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The NPB/NPW Neuropeptide System and Its Role in Regulating Energy Homeostasis, Pain, and Emotion

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Abstract Neuropeptide B (NPB) and neuropeptide W (NPW) are neuropeptides that were recently identified as endogenous ligands for the previously orphan G-protein coupled receptors, GPR7 (NPBWR1) and GPR8 (NPBWR2). This neuropeptide system is thought to have a role in regulating feeding behavior, energy homeostasis, neuroendocrine function, and modulating inflammatory pain. Strong and discrete expression of their receptors in the extended amygdala suggests a potential role in regulating stress responses, emotion, anxiety and fear; however, there have been no functional studies to date to support this possibility. Future studies of NPB/NPW using both pharmacological and phenotypic analysis of genetically engineered mice will lead to further elucidation of the physiological role of this novel neuropeptide system.

1 Introduction

In 1995, O'Dowd et al. reported the existence of human genes encoding two structurally related orphan G protein-coupled receptors, GPR7 and GPR8, which share a 70% nucleotide and a 64% amino acid identity with each other. Among the other GPCR family members, they have high similarities with opioid and somatostatin receptors. Interestingly, GPR8 was not found in the rodent genome, while GPR7 was highly conserved in both human and rodents (O'Dowd et al. 1995).

Although the structures of both receptors suggest that their cognate ligands could be neuropeptides, the endogenous ligands were not identified for some time. Recently, two endogenous peptide ligands for these receptors were identified and named neuropeptide B (NPB) and neuropeptide W (NPW) (Brezillon et al. 2003; Fujii et al. 2002; Shimomura et al. 2002; Tanaka et al. 2003). Following the deorphaning of these receptors, GPR7 and GPR8 were reclassified by IUPHAR as Neuropeptide B/W receptor-1 (NPBWR1) and Neuropeptide B/W receptor-2 (NPBWR2) (Davenport and Singh 2005a,b). In this review, we discuss the discovery of these ligands and the recent findings concerning the pharmacology, histology, and the phenotypic analysis of genetically engi-

neered mice of the NPB/NPW system, and furthermore discuss other potential unexplored physiological roles of this neuropeptide/receptor system.

2 Identification of NPB and NPW

In 2002–2003, three groups independently identified endogenous peptide ligands for GPR7 and GPR8 by reverse pharmacology. To identify the cognate endogenous ligands for GPR7 (NPBWR1) and GPR8 (NPBWR2), Shimomura et al. expressed these receptors in Chinese Hamster Ovary (CHO) cells and used changes in forskolin-induced cAMP production in these cells as the read-out for receptor activation. HPLC fractions from bovine hypothalamic extracts were assayed using this system. While screening the hypothalamic extract fractions, they detected a decrease in forskolin-induced cAMP production, and subsequent purification and structural analysis of the ligands responsible for this inhibition of cAMP production led to the discovery of the novel neuropeptide, NPW. During their purification process, they identified two forms of NPW with different peptide lengths of 23 and 30 amino acid residues and named them NPW23 and NPW30, respectively.

In a similar manner, Fujii et al., Brezillon et al., and Tanaka et al. (Brezillon et al. 2003; Fujii et al. 2002; Tanaka et al. 2003) purified and identified NPB as an additional endogenous ligand for NPBW1 and NPBW2. Fujii et al. first screened the Celera database to identify novel secretory peptides and then expressed the cDNAs of the putative secretory peptides to find novel peptide ligands. Subsequent pharmacological studies and purification of the peptide from bovine hypothalamic extracts led to the identification of the second ligand for GPR7 and GPR8, which was named Neuropeptide B (NPB) due to the bromination of the first tryptophan residue. Brezillon et al. also identified human NPB mRNA with a bioinformatics approach by searching the EST database using the NPW sequence as a query (Brezillon et al. 2003). Tanaka et al. used the melanin-pigment aggregation assay in xenopus melanophore cells expressing GPR7 as the assay system to purify NPB from bovine hypothalamus. This system also detected a decrease in intracellular cAMP levels. Through EST database searches they also identified NPW as a putative paralogous peptide (Tanaka et al. 2003).

3 Structures of NPB and NPW

NPB and NPW belong to a distinct family of peptides that do not display any significant sequence similarity to other previously known peptides, while sharing a high degree of sequence similarity with each other (Fig. 1a)

