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REVIEW

Neuropeptide B and W: neurotransmitters in an emerging G-protein-coupled receptor system

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Deorphanised G-protein-coupled receptors represent new and expanding targets for drug development. Neuropeptide B (NPB) and W (NPW) have recently been identified as the cognate endogenous ligands for the orphan receptor GPR7, now designated as $NPBW_1$. NPB and NPW also bound to a second related orphan receptor, GPR8, now designated as $NPBW_2$ that is present in humans but not rats or mice. In humans, high levels of NPW mRNA have been visualised in the substantia nigra, whereas moderate expression levels have been detected in the amygdala and hippocampus. In peripheral tissues, expression of NPW mRNA has been confirmed in the progenital system, comprising the kidney, testis, uterus, ovary and placenta, and also in stomach homogenates. Immunocytochemical, molecular biological and autoradiography techniques have revealed a discrete CNS distribution for $NPBW_1$ in human, mouse and rat. Highest expression of $NPBW_1$ mRNA and protein was identified in the amygdala and hypothalamic nuclei known to regulate feeding behaviour. [^{125}I]-NPW bound with a single high affinity to rat amygdala, $K_D = 0.44$ nM and 150 fmol mg^{-1} protein. Physiological studies demonstrate that intracerebroventricular infusion of $NPBW_1$ ligands modulates feeding behaviour, regulates the release of corticosterone, prolactin and growth hormone while also manipulating pain pathway. Mouse knockout models of the gene encoding either NPB or $NPBW_1$ have a gender-specific phenotype, with moderate obesity evident in males but not females. Further investigation is required to elucidate the precise physiological role of NPB and NPW as neurotransmitters.
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Abbreviations: ACTH, adrenocorticotrophin hormone; Arc, arcuate nucleus; BST, bed nucleus of the stria terminalis; CRF, corticotrophin-releasing factor; DMH, dorsal medial hypothalamic nucleus; DRN, dorsal raphe nuclei; DVC, dorsal vagal complex; GPCR, G-protein-coupled receptor; i.c.v., intracerebroventricular; i.t., intrathecal; IR, immunoreactivity; LH, lateral hypothalamus; MeA, medial amygdaloid nuclei; MPA, medial preoptic area; NPB, neuropeptide B; $NPBW_1$, (NC-IUPHAR Nomenclature) neuropeptide B/W 1 receptor; $NPBW_2$, (NC-IUPHAR Nomenclature) neuropeptide B/W 2 receptor; NPW, neuropeptide W; NPY, neuropeptide Y; PAG, periaqueductal grey; POMC, proopiomelanocortin; PVN, paraventricular nucleus; SCh, suprachiasmatic nucleus; SFO, subfornical organ; SO, supraoptic nucleus; SuG, superficial grey layer of the superior colliculus; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area

Introduction

G-protein-coupled receptors (GPCRs) represent the largest family of transmembrane spanning proteins. Members of Class 1 GPCRs (Family A) are the targets for a significant proportion of currently prescribed drugs (Wilson & Bergsma, 2000; Maguire & Davenport, 2005). Following the completion of 99% of the human genome (Lander *et al*., 2001), bioinformatics has been applied to identify most of, if not all, the human genes that could potentially encode a GPCR (Foord *et al*., 2005). Recently, about 85 'orphan' Class 1 GPCRs have been identified, so-called because the endogenous ligand(s) activating the receptor is as yet unknown. During the last 10 years, over 50 orphan GPCRs have been deorphanised (paired with their cognate ligands), using reverse pharmacology, with the majority turning out to be peptides (Davenport, 2003). The novel neuropeptide B (NPB) and neuropeptide W (NPW), paired with the previously designated orphan receptors GPR7 and GPR8, have received growing attention as they have been shown to modulate the release of pituitary-derived hormones, regulate feeding behaviour and manipulate pain pathways. Therefore, understanding the role of the NPB/NPW system may provide new insights into human physiology and may offer novel targets for therapeutic drug development. The purpose of this review is therefore to summarise the physiology and pharmacology of the NPB/NPW transmitter system.

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Identification of NPB and NPW as cognate ligands for orphan receptors GPR7 and GPR8

O'Dowd *et al*. (1995) originally identified two genes, GPR7 and GPR8, predicted to encode two GPCRs (designated as orphan receptors). Following pairing of these receptors with

Table 1 Structural information on $NPBW_1$ and $NPBW_2$

Receptor	*Species*	*Amino acid*	*Accession number*	*Chromosomal location*	*Gene name*
$NPBW_1$	Human	328	P48145	8q11.23	GPR7
$NPBW_1$	Rat	329	Q56UD9	5q12	Gpr7
$NPBW_1$	Mouse	146	XP_136404	1 (A1–A2)	Gpr7
$NPBW_2$	Human	333	P48146	20q13.3	GPR8

