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Short communication

Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus

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Abstract

Ghrelin and amylin are gut-derived hormones that stimulate and inhibit food intake, respectively. Feeding is modulated by aminergic neurotransmitters in the hypothalamus. We have evaluated the effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release from rat hypothalamic synaptosomes. We found that ghrelin did not modify dopamine or norepinephrine release, but inhibited serotonin release. On the other hand, amylin inhibited dopamine release, without affecting norepinephrine or serotonin. We conclude that the appetite-stimulating activity of ghrelin could be mediated by inhibited serotonin release, while the anorectic effects of amylin could involve inhibited release of dopamine in the hypothalamus.

Keywords: Amylin; Ghrelin; Hypothalamus; Dopamine; Norepinephrine; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

The appetite regulatory network is modulated at the hypothalamic level by the interaction of hormonal and neuronal signaling (Kalra et al., 1999). Ghrelin is a 28-amino acid peptide hormone, which has been isolated from the rat stomach as the endogenous ligand for the growth hormone (GH) secretagogue receptor (Kojima et al., 1999), but it is also produced in the hypothalamus. Besides its GH-releasing activity, ghrelin stimulates food intake and increases fat stores, either when administered peripherally or intracerebroventricularly, at dosages in the nanomolar or picomolar range, respectively (Tschop et al., 2000). Food intake is markedly stimulated after microinjection into the arcuate nucleus of the hypothalamus, which is potentially accessible to the circulation (Wren et al., 2001). The orexigenic activity of ghrelin is partially mediated by agouti-related peptide (AGRP) and neuropeptide Y (Nakazato et al., 2001), both of which are well known appetite-stimulating signals. Ghrelin can also antagonize the anorectic effect of leptin through the activation of the hypothalamic neuropeptide Y/Y_1 receptor pathway (Shintani et al., 2001).

Amylin is a 37-amino acid peptide hormone that is co-secreted with insulin by pancreatic β-cells following meals, playing a role in glucose homeostasis (Ludvik et al., 1997). Peripheral amylin is able to cross the blood–brain barrier (Banks et al., 1995; Banks and Kastin, 1998), but amylin-immunoreactive neurons are also present in the hypothalamus (D'Este et al., 2001). With respect to feeding behavior, amylin plays a role opposite to ghrelin, in inhibiting food intake following both systemic (10 nmol/kg) and intracerebroventricular administration (100 pmol) (Chance et al., 1991; Rushing et al., 2000).

Aminergic neurotransmitters have long been implicated in feeding control at the hypothalamic level (Kalra et al., 1999), and we have previously found that peptide hormones or neuropeptides such as leptin, thyrotropin-releasing hormone (TRH), cocaine- and amphetamine-regulated transcript (CART) peptide, and the orexins A and B differently modulate hypothalamic dopamine, norepinephrine and serotonin release, which could partially explain their appetite regulatory activity (Brunetti et al., 1999, 2000; Orlando et al., 2001).

In order to further investigate the endocrine link between the digestive tract and the central nervous system, partic-

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ularly with regard to the feeding regulatory mechanisms, in the present study, we have evaluated the effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release from rat hypothalamic neuronal terminals (synaptosomes) in vitro.

2. Materials and methods

As previously described (Brunetti et al., 1999), hypothalamic synaptosomes were prepared from male Wistar rats (200–250 g), loaded with either [^{3}H]dopamine, [^{3}H]norepinephrine or [^{3}H]serotonin, perfused in water-jacketed superfusion chambers with Krebs–Ringer buffer (0.6 ml/min), and perfusate was collected (1 min fractions for serotonin, and 2 min fractions for dopamine and norepinephrine release) to detect released [^{3}H] by liquid scintillation scanning. The European Community guidelines for the use of experimental animals have been adhered to and the protocol was approved by the institutional ethics committee. In a first set of experiments, either ghrelin or amylin were added to the perfusion buffer, in graded concentrations (1–100 nM), for 5 min in the serotonin release experiments, and for 10 min in the dopamine and norepinephrine release experiments, followed by 8 min with Krebs buffer alone. Amine release was calculated as the means ± S.E.M of the percentage of [^{3}H] recovered in the stimulus and return to basal fractions (a total of 11 fractions for serotonin, and 10 fractions for dopamine and norepinephrine), compared to total loaded [^{3}H]. A second set of experiments was run to evaluate the effects of the peptides on neurotransmitter release induced by a mild depolarizing stimulus. After a 30-min equilibration perfusion with buffer alone, a 23-min perfusion with the peptides (1–100 nM) was started, where in the final 3 min, K^+ concentration in the perfusion buffer was elevated to 15 mM (after removal of equimolar concentrations of Na^+). A time–response curve relative to the percentage of [^{3}H] recovered in each perfusate fraction compared to total loaded [^{3}H] was plotted, and amine release was calculated as the area under the time–response curve (AUC) corresponding to 3 min depolarization + return to basal period in Krebs–Ringer buffer (a total of 8 fractions). Preliminary experiments showed that monoamine re-uptake is negligible due to the rapid removal of released amines by perfusion flow, except in the experiments evaluating serotonin release, where a column chromatography of the perfusate was performed to separate serotonin from its metabolites, as previously described (Orlando et al., 2001).

