# Low Baseline and Yohimbine-Stimulated Plasma Neuropeptide Y (NPY) Levels in Combat-Related PTSD

Ann M. Rasmusson, Richard L. Hauger, Charles A. Morgan III, J. Douglas Bremner, Dennis S. Charney, and Steven M. Southwick

**Background:** *Consistent with many studies demonstrating enhanced reactivity of the sympathetic nervous system in posttraumatic stress disorder (PTSD), the administration of yohimbine, a noradrenergic $\alpha_2$-antagonist, has been shown to increase core symptoms of PTSD and to induce greater increases in plasma 3-methyl-4-hydroxy-phenylglycol (MHPG) in subjects with PTSD compared with healthy control subjects. In turn, neuropeptide Y (NPY) has been shown to inhibit the release of norepinephrine from sympathetic noradrenergic neurons.*

**Methods:** *In the following study, plasma NPY responses to yohimbine and placebo were measured in a subgroup of 18 subjects with PTSD and 8 healthy control subjects who participated in the previous study of the effect of yohimbine on plasma MHPG.*

**Results:** *The PTSD subjects had lower baseline plasma NPY and blunted yohimbine-stimulated increases in plasma NPY compared with the healthy control subjects. Within the PTSD group, baseline plasma NPY levels correlated negatively with combat exposure scale scores, baseline PTSD and panic symptoms, and yohimbine-stimulated increases in MHPG and systolic blood pressure.*

**Conclusions:** *This study suggests that combat stress-induced decreases in plasma NPY may mediate, in part, the noradrenergic system hyperreactivity observed in combat-related PTSD. The persistence of this decrease in plasma NPY may contribute to symptoms of hyperarousal and the expression of exaggerated alarm reactions, anxiety reactions, or both in combat veterans with PTSD long after war.* Biol Psychiatry 2000;47:526–539 © *2000 Society of Biological Psychiatry*

**Key Words:** Posttraumatic stress disorder, PTSD, neuropeptide Y, combat stress, anxiety, cardiovascular

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## Introduction

The following study investigates whether alterations in neuropeptide Y (NPY) physiology may contribute to sympathetic nervous system hyperreactivity in posttraumatic stress disorder (PTSD) (APA 1994). Signs and symptoms of sympathetic nervous system hyperreactivity in PTSD include discrete increases in heart rate, blood pressure, and plasma catecholamines in response to stimuli associated with previous traumatic events; time-integrated increases in the release of epinephrine and norepinephrine measured by 24-hour urine and plasma sampling; and decreases in platelet $\alpha_2$-adrenergic receptor number (Blanchard et al 1986, 1991; DeBellis et al 1994; Fraser and Wilson 1918; Grinker and Spiegel 1945; Kardiner and Spiegel 1947; Kosten et al 1987; McFall et al 1990; Orr 1993; Pallmeyer et al 1986; Perry et al 1987; Pitman and Orr 1990; Pitman et al 1987, 1990; Shalev 1993; Yehuda et al 1992, 1998). Therapeutic responses of PTSD patients to pharmacologic agents with $\alpha_2$- and $\beta$-adrenergic receptor activity also suggest a role for hyperreactivity of the peripheral sympathetic system, brain adrenergic system, or both in PTSD (Friedman 1998). Conversely, the intravenous (IV) administration of yohimbine, a noradrenergic $\alpha_2$-antagonist that increases peripheral and central noradrenergic system activity in humans, has been shown to increase flashbacks, intrusive thoughts, emotional numbness, difficulty concentrating, and panic symptoms in combat veterans with PTSD (Charney et al 1982, 1984; Holmberg et al 1962; Peskind et al 1989; Southwick et al 1993, 1997a). In addition, yohimbine has been shown to induce greater increases in plasma 3-methyl-4-hydroxyphenylglycol (MHPG), the major metabolite of norepinephrine, as well as greater reductions in prefrontal cortical metabolism in PTSD patients compared with healthy control subjects (Bremner et al 1997a; Southwick et al 1993).

It has been suggested that the process of sensitization in response to the repeated experience of unconditioned and conditioned stress underlies the hyperreactivity of the noradrenergic system in PTSD (Southwick et al 1993).

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This suggestion is supported by observations of augmented increases in the turnover and release of norepinephrine in chronically stressed animals exposed to subsequent novel stressors or stressors of a lesser intensity (Adell et al 1988; Gresch et al 1994; Irwin et al 1986; Nisenbaum et al 1991). The neural mechanisms underlying sensitization of the noradrenergic system in PTSD, however, have not been defined and are likely to be complex. For example, increases in tyrosine hydroxylase levels, dopamine B-hydroxylase activity, and norepinephrine content have been observed in the brains, sympathetic nerve terminals, and adrenal glands of repeatedly stressed animals and may enable the capacity for increased norepinephrine release (see review by Zigmond et al 1995). Factors that modulate the firing of central and peripheral noradrenergic neurons also may be important. For instance, corticotropin releasing factor (CRF) activates central noradrenergic systems when administered intraventricularly (Dunn and Berridge 1987) and increases the firing of central noradrenergic neurons when applied directly to the locus coeruleus (Curtis et al 1997; Valentino and Foote 1988). In repeatedly stressed animals, CRF messenger RNA (mRNA) and peptide levels are increased in a number of brain regions, including the locus coeruleus (Chappell et al 1986; Imaki et al 1991); in addition, cerebrospinal fluid (CSF) CRF levels have been found to be increased in veterans with combat-related PTSD (Bremner et al 1997b). Increases in central CRF levels thus may contribute to the increases in peripheral sympathetic system reactivity in PTSD because of the tight coupling between the central and peripheral noradrenergic systems (Kopin et al 1983).

