NPY Signalling Pathway in Bone Homeostasis: Y1 Receptor as a Potential Drug Target

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Abstract: Neuropeptide (NPY) is a neurotransmitter widely distributed in central and peripheral nervous system that has been implicated in several physiological processes through activation of five different Y receptors: Y1, Y2, Y4, Y5, and y6. NPY system has been extensively studied for the last decades due to its implications in a wide variety of physiological processes. For this purpose a diversity of sophisticated animal models and receptors agonists and antagonists has been developed to better understand its actions throughout body homeostasis. Consequently, NPY and its receptors have recently emerged as a potential regulator of bone homeostasis. This is supported by the demonstration of an increase of bone mass in mice lacking Y1 or Y2 receptor genes. Recent findings revealed Y1 receptor as a potential drug target candidate for prevention and treatment of bone loss. Indeed, it has been demonstrated that osteoblasts express Y1 receptor while no other Y receptor was detected in these cells, implicating Y1 receptor signalling in the local control of bone turnover. In this review, we have summarized the findings obtained from studies on NPY system in skeletogenesis focusing on Y1 receptor.

Key Words: Neuropeptide Y, Y1 receptor, bone remodelling.

INTRODUCTION

Recent evidences have prompted for a strong relationship between brain and bone, suggesting the concept of a neuro-osteogenic network regulating bone homeostasis. These evidences demonstrated by immunohistochemistry and bone histology studies showed that bone, bone marrow and the periosteum receive a rich supply of neuropeptide fibers [1]. Their phenotyping revealed the presence of several neurotransmitter fibers specifically vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), substance-P (SP) and neuropeptide Y (NPY) fibers. It has also been demonstrated the presence of functional receptors for several neurotransmitters in bone cells such as glutamate and CGRP. Thus, they can affect osteoclast or osteoblast activities.

Although the distribution of VIP and CGRP has been extensively studied in bone, there is a lack of knowledge concerning others neuropeptides also shown to be present, such as NPY. Since its discovery, NPY has been implicated in a large number of physiological actions. Interestingly, recently published articles describing *in vivo* experimental models have confirmed the implication of NPY and Y2 receptors in a central regulation of bone homeostasis [2, 3]. Studies from the same group have also implicated Y1 receptor but rather in the local control of bone turnover. Thus, this review aims to focus in NPY and Y1 receptor as putative key modulator of bone remodelling.

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NEUROPEPTIDE Y FAMILY

Neuropeptide Y (NPY) is a 36-amino acid peptide, which was originally isolated and sequenced from porcine brain [4, 5]. NPY has been found in all mammals as well as in a wide variety of animal species including birds, reptiles, amphibians and fishes. The primary structure of NPY has been well preserved during evolution making NPY one of the most conserved peptides known among species [6].

NPY, together with two other 36-amino acid gut hormones: the intestinal peptide YY (PYY) [7] and the pancreatic polypeptide (PP) [8] form the so called NPY-family [9]. NPY shares a 70% degree of sequence homology with PYY and 50% with PP (Fig. **1**) [5]. Moreover, all members of NPY-family are characterized by exhibiting a common tertiary structure referred to as a PP-fold [11, 12]. This PP-fold consists of an N-terminal polyproline sequence (residues 2-8) and an amphiphilic α-helix (residues 14-32) joined by a type I β-turn, creating a hairpin-like loop [13, 14]. The helices are held in the folded configuration through hydrophobic interactions between side chains of the α-helix interdigitating with the prolines in the N-terminal section [10]. The hairpin-like loop seems to be of critical importance for interaction with Y receptors [15]. Furthermore, studies by Nordmann and colleagues proposed that NPY can adopt two different conformational states in equilibrium: a biologically active PP-fold monomer or a dimmer structure, depending on pH, temperature and NPY concentration [16].

The NPY gene is located on human chromosome 7 at the locus 7p15.1 [12]. The nucleotide coding sequence consists of four exons and three introns coding for a 97 amino acid

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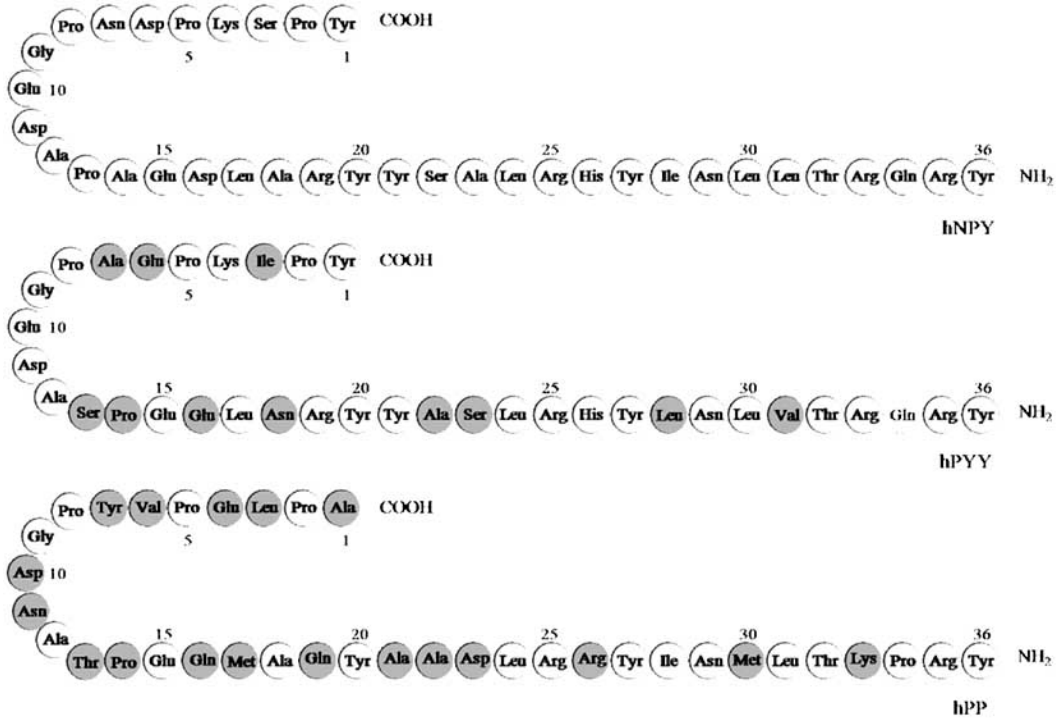

Fig. (1). Schematic structure of the amino acid sequence of human NPY, PYY and PP. The residues which differ from NPY are represented in gray [10].

large peptide, called pre-pro NPY [17]. The pre-pro NPY is in turn proteolytically processed into the C-terminal peptide of NPY (CPON) and the bioactive NPY_{1-36}. The C-terminus of NPY is further amidated which is critical for its biological activity. Furthermore, NPY can be processed to NPY_{3-36} and NPY_{2-36} by two enzymes, dipeptidyl peptidase IV and aminopeptidase P, respectively. Although no functional significance has yet been assigned to CPON, some studies revealed affinity and selectivity of the C-terminal fragments of NPY to Y2 and Y5 receptor subtype [12].

NPY is one of the most abundant peptides in the mammalian brain, e.g. in the hypothalamus, amygdala, hippocampus, nucleus of the solitary tract, locus coeruleus, nucleus acumbens and cerebral cortex [18]. It is widely expressed in the central and peripheral nervous system during development and adulthood [19]. In the periphery, NPY has been shown to be co-stored and co-released with norepinephrine [20]. NPY is also found in non-adrenergic neurons, in which it is co-localized with γ-aminobutyric acid (GABA), somatostatin in agouti-related protein (AGRP) containing neurons, acetylcholine, VIP and peptide histidine isoleucine (PHI) [21]. The adrenal medulla is the primary source of circulating NPY known [22, 23] though it is also expressed in other peripheral regions, e.g. liver, heart, spleen, bone marrow, adipocytes and peripheral blood cells [24-26].

Due to its widespread expression NPY plays an important role in a large range of biological processes such as feeding behaviour, water consumption, learning and memory, locomotion, body temperature regulation, sexual behaviour, emotional behaviour, neuronal excitability, blood pressure regulation, hormone secretion, pain and circadian rhythms. In addition, NPY seems to have direct implication in the pathology of some disorders including obesity, depression and anxiety-related behaviours, epilepsy, memory impairments, alcohol consumption and bone formation (for review see [27]).