Data represent the group means ± S.E.M. of three to five experiments performed in triplicate. Treatment and control group means were compared by the analysis of variance (ANOVA) followed by Student–Newman–Keul's multiple comparison test (GraphPad Prism 2.00 software).

Rat amylin, 1 mg, and rat ghrelin, 0.1 mg, were purchased from American Peptide, USA. [^{3}H]dopamine (40–60 Ci/mmol, 250 μCi pack size), [^{3}H]norepinephrine (30–50 Ci/mmol, 250 μCi pack size) and [^{3}H]serotonin (10–20 Ci/mmol, 1 mCi pack size) were purchased from Amersham Pharmacia Biotech, Italy.

3. Results

Ghrelin, in the dose range 1–100 nM, did not affect basal amine release. Means ± S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions with respect to total loaded [^{3}H] were as follows: [^{3}H]dopamine: control, 1.63 ± 0.02; 1 nM, 1.59 ± 0.02; 10 nM, 1.69 ± 0.04; 100 nM, 1.68 ± 0.06; [^{3}H]norepinephrine: control, 1.28 ± 0.02; 1 nM, 1.28 ± 0.02; 10 nM, 1.29 ± 0.03; 100 nM, 1.32 ± 0.05; [^{3}H]serotonin: control, 1.87 ± 0.02; 1 nM, 1.91 ± 0.04; 10 nM, 1.81 ± 0.04; 100 nM, 1.85 ± 0.03.

On the other hand, ghrelin was able to specifically inhibit depolarization-induced serotonin release (Fig. 1B), with no effect on depolarization-induced dopamine (Fig. 1A) and norepinephrine release. Mean ± S.E.M. of the area under the time–response curve (AUC) of the percentage of [^{3}H]amine recovered with respect to total (fractions + filters) were as follows: control [K^+ (15 mM)], 7.12 ± 0.14; 1 nM, 7.06 ± 0.14; 10 nM, 7.08 ± 0.15; 100 nM, 7.07 ± 0.14.

We also found that amylin, in the dose range 1–100 nM, did not affect basal amine release. Means ± S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions with respect to total loaded [^{3}H] were as follows: [^{3}H]dopamine: control, 1.16 ± 0.03; 1 nM, 1.12 ± 0.03; 10 nM, 1.13 ± 0.03; 100 nM, 1.16 ± 0.06; [^{3}H]norepinephrine: control, 1.28 ± 0.02; 1 nM, 1.26 ± 0.05; 10 nM, 1.28 ± 0.02; 100 nM, 1.23 ± 0.04; [^{3}H]serotonin: control, 1.95 ± 0.05; 1 nM, 2.01 ± 0.02; 10 nM, 2.06 ± 0.07; 100 nM, 2.02 ± 0.07.