In the following study, we investigated whether NPY also may play a role in the hyperreactivity of the noradrenergic system in PTSD. Neuropeptide Y is a 36 amino acid peptide neurotransmitter colocalized with norepinephrine in most sympathetic nerve fibers; NPY is also present in nonadrenergic perivascular, enteric, cardiac nonsympathetic, and parasympathetic nerves (Wahlestedt and Reis 1993). In the brain, NPY is colocalized with norepinephrine in the locus coeruleus; it is also located in the amygdala, cortex, hippocampus, and periaqueductal gray—all structures that play significant roles in the mediation of the mammalian stress response (Heilig and Widerlov 1990). At the synaptic level, NPY has been shown to inhibit the release of neurotransmitters with which it is colocalized, as well as to enhance the postsynaptic receptor response to these neurotransmitters (Colmers and Bleakman 1994). When administered intraventricularly in a variety of animal models, NPY is anxiolytic (Britton et al 1997b; Ehlers et al 1997; Heilig et al 1989, 1993; Wahlestedt and Reis 1993). In addition, centrally administered NPY has been found to counter the anxiogenic effects of CRF (Britton et al 1997a). Finally, in one study of humans with depression, CSF NPY levels were found to correlate negatively with anxiety symptoms (Widerlov et al 1989).

We have previously shown that plasma NPY levels increase in response to yohimbine in healthy subjects (Rasmusson et al 1998); we also observed a trend for a negative correlation between baseline plasma NPY levels and yohimbine-induced increases in plasma MHPG. In the current study, we investigated whether baseline and yohimbine-induced increases in plasma NPY levels differ between combat veterans with PTSD and healthy nontraumatized control subjects. Given the more pronounced plasma MHPG response to yohimbine in combat veterans with PTSD (Southwick et al 1993), as well as the capacity of NPY to inhibit the release of norepinephrine from sympathetic neuronal terminals (Colmers and Bleakman 1994), we hypothesized that baseline plasma NPY levels would be low in PTSD and would correlate negatively with yohimbine-induced increases in plasma MHPG. Finally, we explored relationships between plasma NPY levels and PTSD symptoms, anxiety symptoms, and cardiovascular responses to yohimbine.

## Methods and Materials

### *Subjects*

Eighteen male subjects with combat-related PTSD and 8 healthy nontraumatized control subjects were included in this study of the effects of IV yohimbine on plasma NPY. The PTSD and nontraumatized control subjects comprised all participants from the studies by Southwick et al (1993, 1997a) for whom sufficient plasma was still available for NPY measurement.

The PTSD subjects were evaluated for PTSD and other Axis I diagnoses using the Structured Clinical Interview for DSM-III-R. Eight (44%) of the PTSD subjects met criteria for current major depression and four (22%) met criteria for current panic disorder. Twelve (67%) met criteria for lifetime major depression, 4 (22%) for lifetime panic disorder, and 17 (89%) for lifetime alcohol dependence. No one met criteria for a psychotic disorder. The PTSD subjects exceeded a cutoff score of 107 on the Mississippi PTSD Scale; they had a mean Combat Exposure Scale (CES) score of 32.6 ± 6.0 (SD). The control subjects were screened for current and past psychiatric disorders by use of a Structured Psychiatric Interview for healthy subjects. All subjects were drug and alcohol free for a minimum of 4 weeks before the study and were free of significant medical illnesses, including hypertension.

### *Experimental Procedures*

Each subject received IV infusions of normal saline and yohimbine (0.4 mg/kg) in a double-blind, random fashion at 10:00 AM. Placebo and yohimbine infusions were spaced 4 to 7 days apart. Blood samples were obtained at: −30, −15, +40, +60, +120, and +180 min relative to injection. The first baseline blood

sample was obtained at least 60 min after placement of the IV catheter. Ratings of PTSD symptoms and panic attack symptoms were obtained at −30, −15, +20, +60, +120, and +180 min relative to injection. The PTSD symptom scale consisted of 14 items that queried intrusive traumatic thoughts, flashbacks, startle, hypervigilance, out-of-body feelings, feelings of being distant from other people, emotional numbness, and difficulty with concentration, guilt, anger, grief, helplessness, hopelessness, and sadness. Items were rated on a 5-point scale: 1 = not present, 2 = mild, 3 = moderate, 4 = severe, 5 = worst ever. The Panic Attack Symptom Scale (PASS) consisted of 27 items, including 13 panic attack symptoms specifically set forth in the DSM-III R. These items were rated on a 4-point scale as 1 = not present, 2 = mild, 3 = moderate, and 4 = severe. Both sitting and standing pulse, systolic blood pressure, and diastolic blood pressure were obtained at −30, −15, +40, +60, +120, and +180 min from injection.