NEUROPEPTIDE Y AND Y1 RECEPTOR

General Description of Y Receptors

The NPY-family peptides bind to a family of five receptors, namely Y1, Y2, Y4, Y5 and in the mouse y6, numbered in the chronological order of their discoveries (reviewed by [9, 28]). Each of these receptors is responsible for particular NPY functions and consequently NPY can elicit numerous physiological responses by activating a specific receptor.

They all belong to the rhodopsine-like superfamily of G-protein coupled receptors (GPCRs), with their typical seven-transmembrane (7-TM) helix structure and they can be distinguished by their affinity for NPY, PPY and PP. Interestingly, both NPY and PYY display high affinity for the Y1, Y2 and Y5 receptor subtypes that show itself very low sequence identity to each other (about 30%) while the Y4 receptor preferentially binds PP. Furthermore, the Y-receptors can be distinguished pharmacologically using various synthetic peptides such as Pro^{34} substituted versions of NPY and PYY that have decreased Y2 potency. On the other hand, Y2 binds truncated versions of NPY and PYY such as PPY_{3-36} and NPY_{13-36} with similar affinity as the native peptides [29, 30].

All mammals have the genes for the Y1, Y2, Y4, Y5 and y6 receptor subtypes [31]. However, the y6 receptor subtype

remains has a non-functional receptor in humans and absent in rats thus, it was established as a particular mouse and rabbit receptor subtype [32, 33]. In human, the Y1, Y2 and Y5 genes are located within a relatively short segment of chromosome 4q31-32 in the human genome, while Y4 and y6 are located on two separate chromosomes, chromosome 10q11.2 and 5q23.5, respectively [31, 34]. Although an additional Y3 receptor has been postulated, it is not encoded by a separate gene and no specific agonists or antagonists have been described yet [35].

All of the Y-receptors are expressed both in neuronal and non-neuronal tissues and modulate a variety of pathways through coupling to inhibitory heterotrimeric GTP-binding protein (G_i/G_o), resulting in the inhibition of adenylyl cyclase and thus mediate inhibition of cAMP synthesis [36, 37]. But other particular signal transduction systems may also be triggered. Some studies reported that a protein kinase C (PKC) pathway may also be involved in Y1, Y2, Y4 and Y5 signalling [38]. Furthermore, Y1, Y2, Y4 and Y5 receptors can stimulate the release of Ca^{2+} from intracellular stores [30, 36, 37].

Table **1** summarizes the physiological processes proposed to be mediated by each Y receptors.

Table 1. Succinct Summary of the Major Physiologic Processes where Y Receptors are Described to be Involved

Receptor	Physiologic Processes
Y1	Cardiovascular regulation Energy homeostasis Neuroendocrine regulation Neurogenesis Bone Homeostasis Ethanol consumption Seizure regulation Anxiety regulation Angiogenesis
Y2	Bone Homeostasis Anxiety regulation Cardiovascular regulation Energy homeostasis
Y4	Cardiovascular regulation Energy homeostasis
Y5	Energy homeostasis Seizure regulation Angiogenesis
y6	Energy homeostasis

Detailed Description of Y1 Receptor

The first mammalian NPY receptor to be cloned was the Y1 receptor [36, 37, 39, 40] which displays 90-96% overall identity across mammals [31, 35]. The human Y1 receptor is located on chromosome 4q31.3-32 and is coding for a 384 amino acid protein that has all the characteristics of the GPCR family including glycosylation sites in the N-terminal portion and in the second extracellular loop, four extracellular cysteines which may form two disulfide bridge (Fig. **2**) [28, 39-41]. In contrast to all other known members in this family, the gene for the Y1 receptor is the only one that contains an intron within its coding region [43, 44]. At least three different alternative exons encoding the 5´- untranslated region have been identified. Different promoter regions preceding these exons suggest tissue-specific expression of the receptor mRNAs [45]. In addition, two splice variants of the mouse Y1 receptor have been identified. Although both variants bind NPY, the form with a shortened seventh transmembrane-spanning region and a lacking C-terminal tail does not appear to couple to signal transduction as efficiently as the full length form [41].

It is well documented that after prolonged agonist stimulation, many GPCRs undergo desensitization and internalization. Desensitization, defined as a decrease responsiveness of the receptor to a subsequent ligand, is accompanied by receptor aggregation on the cell surface, receptor phosphorylation on C-terminus serine/threonine residues and further internalization *via* clathrin-coated pits, receptor-mediated endocytic pathway, or *via* an alternative pathway mediated by caveolae (for review see [46, 47]). Clathrin-coated vesicles eventually fuse with endosomes where ligand is separated from the receptor and the receptor is dephosphorylated. Receptors are then recycled to the plasma membrane by a poorly understood mechanism, or are degraded in lysosomes.

Some studies have reported the agonist driven internalization of Y1 receptor in different cell lines, using advanced and sophisticated methods to study the mechanisms underlying the desensitization and internalization of Y1 receptors. About 20-30% of endogenous Y1 receptors expressed in a line of human neuroblastoma cells, SK-N-MC cells [48] and guinea pig Y1 receptors expressed in Chinese hamster ovary cells, CHO cells [49] were internalized after stimulation with NPY followed by rapid recycling to the cell surface. When expressed in HEK293 cells, the Y1 receptor fused to GFP was found to internalize after agonist stimulation through clathrin-coated pits and recycle back to the plasma membrane through both fast and slow routes [50, 51]. In addition, another group investigating Y1 receptor internalization by BET method reported that Y1 displayed a strong and rapid agonist driven interaction with the specific β-arrestin2 [29]. Interestingly, a recent report described the absence of internalization, after agonist-promoted phosphorylation and β-arrestin recruitment for a truncated rat Y1 receptor lacking the last 31 C-terminal amino acids [52]. Furthermore, Ouedraogo and colleagues showed that distinct point mutations in the C-terminus differentially influence transportation of internalized NPY receptors to the plasma membrane [51]. This indicates that internalization of Y1 receptors are mainly mediated by β-arrestins and that the C-terminus of the Y1 receptor is crucial for its phosphorylation and rapid desensitization through fast or slow recycling pathways. However, neither the underlying molecular mechanisms nor the intracellular trafficking pathways involved are completed understood. Further studies must be address to this issue since the

Table 2. Summary of the Peptidic and Non-Peptidic Ligands for Y1 Receptor

		References
Agonists order of affinity (ligand binding profile)	NPY ≈ PYY ≈ [Leu^{31},Pro^{34}]-NPY ≈ [Pro^{34}]-NPY >> C-terminal fragments of NPY/PYY > PP	[39]
Agonists	NPY	[39]
	PYY	[39]
	[Leu^{31},Pro^{34}]-NPY	[39, 61]
	[Pro^{34}]-PYY	[62]
	[Leu^{31},Pro^{34}]-PYY	[62]
	[Arg^{6}, Pro^{34}]-NPY	[63]
	[Phe^{7}, Pro^{34}]-NPY	[63]
	[D-Arg^{25}]-NPY	[64]
	[D-His^{26}]-NPY	[64]
	Des-AA$^{11-18}$[Cys^{7,21}, D-Lys^{9}(Ac), D-His^{26}, Pro^{34}]-NPY	[64]
Antagonists	BIBP3226	[65]
	BIBO3304	[66]
	GR231118	[67, 68]
	GR231118-OMe substituted	[69]
	GI264879A	[70]
	LY357897	[71]
	Fluorescent Nonpeptide Y1 receptor	[72]
	SR120819A	[73]
	J-115814	[74]

Chemical names: BIBO3304, ((R)-N-[[4(Aminocarbonylaminomethyl)phenyl)methyl]N^{2}(diphenylacetyl)-argininamide-trifluoroacetate; BIBP3226, R-N^{2}-(diphenylacetyl)-N-[(4-hydroxy-phenyl)methyl]-D-arginine amide); SR120819A, (R,R) -(1-[2-[2-(2- naphthylsulphamoyl)-3-phenylpropionamido]-3-[4-[N-[4-(di- methylaminomethyl)-cis-cyclohexyl-methyl]amidino]phenyl]propionyl]-pyrrolidine); J-115814, (2)-2-[1-(3-chloro-5-isopropyloxycarbonylaminophenyl)ethylamino]-6-[2-(5-ethyl-4-methyl-1,3-thiazol-2-yl)ethyl]-4-morpholinopyridine; LY357897, 1-(1-[3-((3s)(3-piperidyl))-propyl]-2-[(4-chlorophenoxyl)-methyl]indol-3-yl]-2-(4-piperidylpiperidyl)ethan-1-one; GR231118 (also known as 1229U91 or GW1229), homodimeric Ile-Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-$CONH_2$. GI264879A (non-selective), N-alpha-[3,3-bis(1-Naphthyl)Propionyl]-D-ArginineN-[(S)-1-Benzyl-2-Methoxyethyl] Amide.

possible widespread clinical use of these Y1 receptor ligands as a pharmacologic tool may be compromised by receptor internalization or desensitization.

