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Plasma NPY concentrations during tryptophan and sham depletion in medication-free patients with remitted depression

Christoph Czermak^a, Richard Hauger^b, Wayne C. Drevets^c, David A. Luckenbaugh^c, Marilla Geraci^c, Dennis S. Charney^d, and Alexander Neumeister^{a,*}

^a Department of Psychiatry, Yale University School of Medicine, New Haven, CT and VA Connecticut Healthcare System, West Haven, CT, USA

^b San Diego VA Healthcare System and University of California, San Diego, Department of Psychiatry, School of Medicine, La Jolla, CA, USA

^c Mood and Anxiety Disorders Program, NIMH, Bethesda, MD, USA

^d Mount Sinai School of Medicine, New York, NY, USA

Abstract

Background—Neuropeptide Y (NPY) and serotonergic systems have been implicated in the pathophysiology of depression but have not yet been linked together.

Methods—In a randomized, double-blind crossover study, 28 medication-free patients with remitted depression and 26 healthy control subjects underwent tryptophan depletion (TD) and sham depletion. Plasma NPY concentrations were determined at baseline and at +5, +7, and +24 h during TD and sham depletion, respectively. Hamilton Depression Rating Scale (HDRS, 24-item) scores were assessed at baseline and at +7 and +24 h after TD and sham depletion, respectively.

Results—There was no difference between healthy subjects and patients with remitted depression in baseline plasma NPY concentrations and in plasma NPY concentrations during TD and sham depletion, respectively. Plasma NPY concentrations did not differ between TD and sham depletion. At no time point there was an association between HDRS scores and plasma NPY concentrations in patients with remitted depression.

Limitations—Plasma NPY concentrations in rMDD patients were not obtained during the symptomatic phase of the illness. Only peripheral measurements of NPY were used.

Conclusions—Decreased plasma NPY concentrations, as described previously during a spontaneous episode of major depression, appear as state but not as trait marker in depression. No evidence was found for an involvement of plasma NPY in relapse during TD. There appears no direct functional link between serotonergic neurotransmission and plasma NPY concentrations.

Keywords

Major depressive disorder; Serotonin; Neuropeptide Y; Tryptophan depletion; Neurobiology

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* Corresponding author. Yale University School of Medicine, Molecular Imaging Program of the Clinical Neuroscience Division, 950 Campbell Ave., West Haven, CT 06516, USA. Tel.: +1 203 932 5711x2428; fax: +1 201 937 3481. alexander.neumeister@yale.edu (A. Neumeister).

Conflict of interest

All authors declare that they have no conflicts of interest.

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid, amidated peptide, which is abundantly found in the central and peripheral nervous system (Lundberg, 1996). In the brain, NPY has been found enriched in hypothalamus, neocortex, amygdala, locus coeruleus and hippocampus and has been implicated in modulation of stress response, feeding behavior, circadian rhythms and reproductive behavior (Carrasco and Van de Kar, 2003; Lundberg, 1996). In the periphery, NPY is released into plasma from sympathetic nerve terminals of perivascular fibers (Lundberg, 1996).

Plasma NPY concentrations appear to have particular relevance in stress and stress-related disorders. Plasma NPY concentrations rise after exposure to several stressors and are part of peripheral stress response (Carrasco and Van de Kar, 2003; Lundberg, 1996). Increases in plasma NPY during experimental stress exposure in humans were found negatively correlated with experienced psychologic distress (Morgan et al., 2002). In patients with posttraumatic stress disorder, baseline plasma NPY concentrations were found negatively correlated with the frequency of exposure to traumatic experience (Morgan et al., 2003).

Alterations in stress response have been implicated in the pathophysiology of major depressive disorder (MDD) (Gold and Chrousos, 2002). As plasma NPY is part of the peripheral stress response, alterations of plasma NPY concentrations in MDD may therefore be part of stress response dysregulation in MDD. Also, stress has been shown to often precede relapse in MDD (Kendler et al., 2001). Therefore one may hypothesize that persisting alterations in NPY during remission in MDD may contribute to an enhanced risk of relapse.

Alterations of NPY have been implicated in the pathophysiology of depression. Animal models of depression have reported region-specific alterations in central NPY levels and antidepressant treatment has been shown to alter central NPY synthesis (Heilig, 2004). In humans, decreased cerebrospinal fluid (Heilig et al., 2004) and plasma (Hashimoto et al., 1996) NPY concentrations were found during a spontaneous episode of MDD, irrespective of medication status.

It is unclear however, whether decreased plasma NPY concentrations represent a trait or state marker of MDD. It is also unclear, whether alterations of NPY in depression are linked to serotonergic alterations, which have been centrally implicated in the pathophysiology of depression (Kalia, 2005). There may be a link between serotonergic neurotransmission and plasma NPY concentrations, as release of NPY into plasma depends upon sympathetic activity (Lundberg, 1996), which has been shown to be modulated by serotonergic neurotransmission (Ramage, 2001).

