmission of the anorectic action of CCK. These data not only confirm previous studies demonstrating that the anorectic effect of CCK does not depend on the histaminergic but rather the serotonergic system (33), but also underline the specificity of the observed effects in our present investigations.

In conclusion, we have shown that the histaminergic system is involved in mediating the anorectic effects of
amylin, sCT, and leptin but not CCK through histamine H₁ receptors in mice. Furthermore, H₁Rko mice showed significantly increased body weight gain compared with the WT animals, supporting the role of endogenous histamine in the regulation of food intake and body weight.

**Perspectives**

The results of this and other studies have shown that histamine via H₁ receptors and dopamine via D₂ receptors (14) are involved in mediating the anorectic effect of amylin. As discussed, it is not clear where in the CNS these neurotransmitter systems that are involved in amylin’s satiating effect are located. Some evidence indicates, however, that the amylin-induced activation of AP neurons, which seems to underlie the anorectic action of peripheral amylin (13, 23, 25), leads to a release of dopamine that acts locally on D₂ receptors of other neurons that then directly or indirectly influence hypothalamic histamine release to bring about amylin’s effect by acting on hypothalamic histamine H₁ receptors, e.g., in the VMH.

The findings that the anorectic effects of both amylin and leptin are partly mediated via histamine H₁ receptors (18, 35) raise the question as to whether the central nervous signaling pathways for amylin, which acts on AP neurons (13, 23, 25), and leptin, which acts primarily via arcuate hypothalamic nucleus neurons (31, 34) converge via the histaminergic system in the VMH. It also remains to be investigated whether this convergence could be the neuroanatomical basis for a modulation of the anorectic action of the satiating peptide amylin, a possible signal for direct control of meal size (32), by leptin, a signal for indirect control of meal size (32). Future studies will have to address these open questions by a combination of feeding studies and other experimental techniques to clarify the ascending signaling pathway for the anorectic action of amylin and its interaction with other anorectic agents. It also remains to be investigated whether other neurotransmitters and/or neuropeptides in the CNS feeding centers are affected by amylin.

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