### *Biochemical Methods*

Plasma was stored at −70°C from the time of initial collection. Storage time and conditions did not differ between the PTSD and control groups. Neuropeptide Y was measured after plasma extraction by use of a double antibody radioimmune assay, using $^{125}$I-NPY as the tracer. This radioimmunoassay possesses an assay sensitivity of 20 pg/mL and intra-assay and interassay coefficients of variation of 8% and 10%, respectively (Allen et al 1991). The level of MHPG was measured by mass spectrometry as described in Southwick et al (1993).

### *Statistical Methods*

The average of the −30- and −15-min NPY values was used as a baseline for each condition. Occasional missing NPY values were imputed by carrying the last measured value forward; only one value was missing in the yohimbine condition. A three-way multivariate repeated measures analysis of variance (ANOVA) using the Greenhouse–Geisser estimation of epsilon to correct for correlations among repeated measures assessed the effects of diagnosis (PTSD vs. healthy), condition (placebo vs. yohimbine), and time, as well as their interaction, on plasma NPY levels. Post hoc *T*-tests with Bonferroni corrections were used to determine whether differences between these groups were present at each time point for each condition. The change in plasma NPY following yohimbine or placebo was measured by subtracting the baseline from the NPY level at each time point subsequent to baseline. The change in plasma NPY for the yohimbine condition minus the change in plasma NPY for the placebo condition gave a net effect for yohimbine at each time point. The peak change in plasma NPY for the yohimbine condition minus the peak change in plasma NPY for the placebo condition gave a net peak yohimbine effect. A one-way ANOVA was used to determine differences between the PTSD and healthy control groups for the net yohimbine effect or net peak yohimbine effect on plasma NPY. Student's *t* tests were used to assess differences between diagnostic groups in the peak plasma NPY level after yohimbine, the peak change in plasma NPY after yohimbine, and the peak percent change in plasma NPY after yohimbine.

There was a significant difference in age between the PTSD and healthy control groups, $p = .0001$. The PTSD subjects were 42.8 ± 2.1 SD years old (range: 39.8–48.3 years). The control subjects were 26.2 ± 7.2 SD years old (range: 21.1–42.2 years; all controls but one were less than 30 years old). Using age as a covariate in the repeated measures ANOVA rendered all effects except for diagnosis nonsignificant. There was a trend for a significant effect of diagnosis: $F(1,23) = 3.37, p = .08$; however, because there were such large differences between groups in mean age and age variance (age variance for the control group was 11 times that for the PTSD group), age used as a covariate essentially functioned as a class variable, duplicating the effects of diagnosis. Thus, the current study could not discriminate real effects of age from effects of diagnosis on plasma NPY. We, therefore, turned to independent studies examining relationships between age and plasma NPY (see Discussion) to determine the likelihood that differences in plasma NPY between the groups in the current study could be related to age rather than to PTSD or trauma exposure.

A secondary repeated measures analysis was performed to determine whether the presence of current major depression in 8 of the 18 PTSD subjects affected the group plasma NPY response to yohimbine versus placebo.

A three-way multivariate repeated measures ANOVA was used to assess the effects of diagnosis, condition, time, and their interaction on heart rate and blood pressure. As there may be a modest increase in systolic and diastolic blood pressure in men between the ages of 20 and 42 (Kannel et al 1981), we tested for a correlation between age and mean baseline and post-yohimbine systolic and diastolic blood pressure measures to determine whether age should be entered as a covariate into the multivariate analyses of the blood pressure data.

Correlations between NPY and MHPG measures and other dependent variables of interest were calculated for the yohimbine challenge day only. Pearson product moment correlations or Spearman correlations were calculated depending on whether the data were normally distributed. We did not use Bonferroni corrections in determining the significance of the post hoc exploratory correlations presented in this study, as the risk of Type II error is substantial; we did not want to underrepresent any consistent or mechanistically feasible relationships among the variables of interest in this first report.

Significance was set at $p < .05$. A trend toward significance or near significance was considered present when $.05 < p < .10$. Abacus Concepts, SuperANOVA (Abacus Concepts, Inc., Berkeley, CA, 1989) and SAS (SAS Institute Inc., Cary, NC, 1988) software was used in the statistical analyses.

## Results

### *Differential Effects of Yohimbine on Plasma NPY in the PTSD versus Control Subjects*

Patients with PTSD demonstrated decreased plasma NPY on both placebo and yohimbine-stimulation days compared with the healthy nontraumatized control subjects (Figure 1). Multivariate repeated measures ANOVA revealed an effect of diagnosis: $F(1,24) = 23.13, p = .0001$.