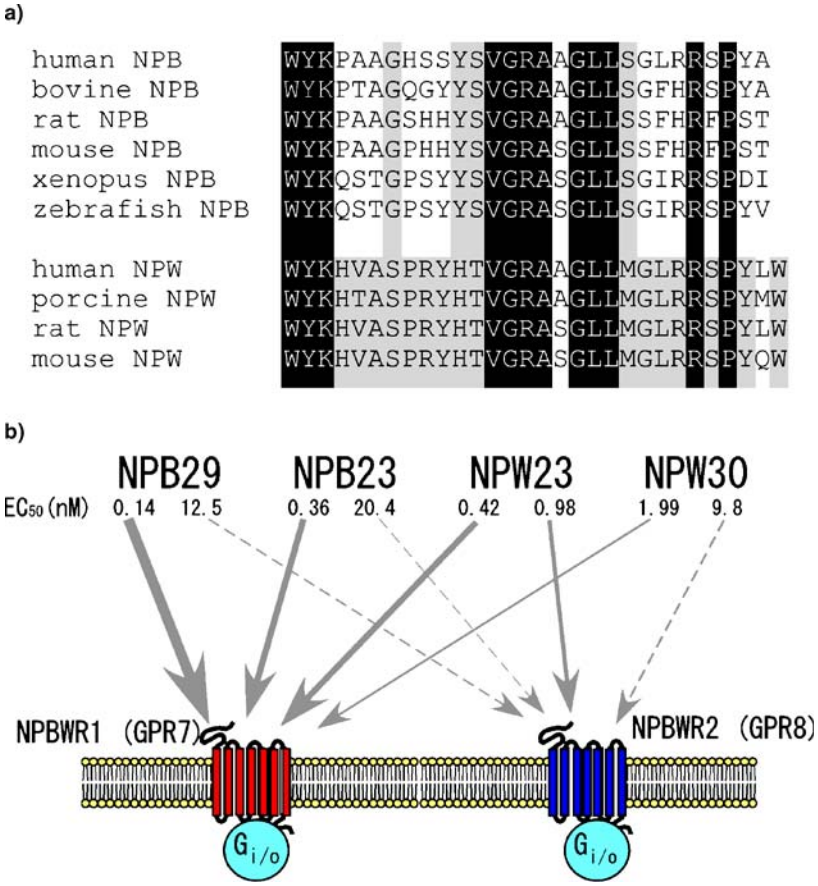

Fig. 1 The NPB/NPW neuropeptide system. **a** Amino acid sequences of NPB and NPW. *Dark shadow* shows amino acid identities between NPB and NPW. *Light shadow* shows conserved amino acids within NPB or NPW. **b** Interaction between NPB/NPW and NPBWR1/NPBWR2. EC_{50} values are determined by assays on inhibition of cAMP production in CHO cells expressing each receptor (Brezillon et al. 2003). These values are dependent on the assay systems, because expression levels of receptors and their abilities to evoke cellular responses vary in each system. However, potency rank orders of these peptides are consistent among the various reports (Brezillon et al. 2003; Fujii et al. 2002; Shimomura et al. 2002; Tanaka et al. 2003)

(Brezillon et al. 2003; Fujii et al. 2002; Shimomura et al. 2002; Tanaka et al. 2003).

NPB has a unique modification at the N-terminus tryptophane residue, C-6-bromination (Fujii et al. 2002; Tanaka et al. 2003). While this represents the first evidence of bromination in mammals, the biological significance of this bromination is unclear as it has been demonstrated that des-Bromo-NPB is equipotent to brominated NPB in in vitro cAMP inhibition assays (Tanaka

et al. 2003). Furthermore, bromination on Trp-1 has not been confirmed in any other mammalian species besides bovine. Further studies are needed to elucidate any possible role for this unique bromine modification in NPB.

By analogy with NPW, Brezillon et al. predicted that two isoforms of NPB, NPB23 and NPB29, could be produced from the processing of a 125-amino acid human precursor through the alternative usage of a dibasic amino acid pair. However, the dibasic motif, Arg24–Arg25, which is seen in human NPB, does not exist in NPB of other mammalian species including bovine, rat, and mouse. Furthermore, both Fujii et al. and Tanaka et al. were only able to isolate NPB29 from bovine hypothalamus extracts during their purification procedures. Therefore, it is unlikely that the NPB23 isoform exists as a mature peptide in mammalian species other than human. NPB (NPB29) binds and activates human NPBWR1 or NPBWR2 with median effective concentrations (EC_{50}) of 0.23 nM and 15.8 nM, respectively (Tanaka et al. 2003). This suggests that NPB is a relatively selective agonist for NPBWR1 (Fig. 1b).

As discussed earlier, peptide purification as well as sequence analysis showed that NPW has two isoforms with lengths of 23 and 30 amino acid residues. These are called neuropeptide W-23 (NPW23) and neuropeptide W-30 (NPW30) respectively, which are processed from the same precursor. NPW30 has a pair of arginine residues in the 24th and 25th positions. NPW23 is produced as a result of proteolytic processing at this site (Brezillon et al. 2003; Fujii et al. 2002; Tanaka et al. 2003). Therefore, the amino acid sequence of NPW23 is completely identical to that of the N-terminal 23 residues of NPW30. In vitro experiments using recombinantly expressed receptors showed that synthetic NPW23 activates and binds to both NPBWR1 and NPBWR2 at similar effective doses, while NPW30 shows slightly lower affinities to both receptors as compared with NPW23 (Fig. 1b) (Brezillon et al. 2003; Tanaka et al. 2003).

The preferred conformations of the desbromo-NPB and NPW have been determined by the combination of ^{1}H NMR, CD, and molecular modeling (Lucyk et al. 2005). NPB consists of a type II beta-turn involving residues Lys-3 to Ala-6. The C-terminal region of NPB exists in a conformational equilibrium between different secondary structures, including an alpha-helix from residues Arg-15 to Ser-21, and a 3-helix from residues Ser-12 to Ser-21s. The N-terminus of NPW exhibits a cation-pi interaction between the Lys-3 side chain and the quadrupole moment of the Trp-1 indole group. At the C-terminus of NPW, a well-defined alpha-helical conformation exists from Arg-15 to Met-21.