Table 2 Distribution of $NPBW_1$ and its homologue in human, mouse and rat

	Human		*Mouse*	*Rat*
	$NPBW_1$	*$NPBW_2$*	*$NPBW_1$*	*$NPBW_1$*
CNS tissues				
Cerebellum	–[a]	+[a]	–[b,c]	–[a,b,c]
Cerebral cortex	ND	ND	–[b,c]	+[a,b,c]
Parietal cortex	–[a]	++[a]	–[b,c]	–[c]
Hippocampus	+++[a]	+++[a]	+[b,c]	+[a,b,c]
Amygdala	+++[a]	+++[1]	+++[b,c]	+++[a,b,c,d]
Hypothalamus	–[a]	–[a]	+++[b,c]	+++[a,b,c,d]
Thalamus	+[a]	++[a]	+[b,c]	++[a,b,c]
Caudate nucleus	–[a]	++[a]	–[b,c]	–[c]
Substantia nigra	+[a]	+[a]	–[b,c]	–[c]
Corpus callosum	+[a]	+[a]	–[b,c]	–[c]
Bed nucleus of the stria terminalis	ND	ND	++[b,c]	+++[c]
Dorsal vagal complex	ND	ND	–[b,c]	++[c]
Periaqueductal grey	ND	ND	–[b,c]	++[c]
Superficial grey layer of superior colliculus	ND	ND	–[b,c]	++[c]
Medulla oblongata	ND	ND	ND	++[a]
Spinal cord	–[a]	–[a]	ND	++[a]
Peripheral tissues				
Pituitary gland	++[a,b]	++[a]	ND	+[a]
Adrenal gland	++[a]	++[a]	ND	–[a]
Testis	+[a]	+[1]	ND	+[a]
Uterus	–[a]	–[a]	ND	+++[a]

Expression of receptors $NPBW_1$ or $NPBW_2$: ND, not determined; –, not detected; +, low levels; ++, moderate levels; +++, high levels, as determined by
[a]RT–PCR.
[b]*In situ* hybridisation.
[c]*In vitro* autoradiography or
[d]Immunocytochemistry. These data are from Brezillon *et al*. (2003), Fujii *et al*. (2002), Lee *et al*. (1999), O'Dowd *et al*. (1995), Singh *et al*. (2004) and Tanaka *et al*. (2003).

NPB and NPW, they were reclassified by IUPHAR as $NPBW_1$ (GPR7) and $NPBW_2$ (GPR8) (see Davenport & Singh, 2005a, b). $NPBW_1$ and $NPBW_2$ (Table 1) are predicted to comprise 328 and 333 amino acids, respectively, with 64% sequence homology. Among other families of GPCRs, $NPBW_1$ and $NPBW_2$ are most closely related to opioid receptors.

Amino-acid analysis of $NPBW_1$ orthologues in rat and mouse has revealed high degree of conservation throughout evolution (Lee *et al*., 1999). In contrast, the gene-encoding $NPBW_2$ has not been detected in rat or mouse (Lee *et al*., 1999) and the receptor may not be present in these species. However, the gene encoding $NPBW_2$ has been discovered in other mammalian species such as rabbit (Lee *et al*., 1999).

Anatomical localisation of mRNA encoding $NPBW_1$ and $NPBW_2$ receptors

The distribution of messenger RNA (mRNA) encoding $NPBW_1$ and $NPBW_2$, determined by RT–PCR or *in situ* hybridisation in a panel of human, rat and mouse tissues, is summarised in Table 2. Although RT–PCR requires homogenisation of tissue resulting in the loss of anatomical structure, it provides crucial evidence that a particular tissue is capable of expressing mRNA encoding one or both receptors. In some cases, results have been confirmed by *in situ* hybridisation that, by retaining the anatomical structure, has given some initial information about the possible physiological function of these receptors.

RT–PCR analysis of a panel of human tissues revealed a predominant central nervous system (CNS) distribution for $NPBW_1$ mRNA (Brezillon *et al*., 2003). Highest expression of $NPBW_1$ mRNA was detected in the hippocampus and amygdala. $NPBW_2$ mRNA was also detected at high levels within these regions of the brain with moderate expression further detected in the thalamus (Brezillon *et al*., 2003). However, the precise anatomical distribution of both $NPBW_1$ and $NPBW_2$ in the human brain is currently unknown and warrants further investigation.

In peripheral tissues, moderate expression levels of $NPBW_1$ and $NPBW_2$ mRNA were reported in the pituitary gland, whereas mRNA for only the latter receptor was detected in the adrenal gland (Brezillon *et al*., 2003). In contrast, Mazzocchi

et al. (2005) were able to confirm mRNA expression of both $NPBW_1$ and $NPBW_2$ to cultured human adrenal zona glomerulosa, zona fasciculata and reticularis cells. Within the pituitary gland, the distribution of $NPBW_1$ was further refined to the lateral wings of the anterior pituitary using *in situ* hybridisation (O'Dowd *et al*., 1995).