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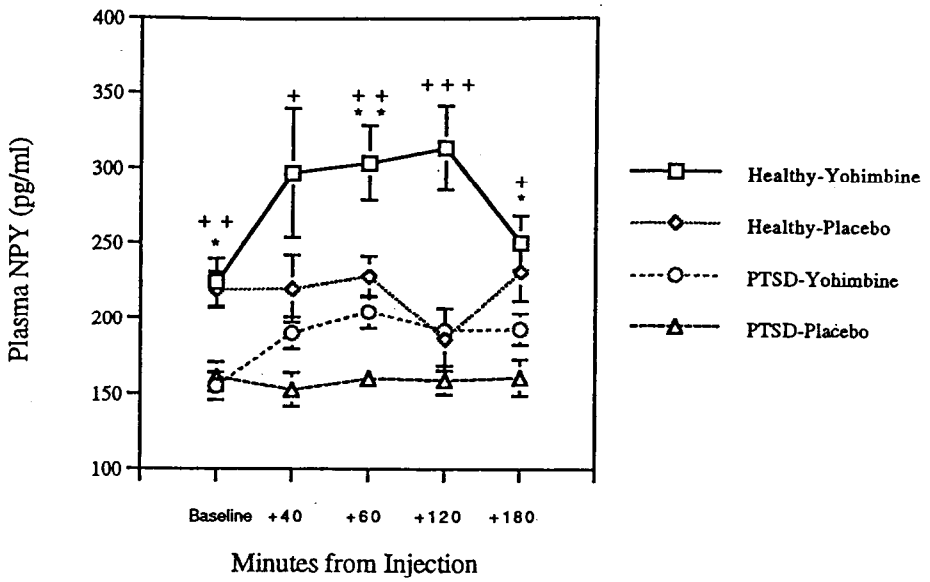


Figure 1. Effects of the intravenous injection of yohimbine (0.4 mg/kg) or normal saline (placebo) on plasma neuropeptide Y (NPY) in veterans with combat-related posttraumatic stress disorder (PTSD) and healthy, nontraumatized control subjects. Each time point represents the mean (± SEM). Multivariate repeated measures ANOVA with Greenhouse–Geiser estimations of epsilon revealed significant effects of diagnosis: $F(1,24) = 23.13$, $p = .0001$; condition: $F(1,24) = 38.21$, $p = .0001$; condition by diagnosis: $F(1,24) = 6.06$, $p = .02$; time: $F(4,96) = 6.08$, $p = .003$; condition by time: $F(4,96) = 11.93$, $p = .0001$; and condition by time by diagnosis: $F(4,96) = 4.33$, $p = .008$. Post hoc $T$-tests with Bonferroni corrections showed significant differences between the PTSD and healthy groups at the time points indicated for placebo: $^{*}p \leq .005$, $^{**}p \leq .001$, $^{***}p \leq .0001$, and yohimbine: $^{+}p \leq .005$, $^{++}p \leq .001$, $^{+++}p \leq .0001$.

Post hoc $T$-tests with Bonferroni corrections revealed significant differences in plasma NPY levels between the PTSD and control groups at baseline, +60 min and +180 min during the placebo condition and at baseline, +40, +60, and +120 min during the yohimbine condition. In addition, there was a significant difference between groups in the peak post-yohimbine plasma NPY level reached (PTSD: 224.9 ± 10.4 pg/mL, controls: 336.9 ± 24.0 pg/mL, $p = .0001$) and the mean post-yohimbine plasma NPY level reached (PTSD: 194.7 ± 8.0 pg/mL, controls: 290.9 ± 22.7 pg/mL, $p = .0001$).

The PTSD and control groups both demonstrated an increase in plasma NPY in response to yohimbine as evidenced by a significant condition by time interaction: $F(4,96) = 11.93$, $p = .0001$. However, the PTSD group NPY response to yohimbine was blunted in comparison with that of the healthy control group. This was evidenced by a significant condition by time by diagnosis interaction: $F(4,96) = 4.33$, $p = .008$, a trend toward a significant time by diagnosis interaction effect for the *net* yohimbine effect on plasma NPY: $F(3,60) = 2.40$, $p = .08$, and a significant difference between groups in the area contained between the placebo and yohimbine condition curves, $p = .009$. There also was a significant difference between groups in the peak and mean changes in plasma NPY in response to yohimbine (peak change: PTSD 69.7 ± 8.3 pg/mL, controls 112.6 ± 13.4 pg/mL, $p = .01$; mean change: PTSD 39.5 ± 5.5 pg/mL, controls 66.6 ± 10.7 pg/mL, $p = .02$).

Multivariate repeated measures ANOVA revealed no effect of depression on plasma NPY levels within the PTSD group: $F(1,16) = 0.13$, $p = .72$. In addition, there was no time by depression by condition effect on the plasma NPY response: $F(4,64) = 1.00$, $p = .39$.

### *Relationships between Plasma NPY and CES Scores*

In the PTSD group, there was a negative correlation between the CES scores and the baseline plasma NPY levels ($r = -.51$, $p = .03$, Spearman) and between the CES scores and the mean post-yohimbine plasma NPY levels ($r = -.55$, $p = 0.01$, Spearman) (Figure 2).

