Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 519 (2005) 48 – 51

www.elsevier.com/locate/ejphar

Peptide YY (3–36) inhibits dopamine and norepinephrine release in the hypothalamus

Luigi Brunetti, Giustino Orlando, Claudio Ferrante, Annalisa Chiavaroli, Michele Vacca *

Department of Drug Sciences, G. D'Annunzio University, School of Pharmacy, via dei Vestini, 66013 Chieti, Italy

Received 1 June 2005; accepted 8 July 2005
Available online 10 August 2005

Abstract

Peptide YY (1–36) and peptide YY (3–36) are gut-derived hormones which are involved in feeding control in the hypothalamus. The hypothalamic mechanisms of feeding have been shown to be modulated by aminergic neurotransmitters, which could mediate the anorectic or orexigenic effects of neuropeptides and hormones. We have investigated the role of peptide YY (1–36) and peptide YY (3–36) on dopamine, norepinephrine, and serotonin release from hypothalamic synaptosomes in vitro. We found that peptide YY (3–36) inhibited depolarization-induced dopamine and norepinephrine release, leaving unaffected serotonin release, while peptide YY (1–36) did not modify either basal or stimulated amine release. We can hypothesize that the effects of peptide YY (3–36) could be mediated by inhibited hypothalamic dopamine and norepinephrine release, which could partially account for the anorectic activity of the peptide. On the other hand, peptide YY (1–36), which has a feeding stimulatory role, does not affect aminergic neurotransmission in the hypothalamus.

Keywords: Peptide YY; Hypothalamus; Dopamine; Norepinephrine; Serotonin

1. Introduction

Peptide YY is a gastrointestinal hormone mainly secreted by L cells of the distal small bowel and colon. There are two main circulating forms, peptide YY (1–36) and peptide YY (3–36), the latter deriving from N-terminal Tyr-Pro cleavage by the enzyme dipeptidyl peptidase IV (DPP-IV). Peptide YY is released in response to food ingestion, and peptide YY (3–36) is the main circulating form in the postprandial state (Grandt et al., 1994). Besides their roles in gastrointestinal functions, both peptides have been shown to be involved in feeding control. In particular, peptide YY (3–36) seems to play an anorectic role after either peripheral or central administration (Batterham et al., 2002), and its levels are decreased in obese patients (Batterham et al., 2003). On the contrary, peptide YY (1–36) administration into cerebral ventricles (Clark et al., 1987), hypothalamic paraventricular nucleus and hippocampus (Stanley et al., 1985) stimulates food ingestion.

The central mechanisms of feeding have been shown to be modulated by aminergic neurotransmitters (Kalra et al., 1999), and we have previously reported that neuropeptides, such as cocaine- and amphetamine-regulated transcript (CART) peptide and thyrotropin releasing hormone (TRH) (Brunetti et al., 2000), and hormones, such as amylin, ghrelin, leptin and resistin (Brunetti et al., 1999, 2002, 2004; Orlando et al., 2001), which play a role in feeding control, also modulate aminergic neurotransmitters in the hypothalamus, which could partially explain their anorectic or orexigenic effects.

In order to further elucidate the mechanisms of the central modulation of feeding, in the present study, we have investigated the role of peptide YY (1–36), peptide YY (3–36), and peptide YY (13–36), a selective neuropeptide Y

* Corresponding author. Tel.: +39 871 355 5233; fax: +39 871 355 5322.
E-mail address: mvacca@unich.it (M. Vacca).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.07.008

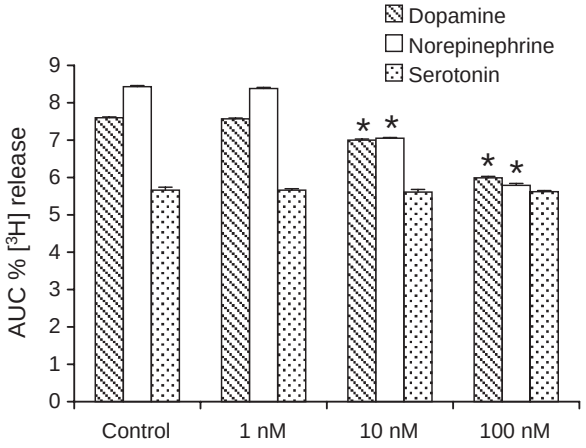

Fig. 1. Effects of peptide YY (3–36) (1–100 nM) on depolarization-induced dopamine, norepinephrine and serotonin release. The synaptosomes were perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min (control) or with graded concentrations of peptide YY (3–36) in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20 min pre-incubation 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, respect to total (fractions+filters); each column represents the mean± S.E.M. of 3–5 experiments performed in triplicate; ANOVA, $P<0.0001$; *$P<0.001$ vs. the respective control.

