Neuropeptide Y effects on vasorelaxation and intestinal contraction in the Atlantic cod Gadus morhua

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Shahbazi, Fatemeh, Susanne Holmgren, Dan Larhammar, and Jörgen Jensen. Neuropeptide Y effects on vasorelaxation and intestinal contraction in the Atlantic cod Gadus morhua. Am J Physiol Regulatory Integrative Comp Physiol 282: R1414–R1421, 2002; 10.1152/ajpregu.00434.2001.—Neuropeptide Y (NPY) has prominent cardiovascular effects in mammals and sharks, but no such effect has previously been demonstrated in any teleost fish. In the Atlantic cod, we found that cod NPY (10−10–10−6 M) relaxed celiac arteries precontracted with epinephrine, and weak contractions were elicited in intestinal ring preparations. A few NPY-immunoreactive nerve fibers were present along small gut arteries. The results suggest that cod NPY produces vasorelaxation both by a direct action on smooth muscle and by release of prostaglandins, but with no involvement of nitric oxide, leukotrienes, or endothelium-derived relaxing factors. An additional indirect effect involving another neurotransmitter may occur. Cod NPY (10−7 M) and human NPY (10−7 M) had identical effects on the vessels. Small differences only in the effects of porcine [Leu31,Pro34]NPY, NPY-(13–36), and cod NPY suggest the presence of a Y1 subfamily receptor, similar to the zebrafish Ya receptor. A physiological role for NPY in teleost vasculature is concluded, but surprisingly the effect, a vasodilation, is opposite to that in mammals and is mediated by prostaglandins.

cod neuropeptide Y; celiac artery; cod intestine; receptor

NEUROPEPTIDE Y (NPY) is a 36-amino acid peptide characterized by its NH2 terminal tyrosine and COOH terminal tyrosine amide residues. NPY was first isolated from porcine brain and belongs to the pancreatic polypeptide family (40). NPY is abundant in the central and peripheral nervous system of vertebrates, often coexisting with norepinephrine (11, 26). This peptide has several biological effects, including stimulation of food intake; hormone release; and cardiovascular functions, particularly vasoconstriction, reduction of coronary blood flow, and reduction of cardiac output (7, 29). The regulation of cardiovascular functions by NPY appears to be common to most vertebrates, including elasmobranch fish (4, 33). A potent vasoconstriction is induced in several vascular beds, such as the mesenteric arteries in pig, the celiac and mesenteric arteries in crocodile, and the dogfish afferent branchial arteries (4, 19, 28). Intravenous injection of dogfish, frog, and human NPY produced similar vasopressor effects in three elasmobranch species, except that human NPY lowered blood pressure in one (Portjackson shark) of the three species (33). In addition, NPY may have vasodilator effects (23, 32, 41, 43). However, to our knowledge, there are so far no reports of a function of NPY in the teleost vasculature. NPY has been isolated from various fish species, including cod, rainbow trout, and dogfish (6, 16), and is one of the most conserved peptides during evolution (5, 24).

Several NPY receptor subtypes have been identified in mammals and classified as Y1, Y2, Y3, Y4, Y5, and y6 (25). Of these, Y1 and Y2 receptors are involved in vascular control (44). Three NPY receptor genes, zYa, zYb, and zYc, have been identified in zebrafish and a Yb receptor gene in the Atlantic cod (1, 34, 38). The zYb and zYc genes show 76% identity and both show high homology to the cod Yb receptor gene (1). The receptor mediating blood pressure responses in elasmobranch species functionally is more like a Y1 than a Y2 receptor (33).

To investigate the vasoactive effect and mechanisms of cod NPY in its native species and to characterize the receptor subtypes in the small arteries from cod, in vitro experiments using a microvascular myograph were performed. Immunohistochemical staining was accomplished to identify possible NPY-containing neurons innervating these arteries. We also examined the biological activities of the cod NPY in a ring preparation of proximal intestine from cod.

MATERIAL AND METHODS

Atlantic cod, Gadus morhua, of both sexes weighing between 150 and 800 g were supplied by local fishermen on the Swedish west coast. The fish were kept unfed (~1 wk before experiments) in aerated, recirculating seawater at 10°C. The cod were killed by a sharp blow to the head, and tissues for vascular and intestinal studies were dissected out.