Different approaches either by PCR analysis of mRNA or immunohistochemistry assays have been conducted to localize and evaluate general organ distribution of Y1 receptor, especially within the nervous system. Y1 receptors are primarily localized to the central nervous system, e.g. anterior thalamus, cerebral cortex, medial geniculate, hypothalamus and the amygdala [53, 54]. Additionally, mRNA for the Y1 receptor has been detected in a number of human, rat, and murine peripheral tissues including the colon, kidney, adrenal gland, gastrointestinal tract, heart, placenta, vascular smooth muscle cells, adipocytes and endothelial cells [41, 55, 56].

There are various cell lines described to robustly express Y1 receptor. These cell lines include human erythroleukemia (HEL) cells [57], human neuroblastoma (SK-N-MC) cells [10, 48] and rat pheochromocytoma (PC-12) cells [58].

Moreover, several groups have generated mutants of the Y1 receptor by site-directed mutagenesis in order to identify the key amino acids responsible for the interaction of NPY with this receptor, the ligand-receptor binding points, the receptor localization [59]. Thus, Y1 receptor expression and distribution provides putative guesses about its physiological functions overall systems.

Accordingly to the literature, Y1 is the receptor subtype for which the greatest range of peptide and non-peptide agonists and antagonists [60] are available (Table **2**), which should help to elucidate the role of Y1 receptor subtype upon NPY-induced actions.

The Y1 receptor subtype exhibits almost equally high affinity for endogenous NPY and PYY, but a very low affinity for PP. The first selective agonist for the Y1 receptor was created by introducing Leu31 and Pro34 into human NPY and PYY, e.g. [Leu^{31}, Pro^{34}]-NPY [61] or [Pro^{34}]-PYY [62]. Additionally, the low affinity of these peptides to Y2 receptor helps distinguishing between the Y1 and Y2 receptor subtypes [75]. However, it also has high affinity for the Y5 subtype and reduced affinity for the Y4 receptor, thereby limiting its use as a selective ligand [9]. Additionally, all N-terminally truncated versions of NPY such as $NPY_{2–36}$, $NPY_{3–36}$ or $NPY_{13–36}$ showed intermediate or no affinity for the Y1 subtype [28].

The first high potency non-peptidic antagonist of Y1 receptor described and extensively studied was BIBP3226 [65,

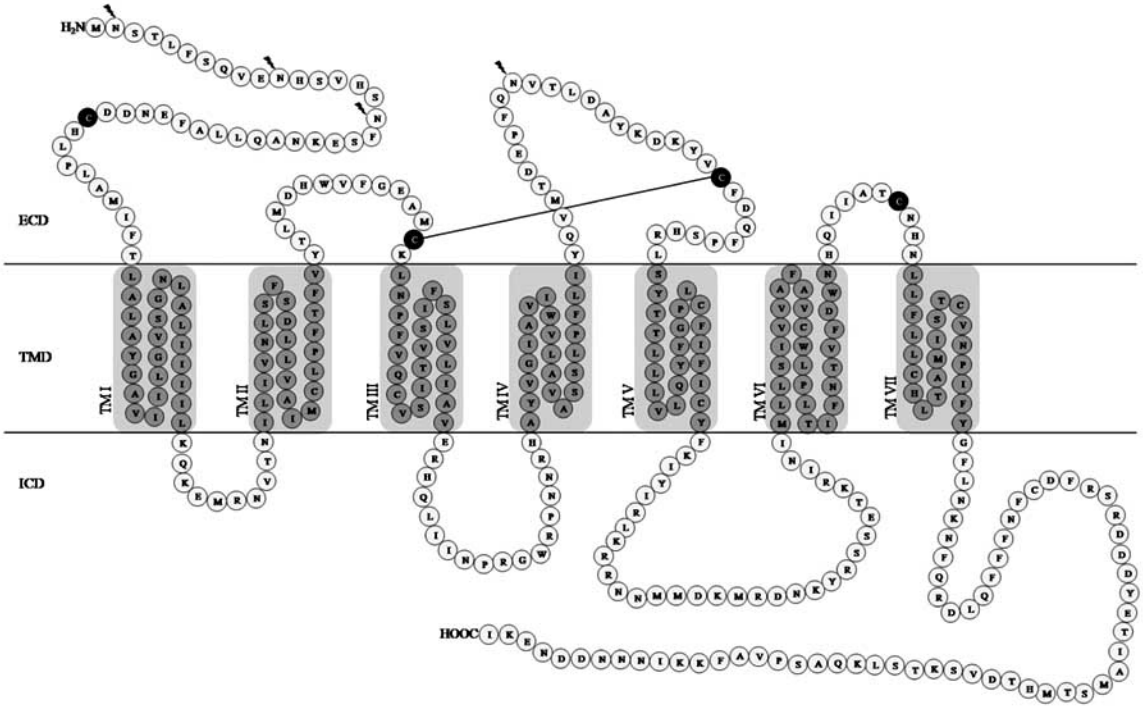

Fig. (2). Schematic representation of the amino acid sequence for human Y1 receptor. The human Y1 receptor is a G-protein coupled receptor, which comprises an extracellular domain (ECD), a transmembrane domain (TMD) and an intracellular domain (ICD). The 384 amino acid protein comprises four potential N-glycosylation sites and four extracellular cysteines which may form disulfide bridges (marked in black) [37, 41].

76, 77], which competitively block Y1 but not Y2 receptors. Others compounds have also been reported, e.g. BIBO3304 [66], SR 120819A, GR 231118 (also known as GW1229 or 1229U91) [67] or LY357897 [71], among many others. However, the selectivity, potency and *in vivo* efficacy of these agents remain unclear due to the synergistic activation with other Y-receptor, specifically with Y4 and Y5 receptors. Therefore, due to a lack of pharmacologically selective agonists and antagonists many researchers have developed knockout and transgenic mice models for the NPY family and its receptors in an attempt to uncover the physiological roles of this complex receptor-ligand system.

Following the generation of NPY knockout mice, several laboratories have reported the generation of Y1 receptor deficient mice using distinct deletion strategies [78, 79]. These mice strains carrying null mutations for Y1 receptors represent powerful tools to determine the physiological and pharmacological roles of this receptor in meditating the effects of NPY.

For instance, a role for the Y1 receptors in the control of food intake and in blood pressure regulation has been postulated. $Y1^{-/-}$ exhibited a mild late-onset obesity, mild hyperinsulinemia, impaired insulin secretion in response to hyperglycemia (obese phenotype) and complete absence of NPY-mediated vasoconstriction [74, 80, 81].

The Y1 receptor has been shown to participate in NPY-induced sensitization to sedation [82] and to regulate voluntary ethanol consumption and some of the intoxicating effects caused by administration of ethanol [83]. Furthermore, it was also reported that Y1 receptor subtype is necessary for the anxiolytic-like effects of icv-administered NPY, reinforcing Y1 receptor as a potential target for novel anxiolytic medication [84].

A role for the Y1 receptor in the immune system was also demonstrated using Y1 receptor deficient mice. T cells from $Y1^{-/-}$ mice were hyper-responsive to activation thus, signalling through Y1 receptor on T cells inhibits T cell activation and controls the magnitude of T cell responses. Although, $Y1^{-/-}$ mice had reduced numbers of T cells effectors due to functionally impaired antigen-presenting cells (APCs). They showed a possible role for the Y1 receptor in the immune system, serving as a strong negative regulator on T cells as well as a key activator of APC function [85].

NPY was recently found to be potently angiogenic and growth promoting in cells [86]. There is growing evidence that NPY induced cell proliferation mainly *via* Y1 receptor in a variety of cell types, including vascular smooth muscle cells, endocardial endothelial cells, neuronal precursors cells, pre-adipocytes, endothelial cells, neuroblasts of olfactory epithelium, hippocampal precursor cells, rat enteric neurons and others [26, 87-94].

However, the single involvement of Y1 receptor in NPY-induced mitogenic effect is still a matter of controversy. In vascular smooth muscle cells and endothelial cells some reports demonstrate that the mitogenic effect of NPY is mediated by synergistic activation of Y1, Y2 and Y5 receptors [42, 86, 95]. Moreover, Y1 receptor has been shown to mediate proliferative and anti-proliferative effects of NPY in prostate cancer cells [96].

