Potential of Neuropeptide Y for Preventing or Treating Post-Traumatic Stress Disorder

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Abstract

There is extensive evidence that NPY in the brain can modulate the responses to stress and play a critical role in **resistance to, or recovery from**, harmful effects of stress. Development of PTSD and comorbid depression following exposure to traumatic stress are associated with low NPY. This review discusses putative mechanisms for NPY's anti-stress actions. Recent preclinical data indicating potential for intranasal delivery of NPY to brain as a promising non-invasive strategy to prevent a variety of neuroendocrine, molecular and behavioral impairments in PTSD model are summarized.

Neuropeptide Y and its receptors

Neuropeptide Y (NPY), so named for the abundance of tyrosine (Y) residues (5/36 amino acids, including the amino and carboxyl terminal residues), has widespread functions in CNS and periphery. These include regulation of feeding behavior, blood pressure, circadian rhythm, reproductive behavior, learning, memory, vascular remodeling, cell proliferation, angiogenesis, as well as behavioral responses to stress, mood disorders and alcoholism (Eaton *et al.* 2007, Heilig 2004, Heilig *et al.* 1989, Hirsch & Zukowska 2012).

In the periphery NPY is expressed primarily in sympathetic ganglia, the adrenal medulla and in platelets. NPY is one of the most widely distributed and abundant neuropeptides in the mammalian brain. NPY immunopositive cell bodies and fibers are generally found in cortical, limbic, hypothalamic, and brainstem regions. Expression of NPY in the human and rodent brain is similar, with abundant NPY mRNA or immunoreactivity located in the neocortex, amygdala, hippocampus, basal ganglia, hypothalamus, periaqueductal grey, dorsal raphe nucleus, and the A1-3 and A6 noradrenergic cells groups in the brainstem [reviewed in (Kask *et al.* 2002b)].

The biological effects of NPY are mediated by activation of at least four G-protein coupled receptor subtypes: Y1, Y2, Y4, and Y5 (Michel *et al.* 1998, Hirsch & Zukowska 2012, Redrobe *et al.* 2002). The Y6 subtype is not present in the rat while the human analogue is truncated and non-functional. Y1, Y2 and Y5 receptors exhibit dense and overlapping gene expression in brain areas implicated in anxiety and depression including the hippocampus, bed nucleus of stria terminalis, hypothalamus, amygdala, locus coeruleus and prefrontal cortex. The distribution of the Y4 receptor is much more limited in brain areas involved in stress. All NPY receptors are G protein-coupled receptors that can regulate several signaling cascades leading to rapid responses as well as changes in gene transcription. Receptors associate with Gi/Go

proteins, which trigger hyperpolarization by inhibiting calcium channels, activating GIRK (G protein-coupled inwardly rectifying potassium) channel activity or I_H channels. They inactivate adenylyl cyclase and thus cAMP dependent pathways and mobilize intracellular calcium by phospholipase C and phosphatidyl inositol kinase. They can lead to changes in gene expression by way of ERK or CREB signaling [rev. in (Brothers & Wahlestedt 2010, Sah & Geraciotti 2013a)].

Neuropeptide Y and Resilience to Harmful Effects of Stress:

There is now abundance of evidence that neuropeptide Y (NPY) can modulate the responses to stress and may play a critical role in resilience to harmful effects of stress. This information is covered in a number of excellent review articles, which show how NPY is inversely related to stress associated neuropsychiatric disorders, including PTSD and comorbid depression, and as well as the selective NPY receptor subtypes implicated in mediating these effects (Eaton *et al.* 2007, Wu *et al.* 2011, Sah & Geraciotti 2013b, Enman *et al.* 2015, Heilig 2004, Kask *et al.* 2002b, Rasmusson *et al.* 2010, Kormos & Gaszner 2013, Bowers *et al.* 2012, Pedrazzini *et al.* 2003, Reichmann & Holzer 2015).