### *Relationships between Plasma NPY and Plasma MHPG in Response to Yohimbine*

As seen in Table 1 and Figure 3, there was a trend for a negative correlation between the baseline plasma NPY level and peak change in plasma MHPG in response to yohimbine when both groups were considered together

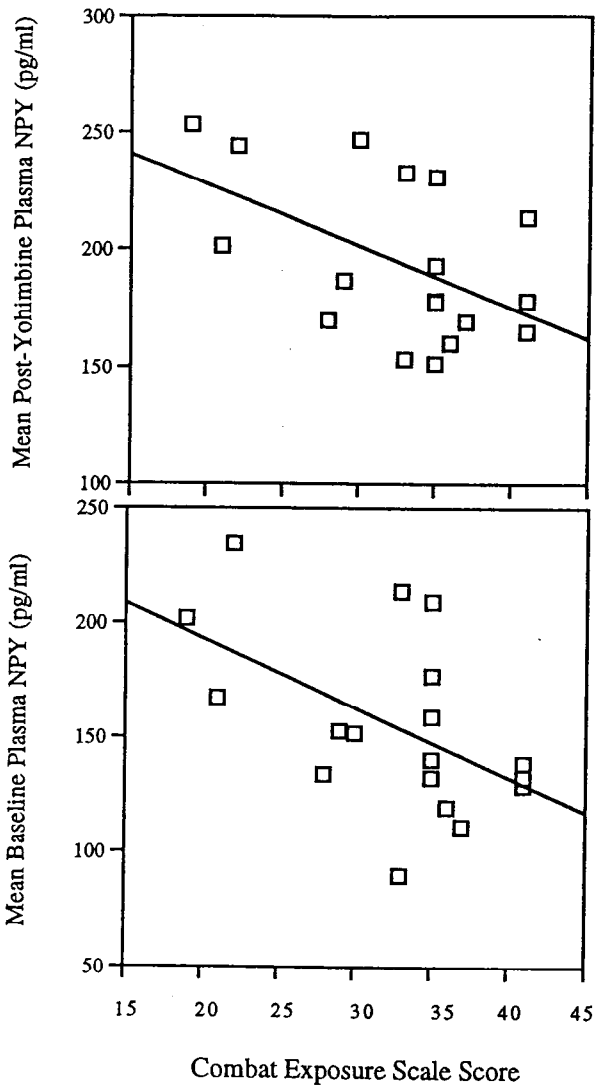


Figure 2. **Top:** The correlation between Combat Exposure Scale (CES) scores and mean post-yohimbine (0.4 mg/kg) plasma NPY levels ($r = -.55, p = .01$, Spearman) in 18 subjects with PTSD. **Bottom:** The correlation between CES scores and mean baseline plasma NPY levels ($r = -.51, p = .03$, Spearman) in the same 18 subjects with PTSD.

(Figure 3). There was a similar relationship between baseline plasma NPY and yohimbine-stimulated changes

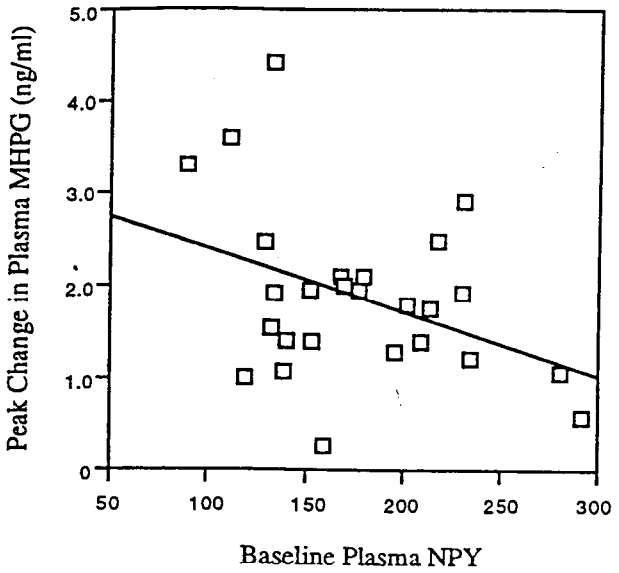


Figure 3. The correlation between baseline plasma neuropeptide (NPY) levels and the yohimbine-stimulated peak change in plasma 3-methyl-4-hydroxyphenylglycol (MHPG) in both PTSD and healthy nontraumatized control subjects: $r = -.38$, $p = .055$.

in plasma NPY in the PTSD group alone but not in the healthy control group. There was a positive correlation between the peak changes in plasma NPY and plasma MHPG within the PTSD group but not the healthy nontraumatized control group.

### *Relationships between Plasma NPY and PTSD or Panic Symptoms*

There was a nonsignificant negative correlation between baseline plasma NPY levels and baseline PTSD symptom scale scores in the PTSD group ($r = -.35, p = .16$). There was a positive correlation between maximum plasma NPY levels reached after yohimbine and the peak change in PTSD symptom scale scores in this group ($r = .55, p = .02$).

When both the PTSD and control groups were considered together, there was a trend for a negative correlation between baseline plasma NPY levels and baseline PASS

Table 1. Correlations between Plasma NPY and Plasma MHPG under Conditions of Yohimbine Stimulation

| | All | PTSD | Healthy |
|---|---|---|---|
| Baseline plasma NPY and Peak change in plasma MHPG | $r = -.38, p = .055$ | $r = -.49, p = .04$ | $r = -.54, p = .16$ |
| Baseline plasma NPY and Peak change in Plasma NPY | $r = .11, p = .52$ | $r = -.49, p = .04$ S | $r = .34, p = .39$ |
| Peak change in plasma NPY and Peak change in plasma MHPG | $r = .32, p = .11$ S | $r = .52, p = .03$ S | $r = -.33, p = .43$ |

scores ($r = -.38, p = .07$). When the PTSD subjects were considered alone, there also was a trend for a negative correlation between baseline plasma NPY levels and baseline PASS scores ($r = -.42, p = 0 < .10$). There was a trend for a positive correlation between the maximum plasma NPY levels reached after yohimbine and the peak change in PASS scores in the PTSD ($r = .48, p = .05$) and healthy control groups ($r = .71, p = .08$).