In the present study we studied baseline plasma NPY concentrations in medication-free, remitted MDD (rMDD) patients and healthy subjects. In addition, we studied plasma NPY concentrations during tryptophan depletion (TD) and sham depletion (SD), respectively.

To test the hypothesis that decreased plasma NPY concentrations represent a trait abnormality in MDD, we hypothesized that rMDD patients would have decreased plasma NPY concentrations relative to healthy subjects. Assuming that there is a functional connectivity between serotonergic neurotransmission and plasma NPY concentrations and that this crosstalk is altered in MDD, we also hypothesized a different pattern of NPY response during TD in rMDD patients and healthy subjects.

During TD, at least a subgroup of rMDD patients shows a transient re-occurrence of depressive symptoms (Neumeister et al., 2004). Assuming that alterations in plasma NPY

concentrations in rMDD patients may be involved in relapse we hypothesized that plasma NPY concentrations in rMDD patients will be associated with re-occurrence of depressive symptoms during TD.

2. Methods and materials

Twenty-eight medication-free fully remitted patients with MDD according to DSM-IV criteria (20 women; mean age=39.5 years [SD=12.5]; mean baseline 24-item Hamilton depression rating scale (HDRS) total score=1.0 [SD=1.2]; age at onset=23.1 years [SD=8.3]; number of previous episodes=3.5 [SD=2.6]) and 26 healthy subjects (18 women; mean age=33.8 years [SD=11.2]; mean baseline 24-item HDRS score=0.4 [SD=0.7]), all non-smokers, participated in the study. Subjects were entered into the study after written informed consent had been obtained as approved by the NIMH Institutional Review Board.

Duration of the depressive illness and number of episodes were estimated from the Past History of MDD addendum of the Structural Clinical Interview for DSM-IV. Information about family history of mental illness (Axis I diagnoses) was obtained from the study participants for all first-degree relatives using the Family Interview for Genetic Studies (Maxwell, 1992). Controls had no personal or first-degree relative history of psychiatric disorders. For rMDD patients, no lifetime diagnosis other than MDD was allowed. Remission was defined as at least 3 months during which the individual did not take an antidepressant agent and had 24-item HDRS scores in the non-depressed range (<8) (Frank et al., 1991). The depressed patients were in remission for a mean of 40.4 months (SD=48.4) and had not been taking anti-depressant medication for a mean of 37.6 months (SD=43.9) at the time of the study. No use of medication was allowed during the study. Subjects were medically healthy as determined by history and results of physical examination, electrocardiogram and laboratory tests, including liver and kidney function tests, hematologic profile, thyroid function tests, urinalysis and toxicology. Women were studied during the follicular phase of the menstrual cycle.

In a randomized, placebo-controlled, balanced, double-blind crossover study, participants received during TD capsules containing an amino acid mixture without tryptophan, and during SD identical capsules containing lactose. Test days were separated by at least 7 days. Plasma NPY concentrations scores were measured at baseline and +5, +7, and +24 h after administration of the capsules. Twenty-four item HDRS scores were assessed at baseline and +7 and +24 h after administration of the capsules.

Plasma concentrations of NPY were measured using our previously developed radioimmunoassay (Allen et al., 1991; Rasmusson et al., 1998). Plasma samples were prepared by completing an acid ethanol extraction. The extraction recovery was ~70%. After lyophilized extracts were reconstituted in assay buffer, NPY was detected using a highly sensitive and specific NPY antibody. The NPY assay working range was 19.5 to 1250 pg/ml, and the assay sensitivity was ~15 pg/ml. The assay intra- and inter-assay coefficients of variation were ~4% and ~14%, respectively.

For plasma tryptophan concentration assessments, plasma was deproteinized for total tryptophan or filtered for free tryptophan measurements before it was subjected to an isocratic reversed-phase high-performance liquid chromatography (waters; excitation/emission wavelengths 300/350 nm).

Baseline plasma NPY concentrations were compared between healthy subjects and rMDD patients using Oneway-ANOVA. Plasma NPY concentrations during TD and SD were analyzed using a General Linear Model for repeated measures. Time and treatment were entered as within-subjects factors, and diagnosis as between-subject factor. Due to an age-

difference, though non-significant, between rMDD patients and healthy subjects, age was entered as covariate. Plasma NPY concentrations were compared to HDRS scores and clinical variables by simple nonparametric correlation analyses using Spearman's rho. Data are presented as means±standard deviation, and results were considered significant at $p<0.05$.