Vascular preparations. Segments (internal diameter 260–1,230 μm, ~2 mm length) of second- to fourth-order branches

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of the celiac artery were gently removed and mounted in a myograph apparatus (J. P. Trading, Aarhus, Denmark) for measurement of vascular wall isometric tension. Tension changes were displayed on a Kipp and Zonen pen recorder. Tissues were maintained at 10°C and aerated with 0.3% CO₂ in air, in a bath containing 12 ml of cod Ringer solution of the following composition (in mM): 150.1 NaCl, 5.2 KCl, 1.9 CaCl₂, 1.8 MgSO₄, 1.9 Na₂HPO₄, 7.0 NaHCO₃, and 5.6 glucose. pH was 7.8–7.9. Each artery was kept under a normalized tension as previously described (31, 36). After normalization of the vessels and 30 min of equilibration, the contractile capacity of the vessels was examined by repeated exposure to a potassium-rich (60 mM KCl) Ringer solution containing epinephrine (10⁻⁵ M) (17).

Initially, cod NPY was given to unstimulated branches from the celiac arteries and caused relaxation. To better study this relaxant activity of NPY, the preparations were precontracted by epinephrine (3 × 10⁻⁷ M), which caused a contraction that remained stable for at least 45–60 min before addition of the peptides. This was repeatable with washout and 20–30 min reequilibration in between. In cod NPY or human NPY was added to the bath in cumulative concentrations (10⁻¹⁰–10⁻⁶ M). Due to tachyphylaxis, it was not possible to repeat cumulative concentration response curves on the same preparation. However, the effect of single additions of cod NPY (10⁻⁷ M) was repeatable and therefore the mechanisms of action of cod NPY were studied in (precontracted) preparations subjected to cod NPY (10⁻⁷ M).

After preparations were washed and reequilibrated, the procedure was repeated a second time in the presence of inhibitors or NPY receptor agonist. The inhibitors were added 5–10 min before the second precontraction with epinephrine, which was equivalent to 55–65 min before the subsequent addition of cod NPY to the bath. In some experiments, responses to cod NPY were compared with responses to human NPY or NPY agonists. The preparations were exposed to cod NPY or general NPY receptor agonists, washed, and reequilibrated and subjected to human NPY or cod NPY, respectively.

In an additional series of experiments, the endothelium was removed from the mounted arteries by rubbing the luminal surface with a fine hair. Responses in endothelium-denuded segments were compared with responses in endothelium-intact segments from the same branch of the celiac artery. The quality of the endothelial abrasion was controlled by light microscopy after staining of the preparation using toluidine blue.

Intestinal preparations. Preparations were made from proximal intestine (ring preparations ~3–5 mm wide and longitudinal preparations ~2 × 10 mm) and cardiac stomach (circular and longitudinal orientation, ~2 × 10 mm). They were mounted in organ baths containing 5 ml cod Ringer solution kept at 10°C and bubbled with 0.3% CO₂ in air. The force developed by the preparations was recorded using FT03 force transducers and a Grass model 7 Polygraph. An initial tension of 10 mN was applied to the preparations, and 1 h was allowed for the preparations to obtain a steady basal tension before the start of the experiments.

Acetylcholine (10⁻⁶ M) was added to test the viability of the preparations, and, after being washed, the preparations were allowed to reestablish their basal tension during 1 h. Cod NPY was added in increasing concentrations until a maximum response was obtained.

Histochromy. Arterial segments for immunohistochemistry (whole mount preparations and sections) and morphological studies (denuded arteries and control arteries) were collected and fixed in Zamboni’s fixative (15% saturated picric acid and 2% formaldehyde in 0.1 M phosphate buffer, pH 7.2). The fixative was washed out with 80% ethanol, and then the preparations were dehydrated, treated with xylene for 20 min, and finally rehydrated. They were kept in PBS containing 30% sucrose for at least 24 h before being quick-frozen in liquid N₂-cooled isopentane. Sections (10 µm thick) were cut on a cryostat and mounted on gelatin-coated slides. For detection of NPY and the adrenergic enzyme dopamine β-hydroxylase (DBH), the preparations were incubated with primary antisera: NPY-A (raised in rabbit, diluted 1:400, Amersham), NPY-AB22 (rabbit, 1:200, Diagnostic), NPY-1236 (goat, 1:100, Affiniti), NPY-6730 (rabbit, 1:50, Biogenesis), NPY-7172 (rabbit, 1:200, Peninsula), or DBH-3960 (rabbit, 1:20, Biogenesis). The incubated preparations were kept at room temperature for 24–48 h in a moist chamber. They were subsequently rinsed 4 × 5 min with high salinity PBS (2.0% NaCl, pH 7.2) and incubated with secondary antibodies (indocarbocyanine, Cy3, conjugated donkey anti-rabbit, 1:800, Jackson; or dichlorotriazinyl amino fluorescein (DTAF)-conjugated donkey anti-goat, 1:100, Jackson) for 1 h at room temperature. After being repeatedly rinsed, the preparations were mounted on slides with carbonate-buffered glycerol and viewed with a fluorescence microscope. Photographs were taken on Kodak T-Max 400 film. For light microscopy viewing (Olympus), the sections were stained with toluidine blue (0.1%) for 7 min and photographs were taken on ILFORD PANF 50 film.