The mechanisms which have been shown to mediate the proliferative effects of NPY through Y1 receptor have extensively be studied in several different cell lines and are generally coupled with the extracellular signal-regulated kinases (ERK 1/2) phosphorylation [87-91, 96], known to be linked to many G protein-linked cell surface receptors. The ERK 1/2 subgroup of the mitogen-activated protein kinases (MAPK) represents the key intracellular signal transducer of mitogenic stimulus implicated in the signalling pathway leading to cellular proliferation (for review see [97, 98]).

Furthermore, the activation of the MAPK pathway through NPY-Y1 receptor has been reported to be regulated by protein kinase C (PKC) [87, 89, 96]. Recently, it was reported that the NPY-proliferative effect in retinal neural and progenitor cells can be also mediated by the activation of the NOS–sGC (nitric oxide synthase-soluble guanylyl cyclase) pathway, that in turns activates ERK 1/2 signalling pathway [42]. Moreover, it is possible that the signalling through ERK1/2 is just one of several pathways that together regulate cellular proliferation (Fig. **3**). Those pathways could either work synergistically or each one could regulate proliferation independently. For certain, the mechanisms underlying the NPY-mitogenic effects through Y1 receptor is still a matter of extensive study. For instance, the fact that NPY can induce proliferation of cancer cells lead researchers to investigate which signal pathway might conduct this effect and a possible blockade of the cascade.

Thus, for the last 20 years multiple investigations approaches have defined important roles for Y1 receptors signalling in the regulation of several physiological and also behavioral functions, including feeding behavior and energy homeostasis, sexual hormone secretion, stress response, emotional behavior, neuronal excitability and ethanol consumption (for more details see [99]).

NPY AND BONE

Bone remodelling is a dynamic physiological process used to maintain a constant bone mass and to renew bone throughout life. This process occurs through two distinct stages – bone resorption and bone formation – that involve the activity of osteoclasts and osteoblasts, respectively. This process of remodelling promotes the removal of old bone and the formation of new bone necessary for an appropriate architecture and constant bone mass during adulthood.

The mechanisms controlling bone homeostasis are traditionally viewed has being regulated by hormonal, autocrine/paracrine and mechanical signals. However, new emergent evidences showed that skeleton metabolism is also controlled by the nervous system [100-102], creating a link between brain and bone.

Early studies have demonstrated the presence of nerve fibres immunoreactive to NPY in bone. The distribution of the NPY-nerve fibers was mostly located in the Volkmann's

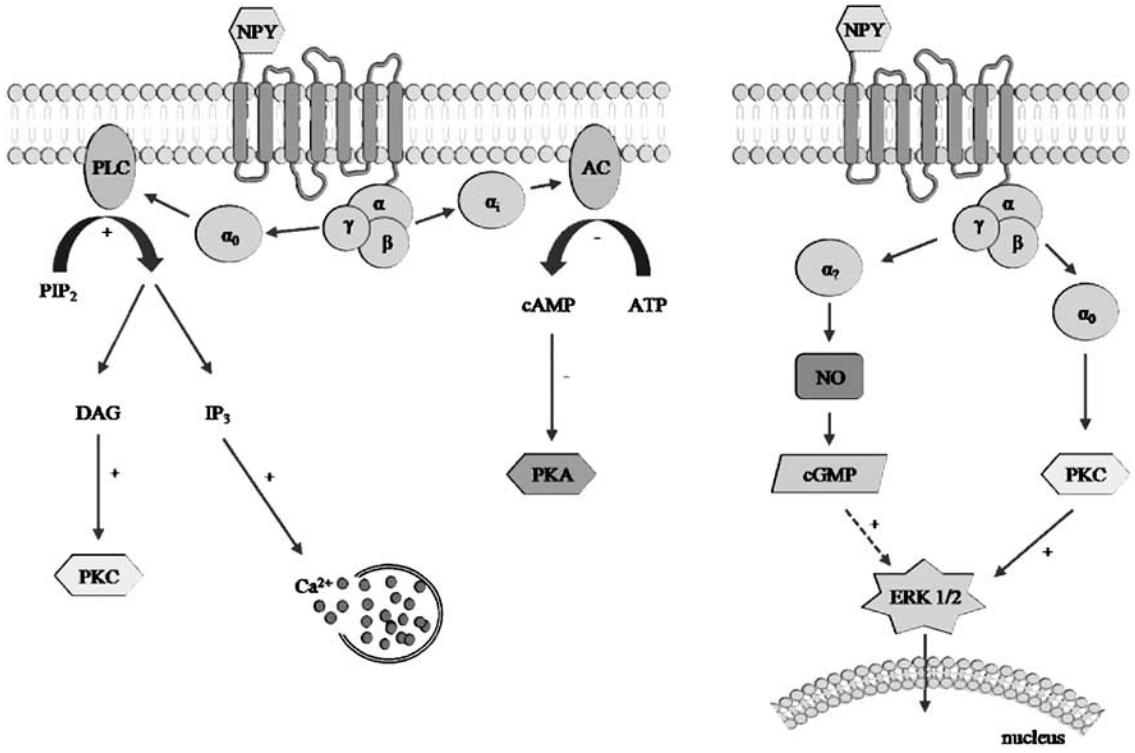

Fig. (3). Schematic illustration of the signal transduction mechanisms activated by NPY through Y1 receptor. NPY can modulate a variety of pathways through activation of G-protein (G_i/G_0) resulting in: a) inhibition of adenylyl cyclase (AC) and thus mediate inhibition of protein kinase A (PKA); b) activation of phospholipase C (PLC) which generates diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) through phosphatidyl inositol 4,5-diphosphate (PIP_2) hydrolyses. DAG activates protein kinase C (PKC) while IP_3 leads to the cytosolic Ca^{2+} elevation. NPY can also modulate mitogenic signals to nucleus through the stimulation of nitric oxide (NO) production by nitric oxide synthase. NO, in turn, activates the synthesis of cGMP that induces phosphorylation of ERK 1/2 and consequently stimulates cell proliferation [23, 42].

canal alongside blood vessels [103, 104], suggesting a NPY vasoregulatory function role in bone. Furthermore, other study provided evidence for cellular immunoreactivity to NPY in large megakaryocytes and mononuclear hematopoietic cells of the bone marrow [105], consistent with another study reporting that NPY is produced by megakaryocytes within bone marrow [25]. And although, NPY-immunoreactive fibers were largely confined to vascular elements, occasional fibers were also observed among the bone-lining cells [104]. Moreover, there are indications that Y receptors may be expressed on bone cells. The Y1 receptor was reported to be present in human osteoblastic and osteosarcoma-derived cell lines and in mouse bone marrow cells [41, 106]. While a study did not detect Y receptor transcripts in primary murine osteoblastic cultures or whole long bone [2], recent studies from the same group have reported the presence of Y1 receptor but not the others Y receptors (Y2, Y4, Y5 and y6) in cultured stromal cells from wild type mice [107, 108].

Recently, studies analyzing germ line or conditional knockout mice lacking leptin, leptin's receptors or Y receptors have revealed the hypothalamus as centrally-controlling osteoblast activity. Thus, two main pathways have been implicated in bone turnover *via* hypothalamic mechanisms, namely Y2 receptors and the adipocytic hormone, leptin [2, 109].

Germ line Y2 receptor knockout mice revealed an increased rate of bone mineralization and elevated bone mass with a two-fold increase in cancellous bone volume, due to elevated osteoblast activity. Furthermore, the fact that no mRNA of Y receptors, were not detected in bone marrow stromal cells collected from mice lacking Y2 receptor ($Y2^{-/-}$), lead the researcher to hypothesize that this effect was mediated through central-controlled mechanism instead of a direct mechanism in bone tissue.

Interestingly, analysis of bone sections from the distal femur of mice with conditional deletion of hypothalamic Y2 receptors revealed an identical increase in trabecular bone volume within 5 weeks of Y2 deletion. This hypothalamus-specific Y2 receptor deletion stimulated osteoblast activity and increased the rate of bone mineralization and formation, although with no effect on osteoblast or osteoclast surface measurements. Moreover, the increase in bone volume observed in both germ line and hypothalamus-specific Y2 receptor deletion was not stimulated by any of the known effectors of bone turnover, such as insulin-like growth factor-1 (IGF-1), free T4, calcium, leptin or testosterone. These findings strongly suggested a key role of hypothalamic Y2 receptors in bone formation *via* modulation of an unknown signal mechanism, other than the direct mediation by traditional bone remodelling effectors [2]. Highlighting this results a further study revealed that Y2 reception ablation also leads to an increased of osteoblast activity and bone mineral content in the cortical bone of $Y2^{-/-}$ long bones [3].