On the other hand, amylin significantly inhibited depolarization-stimulated dopamine release (Fig. 1A), but did not

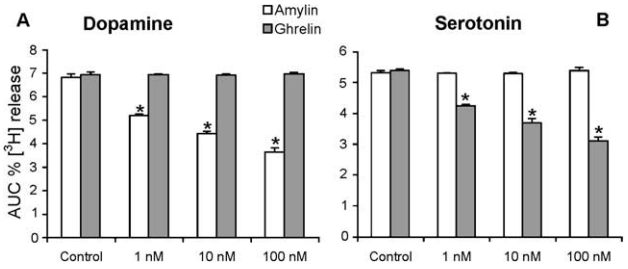

Fig. 1. Effects of amylin and ghrelin on depolarization-induced dopamine (panel A) and serotonin (panel B) release. The control group was perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min; the amylin and ghrelin groups were perfused with graded concentrations of the respective peptides in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20-min preincubation with the peptide in Krebs–Ringer buffer. The columns represent the area under the time–response curve (AUC) of the percentage of [^{3}H]amine recovered, with respect to total (fractions + filters); each column represents the mean ± S.E.M. of three to five experiments performed in triplicate; ANOVA, $P<0.0001$; *$P<0.001$ vs. control.

modify the stimulated release of serotonin (Fig. 1B) and norepinephrine. Mean ± S.E.M. of the area under the time–response curve (AUC) of the percentage of [^{3}H]amine recovered with respect to total (fractions + filters) were as follows: control [K^+ (15 mM)], 7.23 ± 0.01; 1 nM, 7.24 ± 0.04; 10 nM, 7.18 ± 0.11; 100 nM, 7.29 ± 0.08.

4. Discussion

The aminergic system plays a still unsettled role in the fine-tuning of feeding behavior at the hypothalamic level. On one side, dopamine administration into the hypothalamus inhibits food intake (Gillard et al., 1993), and the well-established anorectic effects of amphetamines could be explained by dopamine re-uptake inhibition in the lateral hypothalamus (Samanin and Garattini, 1993). On the other hand, dopamine injection into the lateral hypothalamus stimulates feeding behavior and obese rats have raised hypothalamic dopamine levels (Yang and Meguid, 1995). Moreover, brain dopamine release is associated with rewarding behavior (Robbins and Everitt, 1996), and dopamine is required for the increased food intake that follows leptin deficiency (Szczypka et al., 2000). The present findings, demonstrating that amylin inhibits dopamine release in the hypothalamus (Fig. 1A), together with our previous results, showing that the anorectic peptides leptin, CART peptide-(55–102) and TRH inhibit hypothalamic dopamine release (Brunetti et al., 1999, 2000), further support a role for inhibited dopamine release in mediating the anorectic peptide effects in the hypothalamus. It has been reported that the acute satiety effect of intraperitoneally administered amylin could be blocked by dopamine D_2 receptor antagonists, but this could be explained by extra-hypothalamic effects, probably in the area postrema/nucleus of the solitary tract region (Lutz et al., 2001).

Serotonin plays a well-established role in inhibiting feeding behavior at the hypothalamic level (Leibowitz and Alexander, 1998) and amphetamine-related anorectic drugs, such as fenfluramine, increase serotonin neurotransmission (Davis and Faulds, 1996). We have previously reported that orexin A and orexin B, which play a physiological role of appetite-stimulating peptides, inhibit serotonin release from hypothalamic synaptosomes, and this could account for the feeding stimulatory effects of these peptides. The present results, showing that depolarization-stimulated serotonin release is inhibited by ghrelin and not affected by amylin (Fig. 1B), confirm a role for decreased serotonergic signaling in mediating the acute hypothalamic effects of appetite-stimulating peptides.

Norepinephrine has been found to stimulate food intake via α_2-adrenoceptors (Wellman et al., 1993), and we have previously shown that the leptin acutely inhibits norepinephrine release from hypothalamic synaptosomes, which could partially explain the anorectic effect of leptin (Brunetti et al., 1999). The present findings, showing ghrelin and amylin do not affect norepinephrine release, rule out an involvement of noradrenergic modulatory effects of these peptides in the hypothalamus.

Considering the role played by serotonin and dopamine in the mechanisms of feeding and reward, though in the limited setting of an in vitro superfusion system, we can conclude that the appetite-stimulating activity of ghrelin could be mediated in part by inhibition of serotonin release, while the anorectic effects of amylin could involve inhibited release of dopamine in the hypothalamus.

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References

Banks, W.A., Kastin, A.J., 1998. Differential permeability of the blood–brain barrier to two pancreatic peptides: insulin and amylin. Peptides 19, 883–889.

Banks, W.A., Kastin, A.J., Maness, L.M., Huang, W., Jaspan, J.B., 1995. Permeability of the blood–brain barrier to amylin. Life Sci. 57, 1993–2001.