Specificity tests. Preabsorption tests with cod NPY or human NPY were made with the primary antisera (NPY-AB22 or NPY-A). Addition of antigen (10 µM) to the antisera abolished all visible immune reactivity in the preparations. Incubation of the preparations with only secondary antibodies (DTAF or Cy3) was made, and no spurious binding was detected.

Drugs. Human NPY was supplied by Auspep. Cod NPY was synthesized by Chiron Mimotopes Peptide System. [Pro30,Tyr31,Leu32]NPY-(28–36), Bis (31/31) ([Cys31,Trp32,Nva34]NPY-(31–36)), and 1229U91 were provided by Peptide System. T4-NPY-(33–36) was purchased from Dr. E. Grouzmann. MK-886 was supplied by Calbiochem. Porcine NPY-(13–36) and [Leu31,Pro30]NPY were from Bachem (King of Prussia, PA). Epinephrine bitartrate, indomethacin, N²-nitro-L-arginine methyl ester (l-NAME), and TTX were all from Sigma (St. Louis, MO). Indomethacin, T4-NPY-(33–36) and MK-886 were dissolved in DMSO and diluted in phosphate buffer. DMSO (0.1%, the highest concentration used in the study) had no effect on the response studied. All peptides were dissolved in water and diluted in 0.1% bovine serum albumin for further dilution (0.9% NaCl was used).

Data analysis. All data were collected into a personal computer for online data acquisition using Lab View 5.1 (National Instrument, Austin, TX).

For the vascular preparations, relaxations produced by cod NPY and other peptides were calculated as percent reduction of the contraction produced by epinephrine (3 × 10⁻⁷ M). For the intestinal ring preparations, contractions produced by cod NPY were calculated as percent of the contraction produced by a previous exposure to acetylcholine (10⁻⁶ M). pD₂ values (– log EC₅₀) for cod NPY and human NPY on vascular preparations were calculated from cumulative concentration response curves using a nonlinear regression analysis program (GraphPad Prism, San Diego, CA). Intestinal ring preparations showing no response to acetylcholine were not used for calculation. The nonparametric Wilcoxon’s matched-pair signed ranks test was used for statistical evaluation of the results, because a normal distribution could not be assumed in these groups. The results are presented as means ± SE,
and n represents the number of fish used in each experiment. P < 0.05 was regarded as statistically significant.

RESULTS

Effect of cod NPY and human NPY on the celiac arteries. In precontracted celiac arteries, cod NPY and human NPY (10⁻¹⁰–10⁻⁶ M) induced identical concentration-dependent dilations (pD₂ value 8.72 ± 0.34 and 8.3 ± 0.33 for cod NPY and human NPY, respectively, n = 6, Fig. 1). Threshold concentration was the same (10⁻⁸ M) for cod NPY and human NPY. Repetition of concentration response curves was impossible. Therefore, the use of a single concentration (10⁻⁷ M), which gave a repeatable relaxation with no significant difference in response between first and second exposure (64.6 ± 10.9 and 64.9 ± 9.1% reduction of the contraction, respectively, n = 6), was chosen for further experiments. There was no significant difference in effect between cod NPY (10⁻⁷ M) and human NPY (10⁻⁷ M, 60.2 ± 7.7 and 45.7 ± 11.9% reduction of the contraction, respectively, n = 6).