The second hypothalamic circuit reported to be involve within bone turnover is leptin. Leptin, the product of the *ob* gene, is a small hormone mainly produced by adipocytes that acts *via* binding to specific receptors located in the hypothalamus to regulate energy homeostasis [110]. Mice lacking either leptin gene (ob/ob) or its receptor gene (db/db), were reported to have an increase in trabecular bone volume attributable to an elevation in osteoblast activity, resulting in increased bone mass in the vertebral bodies [111], similarly with the germ line $Y2^{-/-}$ mice. Additionally, icv administration of leptin caused a dose-dependent reduction in trabecular bone volume in both ob/ob and wild type mice. This study identifies leptin as a potent inhibitor of bone formation acting through a central circuit [111].

Hypothalamic levels of NPY mRNA and the secretion of NPY are strongly elevated in ob/ob mice due to an absence of leptin signalling [112]. Moreover, NPY ablation in ob/ob further demonstrated that NPY is a major downstream mediator of leptin's central effects [113], acting as an antiosteogenic effector.

Moreover, Y2 receptors and leptin receptors are co-localized on NPY-ergic neurons in the arcuate nucleus, indicating a role for the Y2 receptor in the regulation of energy homeostasis by leptin [114]. Due to the known interaction of NPY and leptin in energy homeostasis regulation, at first, it was suggested that these two anabolic pathways might regulate osteoblast activity by a common pathway. However, current evidences emphasize NPY and its receptors as a key player in the regulation of bone formation, distinct from that of the centrally regulated pathway mediated by leptin [102].

First, central infusion of NPY for 28 days in wild-type mice had the same inhibitory effect on bone function as leptin had, suggesting that the increased hypothalamic NPY expression of leptin-deficient mice does not mediate the associated increase in bone density [111]. Although, it remains to be elucidated the NPY-effect when injected directly in bone.

Genetic modulation of these pathways has powerful actions in cancellous bone, with a 2-fold increase in the volume of cancellous bone reported in ob/ob and Y2 receptor knockout mutant mouse models [108]. Cortical bone mass was increased in both germ line and hypothalamic Y2 knockout mice because of elevated osteoblast activity, indicating that the $Y2^{-/-}$ pathway has a consistently anabolic action in both cancellous and cortical compartments in bone. In contrast, leptin deficiency was associated with reduced cortical bone mass, indicating that the leptin pathway has contrasting effects on cortical and cancellous bone, with its deficiency resulting in a lowered bone mass phenotype [3]. Thus, these studies show the diversity in hypothalamic control of bone homeostasis. However, future studies remains to be conducted to better understand the interaction between leptin, NPY and bone.

In addition to its role in central nervous system, there are some evidences for a NPY-mediated signalling in bone homeostasis through the peripheral nervous system. As mentioned above, NPY is co-released with noradrenaline from sympathetic nerves [20]. In addition, NPY has been shown *in vitro* to modulate osteoblastic parathyroid hormone (PTH) response through a receptor–receptor interaction [115]. Furthermore, NPY attenuates the effect of noradrenaline on osteoblasts [105, 116]. A recent study, revealed that mouse bone marrow cells constitutively expressed mRNAs for Y1 and β2-adrenergic receptors and that NPY inhibited the isoprenaline-induced formation of osteoclast-like cells from mouse bone marrow cells, suggesting an interaction between

NPY and β-adrenergic stimulation in osteoclastogenesis [117].

Taken altogether, the evaluation of Y receptor knockout models has delineated a key role of NPY in osteoblast regulation through a variety of central and peripheral mechanisms. However, further work is needed to elucidate the direct physiological modulation of osteoblasts and osteoclasts by NPY and its Y receptors.

Y1 RECEPTOR IN THE REGULATION OF BONE HOMEOSTASIS

Alongside Y2 receptors modulation in bone homeostasis, there are increasing evidences implicating another Y receptor in skeletal homeostasis, namely Y1 receptor [107, 117, 118]. Baldock and colleagues investigated the effect of germ line and conditional deletion of Y1 receptors in mice in bone regulation and the potential interaction between Y1 receptor signalling and the previously identified Y2 receptor pathway. This study showed that germ line disruption of Y1 receptor signalling revealed an increase in osteoblast activity on both cancellous and cortical surfaces, with consistent changes in femoral, tibial, and vertebral bones. Importantly, *in vitro* NPY treatment of bone marrow stromal cells derived from $Y1^{-/-}$ mice did not altered the cell number comparing with wild type. Furthermore, quantification of RT-PCR revealed the expression of Y1 receptor gene transcripts in bone marrow stromal cells, while expression of Y2 receptors was not detected. Moreover, conditional deletion of hypothalamic Y1 receptors in mice did not alter bone homeostasis, in contrast to Y2 receptor or germ line Y1 receptor deletion. Thus, these results indicate a possible direct action of NPY on bone cells *via* this Y receptor [118].

It has recently been demonstrated that deletion of Y2 receptors down-regulates Y1 receptor expression in bone marrow stromal cells [107], suggesting a common signalling pathway to regulate bone formation. Furthermore, deletion of both Y1 and Y2 receptors did not produce additive effects in bone [118]. In addition, while the increased in bone volume in the Y2 knockout model is exclusively attributed to increased bone formation, the increase in bone volume in Y1 knockout mice is a result of altered bone turnover, with increased indices of both osteoblast and osteoclast activity [118]. Given the recent evidence for a direct action of NPY in osteoclastogenesis *via* Y1 receptor [117], altogether these studies propose that these two models may act *via* alternative pathways.

Taking into the account that no other Y receptor has been detected in bone cells, it has been hypothesized that Y1 receptor pathway might have a potent direct inhibitory-effects in bone homeostasis, through a non-hypothalamic pathway. Now, if either these effects are mediated *via* a common or an alternative pathway with Y2 receptor it is an issue which remains to be elucidated. Further studies will be required to fully assess NPY and its receptors direct role in bone remodelling.

FUTURE PERSPECTIVES AND THERAPEUTIC APPLICATIONS

Therapeutic application of NPY receptors drugs has arisen as a promising area of research. The vast implication of Y1 receptor in a diversity of centrally physiological actions has prompted Y1 receptor as a novel therapeutic target namely for obesity, eating or anxiety disorders treatment and tumor therapy [83, 84, 96].

The increasing evidences showing the involvement of Y1 receptor signalling in the local control of bone remodelling suggest that an anti-receptor strategy may be a useful approach to prevent and/or reverse bone loss. Due to its wide distribution through CNS and PNS and to its central function in a variety of important regulatory systems, a systemic delivery or administration of Y1 receptor antagonists may offers great disadvantages. In this order of knowledge, a possible therapeutic strategy will be a local and controlled release of Y1 receptor antagonists targeting bone tissue using appropriate biomaterial as drug carrier that allow cell targeting, prolonged half-life of the drug, and controlled drug release.

ABBREVIATIONS

AC	=	Adenylyl cyclase
AGRP	=	Somastotin in agouti-related protein
APC	=	Antigen-presenting cells
ATP	=	Adenosine triphosphate
BET	=	Bioluminescent energy transfer
cAMP	=	3´,5´-Cyclic adenosine monophosphate
cGMP	=	3´,5´-Cyclic guanosine monophosphate
CGRP	=	Calcitonin gene-related peptide
CNS	=	Central nervous system
CPON	=	C-terminal peptide of NPY
DAG	=	Diacylglycerol
db/db	=	Leptin receptor deletion
ECD	=	Extracellular domain
ERK1/2	=	Extracellular signal-regulated kinase 1/2
GABA	=	γ-aminobutyric acid
GFP	=	Green fluorescence protein
GPCRs	=	G-protein coupled receptors
ICD	=	Intracellular domain
icv	=	Intracerebroventricular
IP_3	=	Inositol 1,4,5-triphosphate
MAPK	=	Mitogen-activated protein kinases
mRNA	=	Messenger RNA
NO	=	Nitric oxide
NOS	=	Nitric oxide synthase
NPY	=	Neuropeptide Y
ob/ob	=	Leptin gene deletion
PCR	=	Polymerase chain reaction
PHI	=	Histidine isoleucine
PIP_2	=	Phosphatidyl inositol 4,5-diphosphate
PKA	=	Protein kinase A

PKC = Protein kinase C

PLC = Phospholipase C

PNS = Peripherial nervous system

PP = Pancreatic polypeptide

PTH = Parathyroid hormone

PYY = Peptide YY

RT-PCR = Real time-polymerase chain reaction

sGC = Solube guanylyl cyclase

SP = Substance P

TMD = Transmembrane domain

VIP = Vasoactive intestinal peptide

7-TM = Seven-transmembrane

REFERENCES

[1] Lundberg, P. and Lerner, U.H. (2002) *Microsc. Res. Tech.*, **58**(2), 98-103.