A similar distribution of $NPBW_1$ mRNA was detected in rat tissues using RT–PCR, with almost exclusive expression to the CNS (Fujii *et al*., 2002). Moderate to high levels were present in peripheral tissues such as the pituitary gland and progenital system (Fujii *et al*., 2002). *In situ* hybridisation revealed high levels of $NPBW_1$ mRNA expression in discrete regions of rat brain, including various hypothalamic and amygdaloid nuclei (Lee *et al*., 1999). In particular, $NPBW_1$ mRNA was detected in the ventromedial hypothalamic nuclei (VMH), dorsal medial hypothalamic nucleus (DMH) and paraventricular hypothalamic nucleus (PVN), all of which are strongly associated with the modulation of feeding behaviour and metabolic disorders (Williams *et al*., 2001). $NPBW_1$ mRNA was also found in the arcuate (Arc) and supraoptic nucleus (SO). Interestingly, the former nucleus consists of neurones that contain neuropeptide Y (NPY), a peptide that has pronounced effects on feeding (Woods *et al*., 1998), or contain other neurotransmitters regulating the release of anterior pituitary-derived hormones. In contrast, the SO contains neurones that extend into the posterior pituitary, releasing vasopressin to have peripheral effects (Johnston, 1985). High $NPBW_1$ mRNA levels were also present in the suprachiasmatic nucleus (SCh), which regulates body functions over 24 h in response to the light and dark cycle (Hastings, 1997). Strongest expression of $NPBW_1$ mRNA was in the amygdala, particularly within the medial amygdaloid nucleus, whereas moderate levels were also detected in the posteromedial amygdaloid nucleus. These nuclei represent part of the limbic system, which including areas such as the amygdala, hypothalamus and hippocampus, is responsible for the emotional state of mammals (Sah *et al*., 2003).

Anatomical localisation and characterisation of [^{125}I]-NPW binding by quantitative autoradiography and image analysis

mRNA levels do not necessarily correlate with protein expression (Curtis *et al*., 2000; Greenbaum *et al*., 2003; Neumann *et al*., 2004). Radioligand binding assays using tissue sections followed by image analysis of the resulting autoradiographs provide direct evidence of receptors within discrete anatomical regions and have the advantage of being quantitative. Using [^{125}I]-NPW, highest receptor densities ($\sim$150 fmol mg^{-1} protein) (Figure 1) were measured in the amygdala, and this distribution was confirmed to be the receptor $NPBW_1$ using immunocytochemistry (Singh *et al*., 2004). $NPBW_1$ receptors were also localised in a range of hypothalamic nuclei such as the VMH, DMH and SCh, demonstrating high correlation with $NPBW_1$ mRNA expression. Interestingly, one mismatch between receptor protein and mRNA was observed in the hippocampus, where $NPBW_1$ expression was reported (Lee *et al*., 1999), but protein was not detected by ligand binding (Singh *et al*., 2004). Comparison of the distribution of $NPBW_1$ mRNA and protein in mouse brain revealed a limited distribution of receptors when compared to rats (O'Dowd *et al*., 1995; Tanaka *et al*., 2003; Singh *et al*., 2004). However, the pattern of $NPBW_1$ mRNA in mouse was similar to that observed in rats and humans, with the expression of $NPBW_1$ predominantly in the amygdala and hypothalamus (O'Dowd *et al*., 1995; Tanaka *et al*., 2003; Singh *et al*., 2004).

Identification of NPB and NPW as cognate ligands for orphan receptors by reverse pharmacology

To identify the cognate endogenous ligand for GPR7 ($NPBW_1$) and GPR8 ($NPBW_2$), Shimomura *et al*. (2002) artificially expressed these receptors in Chinese hamster ovary

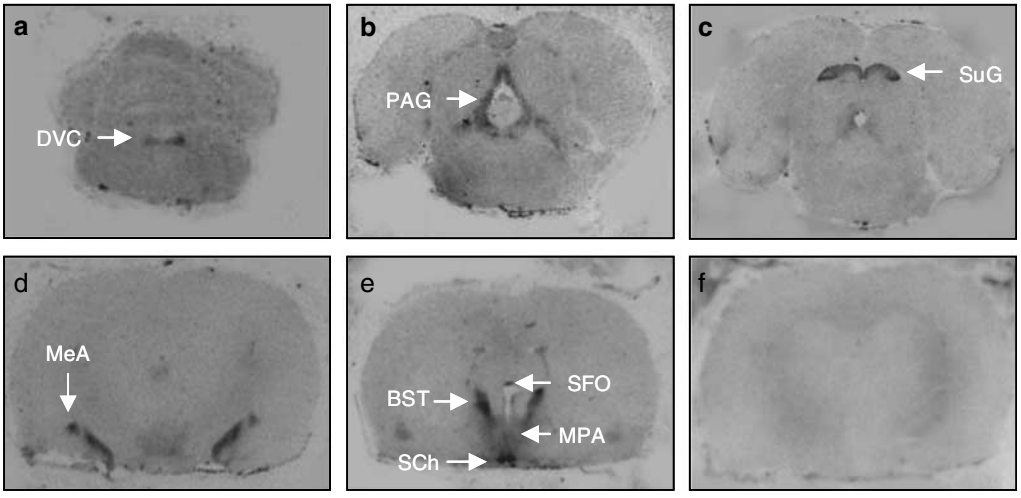

Figure 1 Localisation of $NPBW_1$ in coronal sections of rat brain using [^{125}I]-NPW receptor autoradiography. [^{125}I]-NPW binding was detected to the (a) DVC, dorsal vagal complex, (b) PAG, periaqueductal grey, (c) SuG, superficial grey layer of superior colliculus, (d) MeA, medial amygdaloid nuclei, (e) SFO, subfornical organ, BST, bed nucleus of the stria terminalis, MPA, medial preoptic area and the SCh of rat brain ($n=4$). Specific binding was not detected in the presence of unlabelled NPW (f). Scale bar = 1 cm. Reproduced with permission from Brain Research (Singh *et al*., 2004).