4 Structure-Activity Relationships of NPB and NPW

The rank order of potency has been determined in cell lines expressing NPBWR1 and NPBWR2. Pharmacologically, both NPW and NPB activate

both receptors, however, with varying degrees of affinity. NPBWR1 has a slightly higher affinity for NPB as compared with both forms of NPW, whereas NPBWR2 shows a potency rank order of NPW23 > NPW30 > NPB (Fig. 1b) (Brezillon et al. 2003; Fujii et al. 2002; Shimomura et al. 2002; Tanaka et al. 2003). NPB23 and NPB29 have similar affinities for both receptors. In brief, NPW23 and NPW30 bind to both receptors almost equally, while NPB is a relatively selective agonist for NPBWR1.

Tanaka et al. showed deletion of Trp-1 from NPB or NPW drastically decreased activity, suggesting that the N-terminus is involved in receptor binding (Tanaka et al. 2003). This is consistent with the fact that NPB and NPW have similarity in their sequences in N-terminal regions.

5
Structures and Functions of NPBWR1 and NPBWR2

From the chromosomal assignment by fluorescent in situ hybridization, human NPBWR1 was mapped to chromosome 10q11.2–121.1 and human NPBWR2 to chromosome 20q13.3.

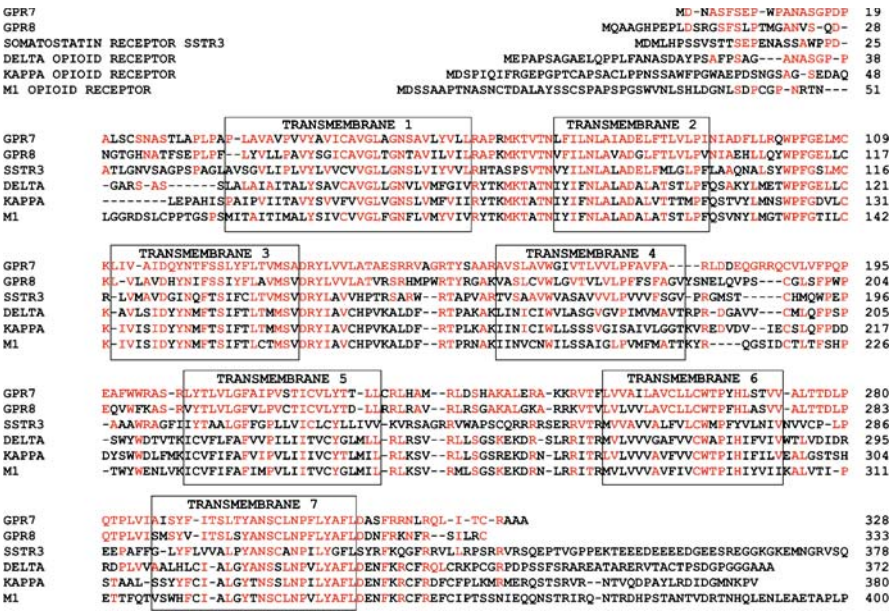

Fig. 2 Sequences of NPBWR1/NPBWR2 compared to somatostatin receptor and opioid receptors. Conserved amino acid residues are shown in *red letters*. Each of the transmembrane domains is boxed

Human NPBWR1 and NPBWR2 are predicted to have 328 and 333 amino acids, respectively and share 64% sequence homology with each other (Fig. 2). Among other family members of GPCRs, NPBW1 and NPBW2 are most closely related to opioid and somatostatin receptors (Fig. 2) (O'Dowd et al. 1995). Amino-acid analysis of NPBW1 orthologues in other mammalian species has revealed a high degree of conservation throughout evolution (Lee et al. 1999). In contrast, while the gene encoding NPBWR2 has been discovered in several mammalian species such as monkey, lemur, bat, shrew and rabbit, it has not been detected in rodents (Lee et al. 1999). This suggests that these two receptors were produced by a phylogenetically relatively recent gene duplication event (Lee et al. 1999).

As suggested by the assay systems used during their purification process, both NPBWR1 and NPBWR2 couple to the Gi-class of G-proteins (Tanaka et al. 2003). This suggests that these neuropeptides have inhibitory properties on neurons via activation of GIRK (Kir3) channels. NPB and NPW were also shown to stimulate Erk p42/p44 activities in human adrenocortical carcinoma-derived NCI-H295 cells (Andreis et al. 2005). These activations are probably mediated by beta/gamma subunits released from G_i-proteins (Tim van et al. 1995).

At present, no synthetic antagonists or agonists have been developed that are selective for either receptor.

6
Tissue Distributions of NPB/NPW and NPBWR1/NPBWR2

6.1
Neuropeptide B

In situ hybridization showed localizations of the prepro-NPB mRNA in several specific regions in the mouse brain such as the paraventricular hypothalamic nucleus (PVN), CA1-CA3 fields of the hippocampus, and several nuclei in the midbrain and brainstem, including the Edinger–Westphal nucleus (EW) as well as the sensory and motor nuclei of the trigeminal nerve, locus coeruleus (LC), inferior olive, and lateral parabrachial nucleus (Fig. 3) (Jackson et al. 2006; Tanaka et al. 2003).