[2] Baldock, P.A.; Sainsbury, A.; Couzens, M.; Enriquez, R.F.; Thomas, G.P.; Gardiner, E.M. and Herzog, H. (2002) *J. Clin. Invest.*, **109**(7), 915-921.

[3] Baldock, P.A.; Allison, S.; McDonald, M.M.; Sainsbury, A.; Enriquez, R.F.; Little, D.G.; Eisman, J.A.; Gardiner, E.M. and Herzog, H. (2006) *J. Bone Miner. Res.*, **21**(10), 1600-1607.

[4] Tatemoto, K.; Carlquist, M. and Mutt, V. (1982) *Nature*, **296** (5858), 659-660.

[5] Tatemoto, K. (1982) *Proc. Natl. Acad. Sci. U.S.A.*, **79**(18), 5485-5489.

[6] Larhammar, D. (1996) *Regul. Pept.*, **62**, 1-11.

[7] Tatemoto, K. (1982) *Proc. Natl. Acad. Sci. U.S.A.*, **79**(8), 2514-2518.

[8] Kimmel, J.R.; Hayden, J. and Pollock, H.G. (1975) *J. Biol. Chem.*, **250**(24), 9369-9376.

[9] Michel, M.C.; Beck-Sickinger, A.; Cox, H.; Doods, H.N.; Herzog, H.; Larhammar, D.; Quirion, R.; Schwatz, T. and Westfall, T. (1998) *Pharmacol. Rev.*, **50**(1), 143-150.

[10] Fuhlendorff, J.; Johansen, N.L.; Melberg, S.G.; Thogersen, H. And Schwartz, T.W. (1990) *J. Biol. Chem.*, **265**(20), 11706-11712.

[11] Blundell, T.L.; Pitts, J.E.; Tickle, I.J.; Wood, S.P. and Wu, C.W. (1981) *Proc. Natl. Acad. Sci. U.S.A.*, **78**(7), 4175-4179.

[12] Cerdá-Reverter, J. and Larhammar, D. (2000) *Biochem. Cell Biol.*, **78**(3): 371-392.

[13] Allen, J.; Novotný, J.; Martin, J. and Heinrich, G. (1987) *Proc. Natl. Acad. Sci. U.S A.*, **84**(8), 2532-2536.

[14] Wan, C.P. and Lau, B.H.S. (1995) *Life Sci.*, **56**(13), 1055-1064.

[15] Schwartz, T.W.; Fuhlendorff, J.; Kjems, L.L.; Kristensen, M.S.; Vervelde, M.; O'Hare, M.; Krstenansky, J.L. and Bjørnholm, B. (1990) *Ann. N.Y. Acad. Sci.*, **611**, 35-47.

[16] Nordmann, A.; Blommers; M.J.J.; Fretz, H.; Arvinte, T. and Drake, A.F. (1999) *Eur. J. Biochem.*, **261**(1), 216-226.

[17] Minth, C.D.; Bloom, S.R.; Polak, J.M. and Dixon, J.E. (1984) *Proc. Natl. Acad. Sci. U.S.A.*, **81**(14), 4577-4581.

[18] Chronwall, B.M.; DiMaggio, D.A.; Massari, V.J.; Pickel, V.M.; Ruggiero, D.A. and O'Donohue, T.L. (1985) *Neuroscience.*, **15**(4), 1159-1181.

[19] Wai, S.M.; Kindler, P.M.; Lam, E.T.; Zhang, A. and Yew, D.T. (2004) *Cell Mol. Neurobiol.*, **24**(5), 667-684.

[20] Sundler, F.; Böttcher, G.; Ekblad, E. and Hakanson, R. (1993) In *The Biology of Neuropeptide Y and Related Peptides*, (Colmers, W.F. and Wahlestedt, C., Ed.), Humana Press, New Jersey, pp. 157-196

[21] Ekblad, E.; Hakanson, R. and Sundler, F. (1984) *Regul. Pept.*, **10**(1), 47-55.

[22] Cavadas, C.; Silva, A.P.; Mosimann, F.; Cotrim, M.D.; Ribeiro, C.A.F.; Brunner, H.R. and Grouzmann, E. (2001) *J. Clin. Endocrinol. Metab.*, **86**(12), 5956-5963.

[23] Silva, A.P.; Cavadas, C. and Grouzmann, E. (2002) *Clin. Chim. Acta*, **326**(1-2), 3-25.

[24] Strand, F.L. Neuropeptides: Regulators of Physiological Processes. MIT Press: Cambridge, 1999.

[25] Ericsson, A.; Schalling, M.; McIntrye, K.R.; Lundberg, J.M.; Larhammar, D.; Seroogy, K.; Hokfelt, T. and Persson, H. (1987) *Proc. Natl. Acad. Sci. U.S.A.*, **84**(16), 5585-5589.

[26] Yang, K.; Guan, H.; Arany, E.; Hill, D.J. and Cao, X. (2008) *FASEB J.*, **22**(7), 2452-2464.

[27] Pedrazzini, T.; Pralong, F. and Grouzmann, E. (2003) *Cell Mol. Life Sci.*, **60**(2), 350-377.

[28] Blomqvist, A.G. and Herzog, H. (1997) *Trends Neurosci.*, **20**(7), 294-298.

[29] Berglund, M.M.; Schober, D.A.; Statnick, M.A.; McDonald, P.H. and Gehlert, D.R. (2003) *J. Pharmacol. Exp. Ther.*, **306**(1), 147-56.

[30] Berglund, M.; Hipskind, P. and Gehlert, D. (2005) in *NPY Family of Peptides in Immune Disorders, Inflammation, Angiogenesis and Cancer*, (Zukowska, Z. and Feuerstein, G.Z., Ed.), Birkhaeuser Verlag, Switzerland, pp. 29-62.

[31] Larhammar, D. and Salaneck, E. (2004) *Neuropeptides*, **38**(4), 141-151.

[32] Gregor, P.; Feng, Y.; DeCarr, L.B.; Cornfield, L.J. and McCaleb, M.L. (1996) *J. Biol. Chem.*, **271**(44), 27776-27781.

[33] Starbäck, P.; Wraith, A.; Eriksson, H. and Larhammar, D. (2000) *Biochem. Biophys. Res. Commun.*, **277**(1), 264-269.

[34] Wraith, A.; Törnsten, A.; Chardon, P.; Harbitz, I.; Chowdhary, B.P.; Andersson, L., Lundin, L.G. and Larhammar, D. (2000) *Genome Res.*, **10**(3), 302-310.

[35] Larhammar, D.; Wraith, A.; Berglund, M.M.; Holmberg, S.K.S. and Lundell, I. (2001) *Peptides*, **22**(3), 295-307.

[36] Larhammar, D.; Blomqvist, A.; Yee, F.; Jazin, E.; Yoo, H. and Wahlested, C. (1992) *Proc. Natl. Acad. Sci. U.S.A.*, **267**(16), 10935-10938.

[37] Herzog, H.; Hort, Y.J.; Ball, H.J.; Hayes, G.; Shine, J. and Selbie, L.A. (1992) *Proc. Natl. Acad. Sci. U.S.A.*, **89**(13), 5794-5798.

[38] Mullins, D.E.; Zhang, X. and Hawes, B.E. (2002) *Regul. Pept.*, **105**(1), 65-73.

[39] Krause, J.; Eva, C.; Seeburg, P.H. and Sprengel, R. (1992) *Mol. Pharmacol.*, **41**(5), 817-821.

[40] Eva, C.; Keinanen, K.; Monyer, H.; Seeburg, P. and Sprengel, R. (1990) *FEBS J.*, **271**(1-2), 81-84.

[41] Nakamura, M.; Sakanaka, C.; Aoki, Y.; Ogasawara, H.; Tsuji, T.; Kodama, H.; Matsumoto, T.; Shimizu, T. and Noma, M. (1995) *J. Biol. Chem.*, **270**(50), 30102-30110.

[42] Álvaro, A.R.; Martins, J.; Araújo, I.M.; Rosmaninho-Salgado, J., Ambrósio, A.F. and Cavadas, C. (2008) *J. Neurochem.* (Epub ahead of print).