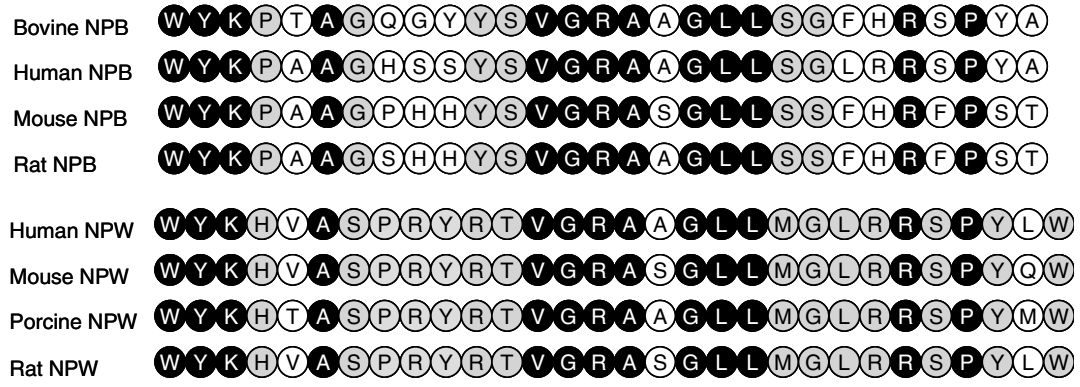

Figure 2 Bovine, human, mouse, porcine and rat amino-acid sequences of NPB and NPW. Sequence similarities between NPB and NPW orthologues are shown in black. Further identical amino acids within the NPB or NPW orthologues are shown in grey.

cells, exposing them to porcine hypothalamus extracts while monitoring alterations in cAMP levels. Hypothalamic extracts were assayed for the endogenous ligand as the receptor $NPBW_1$ mRNA was highly expressed in various hypothalamic nuclei, and in accordance with classical neurotransmitters, the ligands should be in close proximity to their receptors. When these cell lines expressing either $NPBW_1$ or $NPBW_2$ were incubated with the hypothalamic extracts, specific inhibition of forskolin-induced cAMP production was detected. This also suggested that both $NPBW_1$ and $NPBW_2$ are coupled to the hetrotrimeric G_i protein. Purification, isolation and further analysis of the ligand responsible for this inhibition of cAMP production revealed the discovery of the novel peptide, NPW. In a similar manner, Fujii *et al*. (2002), as well as two other independent groups (Brezillon *et al*., 2003; Tanaka *et al*., 2003), identified NPB as an additional endogenous ligand for $NPBW_1$ and $NPBW_2$.

NPB and NPW belong to a distinct family of peptides that do not display any significant sequence similarity to other known peptides while sharing a high degree of sequence similarity with each other (Fujii *et al*., 2002; Shimomura *et al*., 2002; Brezillon *et al*., 2003; Tanaka *et al*., 2003). The amino-acid sequence of the human forms of NPB and NPW are aligned with their respective orthologues in other mammalian species in Figure 2. NPB has a post-translational modification, bromination of the N-terminus tryptophan (Fujii *et al*., 2002) that is presently unique among the mammalian bioactive peptides. Whether this modification is essential for the peptide to function in tissues or stability *in vivo* remains to be determined. However, it has been demonstrated that des-Br-NPB is equipotent in *in vitro* cAMP inhibition assays to brominated NPB (Tanaka *et al*., 2003).

In common with other peptides, NPB and NPW are derived from larger precursor proteins with signal peptides of 24 and 32 amino acids, respectively. These peptides, of separate gene products, are then cleaved at basic pairs of amino acids to yield shorter forms of the peptides, both consisting of 23 amino acids (NPW-23 and NPB-23) (sharing 61% amino-acid sequence similarity), or C-terminally extended forms of 29 amino acids (NPB-29) and 30 amino acids (NPW-30) (sharing 66% amino-acid sequence similarity) (Fujii *et al*., 2002; Brezillon *et al*., 2003). The rank order of potency has been determined in artificial cell lines expressing two different receptors. The four peptides bound with high potency to both receptors but $NPBW_1$ have slightly higher affinity for both forms of NPB, whereas $NPBW_2$ have higher affinity for the short and C-terminal extended forms of NPW (Fujii *et al*., 2002; Shimomura *et al*., 2002; Brezillon *et al*., 2003; Tanaka *et al*., 2003). Consistent with the sequence, NMR conformational analysis of the shorter forms of NPB and NPW (Lucyk *et al*., 2005) reveled that they displayed similar C-terminal secondary structures, whereas the N-terminal secondary structures differed, which may contribute to the slight differences in potencies reported for the peptides. However, these differences are small and it is unlikely that the two receptors can be distinguished pharmacologically using the rank order of these agonists. At present, no antagonists have been developed that are selective for either receptor.