Schulz et al. reported that NPB-immunoreactive cell bodies were observed in many regions within the hypothalamus which also contained high levels of NPBWR1 mRNA and NPB mRNA including the ventromedial hypothalamic nucleus, dorsomedial hypothalamic nucleus, arcuate nucleus, supraoptic retrochiasmatic nucleus, and in the area ventral to the zona incerta (Schulz et al. 2007). Although NPB mRNA was detected in several regions outside the hypothalamus, such as the hippocampus and brain stem, they did not report the existence of NPB-positive neurons in these regions (Jackson et al. 2006;

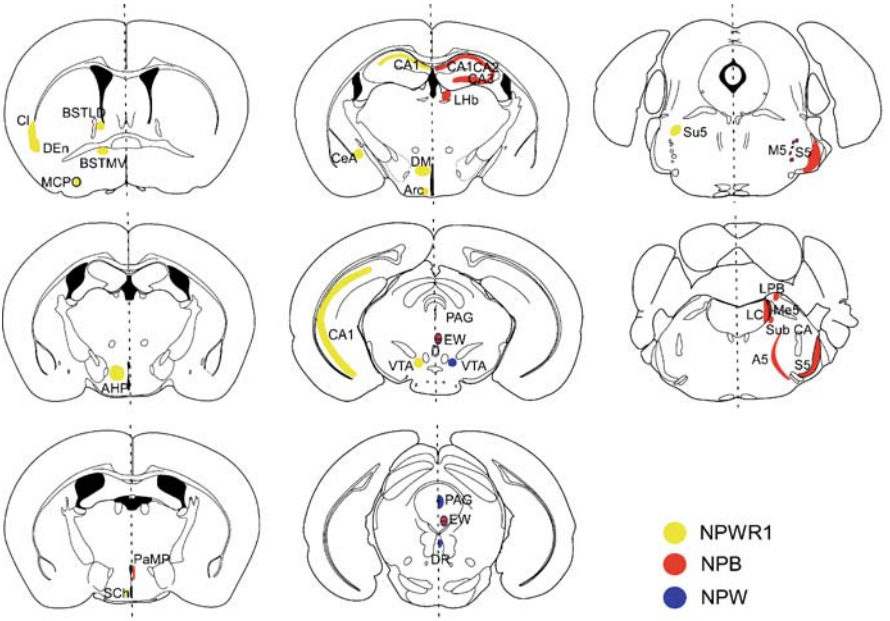

Fig. 3 Schematic representation of the NPB/W-GPR7 system in mouse brain. Distribution of NPB/NPW and NPBWR1 mRNA on mouse brain coronal sections are shown. Distribution of the receptor is shown in the *right* hemisphere, while the distributions of the ligands are shown in the *left*. The distribution of NPB mRNA (shown by *red regions*) included the hippocampus (CA1, CA2, CA3), lateral habenular nucleus (LHb), paraventricular hypothalamic nucleus, medial parvicellular part (PaMP), Edinger–Westphal (EW) nucleus, motor root of the trigeminal nerve (m5), sensory root of the teigeminal nerve (s5), lateral parabrachial nucleus alpha part (Sub CA), locus coeruleus (LC), noradrenergic cell group A5 (A5), and inferior olive subnucleus B (IOB) (Tanaka et al. 2003). The distribution of NPW mRNA (shown by *blue regions*) included the periaqueductal gray matter (PAG), EW nucleus (EW), ventral tegmental area (VTA), dorsal raphe nucleus (DR). The distribution of NPBWR1 mRNA (shown by *yellow regions*) included the claustrum (Cl), dorsal endopiriform nucleus (DEn), bed nucleus of the stria terminals, laterodorsal part (BSTLD), bed nucleus of the stria terminals, medioventrial part (BSTMV), suprachiasmatic nucleus (Sch), magnocellular preoptic nucleus (MCPO), paraventricular hypothalamic nucleus, posterior part (PaPo), dorsomedial hypothalamic nucleus (DM), central amygdala (CeA), CA1 field, hippocampus (CA1), ventral tegmental area (VTA), sensory root trigeminal nerve (Su5), subiculum (S), anterior hypothalamic area, posterior part (AHP), arcuate hypothalamic nucleus (Arc)

Schulz et al. 2007; Tanaka et al. 2003). In peripheral tissues, expression of human NPB mRNA was detected by RT-PCR in kidney, uterus, ovary, testis, and placenta, while murine NPB mRNA was detected by Northern blot at high levels in the stomach, spinal cord, testis and lower levels in the liver and kidney (Brezillon et al. 2003; Tanaka et al. 2003).

6.2 Neuropeptide W

Compared to the relatively widespread expression pattern of NPB mRNA, the expression of NPW mRNA in mouse brain is more confined to specific nuclei in midbrain and brainstem including the EW, ventral tegmental area (VTA), periaqueductal gray (PAG) and dorsal raphe nucleus (DR) (Fig. 3) (Kitamura et al. 2006; Tanaka et al. 2003). In humans, high levels of NPW mRNA were detected in the substantia nigra, and moderate expression levels were detected in the amygdala and hippocampus (Fujii et al. 2002). In peripheral tissues, expression of human NPW mRNA was confirmed by RT-PCR in the progenital system, comprising the kidney, testis, uterus, ovary, placenta, and also in the stomach and respiratory system, while murine NPW mRNA was detected by Northern blot at high levels in the lung and lower levels in the stomach (Brezillon et al. 2003; Tanaka et al. 2003).