3. Results

Plasma NPY concentrations during TD and SD are shown in Table 1.

Healthy subjects did not differ from rMDD patients in baseline plasma NPY concentrations, neither at baseline of TD nor at baseline of SD ($F=2.69$, $df=1$, $p=0.107$, and $F=1.25$, $df=1$, $p=0.27$, respectively). Neither at baseline of TD nor at baseline of SD, plasma NPY levels in rMDD patients were correlated with age of onset of the illness (Spearman's rho=0.03 and 0.20, $p=0.87$ and 0.29, respectively), number of previous episodes (Spearman's rho=0.07 and 0.14, $p=0.74$ and 0.49, respectively), and length of remission (Spearman's rho=-0.12 and -0.04, $p=0.55$ and 0.85, respectively).

TD lowered plasma total and free tryptophan levels (treatment×time: $F=156.6$, $df=3,46$, $p<0.001$, and $F=72.5$, $df=3,49$, $p<0.001$, respectively) with no between group differences. Nadir tryptophan concentrations were found at the +5- and +7-hour time points. During TD, the transient increase in mean HDRS scores for rMDD patients was from 1.0 (SD=1.2) at baseline to 11.2 (SD=5.2) at +7-h and 1.4 (SD=1.5) at +24-h.

Healthy subjects and rMDD patients did not differ in plasma NPY concentrations at any time point following TD or SD. There was no treatment×time ×diagnosis effect ($F=1.67$, $df=3, 48$, $p=0.184$) and no main effect for treatment ($F=0.89$, $df=1, 50$, $p=0.350$). At no time point after TD and SD, HDRS scores in rMDD patients were correlated with plasma NPY concentrations or with respective changes of plasma NPY concentrations from baseline.

4. Discussion

There was no difference between healthy subjects and medication-free rMDD patients in baseline plasma NPY concentrations. Moreover, there was no difference between healthy subjects and rMDD patients in plasma NPY concentrations during TD and SD, and no association was found between plasma NPY concentrations and HDRS scores during TD in rMDD patients. There was no difference between TD and SD in their respective effect on plasma NPY concentrations.

Our results support that decreased plasma NPY concentrations, as reported previously for patients during a spontaneous episode of MDD (Hashimoto et al., 1996), are a state but not trait abnormality of MDD. Also, a reoccurrence of depressive symptoms during TD in rMDD patients was not associated with plasma NPY concentrations, indicating that plasma NPY plays no critical role in TD-induced relapse in MDD. There was no significant main effect for treatment, indicating that there is no direct functional link between serotonergic neurotransmission and plasma NPY concentrations, which can be uncovered by tryptophan depletion.

The study has limitations. Plasma NPY concentrations in rMDD patients were not obtained during the symptomatic phase of the illness. The results of the study call for a longitudinal assessment of plasma NPY concentrations in MDD patients during the symptomatic phase of the illness and during remission. Peripheral NPY measurements were obtained, and it is unclear to which extent peripheral NPY concentrations correspond to brain concentrations. It has been suggested, however, that alterations in plasma NPY concentrations may parallel

respective changes of NPY levels in the brain (Morgan et al., 2002), and that peripheral plasma NPY may exert central effects via its interaction with NPY-4 receptors located in blood-brain-barrier-free areas of the brainstem (Larsen and Kristensen, 1997). Also, the relatively small sample size calls for a confirmation study in a larger sample.

In summary, our results support that low plasma NPY concentrations are a state characteristic for MDD (Hashimoto et al., 1996) and levels return back to normal in medication-free, remitted MDD patients. The results also support no direct functional link between serotonergic neurotransmission and plasma NPY concentrations. Future studies may focus on other components of the stress response and potential respective persisting alterations during remission in MDD, posing possible biological risk factors for relapse.

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Table 1

Plasma NPY (pg/ml, mean±standard deviations) concentrations during tryptophan and sham depletion in healthy subjects and medication-free patients with remitted major depressive disorder (rMDD)

	Tryptophan depletion			Sham depletion				
	BL	+5	+7	+24	BL	+5	+7	+24
Healthy subjects (<i>n</i> =26)	99.9±25.1	80.3±23.7	90.5±31.7	84.9±24.2	103.7±30.1	88.6±24.5	96.9±28.8	86.0±20.2
rMDD patients (<i>n</i> =28)	89.3±28.3	84.0±29.6	82.5±32.3	87.3±27.6	96.7±24.1	84.9±29.9	91.6±32.8	83.6±29.7

Plasma NPY concentrations were determined at baseline and at +5, +7, and +24 h following tryptophan and sham depletion, respectively. Healthy subjects and rMDD patients did not differ in plasma NPY concentrations at any time point following tryptophan or sham depletion, respectively.