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SINGLE INTRANASAL NEUROPEPTIDE Y INFUSION ATTENUATES DEVELOPMENT OF PTSD-LIKE SYMPTOMS TO TRAUMATIC STRESS IN RATS

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Abstract—Exposure to severe stress leads to development of neuropsychiatric disorders, including depression and Post-Traumatic Stress Disorder (PTSD) in at-risk individuals. Neuropeptide Y (NPY) is associated with resilience or improved recovery. Therefore exogenous administration to the brain has therapeutic potential although peripheral administration can trigger undesirable side effects. Here, we established conditions with intranasal (IN) NPY infusion to rats to obtain CSF concentrations in the proposed anxiolytic range without significant change in plasma NPY. Rats were pretreated with IN NPY or vehicle before exposure to single prolonged stress (SPS) animal model of PTSD and compared to untreated controls. The IN NPY appeared to lessen the perceived severity of stress, as these animals displayed less time immobile in forced swim part of the SPS. Thirty minutes after SPS the elevation of plasma adrenocorticotropic hormone (ACTH) and corticosterone was not as pronounced in NPY-infused rats and the induction of tyrosine hydroxylase (TH) in locus coeruleus (LC) was attenuated. Seven days after SPS, they displayed lower depressive-like behavior on Forced Swim Test and reduced anxiety-like behavior on Elevated Plus Maze. The prolonged effect of SPS on Acoustic Startle Response was also lower in NPY-infused rats. Plasma ACTH, corticosterone, and hippocampal glucocorticoid receptor levels were significantly above controls only in the vehicle – but not IN NPY-treated group 1 week after SPS. Baseline TH mRNA levels in LC did not differ among groups, but increased with forced swim in the vehicle – but not NPY-pretreated animals. Administration of IN NPY after exposure to SPS led to similar, but not identical, reduction in development of anxiety, depressive-like behavior and hyperarousal. The results show that single IN NPY can alter stress-triggered dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and activation of central noradrenergic activity. These findings provide proof of concept for potential of IN NPY for non-invasive prophylactic treatment or early intervention in response to traumatic stress.

Key words: single prolonged stress, locus coeruleus, HPA axis, TH mRNA, glucocorticoid receptor, intranasal neuropeptide Y administration.

INTRODUCTION

Exposure to severe life-threatening or traumatic events increases the risk of neuropsychiatric disorders, including emergence of symptoms of Post-Traumatic Stress Disorder (PTSD) in subpopulation of these individuals (Kessler, 2000; Lupien et al., 2009; Skelton et al., 2012). PTSD is a debilitating disorder associated with functional impairments, physical health concerns and mental health comorbidities, such as depression, with a sixfold higher risk of suicide. The treatment of PTSD is extremely challenging, and may include many years of individual and group therapy and medications such as antidepressants, anxiolytic drugs, β-adrenergic antagonists, opiates, or cortisol with variable results [rev. in Bowirrat et al., 2010; Kar, 2011]. Better therapies are clearly needed as are PTSD prevention techniques (Baker et al., 2009; Sones et al., 2011). Because PTSD results from a specific identifiable event, the disorder lends itself to the application of prevention strategies for at-risk individuals.

Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and increased noradrenergic activity, both centrally and peripherally, are usually observed in PTSD patients (Southwick et al., 1999; Yehuda, 2002, 2005; O'Donnell et al., 2004; Strawn and Geracioti, 2008; Pervanidou and Chrousos, 2010). In contrast, increased neuropeptide Y (NPY) in the CNS and plasma is associated with resilience or improved recovery from harmful effects of traumatic stress (Eaton et al., 2007; Wu et al., 2011; Sah and Geracioti, 2012). Men with combat-related PTSD display significantly lower CSF concentrations of NPY than control subjects (Rasmusson et al., 2000; Sah et al., 2009). In trauma-exposed veterans plasma NPY levels are associated with positive coping mechanisms (Yehuda et al., 2006).

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Abbreviations: ASR, Acoustic Startle Response; ACTH, adrenocorticotropic hormone; CA, closed arms; CRH, corticotropin-releasing hormone; DBH, dopamine beta-hydroxylase; EDTA, Ethylenediaminetetraacetic acid; EPM, Elevated Plus Maze; FS, forced swim; FST, Forced Swim Test; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; icv, intracerebroventricular; IN, intranasal; LC, locus coeruleus; mPFC, medial prefrontal cortex; MR, mineralocorticoid receptors; NE, norepinephrine; NPY, Neuropeptide Y; OA, open arms; PCR, polymerase chain reaction; PTSD, Post-Traumatic Stress Disorder; PVN, paraventricular nucleus; TBS, Tris-buffered saline; TH, tyrosine hydroxylase; SPS, single prolonged stress.

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Moreover, decreased NPY levels in the CSF (Heilig and Widerlöv, 1990; Hou et al., 2006) and plasma (Nilsson et al., 1996) were also found in depressed patients.