Consistent with its mRNA distribution, NPW-immunoreactive (ir) cells were also exclusively detected in EW, VTA, PAG, and DR in rats (Kitamura et al. 2006). NPW-ir fibers were observed in several brain regions in rats including the lateral septum, bed nucleus of the stria terminalis (BNST), dorsomedial and posterior hypothalamus, CeA, CA1 field of hippocampus, interpeduncular nucleus, inferior colliculus, lateral parabrachial nucleus, facial nucleus, and hypoglossal nucleus. Among these regions, NPW-ir fibers were most abundantly observed in the CeA and the BNST, the output nuclei of the extended amygdala, which are regions implicated in fear and anxiety. These observations suggest that NPW-producing neurons are exclusively localized to the mid brain, and they project mainly to the limbic system, especially the CeA and BNST (Fig. 3).

Some reports showed the existence of NPW-ir cell bodies in the hypothalamic paraventricular nucleus (PVN) in rats and mice (Dun et al. 2003). However, a recent study suggested that the staining of NPW like immunoreactivity-positive cells in the PVN is probably due to non-specific staining for two main reasons (Kitamura et al. 2006). First, the PVN immunoreactivity is observed in the $NPW^{-/-}$ mice using many of the commercially available antibodies, and second the NPW mRNA is not expressed in the PVN in both mice and rats (Kitamura et al. 2006).

6.3 NPBWR1 (GPR7)

In situ hybridization and tissue binding studies showed that the CeA and BNST expresses the highest levels of NPBWR1 mRNA and binding signals (Fig. 3) (Jackson et al. 2006; Singh et al. 2004; Tanaka et al. 2003). Other nuclei with high levels of NPBWR1 expression and binding are the suprachiasmatic (SCN) and the ventral tuberomamillary nuclei of the hypothalamus.

Moderate levels are seen in the CA1-CA3 regions of the hippocampus, dorsal endopiriform, dorsal tenia tecta, bed nucleus, and the red nucleus. Low levels of expressions are seen in the olfactory bulb, parastrial nucleus, hypothalamus, laterodorsal tegmentum, superior colliculus, locus coeruleus, and the nucleus of the solitary tract.

Collectively, these observations suggest that NPBWR1 is most abundantly observed in the CeA and BNST. These results suggest that NPBWR1 might be involved in the regulation of stress and emotive responses, especially in fear and anxiety-related physiological and behavioral functions, which is discussed in detail later (Kitamura et al. 2006). Expression of NPBWR1 in the SCN suggests that they might be involved in the regulation of the circadian clock. However, as discussed later, *NPBWR1*$^{-/-}$ mice did not show any behavioral abnormalities in circadian behavioral pattern (our unpublished observation).

6.4
NPBWR2 (GPR8)

Only limited information about tissue distribution of NPBWR2 has been available. RT-PCR analysis showed that NPBWR2 mRNA is strongly expressed in human amygdala and hippocampus. Lower levels of expression were also detected in corpus callosum, cerebellum, substantia nigra, and caudate nucleus (Brezillon et al. 2003).

7
Pharmacological Activities of NPB as NPW

7.1
Feeding and Energy Homeostasis

From the distribution of NPBWR1, it was initially hypothesized that the NPB/W system may modulate feeding behavior (Shimomura et al. 2002; Tanaka et al. 2003). Therefore, many studies have focused on the roles of NPB/W in the regulation of feeding and energy homeostasis. The first physiological study on the action of NPW reported acute hyperphagia in male rats when NPW was administered intracerebroventricularly (i.c.v.) (Shimomura et al. 2002; Tanaka et al. 2003). However, Tanaka et al. showed that the effect of NPB in mice on feeding behavior is not simple (Tanaka et al. 2003). When NPB was i.c.v. injected during the light period, no significant effect of NPB on feeding was observed (Tanaka et al. 2003). In contrast, in the dark period, i.c.v. administration of 3 nmol of NPB increased feeding, but only within the first 2 h. A higher dose of NPB suppressed food intake in this interval. After 2 h, both doses of NPB decreased food intake. This bipha-

sic (early orexigenic followed by delayed anorexic) effect of NPB is different from the initially reported orexigenic action of NPW (Shimomura et al. 2002). Because rodents do not express NPBWR2 and only have NPBWR1, which accepts both peptides with relatively high affinities, these differences can not simply stem from the different potency rank orders of the two peptides on NPBWR1 and NPBWR2. In fact, we also observed a similar biphasic action of NPW in feeding behavior when administered i.c.v in mice or rats (our unpublished observation). Mondal et al. also reported anorexic effects of NPW (Mondal et al. 2003). These findings suggest a complex role for NPB and NPW in the regulation of food intake.