A radiolabelled analogue of NPW-23 ($[^{125}I]$-NPW), which binds saturably, specifically and with high affinity to $NPBW_1$ in rat amygdala (K_D of 0.44 nM) has been synthesised, thus providing a potentially useful pharmacological tool to quantify receptor density (Singh *et al*., 2004). Fixed concentrations of NPB-23 were found to compete with $[^{125}I]$-NPW binding at levels comparable to that seen for nonspecific binding (Singh *et al*., 2004). As $NPBW_2$ is not expressed in rat, the results represent binding to $NPBW_1$ and provide pharmacological evidence that NPB and NPW, at least in rat brain, share one common receptor. Similarly, $NPBW_2$ is also not detected in mice (Lee *et al*., 1999). C-terminally truncated forms of NPB or depletion of Trp^1 in either peptide resulted in the loss of potency, suggesting that the C- and N-terminus comprise regions consisting of critical amino acids for the efficacy and binding of the peptides to their receptors (Brezillon *et al*., 2003; Tanaka *et al*., 2003).

Anatomical localisation of the endogenous peptide ligands, NPB and NPW

In humans, high levels of NPW mRNA were visualised in the substantia nigra, whereas moderate expression levels were detected in the amygdala and hippocampus (Fujii *et al*., 2002). In peripheral tissues, expression of NPW mRNA was confirmed in the progenital system, comprising the kidney, testis, uterus, ovary and placenta, and also in stomach homogenates (Fujii *et al*., 2002). The distribution of NPB

mRNA was similar to NPW mRNA, but with higher expression within the CNS. Unlike NPW, NPB mRNA was detected in the thalamus and hypothalamus (Table 3).

Using immunocytochemical techniques, NPW-like immunoreactivity (IR) has been detected in the dorsal raphe nuclei (DRN) and ventral tegmental area (VTA) (Singh *et al*., 2004) as well as various hypothalamic nuclei such as the PVN, SO, dorsal hypothalamic areas, Arc and accessory neurosecretory nuclei of rat brain (Dun *et al*., 2003). NPW-IR was also detected in the cell processes of the basomedial and central nuclei of the amygdala (Dun *et al*., 2003). Although the method of immunocytochemistry employed was not quantitative, it was observed that NPW-IR was more abundant in male hypothalamus compared to their female counterparts (Dun *et al*., 2003). IR to NPW was also detected in the pituitary gland, both the anterior and posterior parts with dense network of NPW-IR fibres visualised within the median eminence (Dun *et al*., 2003). A similar distribution for NPB-IR was reported in rat brain (Dun *et al*., 2005).

In situ hybridisation techniques compared the distribution of NPB and NPW mRNA in mouse brain. NPW mRNA was detected in the periaqueductal grey, VTA and the DRN (Tanaka *et al*., 2003). In contrast, NPB mRNA was more abundant and detected in the Edinger Westphal nuclei as well as the sensory and motor root of the trigeminal nerve. The former nuclei of the trigeminal nerve is known to provide preganglionic parasympathetic inputs to the eye, which results in the control of pupil formation in order to accommodate the lens, whereas the latter nerve is responsible for receiving sensory information from the face and providing motor connections to the muscles involved in mastication (Trimarchi, 1992; Lund *et al*., 1998). NPB mRNA was also detected in the hypothalamus, particularly the medial parvicellular region of the PVN (Tanaka *et al*., 2003).

Physiological role of $NPBW_1$

Feeding behaviour, metabolic rate and obesity

From the distribution of $NPBW_1$, it was hypothesised that $NPBW_1$ may modulate feeding behaviour (Shimomura *et al*., 2002). The distribution of the receptor and the ligands were also comparable with the distribution of substance P, corticotrophin-releasing factor (CRF) and NPY, all of which are well-established regulators of food consumption (Holmes *et al*., 2003). The first physiological study on the action of NPW reported acute hyperphagia in male rats when NPW was administered intracerebroventricular (i.c.v.) (Shimomura *et al*., 2002). These initial observations were confirmed by other groups who studied the actions of $NPBW_1$ activation over 24 h by i.c.v. application of NPB or NPW (Baker *et al*., 2003; Samson *et al*., 2004; Levine *et al*., 2005). In agreement and further exploring the site of NPW action, Levine *et al*. (2005) demonstrated that administration of NPW into the PVN and lateral hypothalamus (LH) resulted in hyperphagia. However, Tanaka *et al*. (2003) showed that, although the acute effects of NPB administration were to induce hyperphagia, the long-term effect was to significantly attenuate feeding. Significant hypophagia was also reported by Mondal *et al*. (2003), with the effect of high doses lasting for up to 48 h. Interestingly, a recent study identified a source of endogenous NPW from rat stomach antral cells and reported decreased levels of NPW in fasted animals in which, when re-fed, levels of NPW increase (Mondal *et al*., 2006), consistent with the notion that NPW may act as a suppressant to feeding.