Mechanisms of action of cod NPY on the celiac arteries. Indomethacin (10⁻⁶, n = 7), a prostaglandin synthesis (cyclooxygenase) inhibitor, abolished the relaxation induced by cod NPY (10⁻⁷ M, Fig. 2). The responses induced by cod NPY (10⁻⁷ M) were not significantly influenced by MK-886 (10⁻⁵ M, 58.4 ± 12.3 and 45.9 ± 10.6% reduction of the contraction before and after MK-886, respectively, n = 6), a specific inhibitor of leukotriene biosynthesis (9). Treatment of the vessels with the inhibitor of nitric oxide synthesis, L-NAME (3 × 10⁻⁴ M, 74.1 ± 7.7 and 71.4 ± 8.1% reduction of the contraction before and after treatment, respectively, n = 8) or removal of the endothelium had no significant effect on the response to cod NPY (Fig. 2). Mechanical removal of the endothelium, however, reduced the magnitude of the precontraction (37% of first exposure to epinephrine). Incubation of the vessels with TTX (10⁻⁶ M, n = 6), a blocker of voltage-gated sodium channels, significantly reduced the effect of cod NPY (Fig. 2). TTX had no effect on the baseline tone and precontraction with epinephrine.

Pharmacological characterization of NPY receptors in the cod celiac arteries. Porcine [Leu³¹, Pro³⁴]NPY (10⁻⁷ M, n = 6), which is selective for non-Y₂ receptors, produced relaxation of the precontracted celiac arteries. The effect was significantly less (48.7 ± 21.3%) than that of cod NPY. The Y₂ receptor agonist porcine NPY-(13–36), given in the same concentration, produced an even smaller relaxation, 19.5 ± 4% of the cod NPY response (n = 8, Fig. 3A).

The NPY Y₁ receptor antagonists, 1229U91 (which is also a Y₄ agonist) (3 × 10⁻⁶ M, n = 6), [Pro³⁰, Tyr³², Leu³⁴]NPY-(28–36) (10⁻⁶ M, n = 6), or Bis (31/31) (3 × 10⁻⁶ M, n = 6) all significantly reduced the dilation

Fig. 1. Concentration response curves for the relaxing effect of cod neuropeptide Y (NPY; n = 6) and human NPY (n = 6) on branches of cod celiac artery precontracted with epinephrine (3 × 10⁻⁷ M). The responses are expressed as percent relaxation of precontraction.

![Figure 1](http://ajpregu.physiology.org/)

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Fig. 2. The effect of cod NPY (10⁻⁷ M) on branches of the celiac artery precontracted with epinephrine (3 × 10⁻⁷ M) before and after pretreatment with indomethacin (10⁻⁶ M, n = 7), TTX (10⁻⁶ M, n = 6), or removal of the endothelium (n = 6). *Statistically significant difference from control.

![Figure 2](http://ajpregu.physiology.org/)

Fig. 3. The effect of cod NPY (10⁻⁷ M) on branches of the cod celiac artery precontracted with epinephrine (3 × 10⁻⁷ M) compared with the effect of the Y₁ receptor agonist porcine [Leu³¹, Pro³⁴]NPY (10⁻⁷ M, n = 6) and the Y₂ receptor agonist porcine NPY-(13–36) (10⁻⁷ M, n = 8) (A) or the effect of cod NPY in the presence of NPY Y₁ receptor antagonists 1229U91 (3 × 10⁻⁶ M, n = 6), [Pro³⁰, Tyr³², Leu³⁴]NPY-(28–36) (10⁻⁶ M, n = 6), or Bis (31/31) (3 × 10⁻⁶ M, n = 6) all significantly reduced the dilation

![Figure 3](http://ajpregu.physiology.org/)
produced by cod NPY (Fig. 3B). The Y₂ receptor antagonist (T4-[NPY-(33–36)] 4, 10⁻⁶ M, n = 5) had no significant effect on the relaxation.

Effects of cod NPY on the ring preparation of the cod proximal intestine. Cod NPY (test range 10⁻¹⁰–10⁻⁶ M) induced contraction of the ring preparations from cod proximal intestine in five of eight fish, but only at higher concentrations (10⁻⁷ and 10⁻⁶ M). The contractions were 70.9 ± 24 and 150.7 ± 45% of the effect of acetylcholine (10⁻⁶ M), respectively (Fig. 4). Contractions induced in the longitudinal muscle from stomach and intestine and on the circular muscle from the stomach were very weak.