Interestingly, the anorexic effect of NPB was markedly enhanced when corticotrophin-releasing factor (CRF), a known anorexic peptide, was co-administered (Tanaka et al. 2003). The i.c.v. administration of these two peptides almost completely suppressed the food intake over 4 h. The biphasic effects of NPB/W on feeding behavior, and synergistic anorexic effects of NPB and CRF suggest the complex roles of these peptides in regulation of feeding behavior. The synergic effect of NPB with CRF in suppression of feeding suggests that this neuropeptide is implicated in inhibition of feeding under stressful conditions.

Continuous i.c.v. infusion of NPW using an osmotic minipump suppressed feeding and body weight gain over the infusion period (Mondal et al. 2003). Conversely, i.c.v. administration of anti-NPW IgG stimulated feeding suggesting that endogenous NPW play an inhibitory role in feeding behavior (Mondal et al. 2003). However, unlike the results from continuous i.c.v. infusion of NPW, bolus intra-PVN injection of NPW23 at doses ranging from 0.1 to 3 nmol increased feeding for up to 4 h, and bolus doses ranging from 0.3 to 3 nmol was reported to increase feeding for up to 24 h (Levine et al. 2005). This observation suggests that orexigenic versus anorectic effects of NPB/W could stem from different sites of action. When these peptides are injected into the lateral ventricles, they might be initially acting on the PVN, followed by acting on other regions implicated in the suppression of feeding. Alternatively, delayed inhibition of feeding by NPB/W might result from the production of other anorectic factors that are stimulated by NPB or NPW.

I.c.v. administration of NPW also increased body temperature and heat production (Mondal et al. 2003). These effects suggest that endogenous NPB/W might affect energy expenditure, which is consistent with the late onset obesity seen in male *NPBWR1*$^{-/-}$ mice and *NPB*$^{-/-}$ mice (Ishii et al. 2003; Kelly et al. 2005).

7.2 Effect on Inflammatory Pain

Initially, i.c.v. administration of NPB was reported to produce analgesia to subcutaneous formalin injection in rats (Tanaka et al. 2003). It was subse-

quently reported that intrathecal (i.t.) injection of either NPW23 or NPB decreased the number of agitation behaviors induced by paw formalin injection and attenuated the level of mechanical allodynia (Yamamoto et al. 2005). The effects were not antagonized by naloxone, suggesting that this effect is not mediated through the opioid receptor system. While i.t. injection of either NPW23 or NPB did not show any effect in the hot-plate test or mechanical nociceptive test, i.t. injection of either NPW23 or NPB significantly suppressed the expression of Fos-like immunoreactivity of the L4-5 spinal dorsal horn induced by paw formalin injection. These data suggest that both spinally applied NPW23 and NPB suppressed the input of nociceptive information to the spinal dorsal horn, and produced an analgesic effect in inflammatory pain, but not mechanical or thermal pain (Yamamoto et al. 2005). Consistent with the pharmacological studies, $NPB^{-/-}$ mice exhibited hyperalgesia to inflammatory pain, while they show normal responses for mechanical or thermal pain (Kelly et al. 2005). These observations suggest that NPB in the brain and/or spinal cord inhibits allodynia and modulates pain in a modality-specific manner.

Low levels of NPBWR1 were observed in Schwann cells in both normal human and rat nerves as well as in primary rat Schwann cell cultures. Peripheral nerve samples taken from patients exhibiting inflammatory/immune-mediated neuropathies showed a dramatic increase of NPBWR1 expression restricted to myelin-forming Schwann cells. Complementary animal models of immune-inflammatory and ligation-induced nerve injury and neuropathic pain similarly exhibited an increased myelin-associated expression of NPBWR1 (Zaratin et al. 2005). These observations suggest that NPBWR1 is involved in regulation of inflammatory pain in part by modulating Schwann cell function.

7.3 Neuroendocrine Regulation

When injected into the lateral cerebroventricle of conscious, unrestrained male rats, both NPB and NPW elevated corticosterone levels in circulation (Samson et al. 2004; Taylor et al. 2005). NPB was also reported to increase prolactin and decrease growth hormone levels (Samson et al. 2004). Pretreatment with a polyclonal anti-CRF antiserum or CRF antagonists completely blocked the ability of NPB or NPW to stimulate ACTH release and significantly inhibited the effect of NPB/W on plasma corticosterone levels (Samson et al. 2004; Taylor et al. 2005). These observations suggest that NPW and NPB may play a physiologically relevant role in the neuroendocrine response to stress via an activation of the hypothalamus-pituitary-adrenal (HPA) axis. Consistent with these observations, whole cell patch-clamp recording from hypothalamic slice preparation showed that bath application of NPW depolarized and increased the spike frequency of the majority of electrophysiologically

identified putative neuroendocrine PVN neurons. The effects on membrane potential were maintained in the presence of TTX suggesting that they are direct postsynaptic actions on these neuroendocrine cells (Taylor et al. 2005). These observations indicate that NPB/W may play an important role in the hypothalamic function in the endocrine response to stress by modulating the HPA axis.