Brunetti, L., Michelotto, B., Orlando, G., Vacca, M., 1999. Leptin inhibits norepinephrine and dopamine release from rat hypothalamic neuronal endings. Eur. J. Pharmacol. 372, 237–240.

Brunetti, L., Orlando, G., Michelotto, B., Recinella, L., Vacca, M., 2000. Cocaine- and amphetamine-regulated transcript peptide-(55–102) and thyrotropin releasing hormone inhibit hypothalamic dopamine release. Eur. J. Pharmacol. 409, 103–107.

Chance, W.T., Balasubramaniam, A., Stallion, A., Fischer, J.E., 1991. Anorexia following the systemic injection of amylin. Brain Res. 607, 185–188.

Davis, R., Faulds, D., 1996. Dexfenfluramine. An updated review of its therapeutic use in the management of obesity. Drugs 52, 696–724.

D'Este, L., Wimalawansa, S.J., Renda, T.G., 2001. Distribution of amylin-immunoreactive neurons in the monkey hypothalamus and their relationships with the histaminergic system. Arch. Histol. Cytol. 64, 295–303.

Gillard, E.R., Dang, D.Q., Stanley, B.G., 1993. Evidence that neuropeptide Y and dopamine in the perifornical hypothalamus interact antagonistically in the control of food intake. Brain Res. 628, 128–136.

Kalra, S.P., Dube, M.G., Pu, S., Xu, B., Horvath, T.L., Kalra, P.S., 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr. Rev. 20, 68–100.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660.

Leibowitz, S.F., Alexander, J.T., 1998. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biol. Psychiatry 44, 851–864.

Ludvik, B., Kautzky-Willer, A., Prager, R., Thomaseth, K., Pacini, G., 1997. Amylin: history and overview. Diabet. Med. 14, S9–S13.

Lutz, T.A., Tschudy, S., Mollet, A., Geary, N., Scharrer, E., 2001. Dopamine D_2 receptors mediate amylin's acute satiety effect. Am. J. Physiol., Regul. Integr. Comp. Physiol. 280, R1697–R1703.

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa,

K., Matsukura, S., 2001. A role for ghrelin in the central regulation of feeding. Nature 409, 194–198.

Orlando, G., Brunetti, L., Di Nisio, C., Michelotto, B., Recinella, L., Ciabattoni, G., Vacca, M., 2001. Effects of cocaine- and amphetamine-regulated transcript peptide, leptin, and orexins on hypothalamic serotonin release. Eur. J. Pharmacol. 430, 269–272.

Robbins, T.W., Everitt, B.J., 1996. Neurobehavioural mechanisms of reward and motivation. Curr. Opin. Neurobiol. 6, 228–236.

Rushing, P.A., Hagan, M.M., Seeley, R.J., Lutz, T.A., Woods, S.C., 2000. Amylin: a novel action in the brain to reduce body weight. Endocrinology 850, 850–853.

Samanin, R., Garattini, S., 1993. Neurochemical mechanisms of action of anorectic drugs. Pharmacol. Toxicol. 73, 63–68.

Shintani, M., Ogawa, Y., Ebihara, K., Aizawa-Abe, M., Miyanaga, F., Takaya, K., Hayashi, T., Inoue, G., Hosoda, K., Kojima, M., Kangawa, K., Nakao, K., 2001. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50, 227–232.

Szczypka, M.S., Rainey, M.A., Palmiter, R.D., 2000. Dopamine is required for hyperphagia in *Lep*$^{ob/ob}$ mice. Nat. Genet. 25, 102–104.

Tschop, M., Smiley, D.L., Heiman, M.L., 2000. Ghrelin induces adiposity in rodents. Nature 407, 908–913.

Wellman, P.J., Davies, B.T., Morien, A., McMahon, L., 1993. Modulation of feeding by hypothalamic paraventricular nucleus α_1- and α_2-adrenergic receptors. Life Sci. 53, 669–679.

Wren, A.M., Small, C.J., Abbott, C.R., Dhillo, W.S., Seal, L.J., Cohen, M.A., Batterham, L.R., Taheri, S., Stanley, S.A., Ghatei, M.A., Bloom, S.R., 2001. Ghrelin causes hyperphagia and obesity in rats. Diabetes 50, 2540–2547.

Yang, Z.J., Meguid, M.M., 1995. LHA dopamine activity in obese and Zucker rats. NeuroReport 6, 1191–1194.