In soldiers or in trauma exposed veterans increased plasma NPY levels are associated with positive coping mechanisms (Morgan *et al.* 2001, Morgan *et al.* 2002, Yehuda *et al.* 2006). Significantly lower plasma and CSF concentrations of NPY were found in individuals with combat-related PTSD than in control subjects (Sah *et al.* 2009, Rasmusson *et al.* 2000). Depression is commonly co-morbid with PTSD and about half the patients with PTSD also have symptoms of depression. In this regard, decreased NPY levels in CSF (Heilig & Widerlöv 1990, Hou *et al.* 2006) and plasma (Nilsson *et al.* 1996) are also observed in depressed patients.

Genetic studies in humans and rodents found that lower NPY levels are associated with more anxiety and higher reactivity to emotional and stressful challenges (Bannon *et al.* 2000, Heilig 2004, Zhou *et al.* 2008, Mickey *et al.* 2011, Domschke *et al.* 2010). Conversely, overexpression of NPY in hippocampus or amygdala in transgenic rats or with viral vectors produced anxiolytic-like effects (Thorsell *et al.* 2000, Christiansen *et al.* 2014).

A number of pharmaceutical studies have demonstrated that injections of NPY into the brain ventricles or locally into hippocampus, amygdala or locus coeruleus, has anxiolytic and anti-depressive effects, and inhibits many stress induced behaviors (Kask *et al.* 2002a, Sajdyk *et al.* 2002a, Sajdyk *et al.* 2002b, Primeaux *et al.* 2005, Kask *et al.* 1998). Overall the studies suggest that NPY acts with a high potency on core mechanisms of emotionality and behavioral stress responses (Heilig & Thorsell 2002).

This review will concentrate on discussing the potential mechanisms for NPY's anti-stress actions and summarize recent data indicating its potential for preventing PTSD and comorbid disorders.

Putative Mechanisms for NPY's Anti-stress Effects

Counteracting actions of pro-stress transmitters in various brain regions

A number of brain areas are implicated in mediating NPY's anti-stress effects. It has been proposed that NPY is needed to adequately terminate corticotrophin releasing hormone (CRH) triggered responses to stress (Heilig 2004, Palkovits 2008). NPY and CRH co-localize in stress regulatory brain regions, such as the amygdala, hypothalamus and the bed nucleus of stria terminalis. NPY can compete with CRH reducing its anxiogenic effect in the extrahypothalamic

regions involved in regulation of anxiety and fear [rev. in (Thorsell 2010, Heilig *et al.* 1994, Sajdyk *et al.* 2004).

Amygdala: Functional antagonism between NPY and CRH has been observed in amygdala (Kask *et al.* 2002b, Giesbrecht *et al.* 2010) where both peptides regulate GABA neurotransmission in an opposite manner via their respective receptors (Kash & Winder 2006). The balance of NPY and CRH in the amygdala may be important in fear modulation and anxiety.

Hypothalamus: The relationship between CRH and NPY in the hypothalamus may be more complex. The hypothalamic CRH producing paraventricular nucleus (PVN) has a high density of NPY-containing nerve terminals (Liposits *et al.* 1988). NPY projections from both the brain stem and arcuate nucleus are in close apposition to CRH cell bodies and fibers. Under certain conditions NPY increases CRH gene expression and release (Dimitrov *et al.* 2007, Wahlestedt *et al.* 1987, Suda *et al.* 1993, Pronchuk *et al.* 2002). However its inhibitory effects on the CRH-producing neurons in the PVN have also been demonstrated (Horvath *et al.* 1997). Accordingly, NPY administered continuously for 3 days into the cerebral ventricle reduced CRH mRNA in the PVN (Füzesi *et al.* 2007). Our studies revealed that intranasal NPY given at end of traumatic stressors in single prolonged stress (SPS) rodent PTSD model prevented the SPS-elicited rise of CRH and FKBP5 mRNAs in the mediobasal hypothalamus measured a week later, a time for manifestation of behavioral impairments (See table). Thus NPY may enable proper HPA feedback regulation (Laukova *et al.* 2014, Serova *et al.* 2013b). Overall, NPY may elicit diverse effects on the HPA axis depending upon the origin of NPY input and nature of the specific physiological stimuli (Palkovits 2008).