Histochemistry. For immunohistochemistry, five different NPY antibodies, which target different areas of the NPY molecule, NPY-A, NPY-G, NPY-AB22, NPY-6730, and NPY-7172, were used. Among them, only NPY-A and NPY-AB22 detected NPY-immunoreactive (IR) fibers. A moderate number of NPY-IR (Fig. 5c) and DBH-IR nerve fibers were found in the wall of the small arteries in whole mount preparations.

Efficacy of the denuding process was confirmed by preparing light microscopy images of control vessels (Fig. 5a) and denuded vessels (Fig. 5b). In control vessels, the endothelial cells were intact, except for the regions where the wires had been attached, whereas in the denuded vessels the endothelial layer was almost completely destroyed.

DISCUSSION

This is the first study showing a vasorelaxation induced by NPY via release of a prostaglandin in a vertebrate. It is also the first report demonstrating the presence of perivascular NPY-IR nerve fibers in a teleost. Previous studies have found a dense innervation by NPY-IR fibers of the circulatory system of elasmobranchs (especially skates) but could not show NPY-IR nerve fibers in the circulatory system of teleosts (3, 33). There is a possibility that the differences in immunoreactivity in elasmobranchs and teleosts can be due to the antibody-antigen interaction rather than the concentration of antigen (NPY) present. For some reason

![Fig. 4](image-url)  
**Fig. 4.** Contractile effect of single additions of cod NPY (n = 5) on the intestinal ring preparations from cod proximal intestine. The data are presented as means ± SE and expressed as percent response of contraction obtained by acetylcholine (10⁻⁶ M).

![Fig. 5](image-url)  
**Fig. 5.** Photomicrographs showing the morphology of “intact” (A) and denuded (B) cod celiac arteries used in the myograph. In intact vessels, the endothelium is disrupted on each side of the vessels (stars) by the mounting wires, whereas the rest of the endothelial lining (arrows) is still intact (colored by toluidine blue). In denuded vessels, all of the lining is disrupted. C: immunohistochemistry shows NPY-immunoreactive nerve fibers in the wall of small arteries of the cod. Other structures in the vessel walls are autofluorescent. Calibration bars, 100 µm.
that is not immediately obvious from comparison of NPY sequences, the antibodies raised against mammalian NPY are unable to strongly detect teleost NPY and have a better recognition of elasmobranch NPY.

Coexistence of NPY and epinephrine has been demonstrated in mammals (11) and in fish (21). Our set of antisera did not allow determination of coexistence in the perivascular nerves of the cod vessel, but the study by Karila et al. (21) demonstrates that the adrenergic neurons in the cod gut that show NPY-IR are more likely to project to the submucosa than to the enteric vasculature.

NPY is a potent vasoconstrictor of peripheral blood vessels in several mammalian species (28, 30). In contrast, cod NPY produced vasorelaxation in the cod celiac arteries. In concert with the present results, there are a few reports that show that NPY may cause vasodilation in some vascular beds in mammals, e.g., in human subcutaneous arteries (32) and feline cerebral arteries (23), and a transient relaxation is described in guinea pig basilar arteries (43). In vivo experiments on the estuarine crocodile have shown that NPY increased the flow in the arterial anastomosis, the celiac arteries, and right aorta (22). Physiological studies in dogfish species suggest different effects of NPY depending on the vascular bed. For example, NPY decreased celiac vascular resistance and increased blood flow to the gut of Scyliorhinus canicula (14, 15) but produced contraction in afferent gill arteries from Scyliorhinus canicula (4). Similar vasoconstrictor effects due to intravenous injection of dogfish, frog, or human NPY were seen in three different elasmobranch species, whereas human NPY lowered blood pressure in one species (Portjackson shark) (33). NPY can also have dual effects on the vascular tone of one vessel. In isolated rat middle cerebral arteries, extraluminal administration (smooth muscle side) of NPY constricted the arteries, whereas addition of NPY to the luminal side selectively caused dilation through an effect on endothelial cells and release of nitric oxide (42).