Y2 receptor agonist (Wahlestedt et al., 1990), on dopamine, norepinephrine, and serotonin release from hypothalamic synaptosomes.

2. Materials and methods

Hypothalamic synaptosomes were obtained from male Wistar rats (200–250 g), as previously described (Brunetti et al., 1999). They were 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 peptide YY (1–36), peptide YY (3–36), or peptide YY (13–36) 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 (0.1–100 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 reuptake is negligible due to the rapid removal of released amines by perfusion flow, intrasynaptosomal metabolism is negligible for dopamine and norepinephrine, while in the experiments evaluating serotonin release, a column chromatography of the perfusate proved necessary to separate serotonin from its metabolites, as previously described (Orlando et al., 2001).

Data represent the group means± S.E.M. of 3–5 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 peptide YY (1–36) and peptide YY (3–36), 0.2 mg/vial, were purchased from Phoenix Pharmaceuticals, USA. Rat peptide YY (13–36), 10 mg/vial were purchased from Tocris Cookson Ltd., UK. [^{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

Peptide YY (1–36), peptide YY (3–36) and peptide YY (13–36) did not modify basal amine release.

3.1. PYY (1–36)

Means± S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions respect to total loaded [^{3}H]. [^{3}H]dopamine: control, 1.87±0.03; 1 nM, 1.88±0.03; 10 nM, 1.90±0.04; 100 nM, 1.89±0.02; [^{3}H]norepinephrine: control, 1.48±0.03; 1 nM, 1.46±0.04; 10 nM, 1.49±0.02; 100 nM,

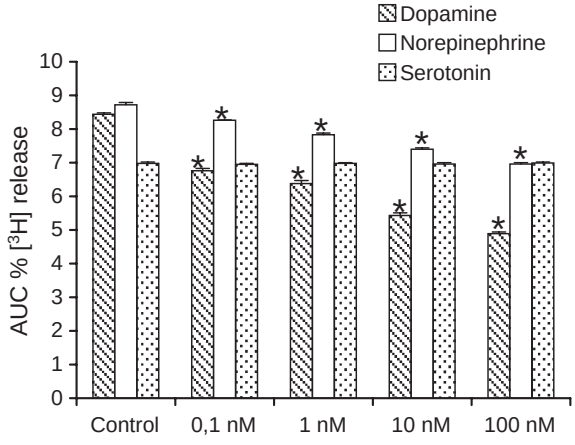

Fig. 2. Effects of peptide YY (13–36) (0.1–100 nM) on depolarization-induced dopamine, norepinephrine and serotonin release. The synaptosomes were perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min (control) or with graded concentrations of peptide YY (13–36) in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20 min pre-incubation 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, respect to total (fractions+filters); each column represents the mean± S.E.M. of 3–5 experiments performed in triplicate; ANOVA, $P<0.0001$; *$P<0.001$ vs. the respective control.

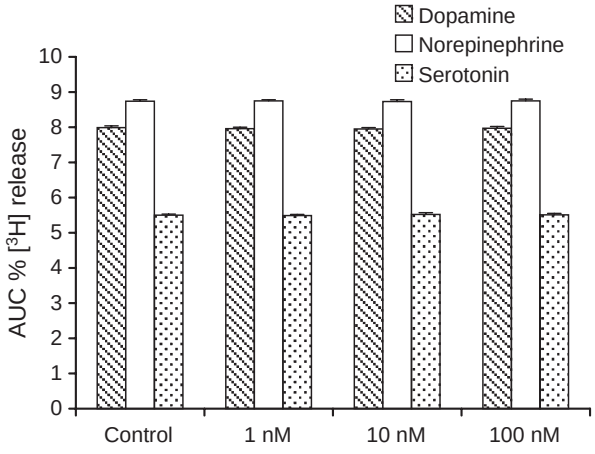

Fig. 3. Effects of peptide YY (1–36) (1–100 μM) on depolarization-induced dopamine, norepinephrine and serotonin release. The synaptosomes were perfused with K^+ (15 mM) in Krebs–Ringer buffer for 3 min (control) or with graded concentrations of peptide YY (1–36) in K^+ (15 mM) Krebs–Ringer buffer for 3 min, after a 20 min pre-incubation 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, respect to total (fractions+filters); each column represents the mean± S.E.M. of 3–5 experiments performed in triplicate.