Hippocampus: NPY may also be acting via modulation of expression of glucocorticoid receptors (GR) and as a consequence GR dependent regulation of transcription of GR responsive genes. In the hippocampus, a key region in controlling learning and memory, GR mRNA and protein levels are up-regulated after exposure to severe traumatic stress (Liberzon *et al.* 1999, Kohda *et al.* 2007, Li *et al.* 2011, Serova *et al.* 2013b) and intranasal infusion of NPY (Serova *et al.* 2013a) (see table) or injections of NPY or NPY-Y1 agonist directly into the hippocampus (Cohen *et al.* 2011) attenuated the rise in GR.

Locus Coeruleus: NPY may likewise be able to alter the response of the noradrenergic system to stress. This effect of NPY is at least partially mediated by activation of pre-synaptic Y2 receptors therefore depressing the post-synaptic potential of the noradrenergic locus coeruleus (LC) neurons (Finta *et al.* 1992, Illes *et al.* 1993, Kask *et al.* 2002b). Attenuation of LC activation will lead to decreased activity of numerous regions innervated by the LC which are implicated in arousal, memory acquisition, attention, vigilance, etc. in response to stress (Foote *et al.* 1983, Valentino & Van Bockstaele 2008).

In sum, NPY likely acts in multiple brain regions to counteract the prolonged activity of pro-stress transmitters CRH and norepinephrine and consequently reduce fear and anxiety, improve stress coping and enable resilience to pathophysiological states such as PTSD.

Effects of stress on NPY gene expression

One of the issues regarding association of low NPY with sensitivity to stress, is whether these individuals have low NPY to begin with and thus are unable to overcome the trauma. Alternatively are they less able to elevate NPY neurotransmission in response to stress and thus need “assistance” during the time when NPY would normally be induced by the stress. Support

for the later idea comes from studies with functional rhesus monkey NPY promoter polymorphism (Lindell *et al.* 2010). A single nucleotide polymorphism (SNP) in the NPY promoter in rhesus macaques (-1002) was found to affect NPY expression as a function of early rearing history, with G, but not T allele showing lower NPY levels, as well as higher alcohol consumption and more arousal to separation stress. This promoter variation predicted loss of a glucocorticoid response element half site. Gel shift studies showed altered binding to several transcription factors including decreased GR binding. These studies suggest that induction of NPY transcription by glucocorticoids may interact with early life adversity and be important for mounting induction of NPY transcription with stress. NPY expression in CSF of these animals was reduced only in animals with G alleles who had early life adversity (reared by peers rather than mothers) as was stress reactivity and alcohol consumption.

Functional promoter polymorphisms in NPY were also found in Flinders genetic model of depression. Binding of the transcription factor CREB2 (ATF4) and a histone acetyltransferase (Ep300) were observed only at the SNP locus in Flinders Resistant but not in Flinders sensitive rats (Melas *et al.* 2013). Impairments of NPY and Y1 gene expression were seen in selective brain regions and the antidepressant fluoxetine treatment normalized NPY-related gene expression selectively in this strain (Caberlotto *et al.* 1998).

A number of studies have examined stress specific changes in NPY gene expression in the brain [reviewed in (Reichmann & Holzer 2015)] although most have not taken into account early life experience. Immobilization or restraint stress was found to elevate preproNPY mRNA in the rat arcuate nucleus within several hours (Makino *et al.* 2002, Conrad & McEwen 2000). Other studies found significant rise in preproNPY mRNA levels after acute and chronic restraint stress, especially in the arcuate nucleus, with significant changes also observed in the

hippocampus, although the response differed between normotensive and hypertensive rats (Sweerts *et al.* 2001).

Acute restraint stress suppressed preproNPY mRNA levels in the rat amygdala within 1 hour. However with repeated exposure to restraint preproNPY mRNA levels were instead up-regulated (Thorsell *et al.* 1998, Thorsell *et al.* 1999). Following chronic variable stress, there was a reduction in NPY protein and mRNA in the amygdala. Seven days later in recovery from chronic mild stress there was significant reduction of NPY protein in the amygdala with large increase in NPY protein and mRNA in the prefrontal cortex (McGuire *et al.* 2011). **Thus while stress can change NPY gene expression in the brain, the direction as well as the magnitude varies depending on the brain region, the type and duration of the stress.**

In addition to regulation of stress triggered changes in NPY gene expression, it is important to consider that expression of selective NPY receptors may also be affected by stress. The promoter for the Y1 receptor contains two cyclic AMP response elements and several glucocorticoid response elements (Bournat & Allen 2001) and indeed Y1 receptor subtype expression is modulated by stress and other brain insults [reviewed in (Eva *et al.* 2006)].