Several blockers of different signal transduction pathways were investigated. The prostaglandin antagonist indomethacin abolished the effect of cod NPY, which suggests that the cod NPY-induced relaxation is mediated by release of prostaglandins. Similarly, bradykinin relaxation of cod celiac arteries and vasoactive intestinal polypeptide relaxation of rainbow trout celiac arteries is mediated by release of prostaglandins (17, 36). In addition to prostaglandins, arachidonic acid metabolism produces leukotrienes. MK-886, a potent and specific inhibitor of leukotriene biosynthesis, failed to significantly reduce the responses of cod NPY, suggesting that leukotriene synthesis is not involved in this relaxation.

A few reports have demonstrated an involvement of the endothelium and of an nitric oxide pathway in producing vasodilation, e.g., in guinea pig basilar arteries, human subcutaneous arteries, and transient vasodilation in cat carotid artery (23, 32, 43). However, neither mechanical elimination of the endothelium nor pretreatment of the vessels with the nitric oxide synthesis inhibitor L-NAME had a significant effect on the cod NPY-induced relaxation, suggesting that this vasorelaxation is endothelium independent and does not involve nitric oxide synthesis. Mechanical removal of the endothelium, however, reduced the magnitude of precontraction, which may be due to disruption of the adjacent smooth muscle, as has been suggested in other fish species (17, 18, 39).

Treatment of the vessels with TTX significantly reduced the effect of cod NPY, suggesting that cod NPY produced vasodilation both by a direct action on the smooth muscle and release of prostaglandins and indirectly by release of another neurotransmitter. If so, this neurotransmitter probably also acts through prostaglandins, because indomethacin almost completely abolished the response to cod NPY.

The NH2 terminally truncated agonist for mammalian Y2 receptors, porcine NPY-(13–36), induced a smaller response than cod NPY. Apparently, the NH2 terminal amino acids are necessary for full stimulation of NPY receptors in cod arteries. There are five NPY receptor subtypes cloned in mammals, and of these the Y1 receptor is predominant in vessels (10). NH2 terminally truncated versions of NPY, such as NPY-(2–36), NPY-(3–36), and NPY-(13–36), have no or low affinity for the NPY Y1 receptor (10). In arteriovenous anastomoses in the rat tail, NPY-induced vasodilation was invoked by the Y1 receptor agonist [Leu31,Pro34]NPY but not by NPY-(13–36) (which is considered a Y2-specific relative of Y1) (13). Similarly, vasodilation in human subcutaneous arteries is mediated via NPY Y1 receptors (32). In contrast, dilation induced by luminal NPY or luminal [Leu31,Pro34]NPY in the rat middle cerebral arteries is not mediated through Y1 receptors (42).

In lampreys, which are agnathan vertebrates, both the modified Y1 agonist porcine [Leu31,Pro34]NPY and the Y2 agonist NPY-(13–36) bind to a cloned lamprey Y receptor (35). In dogfish afferent gill arteries, NPY

Table 1. Comparison of amino acid sequences of five different NPYS

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NPY, neuropeptide Y; PYY, peptide YY.
produced vasoconstriction, and these arteries were sensitive to [Leu\textsuperscript{31},Pro\textsuperscript{34}]NPY but showed no response to NPY-(13–36). The presence of a \(Y_1\)-like receptor was suggested (4). In the cod, porcine [Leu\textsuperscript{31},Pro\textsuperscript{34}]NPY produced a larger response (albeit a relaxation) of celiac arteries than porcine NPY-(13–36), but the difference was small. The \(Y_1\) receptor antagonists significantly reduced the effect of cod NPY, whereas the \(Y_2\) receptor antagonist T4-[NPY-(33–36)] failed to reduce the effect of cod NPY. This would suggest that the cod vascular receptors are more similar to the mammalian \(Y_1\) than the \(Y_2\) receptor.

However, although it is likely that the cod NPY receptor shares its ancestor with mammalian \(Y_1\) (and \(Y_4\) and \(y_6\) receptors) rather than \(Y_2\) or \(Y_5\) receptors, the fish NPY receptors sequenced to date all have properties making them distinct from mammalian NPY receptors. The \(Y_1\) receptor subfamily lineage includes several gene duplications, and different subtypes have evolved in different vertebrate groups. In teleost fish, three or even four duplications have led to \(zYa\), \(zYb\), and \(zYc\) receptors in zebrafish (27, 34, 38) and a \(Yb\) receptor in cod (1, 37). These are all structurally distinct from the mammalian NPY receptors. The cod \(Yb\) receptor shows 50% identity to \(Y_1\), \(Y_4\), and \(y_6\) and only 30% to \(Y_2\) and \(Y_5\) (37). The binding properties of the \(Yb\) and \(Yc\) receptors most resemble those of \(Y_1\), whereas \(Ya\) display a strikingly indiscriminate binding profile (2, 35). The cod \(Yb\) receptor shows a binding profile that is similar to both \(Yb\) and \(Yc\) (37). The pharmacological results from our studies, with only a small difference in effect of porcine [Leu\textsuperscript{31},Pro\textsuperscript{34}]NPY and porcine NPY-(13–36), may suggest an interaction with a (hitherto uncloned) receptor more like the \(Ya\) than the cod \(Yb\) receptor (2, 37), but an interaction with a mixture of the cod \(Yb\) receptor and a \(Ya\)-like receptor in the cod vasculature cannot be excluded.