1.46±0.03; [^{3}H]serotonin: control, 1.50±0.05; 1 nM, 1.49±0.04; 10 nM, 1.47±0.04; 100 nM, 1.50±0.06.

3.2. PYY (3–36)

Means±S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions respect to total loaded [^{3}H]. [^{3}H]dopamine: control, 1.52±0.03; 1 nM, 1.55±0.06; 10 nM, 1.54±0.04; 100 nM, 1.56±0.03; [^{3}H]norepinephrine: control, 1.79±0.03; 1 nM, 1.81±0.06; 10 nM, 1.78±0.04; 100 nM, 1.82±0.03; [^{3}H]serotonin: control, 1.58±0.05; 1 nM, 1.57±0.04; 10 nM, 1.55±0.04; 100 nM, 1.57±0.02.

3.3. PYY (13–36)

Means±S.E.M. of the percentage of [^{3}H]amine recovered in the stimulus and return to basal fractions respect to total loaded [^{3}H]. [^{3}H]dopamine: control, 2.12±0.04; 1 nM, 2.06±0.03; 10 nM, 2.07±0.04; 100 nM, 2.11±0.03; [^{3}H]norepinephrine: control, 1.31±0.04; 1 nM, 1.33±0.02; 10 nM, 1.34±0.02; 100 nM, 1.34±0.02; [^{3}H]serotonin: control, 1.72±0.02; 1 nM, 1.74±0.01; 10 nM, 1.71±0.02; 100 nM, 1.73±0.01.

After preincubating the synaptosomes with graded concentrations of peptide YY (1–36), peptide YY (3–36), or peptide YY (13–36) and then perfusing with depolarizing buffer (K^+ 15 mM), we found that peptide YY (3–36) (Fig. 1) and peptide YY (13–36) (Fig. 2) inhibited the stimulated release of dopamine and norepinephrine, leaving unaffected serotonin release, while peptide YY (1–36) did not modify either amine release (Fig. 3).

4. Discussion

Gut-derived hormones are powerful modulators of feeding both peripherally and at the central nervous system level (Wynne et al., 2004), where the hypothalamus plays a pivotal role in the cross talk signaling between hormones and neurotransmitters (Kalra et al., 1999). The aminergic system is deeply involved in translating peripheral afferents into satiety and feeding signals in the central nervous system. The role played by dopamine in feeding behavior is still unclear. On one side, dopamine administration into the hypothalamus inhibits feeding (Gillard et al., 1993), and amphetamines have anorectic effects through inhibition of presynaptic dopamine reuptake in the lateral hypothalamus (Samanin and Garattini, 1993). On the other hand, dopamine administered into the lateral hypothalamus stimulates feeding, and obese rats have increased hypothalamic dopamine levels (Yang and Meguid, 1995). Furthermore, 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). We have previously found that neuropeptides, such as CART peptide (55–102) and TRH (Brunetti et al., 2000), and hormones, such as leptin and amylin (Brunetti et al., 1999, 2002), all of which have anorectic effects in the central nervous system, also inhibit hypothalamic dopamine release, while ghrelin, which stimulates feeding, has not such an effect (Brunetti et al., 2002). It has been shown that peptide YY (3–36) modulatory effects in the hypothalamus include inhibition of the orexigenic neuropeptide Y and stimulation of the anorectic proopiomelanocortin (POMC) systems, possibly through neuropeptide Y Y2 inhibitory presynaptic receptors (Batterham et al., 2002). The present findings, showing peptide YY (3–36) and peptide YY (13–36), a selective neuropeptide Y Y2 receptor agonist (Wahlestedt et al., 1990), inhibit dopamine release (Figs. 1 and 2), support a central anorectic effect of peptide YY (3–36), possibly partially mediated by inhibited dopamine release through neuropeptide Y Y2 receptors. On the contrary, peptide YY (1–36), which seems to play a feeding stimulatory role, does not modify dopamine release (Fig. 3).