NPY as a Potential **Preventative or** Therapeutic Agent

NPY delivery to the brain

While the NPYergic system is a promising target for therapeutic interventions efforts to develop pharmacological agents with clinical relevance circumscribed NPY receptor mediated effects have so far been unsuccessful (Pitman *et al.* 2012). NPY administered peripherally can enter the brain, although not very effectively (Kastin & Akerstrom 1999). Additionally, it will likely have undesirable side effects, especially on the cardiovascular system. NPY is a co-

transmitter in sympathetic nerves and enhances the vasoconstrictive properties of norepinephrine (Zukowska-Grojec 1995, Edvinsson *et al.* 1984). Moreover, release of NPY from sympathetic nerve terminals in white fat stimulates fat angiogenesis, macrophage infiltration and the proliferation and differentiation of new adipocytes, resulting in abdominal obesity and a metabolic syndrome-like condition (Kuo *et al.* 2007). The effects of NPY in the periphery, including effects in sympathetic nerves, macrophages, endothelium, adipocytes and the gut were recently reviewed (Hirsh and Zulowska, 2012).

Brain penetrant NPY agonists/antagonists have potential to regulate the NPY system in the brain and thereby responses to stress. Several Y2 receptor antagonists have been reported to have good blood-brain penetration and function in the brain (Brothers *et al.* 2010, Shoblock *et al.* 2010). They might enhance NPYergic neurotransmission by antagonizing Y2 presynaptic autoreceptors thus relieving their inhibition of endogenous NPY release.

Intranasal infusion represents a non-invasive approach for the rapid delivery of peptides to the brain that can avoid side effects elicited by peripheral administration. This method allows peptides to rapidly and directly enter the central nervous system via intracellular neuronal olfactory and extracellular trigeminal-associated pathways bypassing the blood–brain barrier to affect multiple sites within the brain (Dhuria *et al.* 2010, Ionescu *et al.* 2012, Thorne *et al.* 1995, Thorne *et al.* 2004). The beneficial effects of NPY delivered into the brain by intranasal infusion are likely achieved by influencing NPY responsive systems in all regions responsible for pathophysiological features of PTSD. Therefore it can modulate their functional activity and lead to behavioral and neuroendocrine improvements. Thus, NPY could help the organism cope with the excessive stress.

To show the potential of intranasal NPY, rats were infused with several doses of NPY and CSF and blood were collected thirty minutes later. The concentration of NPY in the CSF from infusion of 90 µg NPY was increased to the proposed anxiolytic range based on icv injections (Gutman *et al.* 2008) without a significant change in plasma NPY (Serova et al. 2013b). Delivery of NPY to specific brain areas was shown after infusion of fluorescent-labeled NPY (FAM-NPY, Phoenix Pharmaceuticals). FAM-NPY was found in many brain regions, including olfactory bulbs, hypothalamus, hippocampus, locus coeruleus and amygdala 30 min later (Fig. 1).

Intranasal NPY attenuates response to traumatic stress in rats

Recent research demonstrates proof of concept that rapid passage of NPY into the brain by intranasal infusion modifies behavioral neurochemical and hormonal responses and reduces deleterious effects of traumatic stress (Serova et al. 2013a, Serova et al. 2013b, Laukova et al. 2014). Using two paradigms of NPY infusion, 30 min before (prophylactic), or immediately after traumatic stress (earlier intervention) our laboratory demonstrated that rapid delivery of NPY to rat brain has a pronounced, long lasting resilient effect and ameliorates development of many PTSD-comorbid behavioral symptoms (Serova et al. 2013b). These findings are summarized in Table 1. NPY administration normalized functional activities of both the hypothalamic-pituitary-adrenal axis (HPA) and the locus coeruleus noradrenergic system (Serova et al. 2013b, Sabban *et al.* 2015, Laukova et al. 2014), two major systems pivotal in the pathophysiology of PTSD

Encouragingly, intranasal NPY appears to be effective even when administered to rats after manifestation of PTSD-like symptoms (Serova et al. 2013a).