In all studies, animals had free access to food and water and unless stated were injected i.c.v.; therefore, availability of food and route of administration cannot account for the observed differences. Furthermore, administration of different doses of NPB/NPW did not result in different physiological responses. The variations in response to NPB/NPW administration could

Table 3 CNS distribution of NPB and NPW in human, mouse and rat

	Human		*Mouse*		*Rat*	
	NPB	*NPW*	*NPB*	*NPW*	*NPB*	*NPW*
Cerebellum	++[a]	++[a]	−[b]	−[b]	+[a]	ND
Parietal cortex	+[a]	++[a]	−[b]	−[b]	ND	ND
Hippocampus	++[a]	++[a]	++[b]	−[b]	++[a,c]	ND
Amygdala	++[a]	++[a]	−[b]	−[b]	ND	++[c]
Hypothalamus	++[a]	−[a]	+++[b]	−[b]	++[a,c]	++[c]
Thalamus	+[a]	−[a]	+[b]	−[b]	+[a]	ND
Caudate nucleus	+[a]	−[a]	−[b]	−[b]	ND	ND
Substantia nigra	+++[a]	+++[a]	−[b]	−[b]	++[c]	ND
Corpus callosum	++[a]	−[a]	−[b]	−[b]	ND	ND
Chorid plexus	+[a]	++[a]	−[b]	−[b]	ND	ND
Periaqueductal grey	ND	ND	−[b]	++[b]	ND	ND
Ventral tegmental area	ND	ND	−[b]	+[b]	ND	++[c]
Dorsal raphe nuclei	ND	ND	−[b]	+++[b]	++[c]	+++[c]
Edinger Westphal nuclei	ND	ND	+[b]	++[b]	++[c]	ND
Spinal cord	+++[a]	−[a]	ND	ND	+[a]	ND

Expression of NPB or NPW: ND, not determined; −, not detected; +, low levels; ++, moderate levels; +++, high levels, as determined by
[a]RT–PCR.
[b]*In situ* hybridisation, or
[c]Immunocytochemistry. These data are from Brezillon *et al*. (2003), Dun *et al*. (2005), Dun *et al*. (2003), Fujii *et al*. (2002), Singh *et al*. (2004) and Tanaka *et al*. (2003).

in part be attributed to circadian rhythm, as $NPBW_1$ receptors have been localised to the SCh (Lee *et al*., 1999; Singh *et al*., 2004). Thus, the effect of NPB/NPW treatment may depend on the stage of light/dark cycle. However, there is little evidence suggesting that feeding behaviour in male rats administered NPB/NPW differs during the light/dark cycle and requires further investigation (Tanaka *et al*., 2003).

The chronic effects of $NPBW_1$ receptor modulation had not been evaluated until Ishii *et al*. (2003) generated mice with targeted disruption of the gene encoding $NPBW_1$ ($NPBW_1-/-$). Intriguingly, female $NPBW_1-/-$ mice remained similar to wild type in all phenotypes examined, whereas moderate adult onset obesity was evident in $NPBW_1-/-$ male mice, greatly exaggerated when animals were fed with a high-fat diet (Ishii *et al*., 2003). This was further emphasised by the generation of a strain of NPB−/− mice, in which adult onset obesity was specifically observed in males but not females (Kelly *et al*., 2005). The disruption to the receptor or peptide did not cause fatalities or attenuation of growth in either sex. The mechanism of obesity was further investigated and importantly shown to be independent of the leptin and melanocortin signalling pathway (Ishii *et al*., 2003). Furthermore, mRNA levels of NPY were reduced, whereas proopiomelanocortin (POMC) was increased compared to wild-type male mice, indicative of a lean rather than obese state. Importantly, POMC is the precursor to opioid peptides and melanocortin (Low, 2004). Although melanocortin is thought to be an anti-orexigenic peptide (O'Rahilly *et al*., 2003), evidence suggests it may also act as an orexigen (Appleyard *et al*., 2003).

In $NPBW_1$ −/− male mice, spontaneous locomotor activity was decreased as were both resting O_2 consumption and CO_2 production (Ishii *et al*., 2003). Although the genes for NPB, NPW and $NPBW_1$ have not been manipulated in rats, male rats administered NPB show significantly increased locomotor activity (Baker *et al*., 2003), which correlates with the data from the mouse $NPBW_1$ knockout studies (Ishii *et al*., 2003). Interestingly, i.c.v. administration of NPW in rats increased c-Fos IR in neurones expressing hypocretins in the LH (Levine *et al*., 2005). The hypocretins are peptides, which are associated with wakefulness (Berridge & Espana, 2005), and it can be speculated that the hypocretins may be involved in NPB/NPW-induced increases in spontaneous locomotor activity, which may in turn cause acute hyperphagia (Williams *et al*., 2001). Hence, this is consistent with the development of obesity if the $NPBW_1$ receptor is blocked or expression is disrupted, as chronic calorie intake would exceed energy expenditure. However, Mondal *et al*. (2003) were not able to detect enhanced locomotor activity when NPW was administered to rats, but they did report increased O_2 consumption and increased CO_2 production while also demonstrating an increase in body temperature when compared with rats administered vehicle alone. This latter effect, independent of locomotor activity, may result from protein and triacylglycerol catabolism induced by corticosterone.