In the paraventricular nucleus of the hypothalamus norepinephrine released from presynaptic terminals modulates feeding in opposite ways, inhibiting through α_2- and stimulating through α_1-adrenoceptors (Wellman et al., 1993), while chronic infusion of norepinephrine into the ventromedial hypothalamus induces obesity in rats (Shimazu et al., 1986). Our previous experiments have shown that among several peptides which are involved in feeding control, the adipose tissue hormones leptin and resistin inhibit norepinephrine release from hypothalamic synaptosomes (Brunetti et al., 1999, 2004), while adiponectin, amylin, CART peptide, ghrelin and TRH do not affect hypothalamic norepinephrine release (Brunetti et al., 2000, 2002, 2004). The present findings, showing that peptide YY (3–36) and peptide YY (13–36) inhibit both dopamine and norepinephrine release (Figs. 1 and 2), as much as the anorectic hormone leptin does in the hypothalamus, further support a role for both catecholamines as central mediators

of anorectic hormone signaling. On the other hand, peptide YY (1–36), which stimulates feeding after intrahypothalamic administration, neither affected norepinephrine nor dopamine release (Fig. 3), which is consistent with the effects observed with the orexigenic gastric hormone ghrelin (Brunetti et al., 2002).

Serotonergic afferents from the dorsal raphe nucleus to the hypothalamus inhibit feeding, an effect mimicked by direct microinjection of serotonergic agents in the hypothalamus (Leibowitz and Alexander, 1998). We have previously reported that anorectic peptides such as leptin, CART peptide and amylin have no effect on hypothalamic serotonin release, while orexin-A, orexin-B and ghrelin inhibit serotonin release, which could account for their feeding stimulatory role in the CNS (Orlando et al., 2001; Brunetti et al., 2002). The present findings rule out an involvement of serotonin in the hypothalamic effects of peptide YY (3–36), peptide YY (13–36), and peptide YY (1–36) (Figs. 1–3).

In conclusion, the central nervous system effects of peptide YY (3–36) could be mediated by inhibition of hypothalamic dopamine and norepinephrine release, through a selective neuropeptide Y Y2 receptor agonism, which could partially account for the anorectic activity of the peptide in vivo. On the other hand, peptide YY (1–36), which mostly have a central feeding stimulatory role, does not affect aminergic neurotransmission in the hypothalamus.

Acknowledgements

This work was supported by MIUR and COFIN 2003 grants.

References

Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A., Cone, R.D., Bloom, S.R., 2002. Gut hormone PYY (3–36) physiologically inhibits food intake. Nature 418, 650–654.

Batterham, R.L., Cohen, M.A., Ellis, S.M., Le Roux, C.W., Withers, D.J., Frost, G.S., Ghatei, M.A., Bloom, S.R., 2003. Inhibition of food intake in obese subjects by peptide YY3–36. N. Engl. J. Med. 349, 941–948.

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.

Brunetti, L., Recinella, L., Orlando, G., Michelotto, B., Di Nisio, C., Vacca, M., 2002. Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus. Eur. J. Pharmacol. 454, 189–192.

Brunetti, L., Orlando, G., Recinella, L., Michelotto, B., Ferrante, C., Vacca, M., 2004. Resistin, but not adiponectin, inhibits dopamine and norepinephrine release in the hypothalamus. Eur. J. Pharmacol. 493, 41–44.

Clark, J.T., Sahu, A., Kalra, P.S., Balasubramaniam, A., Kalra, S.P., 1987. Neuropeptide Y (NPY)-induced feeding behavior in female rats: comparison with human NPY ([Met17]NPY), NPY analog ([nor-Leu4]NPY) and peptide YY. Regul. Pept. 17, 31–39.

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.

Grandt, D., Schimiczek, M., Beglinger, C., Layer, P., Goebell, H., Eysselein, V.E., Reeve Jr., J.R., 1994. Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1–36 and PYY 3–36. Regul. Pept. 51, 151–159.

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.

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

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.

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

Shimazu, T., Noma, M., Saito, M., 1986. Chronic infusion of norepinephrine into the ventromedial hypothalamus induces obesity in rats. Brain Res. 369, 215–223.

Stanley, B.G., Daniel, D.R., Chin, A.S., Leibowitz, S.F., 1985. Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides 6, 1205–1211.

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.

Wahlestedt, C., Grundemar, L., Hakanson, R., Heilig, M., Shen, G.H., Zukowska-Grojec, Z., Reis, D.J., 1990. Neuropeptide Y receptor subtypes, Y1 and Y2. Ann. N. Y. Acad. Sci. 611, 7–26.

Wellman, P.J., Davies, B.T., Morien, A., McMahon, L., 1993. Modulation of feeding by hypothalamic paraventricular nucleus α1- and α2-adrenergic receptors. Life Sci. 53, 669–679.

Wynne, K., Stanley, S., Bloom, S., 2004. The gut and regulation of body weight. J. Clin. Endocrinol. Metab. 89, 2576–2582.

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