[43] Herzog, H.; Baumgartner, M.; Vivero, C.; Selbie, L.A.; Auer, B. and Shine, J. (1993) *J. Biol. Chem.*, **268**(9), 6703-6707.

[44] Marklund, U.; Byström, M.; Gedda, K.; Larefalk, A.; Juneblad, K.; Nyström, S. and Ekstrand, A.J. (2002) *Mol. Cell Endocrinol.*, **188**(1-2), 85-97.

[45] Ball, H.J.; Shine, J. and Herzog, H. (1995) *J. Biol. Chem.*, **270**(45), 27272-27276.

[46] Ferguson, S.S. (2001) *Pharmacol. Rev.*, **53**(1), 1-24.

[47] Okamoto, T.; Schlegel, A.; Scherer, P.E. and Lisanti; M.P. (1998) *J. Biol. Chem.*, **273**(10), 5419-5422.

[48] Fabry, M.; Langer, M.; Rothen-Rutishauser, B.; Wunderli-Allenspach, H.; Höcker, H. and Beck-Sickinger, A.G. (2000) *Eur. J. Biochem.*, **267**(17), 5631-5637.

[49] Parker, S.L.; Kane, J.K.; Parker, M.S.; Berglund, M.M.; Lundell, I.A. and Li, M.D. (2001) *Eur. J. Biochem.*, **268**(4), 877-886.

[50] Gicquiaux, H.; Lecat, S.; Gaire, M.; Dieterlen, A.; Mély, Y.; Takeda, K.; Bucher, B. and Galzi, J.L. (2002) *J. Biol. Chem.*, **277**(8), 6645-6655.

[51] Ouedraogo, M.; Lecat, S.; Rochdi, M.D.; Hachet-Haas, M.; Matthes, H.; Gicquiaux, H.; Verrier, S.; Gaire, M.; Glasser, N.; Mély, Y.; Takeda, K.; Bouvier, M.; Galzi, J.L. and Bucher, B. (2008) *Traffic*, **9**(3), 305-324.

[52] Holliday, N.D.; Lam, C.W.; Tough, I.R. and Cox, H.M. (2005) *Mol. Pharmacol.*, **67**(3), 655-664.

[53] Broberger, C.; Visser, T.J.; Kuhar, M.J. and Hökfelt, T. (1999) *Neuroendocrinology*, **70**(5), 295-305.

[54] Kopp, J.; Xu, Z.Q.; Zhang, X.; Pedrazzini, T.; Herzog, H.; Kresse, A.; Wong, H.; Walsh, J.H. and Hökfelt, T. (2002) *Neurosci.*, **111**(3), 443-532.

[55] Larsen, P.J.; Tang-Christensen, M.; Stidsen, C.E.; Madsen, K.; Smith, M.S. and Cameron, J.L. (1999) *J. Clin. Endocrinol. Metab.*, **84**(10), 3781-3791.

[56] Wharton, J.; Gordon, L.; Byrne, J.; Herzog, H.; Selbie, L.A.; Moore, K.; Sullivan, M.H.; Elder, M.G.; Moscoso, G.; Taylor, K.M.; Shine, J. and Polak, J.M. (1993) *Proc. Natl. Acad. Sci. U.S.A.*, **90**(2), 687-691.

[57] Feth, F.; Rascher, W. and Michel, M.C. (1992) *Br. J. Pharmacol.*, **105**(1), 71-76.

[58] DiMaggio, D.A.; Farah, J.M. Jr and Westfall, T.C. (1994) *Endocrinology*., **134**(2), 719-727.

[59] Dumont, Y. and Quirion, R. (2006) in *Progress in Inflammation Research*, (Feuernstein, G. and Zukowska-Grojec, Z., Ed.), Birkhaeuser Verlag, Switzerland, pp. 7-33.

[60] Balasubramaniam, A. (2003) *Curr. Pharm. Des.*, **9**(15), 1165-1175.

[61] Fuhlendorff, J.; Gether, U.; Aakerlund, L.; Langeland-Johansen, N.; Thogersen, H.; Melberg, S.G.; Olsen, U.B.; Thastrup, O. and Schwartz, T.W. (1990) *Proc. Natl. Acad. Sci. U.S.A.*, **87**(1), 182-186.

[62] Grandt, D.; Feth, F.; Rascher, W.; Reeve, JR Jr.; Schlicker, E.; Schimiczek, M.; Layer, P.; Goebell, H.; Eysselein, V.E. and Michel, M.C. (1994) *Eur. J. Pharmacol.*, **269**(2), 127-132.

[63] Söll, R.M.; Dinger, M.C.; Lundell, I.; Larhammer, D. and Beck-Sickinger, A.G. (2001) *Eur. J. Biochem.*, **268**(10), 2828-2837.

[64] Mullins, D.; Kirby, D.; Hwa, J.; Guzzi, M.; Rivier, J. and Parker, E. (2001) *Mol. Pharmacol.*, **60**(3), 534-540.

[65] Rudolf, K.; Eberlein, W.; Engel, W.; Wieland, H.A.; Willim, K.D.; Entzeroth, M.; Wienen, W., Beck-Sickinger, A.G. and Doods, H.N. (1994) *Eur. J. Pharmacol.*, **271**(2-3), R11-R13.

[66] Wieland, H.A.; Engel, W.; Eberlein, W.; Rudolf, K. and Doods, H.N. (1998) *Br. J. Pharmacol.*, **125**(3), 549-555.

[67] Daniels, A.J.; Matthews, J.E.; Slepetis, R.J.; Jansen, M.; Viveros, O.H.; Tadepalli, A.; Harrington, W.; Heyer, D.; Landavazo, A.; Leban, J.J. and Spaltenstein, A. (1995) *Proc. Natl. Acad. Sci. U.S.A.*, **92**(20), 9067-9071.

[68] Schober, D.A.; Van Abbema, A.M.; Smiley, D.L.; Bruns, R.F. and Gehlert, D.R. (1998) *Peptides*, **19**(3), 537-542.

[69] Balasubramaniam, A.; Dhawan, V.C.; Mullins, D.E.; Chance, W.T.; Sheriff, S.; Guzzi, M.; Prabhakaran, M. and Parker, E.M. (2001) *J. Med. Chem.*, **44**(10), 1479-1482.

[70] Daniels, A.J.; Chance, W.T.; Grizzle, M.K.; Heyer, D. and Matthews, J.E. (2001) *Peptides*, **22**(3), 483-491.

[71] Hipskind, P.A.; Lobb, K.L.; Nixon, J.A; Britton, T.C.; Bruns, R.F.; Catlow, J.; Dieckman-McGinty, D.K.; Gackenheimer, S.L.; Gitter, B.D.; Iyengar, S.; Schober, D.A.; Simmons, R.M.; Swanson, S.; Zarrinmayeh, H.; Zimmerman, D.M. and Gehlert, D.R. (1997) *J. Med. Chem.*, **40**(23), 3712-3714.

[72] Schneider, E.; Keller, M.; Brennauer, A.; Hoefelschweiger, B.K.; Gross, D.; Wolfbeis, O.S.; Bernhardt, G. and Buschauer, A. (2007) *ChemBioChem*, **8**(16), 1981-1988.

[73] Serradeil-Le, G.C.; Valeet, G.; Rouby, P.E.; Pellet, A.; Oury-Donat, F.; Brossard, G.; Lespy, L.; Marty, E.; Neliat, G.; de Cointet, P.; Maffrand, J.P. and LeFur, G. (1995) *FEBS Lett.*, **362**(2), 192-196.

[74] Kanatani, A.; Hata, M.; Mashiko, S.; Ishihara, A.; Okamoto, O.; Haga, Y.; Ohe, T.; Kanno, T.; Murai, N.; Ishii, Y.; Fukuroda, T.; Fukami, T. and Ihara, M. (2001) *Mol. Pharmacol.*, **59**(3), 501-505.

[75] Wahlestedt, C. and Håkanson, R. (1986) *Med. Biol.*, **64**(2-3), 85-88.

[76] Doods, H.N.; Wieland, H.A.; Engel, W.; Eberlein, W.; Willim, K.D.; Entzeroth, M.; Wienen, W. and Rudolf, K. (1996) *Regul. Pept.*, **65**(1), 71-77.

[77] Gerald, C.; Walker, M.W.; Criscione, L.; Gustafson, E.L.; Batzl-Hartmann, C.; Smith, K.E.; Vaysse, P.; Durkin, M.M.; Laz, T.M.; Linemeyer, D.L.; Schaffhauser, A.O.; Whitebread, S.; Hofbauer, K.G.; Taber, R.I.; Branchek, T.A. and Weinshank, R.L. (1996) *Nature*, **382**(6587), 168-171.