7.4 Autonomic Regulation

I.c.v. administration of NPW30 was reported to increase the arterial blood pressure (ABP), heart rate (HR), and plasma catecholamine concentrations in conscious rats (Yu et al. 2007). The same report showed that most of the PVN neurons are excited, while a subset of smaller populations of PVN neurons are inhibited by NPW30; however, the chemical identities of these neurons was not shown. These observations suggest that NPB/W modulate PVN neuronal activities, which might be involved in the regulation of autonomic nervous system as well as the HPA axis (Yu et al. 2007). NPB is more likely to be involved in this role in vivo, due to the expression of NPB mRNA in the PVN. However, *NPBWR1*$^{-/-}$ mice have normal blood pressure and heart rate in basal states (our unpublished observation).

8 Emotion and Behavior

Amygdala is a well-defined subcortical nuclear group that is the center of emotion including fear (Phelps and LeDoux 2005). A sensory stimulus that predicts an aversive outcome will change neural transmission in the amygdala to produce the somatic, autonomic, and endocrine signs of fear, as well as increased attention to that stimulus. Fear learning involves the lateral and basolateral amygdala (BLA), where the association between incoming sensory stimuli leads to potentiation of synaptic transmission. The BLA receives sensory information from the thalamus, hippocampus, and cortex and then activates or modulates synaptic transmission in target areas appropriate for the reinforcement signal with which the sensory information has been associated. The BLA projects to the CeA and BSNT, whose efferents to the hypothalamus and brainstem trigger the expression of fear. NPW-ir and NPBWR1 are strongly expressed in the CeA and BSNT. However, despite the discrete and strong expression pattern of NPBWR1 in the CeA, BST, and hippocampus, the role of NPBWR1 regarding these functions has not been elucidated. These anatomical evidences suggest that NPB/W systems might have important roles in the modulation of output from the extended amygdala (Kitamura et al. 2006) (Fig. 4). Studying the behavioral phenotypes of

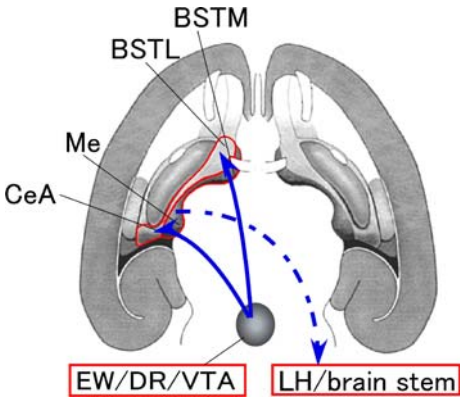

Fig. 4 Schematic representation of the NPW neuropeptide system. NPW neurons are localized in discrete areas in the brain stem, including the VTA, PAG, and EW, which send out projections to the lateral extended amygdala (CeA and BNST). NPBWR1 is localized in the CeA and BNST. These observations suggest that NPW neurons in the brain stem send feedback information to the output regions of the extended amygdala

NPBWR1$^{-/-}$, *NPB*$^{-/-}$, and *NPW*$^{-/-}$ mice would help to clarify the potential role for NPB/W in regulating these behaviors.

8.1 Effects on Circadian Rhythm

NPBWR1 are abundantly expressed in the suprachiasmatic nucleus. This strongly suggests that this neuropeptide/receptor system has a role in regulating circadian rhythm (Lee et al. 1999; Singh et al. 2004; Tanaka et al. 2003). However, we did not observe any effects of NPB/W on circadian activity in rats or mice when administered by i.c.v. injection (our unpublished observations). Furthermore, *NPBWR1*$^{-/-}$ mice displayed a normal circadian pattern of behavior in both light-dark and constant dark conditions. Both light-entrainable and food entrainable oscillation were also normal in these mice (our unpublished observations).

9 Peripheral Actions

Expressions of NPB, NPW, and NPBWR1 mRNAs in both adrenal cortex and adrenal medulla have been reported (Andreis et al. 2005; Hochol et al. 2007; Mazzocchi et al. 2005). NPB and NPW were shown to stimulate adrenal glucocorticoid secretion by an ACTH-independent mechanism when administered intravenously. It was also reported that NPW stimulates in vitro aldosterone

Table 1 In vivo pharmacological effects of NPB/NPW

Substance	Effects	Animal	Refs.
NPW (i.c.v.)	Food intake ↑ Body weight ↑	Rats (male)	Shimomura et al. Tanaka et al.
NPW (i.c.v.)	Body temperature ↑ Heat production ↑	Rats	Mondal et al.
NPW	ACTH ↑ Estradiol ↑	Rats	Hochol et al.
NPW30 (i.c.v.)	Arterial blood pressure (ABP) ↑ Heart rate (HR) ↑ Plasma catecholamine concentration ↑	Rats	Yu et al.
NPB (i.c.v.)	Food intake (light period) — Food intake (dark period) ↑	Mice	Tanaka et al.
NPB (i.c.v.)	Prolactin ↑ Growth hormone ↓	Rats (male)	Samson et al.
NPW23/NPB (i.t.)	Inflammatory pain ↓	Rats	Yamamoto et al.
NPW/NPB (i.c.v.)	Corticosterone in circulation ↑	Rats (male)	Samson et al., Taylor et al.
NPW/NPB (i.p.)	(plasma level) Parathyroid hormone ↑ (plasma level) Corticosterone ↑ (plasma level) Testosterone ↑	Rats	Hochol et al.
NPW/NPB (i.c.v.)	Circadian rhythm —	Rats/mice	Our unpublished observations

secretion by enhancing the release of medullary catecholamines, which activate beta-adrenoceptors located on zona glomerulosa cells (Hochol et al. 2007).