NPY, one of the most abundant and widely distributed neuropeptides in the mammalian brain, has diverse functions in the CNS and periphery including regulation of feeding behavior, blood pressure, circadian rhythm, reproductive behavior, as well as behavioral responses to stress (Heilig et al., 1989; Heilig, 2004; Eaton et al., 2007; Hirsch and Zukowska, 2012). Some of the best evidence for beneficial effects of NPY in coping with stress is derived from genetic studies. Human NPY gene haplotypes with lower NPY expression are associated with trait anxiety and predict higher amygdala reactivity to emotional and stressful challenges (Zhou et al., 2008). Higher anxiety levels were also found in NPY deficient mice (Bannon et al., 2000; Heilig, 2004). In contrast, overexpression of NPY in the hippocampus of transgenic rats markedly reduces sensitivity to anxiogenic-like effects of stress (Thorsell, 2010).

Delivery of exogenous NPY to rodents by intracerebroventricular (icv) injections or locally into several brain areas, attenuated some of the behavioral responses related to stress-associated neuropsychiatric disorders. It exerts antidepressant-like effects on Forced Swim Test (FST) (Stogner and Holmes, 2000), inhibited the expression and extinction of fear-potentiated startle (Gutman et al., 2008), blunted the tachycardial response to fear conditioned and unconditioned auditory stimulation (Tovote et al., 2004) and has anxiolytic-like effects in animal anxiety models (Heilig et al., 1989; Karlsson et al., 2005). Local infusion of NPY or receptor agonists into the amygdala or locus coeruleus (LC) also reduced anxiety-like behavior (Kask et al., 1998, 2002a; Sajdyk et al., 2002a,b; Primeaux et al., 2005). However, these studies examined short-term responses to exogenous NPY from 15 min to 1–2 days after administration. The ability of NPY to change the long-term trajectory of stress responses is unclear. Recently, infusion of NPY into the rat hippocampus 1 h after predator-scent stress, one of the animal models of PTSD, reduced some of the PTSD-associated symptoms 1 week after the stress (Cohen et al., 2012).

While the NPYergic system is a promising target for therapeutic interventions (Wu et al., 2011; Sah and Geracioti, 2012), efforts to develop pharmacological agents with clinical relevance circumscribed by NPY receptor-mediated effects have so far been unsuccessful (Pitman et al., 2012). NPY administered peripherally can enter the brain (Kastin and Akerstrom, 1999). However, 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 (NE) (Edvinsson et al., 1984; Zukowska-Grojec, 1995). Indeed, transgenic mice with over-expression of NPY in noradrenergic neurons display stress-induced hypertension and increased sympathetic activity, although they have somewhat lower anxiety (Ruohonen et al., 2009).

Here, we test the ability of NPY to attenuate the development of prolonged behavioral and biochemical consequences of traumatic stress with the single prolonged stress (SPS) animal model of PTSD. SPS combines psychological, physiological and endocrine components of stress, to provide an animal model with both high face and construct validity (Khan and Liberzon, 2004; Liberzon and Sripada, 2008; Yamamoto et al., 2009). NPY is administered by intranasal (IN) infusion, a non-invasive way to deliver drugs to the brain allowing a viable and very attractive approach especially for peptides (Fehm et al., 2000; Born et al., 2002; Costantino et al., 2007). This method allows drugs to rapidly and directly enter the CNS via intracellular neuronal olfactory and extracellular trigeminal-associated pathways bypassing the blood–brain barrier to affect multiple sites within the brain (Thorne et al., 1995, 2004; Dhuria et al., 2010; Ionescu et al., 2012).

Our results revealed, for the first time, that single IN NPY administration before or shortly after SPS can ameliorate many of the behavioral and molecular changes to traumatic stress. It attenuated the prolonged SPS-triggered rise in anxiety, depressive-like behavior, and acoustic startle. These behavioral changes were associated with both short-term and prolonged changes in the HPA axis and central noradrenergic activity.

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by Institutional Animal Care and Use Committee at NYMC. Male Sprague–Dawley rats from Charles River (Wilmington, MA, USA) weighing 150–160 g at arrival were housed in a barrier area on 12-h light/dark cycle at 23 ± 2 °C with *ad libitum* access to food and water. Animals were acclimated for 14 days, four per cage, and then randomly assigned to the experimental or control groups (8–12 animals each) and housed two per cage. Controls without any treatment were handled for 4 days, 5 min/day before behavioral or biochemical evaluations. The body weight was monitored during the experimental procedures. All efforts were made to minimize the number of animals used and their suffering.

Overall experimental design

Animals were treated with IN infusion of NPY or vehicle 30 min before or shortly after SPS and left undisturbed for 7 days for the symptoms to develop (Liberzon et al., 1999b; Kohda et al., 2007). Then the SPS-treated animals and untreated controls were evaluated for the following behavioral tests: FST, Elevated Plus Maze (EPM) or Acoustic Startle Response (ASR). To avoid carryover from one test to another, each behavioral test was performed on separate groups of animals. To study the mechanism and changes in response of the HPA axis and the central noradrenergic system, animals pretreated with NPY or vehicle were euthanized 30 min or 7 days after the SPS. Corticosterone and adrenocorticotropic hormone (ACTH) levels were determined in the plasma and glucocorticoid receptor (GR) protein levels in several brain locations. Levels of tyrosine hydroxylase (TH) mRNA were evaluated in LC 30 min after SPS, as well as 7 days post-SPS under basal conditions and subsequent exposure to forced swim (FS).