Despite the six amino acid differences in sequences between the cod NPY and human NPY (Table 1), they were almost functionally equipotent in producing vasorelaxation in cod celiac arteries. Seven amino acids differ between cod NPY and porcine [Leu\textsuperscript{31},Pro\textsuperscript{34}]NPY. Five of these coincide with the differences between cod NPY and human NPY. We suggest that the remaining two amino acids, which are isoleucine at position 31 and glutamine at position 34 in cod NPY, contribute to the lower effect in the cod vessel. In mammals, it has been shown that the presence of Pro\textsuperscript{34} rather than Leu\textsuperscript{31} in the porcine [Leu\textsuperscript{31},Pro\textsuperscript{34}]NPY molecule is important for \(Y_1\) receptor interaction (12, 45).

Among the cod gut preparations, NPY induced a contractile effect in intestinal ring preparations at high concentrations only. In the rat, the contraction produced by NPY in the proximal colon is mediated by \(Y_2\) receptors and mainly does not involve the release of a second neurotransmitter. In contrast, porcine [Leu\textsuperscript{31},Pro\textsuperscript{34}]NPY, rat PP, human PP, and [\(\mu\)-Trp\textsuperscript{32}]NPY induced contractile responses through activation of \(Y_4\) and possibly \(Y_5\) receptors, which were sensitive to TTX, indicating an action via a second transmitter (8). In the cod, NPY-like immunoreactivity, probably peptide YY (PYY), has been found in endocrine cells in the intestinal mucosa (3, 5), and NPY-IR nerve fibers are present and more abundant in the longitudinal muscle layer than in the circular layer in both stomach and intestine (20).

The discrepancy between the immunohistochemical findings and the very modest effect of cod NPY may suggest that the experimental protocol used does not reveal an effect of NPY on the gut. Another possibility is that the antibodies used by Karila et al. (20) may have detected PYY or even PY, a peptide identified in several species of acanthomorph fishes although not yet the Atlantic cod (5), whereas NPY does not activate the receptor(s) that respond to PYY and/or PY.

Conclusions and Perspectives

The data from the present study suggest that cod NPY causes vasodilation in the cod celiac artery via release of prostaglandins and that the relaxation is neither endothelium dependent nor is it caused by nitric oxide production. Possibly the responses of the cod artery may be accounted for by NPY receptor subtypes that may be unique to teleost fishes. The receptor(s) may belong to the \(Y_1\) subfamily, possibly a \(Ya\)-like receptor, or a mixture of \(Ya\)-like and cod \(Yb\) receptors. The contraction of the intestinal preparations caused by cod NPY may be through activation of receptors normally stimulated by another NPY-related peptide.

Although we suggest a vascular role for NPY in cod as in mammals, our results revealed some interesting differences between these classes of vertebrates. The vasodilatory effect is uncommon, but not unique, to cod (23, 32, 41, 43) and demonstrates the diversified use of the same precious signal substance in different organisms. NPY is highly conserved (5, 24), which implies its crucial importance whether it is inhibitory or excitatory.

The functional significance of the clear-cut vasodilatory mechanism of NPY in a teleost species (and possibly in other fish species, as well as in certain vascular beds even in mammals) can only be speculated about. A controlled increase in blood flow to the gut is essential to ensure an optimal blood supply during digestion, but NPY seems not to provide the only mechanism for this. Other neuropeptides (vasoactive intestinal polypeptide, calcitonin gene-related peptide) have similar effects in the cod gut vasculature. The vasodilatory effect of NPY in the cod gut may be just another example of the amazing redundancy of physiological systems.

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