### *Plasma NPY and Weight*

The groups did not differ by weight: 88.7 ± 3.3 kg for the PTSD group and 84.4 ± 5.0 kg for the control group. There was no correlation between weight and either baseline plasma NPY or post-yohimbine plasma NPY measures when all subjects were considered together; however, within the PTSD group, there was a positive correlation between weight and baseline plasma NPY ($r = .61, p = .007$), peak post-yohimbine plasma NPY ($r = .47, p < .05$), and mean post-yohimbine plasma NPY ($r = .60, p = .009$).

### *Plasma NPY and Cardiovascular Responses to Yohimbine*

There were no differences between the diagnostic groups in the net or net peak effect of yohimbine on systolic blood pressure, diastolic blood pressure, or pulse. However, there were baseline differences between the groups in pulse and diastolic blood pressure (Figure 4). Table 2 presents correlations between plasma NPY or MHPG and vital signs. Of note, absolute levels of plasma NPY correlated negatively with yohimbine-induced changes in systolic blood pressure in both groups. However, absolute levels of plasma NPY, but not MHPG, correlated with absolute measures of blood pressure in the PTSD group, whereas absolute levels of plasma MHPG, rather than NPY, correlated with absolute measures of blood pressure in the healthy control subjects. In contrast, NPY responses to yohimbine were highly correlated with heart rate in the healthy group, but not in the PTSD group.

## Discussion

### *Low Plasma NPY in PTSD*

This study demonstrated low baseline and blunted yohimbine-stimulated plasma NPY levels in combat veterans with PTSD compared with healthy, nontraumatized control subjects. In addition, a negative correlation between the degree of combat exposure and the PTSD subjects' baseline and post-yohimbine plasma NPY levels was observed. These findings are consistent with the results of a preclinical study in which rats exposed to chronic stress developed lower circulating plasma NPY levels and a blunted increase in plasma NPY in response to acute footshock (Corder et al 1992). The data therefore suggest that plasma NPY levels in humans with PTSD may decrease as a consequence of severe chronic stress and that this adaptation may persist indefinitely. In addition, this study found that low NPY levels within the PTSD group were not related to the presence or absence of major depression.

Given the mismatch in age between the PTSD and control groups in the current study (see the Statistical Methods section above), it is necessary to consider whether age may have mediated the differences in baseline and yohimbine-stimulated increases in plasma NPY levels between these groups. For instance, an age-related decline in NPY-containing superior cervical ganglion cells and NPY immunoreactivity within ganglion cells has been found in rats between the ages of 8 and 60 weeks (Gurusinghe et al 1990). Dotsch et al (1997), however, reported no relationship between age and baseline plasma NPY levels in 35 human male and female subjects aged 50 ± 17 SD years. In addition, our group has found no significant relationship between age and baseline plasma NPY level in two independently recruited groups of healthy male subjects comprising the same age range as the subjects in the current study: 1) There was not a significant correlation between baseline plasma NPY and age in a group of 20 healthy civilian subjects participating in a recent study of the effects of yohimbine on memory ($r = -.20, p = .41$) (age: 36.8 ± 11.2 SD years, range: 20.0–52.3 years) (S. Southwick, personal communication), and 2) there was no correlation between age and baseline plasma NPY in a group of 83 healthy male military personnel participating in an intense training exercise ($r = -.08, p = .48$, age: 28.7 ± 4.6 SD years, age range: 19.0–41.0 years; Morgan et al, in press). Thus, it appears unlikely that a difference in age rather than differential exposure to severe chronic stress or a diagnosis of PTSD accounts for the difference in baseline plasma NPY levels between the PTSD subjects and healthy controls in the current study. In addition, Morgan et al (in press) demonstrated no relationship between age and stress-stimulated absolute plasma NPY levels ($r = -.02, p = .95$) or between age and changes in plasma NPY levels ($r = .09, p = .75$; age: 27.6 ± 4.1 SD years, age range: 19–33 years; $n = 15$). Thus, it appears unlikely that a difference in age rather than differential exposure to severe chronic stress or a diagnosis of PTSD accounts for the difference in yohimbine-stimulated plasma NPY responses between the PTSD subjects and healthy control subjects in the current study. However, it remains possible that yohimbine administration and naturalistic stressors may increase plasma NPY via different mechanisms that could be differentially sensitive to age.