[78] Lin, S.; Boey, D. and Herzog, H. (2004) *Neuropeptides*, **38**(4), 189-200.

[79] Pedrazzini, T. (2004) *Neuropeptides*, **38**(4), 267-275.

[80] Pedrazzini, T.; Seydoux, J.; Künstner, P.; Aubert, J.F.; Grouzmann, E.; Beermann F. and Brunner, H.R. (1998) *Nat. Med.*, **4**(6), 722-726.

[81] Kushi, A.; Sasai, H.; Koizumi, H.; Takeda, N.; Yokoyama, M. and Nakamura, M. (1998) *Proc. Natl. Acad. Sci. U.S.A.*, **95**(26), 15659-15664.

[82] Naveilhan, P.; Canals, J.M.; Arenas, E. and Ernfors, P. (2001) *J. Neurochem.*, **78**(6), 1201-1207.

[83] Thiele, T.E.; Koh, M.T. and Pedrazzini, T. (2002) *J. Neurosci.*, **22**(3), RC208-RC214

[84] Karlsson, R.M.; Choe, J.S.; Cameron, H.A.; Thorsell, A.; Crawley, J.N.; Holmes, A. and Heilig, M. (2008) *Psychopharmacology (Berlin, Ger.)*, **195**(4), 547-557.

[85] Wheway, J.; Mackay, C.R.; Newton, R.A.; Sainsbury, A.; Boey, D.; Herzog, H. and Mackay, F. (2005) *J. Exp. Med.*, **202**(11), 1527-1538.

[86] Zukowska-Grojec, Z.; Karwatowska-Prokopczuk, E.; Fisher, T.A. and Ji, H. (1998) *Regul. Pept.*, **75-76**, 231–238.

[87] Hansel, D.E.; Eipper, B.A. and Ronnett, G.V. (2001) *Nature*, **410**(6831), 940-944.

[88] Howell, O.W.; Doyle, K.; Goodman, J.H.; Scharfman, H.E.; Herzog, H.; Pringle, A.; Beck-Sickinger, A.G. and Gray. W.P. (2005) *J. Neurochem.*, **93**(3), 560-570.

[89] Cho, Y.R. and Kim, C.W. (2004) *Biochem. Biophys. Res. Commun.*, **314**(3), 773-780.

[90] Milenkovic, I.; Weick, M.; Wiedemann, P.; Reichenbach, A. and Bringmann, A. (2004) *Graefe's Arch. Clin. Exp. Ophthalmol.*, **242**(11), 944-950.

[91] Anitha, M.; Chandrasekharan, B.; Salgado, J.R.; Grouzmann, E.; Mwangi, S.; Sitaraman, S.V. and Srinivasan, S. (2006) *Gastroenterology.*, **131**(4), 1164-1178.

[92] Doyle, K.L.; Karl, T.; Hort, Y.; Duffy, L.; Shine, J. and Herzog, H. (2008) *J. Neurochem.*, **105**(3), 641-652.

[93] Jacques, D. and Abdel-Samad, D. (2007) *Can. J. Physiol. Pharmacol.*, **85**(1), 43-53.

[94] Agasse, F.; Bernardino. L.; Kristiansen, H.; Christiansen, S.H.; Ferreira, R.; Silva, B.; Grade, S.; Woldbye, D.P. and Malva, J.O. (2008) *Stem Cells*, **26**(6), 1636-1645

[95] Movafagh, S.; Hobson, J.P.; Spiegel, S.; Kleinman, H.K. and Zukowska, Z. (2006) *FASEB J.*, **20**(11), 1924-1926.

[96] Ruscica, M.; Dozio, E.; Boghossian, S.; Bovo, G.; Riaño, V.M.; Motta, M. and Magni, P. (2006) *Endocrinology*, **147**(3), 1466-1473.

[97] Seger, R. and Krebs, E.G. (1995) *FASEB J.*, **9**(9), 726-735.

[98] Kholodenko, B.N. (2002) *Trends Cell Biol.*, **12**(4), 173-177.

[99] Eva, C.; Serra, M.; Mele, P.; Panzica, G.C. and Oberto, A. (2006) *Front. Neuroendocrinol.*, **27**(3), 308-339.

[100] Elefteriou, F. (2005) *Bone Cell Mol. Life Sci.*, **62**(19-20), 2339-2349.

[101] Chenu, C. (2004) *J. Musculoskcl. Neuron. Interact.*, **4**(2), 132-134.

[102] Allison, S.J.; Baldock, P.A. and Herzog, H. (2007) *Peptides*, **28**(2), 320-325.

[103] Bjurholm, A.; Kreicbergs, A.; Terenius, L.; Goldstein, M. and Schultzberg, M. (1988) *J. Auton. Nerv. Syst.*, **25**(2-3), 119-125.

[104] Hill, E.L. and Elde, R. (1991) *Cell Tissue Res.*, **264**(3), 469-80.

[105] Ahmad, T.; Ugarph-Morawski, A.; Li, J.; Bileviciute-Ljungar, I.; Finn, A.; Ostenson, C.G. and Kreicbergs, A. (2004) *Regul. Pept.*, **119**(1-2), 61-67.

[106] Togari, A.; Arai, M.; Mizutani, S.; Mizutani, S.; Koshihara, Y. and Nagatsu, T. (1997) *Neurosci. Lett.*, **233**(2-3), 125-128.

[107] Lundberg, P.; Allison, S.J.; Lee, N.J.; Baldock, P.A.; Brouard, N.; Rost, S.; Enriquez, R.F.; Sainsbury, A.; Lamghari, M.; Simmons, P.; Eisman, J.A.; Gardiner, E.M. and Herzog, H. (2007) *J. Biol. Chem.*, **282**(26), 19082-19091.

[108] Baldock, P.A.; Sainsbury, A.; Allison, S.; Lin, E.J.; Couzens, M.; Boey, D.; Enriquez, R.; During, M.; Herzog, H. and Gardiner, E.M. (2005) *J. Bone Miner. Res.*, **20**(10), 1851-1857.

[109] Ducy, P.; Schinke, T. and Karsenty, G. (2000) *Science*, **289**(5484), 1501-1504.

[110] Takeda, S. and Karsenty, G. (2001) *J. Bone Miner. Metab.*, **19**(3), 195-198.

[111] Ducy, P.; Amling, M.; Takeda, S.; Priemel, M.;Schilling, A.F.; Beil, F.T.; Shen, J.; Vinson, C.; Rueger, J.M. and Karsenty, G. (2000) *Cell*, **100**(2), 197-207.

[112] Stephens, T.W.; Basinski, M.; Bristow, P.K.; Bue-Valleskey, J.M.; Burgett, S.G.; Craft, L.; Hale, J.; Hoffmann, J.; Hsiung, H.M.; Kriauciunas, A.; Mackellar, W.; Rosteck Jr, P.R.; Schoner, B.; Smith, D.; Tinsley, F.C.; Zhang, X.Y. and Heiman, M. (1995) *Nature*, **377**(6549), 530-532.

[113] Erickson, J.C.; Hollopeter, G. and Palmiter, R.D. (1996) *Science*, **274**(5293), 1704-707.

[114] Allison, S.J.; Baldock, P.; Sainsbury, A.; Enriquez, R.; Lee, N.J.; Lin, E.J.; Klugmann, M.; During, M.; Eisman, J.A.; Li, M.; Pan, L.C.; Herzog, H. and Gardiner, E.M. (2006) *J. Biol. Chem.*, **281**(33), 23436-23444.

[115] Bjurholm, A.; Kreicbergs, A.; Schultzberg, M. and Lerner, U.H. (1992) *J. Bone Miner. Res.*, **7**(9), 1011-1019.

[116] Bjurholm, A.; Kreicbergs, A.; Schultzberg, M. and Lerner, U.H. (1998) *Acta Physiol. Scand.*, **134**(3), 451-462.

[117] Amano, S.; Arai, M.; Goto, S. and Togari, A. (2007) *Biochim. Biophys. Acta*, **1770**(6), 966-973.

[118] Baldock, P.A.; Allison, S.J.; Lundberg, P.; Lee, N.J.; Slack, K.; Lin, E.J.; Enriquez, R.F.; McDonald, M.M.; Zhang, L.; During, M.J.; Little, D.G.; Eisman, J.A.; Gardiner, E.M.; Yulyaningsih, E.; Lin, S.; Sainsbury, A. and Herzog, H. (2007) *J. Biol. Chem.*, **282**(26), 19092-19102.

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