A second significant phenotypic characteristic of $NPBW_1-/-$ male mice is that they become progressively hyperglycaemic and hyperinsulinaemic (Ishii *et al*., 2003). As indicated previously, these effects were specific to male mice, as female $NPBW_1$ −/− mice did not differ from their wild type (Ishii *et al*., 2003). The reason for these sexual dimorphic effects could, in part, be explained by the reduced localisation of NPW-like IR in female rats when compared to male rats (Dun *et al*., 2003). Although immunocytochemistry is not quantitative, it allows speculation that NPW (and NPB) may be more abundant in male mice in relation to their female counterparts. However, the sexual dimorphic results demonstrated for $NPBW_1$ are not unique. Female wild-type rats with lesions to the amygdala complex have been shown to gain significantly more weight owing to hyperphagia compared to male rats (King *et al*., 1999). It is possible that $NPBW_1$ may be a sex-specific receptor, regulating a novel-independent arm in the complex process of energy homeostasis. It should be emphasised that these results are from only a limited number of papers and further studies are required to support these hypotheses.

Hormone release

The distribution of $NPBW_1$ in the PVN, SO, Arc and SCh (Lee *et al*., 1999) combined with the detection of $NPBW_1$ in the pituitary gland (O'Dowd *et al*., 1995; Fujii *et al*., 2002; Brezillon *et al*., 2003) strongly suggest the NPW transmitter system to regulate the release of pituitary gland-derived hormones (O'Dowd *et al*., 1995; Shimomura *et al*., 2002). NPB/NPW infusion into rats has since been demonstrated to induce secretion of corticosterone and prolactin while inhibiting growth hormone release dose dependently (Baker *et al*., 2003; Samson *et al*., 2004). NPB or NPW did not affect the release of other anterior pituitary-derived hormones. These endocrine effects of NPB/NPW administration were not mediated by direct binding of ligand to $NPBW_1$ on the pituitary but indirectly, possibly by regulating the release of CRF, dopamine and GHRH/somatostatin (Baker *et al*., 2003; Samson *et al*., 2004). One mechanism of NPB-mediated release of corticosterone was attributed to the effects of the release of CRF (which normally induces adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, which in turn promotes corticosterone secretion from the adrenal cortex) as neutralisation using anti-CRF serum resulted in significant attenuation of NPB-mediated release of corticosterone (Samson *et al*., 2004). This is supported by the observation that PVN cells depolarise in response to NPW application, resulting in increased plasma corticosterone (Taylor *et al*., 2005). This effect is attenuated when rats are pretreated with a CRF antagonist (Taylor *et al*., 2005). Thus, there is strong evidence suggesting that NPW induced secretion of corticosterone is, in part, mediated through $NPBW_1$ receptors in the PVN, which induce CRF release into the median eminence; CRF then acts on the endocrine cells of the anterior pituitary to release ACTH, which in turn results in the secretion of corticosterone from the adrenal gland (Figure 3). This latter hormone then has effects on various organs of the body, most prominently to induce gluconeogenesis among other important effects such as modulating the body's response to stress and regulating the immune system (Dallman *et al*., 2004).

Pain

This novel transmitter system has also been implicated in modulating responses to acute inflammatory pain. Originally, Tanaka *et al*. (2003) demonstrated an analgesic effect of i.c.v. injection of NPB in rats. This initial work has been progressed and intrathecal (i.t.) injection of either NPB or NPW at the level of the lumbar enlargement has been shown to induce an