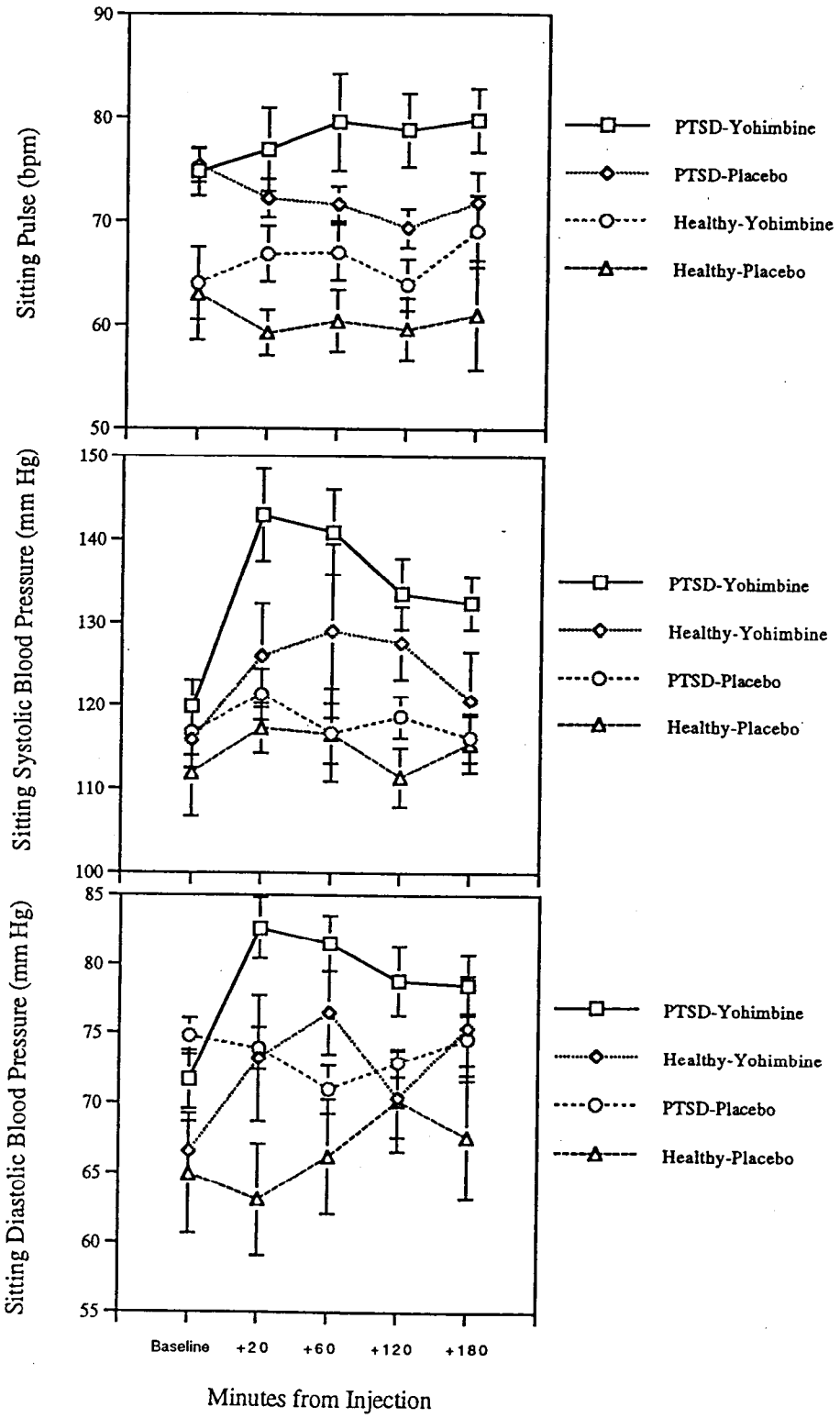


Figure 4. Effects of the intravenous injection of yohimbine (0.4 mg/kg) and placebo on vital signs in veterans with combat-related posttraumatic stress disorder (PTSD) and healthy, nontraumatized control subjects. Each point represents the mean (± SEM). *Top panel:* Multivariate repeated measures analysis revealed a significant effect of diagnosis on sitting pulse: $F(1,23) = 10.62, p = .004$. There also was a significant effect of condition: $F(1,23) = 7.08, p = .01$, and a significant condition by time interaction effect: $F(4,92) = 3.09, p = .02$. There was not a significant diagnosis by condition by time effect, nor was there a group difference in the net or peak yohimbine effect on pulse. There was a significant difference between groups in the baseline pulse calculated as the mean between the yohimbine and placebo conditions (PTSD: 75 bpm, controls: 61 bpm, $p = .0001$). *Middle panel:* There was no correlation between age and baseline ($r = -.07, p = .72$) or mean post-yohimbine ($r = .26, p = .19$) sitting systolic blood pressure (SBP). Thus age was not entered into the multivariate repeated measures analysis which revealed no effect of diagnosis on SBP. There were significant effects of condition: $F(1,23) = 23.96, p = .0001$, and time: $F(4,92) = 10.36, p = .0001$, and a condition by time interaction effect: $F(4,92) = 4.35, p = .003$, on SBP. There was not a significant diagnosis by condition by time effect, nor were there net or peak yohimbine effects on SBP. *Bottom panel:* There was no correlation between age and either baseline ($r = -.05, p = .82$) or mean post-yohimbine ($r = .23, p = .26$) sitting diastolic blood pressure (DBP). Thus, age was not used as a covariate. Multivariate repeated measures analysis revealed a significant effect of diagnosis on DBP: $F(1,23) = 6.27, p = .02$. There also were effects of condition: $F(1,23) = 12.49, p = .002$, and time: $F(4,92) = 4.76, p = .002$, and a condition by time effect: $F(4,92) = 6.97, p = .0001$ on DBP. There was not a significant diagnosis by condition by time effect, nor was there a group difference in the net or peak yohimbine effect on pulse. There was a significant difference between groups in the baseline DBP calculated as the mean between the yohimbine and placebo conditions (PTSD: 73 mm Hg, controls: 66 mm Hg, $p = .01$).