Translation implications

These exciting findings indicate that intranasal NPY is a promising potential non-invasive prophylactic treatment for individuals likely to be exposed to traumatic stress, such as early responders, warfighters and other military personnel, or to individuals soon after traumatic stress such as rape, car accident, terror attack or battle. Intranasal NPY **should be able to be** easily applied, or even self-administered, in the field during deployment. **However, translation of intranasal delivery of NPY to the human brain needs to take into that contrast to rodents, where olfaction plays a key sensory role, the human olfactory region of the nasal cavity is less prominent and not as easily accessible [reviewed in (Dhuria et al. 2010, Chapman *et al.* 2012)].**

Several studies have already administered exogenous NPY by intranasal infusion to humans. Intranasal NPY attenuated allergen evoked nasal responses by lowering airway resistance; vascular permeability and vascular blood flow suggesting its potential usefulness for congested nose in allergic or vascular rhinitis (Lacroix & Mosimann 1996, Lacroix *et al.* 1996, Cervin *et al.* 1999, Hallschmid *et al.* 2003, Hallschmid *et al.* 2004). Intranasal NPY acutely attenuated electrocortical signs of meal related satiety (Hallschmid et al. 2003, Hallschmid et al. 2004). A clinical trial (NCT 01533519) is currently underway with dose escalation of intranasal NPY to PTSD patients primarily to ascertain safety and any potential side effects .

Thus, intranasal administration of NPY or selective NPY receptor agonists may provide a new preventative as well as therapeutic approach for PTSD and comorbid disorders. The spectrum of traumatic stress elicited impairments which can be attenuated by NPY as well as the adoptability of intranasal NPY to humans are pivotal issues which need to be addressed.

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Figure Legends

Fig. 1. Fluorescence in various brain regions 30 min after intranasal infusion of FAM-labelled NPY. White arrows show selective labelled cells. Bar=100µm.

Table: Beneficial effects of intranasal NPY infusion. Results from rats given intranasal NPY or vehicle (V) on development of single prolonged stress (SPS) elicited impairments are summarized (Serova et al. 2013b, Laukova et al. 2014, Sabban et al. 2015).

Figure 1

Olfactory bulbs

Ventral hippocampus

Paraventricular
nucleus (PVN)

Locus coeruleus
(LC)

Amygdala

Central

Basolateral

Table(s)

Time after SPS Stressors		Intranasal Vehicle	Intranasal NPY
Stress hormones in plasma:			
Corticosterone	30 min	↑↑↑	↑↑
	7 days	↑	–
	30 min	↑↑↑	↑↑
	7 days	↑	–
Behavior:			
Anxiety (EPM)	7 days	↑	–
Anxiety (OF)	7 days	↑	–
Risk assessment (EPM)	7 days	↓	–
Grooming(EPM)	7 days	↑	↑
Depressive (FST)	7 days	↑	–
Hyperarousal (ASR)	7 days	↑ (high dB)	–
Locomotion (EPM, OF)	7 days	↓	↓
Body weight:	7-14 days	↓	↓
Mediobasal Hypothalamus:			
CRH mRNA	7 days	↑	–
CRH immunofluorescence	7 days	↓	↓
GR mRNA	7 days	–	–
pGRSer232	7 days	↓	–
FKBP5 mRNA	7 days	↑	–
Hippocampus:			
GR protein	7 days	↑	–
pGRSer232	7 days	↑	–
Central Amygdala:			
CRH immunofluorescence	7 days	↑	–
Locus Coeruleus:			
TH mRNA	30 min	↑↑	↑
TH mRNA	7 days	–	–
TH protein	7 days	↑	–
CRHR1 mRNA	7 days	↑ (subset)	–
Y2R mRNA	7 days	↓	–