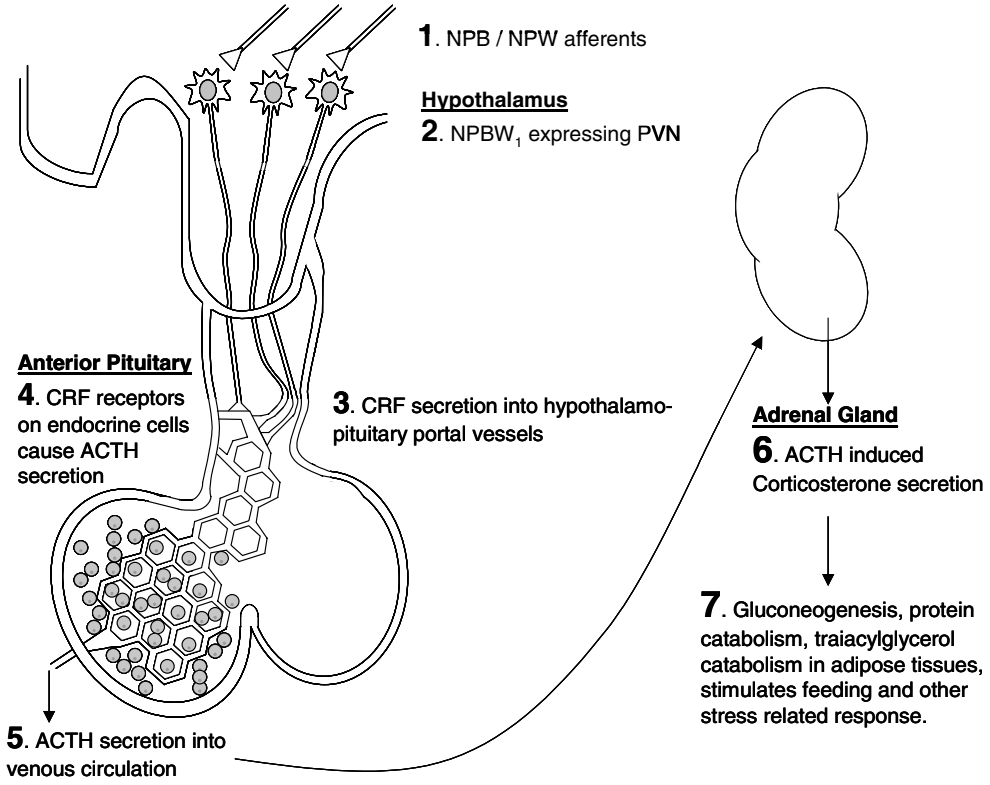

Figure 3 Mechanism of corticosterone secretion induced by NPB/NPW. NPB/NPW released from afferents terminating at the PVN cause depolarisation, resulting in the release of CRF from the PVN into the hypothalamopituitary portal vessels. Once arriving at the anterior pituitary, CRF acts on the cells to induce secretion of ACTH, which is then released in the venous circulation. On arriving to the adrenal cortex, ACTH acts to promote the secretion of corticosterone, which then has its effects on glucose production, protein and triacylglycerol catabolism as well as stimulating appetite (Dallman *et al*., 2004; la Fleur *et al*., 2004).

analgesic effect on pain caused by subcutaneous application of formalin (Yamamoto *et al*., 2005). Interestingly, i.t. injection of these peptides at the mid-thoracic level did not show any significant effects when compared to vehicle controls (Yamamoto *et al*., 2005). Similarly, NPB and NPW exhibited a significant anti-allodynia effect on rats challenged with carrageenan (Yamamoto *et al*., 2005). These effects were not attenuated by the opioid receptor antagonist naloxone, suggesting an independent analgesic and anti-allodynia pathway compared to the opioid peptides (Yamamoto *et al*., 2005). On the contrary, both NPB and NPW were ineffective in reducing the pain response to thermal hyperalgesia or mechanical nociceptive tests. Mouse models in which the mRNA encoding NPB has been knocked out show a typical phenotype expected from the above-described physiology (Tanaka *et al*., 2003; Kelly *et al*., 2005; Yamamoto *et al*., 2005). These mice demonstrated significant hyperalgesia to acute inflammatory stimulus, induced by intraperitoneal application of acetic acid, when compared with wild-type mice (Kelly *et al*., 2005). In agreement with results obtained in rats, these NPB knockout mice did not demonstrate hyperalgesia to chemical or thermal pain (Kelly *et al*., 2005). Therefore, activation of $NPBW_1$ at the spinal level has been shown to produce an analgesic and anti-allodynia effect in response to acute inflammatory pain (Yamamoto *et al*., 2005, 2006), consistent with the reported effect of upregulation of $NPBW_1$ in nerves of the peripheral nervous system during the pathogenesis of inflammatory neuropathies (Zaratin *et al*., 2005).

Future directions

Two components of the NPB/NPW system have been disrupted using knockout mice: the ligand NPB and the receptor $NPBW_1$. Further studies on $NPBW_1-/-$ mice will advance the understanding of the role of this receptor. As both peptides are able to bind this receptor, interpreting the results of deleting the *NPB* gene is more difficult because the receptor may be activated by NPW. Future studies are required in which NPW is deleted. Interestingly, preliminary studies suggest that NPW knockouts (Kelly *et al*., 2005) may have a different phenotype to that of NPB. This suggests that the two peptides might be present in distinct compartments and have different physiological roles. Crosses between the two phenotypes may elucidate these functions. Development of an antagonist for $NPBW_1$ is crucial in clarifying the physiological function of this novel transmitter system. Furthermore, the role of $NPBW_2$ also requires investigation, as this is one of a small number of GPCRs that have no rat or mouse orthologue.

Orphan GPCRs from Class 1, recently paired with their cognate ligands, are emerging as important targets for the development of novel drugs (Wilson *et al*., 1998; Lin & Civelli, 2004). $NPBW_1$ has been demonstrated to modulate feeding, metabolism and to be involved in obesity, which intriguingly appears to be specific to male mice. Whether this is also true in humans remains to be discovered. Interestingly, in humans, abdominal obesity is more prevalent in males and is an

independent risk factor for coronary heart disease (Lakka *et al*., 2002). Thus far, activating or disrupting the $NPBW_1$ transmitter system (Ishii *et al*., 2003; Kelly *et al*., 2005), at least in mice, did not have pronounced effects on growth and mortality, which is an important consideration for initiating a drug discovery programme based on this emerging transmitter system whether as a potential target for treatment of inflammatory mediated pain or for modulating feeding behaviour.

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