Bolus intraperitoneal (i.p.) injection of NPB or NPW increased the plasma levels of parathyroid hormone, corticosterone and testosterone. NPB was also reported to increase the blood concentration of thyroxine, and NPW was shown to increase ACTH and estradiol levels. These findings suggest that NPB and NPW play a role in the regulation of the endocrine system (Hochol et al. 2006).

Existence of NPW in rat stomach antral cells was reported. It was also reported that levels of NPW in stomach is decreased in fasted animals, while it was increased by re-feeding (Mondal et al. 2006), which is consistent with the notion that NPW may act as a suppressant to feeding. However, we did not observe any effects on feeding in mice when NPB or NPW were intravenously administered suggesting that peripheral NPB/W has limited, if any, significant role in modulating feeding behaviors (our unpublished observations).

10
Phenotypes of NPBWR1- and NPB-Deficient Mice

Genetically engineered mice are powerful tools for elucidating the physiological roles of particular genes. In this next section, we discuss the phenotypes of *NPBWR1*$^{-/-}$ mice and *NPB*$^{-/-}$ mice.

10.1
NPBWR1-Deficient Mice

Male *NPBWR1*$^{-/-}$ mice develop an adult-onset obesity that progressively worsens with age and was greatly exacerbated when animals are fed a high-fat diet (Ishii et al. 2003). These mice were hyperphagic and had decreased energy expenditure and locomotor activity resulting in obesity. *NPBWR1*$^{-/-}$ male mice showed decreased hypothalamic neuropeptide Y mRNA levels and increased proopiomelanocortin mRNA levels, a set of effects opposite to those evident in *ob/ob* mice. Furthermore, *ob/ob NPBWR1*$^{-/-}$ and *Ay/a NPBWR1*$^{-/-}$ double mutant male mice had an increased body weight compared with normal *ob/ob* or *Ay/a* male mice, suggesting that the obesity of *NPBWR1*$^{-/-}$ mice is independent of leptin and melanocortin signaling. Female mice did not show any significant weight increase or associated metabolic defects. These data suggest a potential role for NPBWR1 and NPB/W in regulating energy homeostasis independent of leptin and melanocortin signaling in a sexually dimorphic manner (Ishii et al. 2003).

10.2
NPB-Deficient Mice

Consistent with the phenotype of the *NPBWR1*$^{-/-}$ mice, *NPB*$^{-/-}$ mice also manifest mild adult-onset obesity. NPB-deficient mice also exhibit hyperalgesia in response to inflammatory pain. Hyperalgesia was not observed in response to chemical pain, thermal pain, or electrical stimulation. NPB-deficient mice demonstrated intact behavioral responses to pain, and learning from the negative reinforcement of electrical stimulation was unaltered. Baseline anxiety was also unchanged as measured in both the elevated plus maze and time spent immobile in a novel environment (Kelly et al. 2005). These data support the idea that NPB can modulate the responses to inflammatory pain and body weight homeostasis.

11 Discussion

From the hypothalamic distribution of NPBWR1, NPB and NPW were initially hypothesized to modulate feeding behavior (Shimomura et al. 2002; Tanaka et al. 2003), and many studies have focused on the roles of NPB/NPW in the regulation of feeding and energy homeostasis. The biphasic (early orexigenic followed by anorexic) effect of NPB/NPW suggest a complex role for NPB and NPW in the regulation of food intake. Both *NPB*$^{-/-}$ and *NPBWR1*$^{-/-}$ mice both show late onset obesity and hyperphagia, suggesting that the endogenous NPB-NPBWR1 pathway negatively regulates feeding behavior and positively regulates energy expenditure (Ishii et al. 2003; Kelly et al. 2005). This notion is further supported by several pharmacological studies that show that i.c.v. NPB/W increase heat production and sympathetic outflow (Yu et al. 2007).

Many studies have also shown that the NPB/W system is involved in the modulation of inflammatory pain. I.c.v. or i.t. administered NPB/W decreased sensitivity to inflammatory pain, while having no significant effect on chemical pain, heat sensation, or nociception. Consistent with these results, *NPB*$^{-/-}$ mice are hypersensitive to inflammatory pain but display no significant differences in chemical or thermal pains. These data in aggregate strongly support a physiological role for central NPB in pain regulation, and agonists for NPBWR1 or NPBWR2 might be good candidates for analgesic drugs for chronic inflammatory pain.

Finally, strong and discrete expression of NPBWR1 in the CeA and BNST, and abundant projection of NPW fibers in these regions suggest that this neuropeptide system has a role in the regulation of fear and anxiety. The CeA and BNST are the output nuclei of the extended amygdala, which has been implicated in a variety of emotional functions including expression of fear, modulation of memory, and mediation of social communication (Davis and Shi 1999) (Figs. 3, 4). Therefore, the expression of GPR7 in the CeA and BST suggests modulatory roles of GPR7 in these functions. Studies of these functions using genetically engineered mice in NPB/W and their receptors would help to clarify these roles.

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