Table 2. Correlations between Plasma NPY or MHPG and Vital Signs before and after Yohimbine

a. The relationship between plasma NPY and yohimbine *stimulated increases* in systolic blood pressure.

| | All | PTSD | Healthy |
|---|---|---|---|
| Baseline plasma NPY and | | | |
| Peak change in sitting SBP in response to yohimbine | $r = -.44, p = .02$ | $r = -.33, p = .19$ | $r = -.26, p = .53$ |
| Maximum post-yohimbine plasma NPY and | | | |
| Peak change in sitting SBP in response to yohimbine | $r = -.42, p = .03$ | $r = -.34, p = .17$ | $r = -.39, p = .34$ |

b. The relationship between plasma NPY and *static measures* of systolic blood pressure.

| | PTSD | Healthy |
|---|---|---|
| Baseline plasma NPY and | | |
| Baseline sitting SBP | $r = .47, p < .05$ | $r = .23, p = .58$ |
| Baseline sitting DBP | $r = .16, p = .52$ | $r = .05, p = .90$ |
| Mean plasma NPY after yohimbine and | | |
| Mean sitting SBP after yohimbine | $r = .31, p = .20$ | $r = -.07, p = .87$ |
| Mean sitting DBP after yohimbine | $r = .42, p = .08$ | $r = .14, p = .74$ |

c. The relationship between plasma MHPG and *static measures* of systolic blood pressure.

| | PTSD | Healthy |
|---|---|---|
| Baseline plasma MHPG and | | |
| Baseline sitting SBP | $r = -.01, p = .97$ | $r = .54, p = .17$ |
| Baseline sitting DBP | $r = -.27, p = .27$ | $r = -.31, p = .46$ |
| Mean MHPG after Yohimbine and | | |
| Mean sitting SBP after yohimbine | $r = .13, p = .62$ | $r = .73, p = .04$ |
| Mean sitting DBP after yohimbine | $r = -.03, p = .90$ | $r = .60, p = .11$ |

d. Relationships between plasma NPY and pulse.

| | PTSD | Healthy |
|---|---|---|
| Baseline plasma NPY and | | |
| Baseline sitting pulse | $r = .17, p = .49$ | $r = .27, p = .52$ |
| Peak plasma NPY after yohimbine and | | |
| Peak sitting pulse after yohimbine | $r = .20, p = .42$ | $r = .62, p < .10$ |
| Peak change in plasma NPY after yohimbine and | | |
| Peak sitting pulse after yohimbine | $r = .01, p = .96$ | $r = .82, p = .01$ |

DBP, diastolic blood pressure; SBP, systolic blood pressure.

The means by which lower basal plasma NPY levels are achieved and maintained after chronic stress are not clear. Alterations in the regulation of NPY synthesis or release or both may play a role. Recent research also suggests that changes in NPY clearance via enzymatic degradation may be involved (Zukowska-Grojec 1995). Neuropeptide Y-specific protease release increases in response to acute stress in parallel with NPY release (Mentlein et al 1993; Zukowska-Grojec 1995). This may increase the rate of NPY degradation and mediate the production of an NPY fragment ($NPY_{3-36}$) with $Y_2$ receptor activity that, in turn, may increase negative feedback inhibition of noradrenergic neuronal activity and neurotransmitter release. It is also possible that more frequent phasic release of NPY because of a lowered threshold for activation of the sympathetic nervous system or more frequent triggering of the sympathetic nervous system in response to conditioned trauma-related cues in PTSD may result in depletion of NPY stores and blunted NPY responses to stimulation in the PTSD group. Such would contrast with the physiology of NPY in normal animals wherein acute and chronic stress appear to increase NPY immunoreactivity and mRNA in the adrenal medulla and sympathetic ganglia even while decreasing plasma NPY levels (Corder et al 1992; Han et al 1997, 1998a; Nankova et al 1996).

### *The Relationship between Plasma NPY and Plasma MHPG in Subjects with PTSD*

Southwick et al (1993) have previously shown that combat veterans with PTSD have increased MHPG responses to yohimbine compared with healthy nontraumatized control subjects. In the current study, a negative correlation between baseline plasma NPY levels and increases in plasma MHPG in response to yohimbine has been demonstrated, suggesting that combat stress-induced decreases

in plasma NPY may mediate, in part, the noradrenergic system hyperreactivity previously observed in combat-related PTSD (Southwick et al 1993). This would be consistent with numerous preclinical studies demonstrating the capacity of NPY to inhibit the release of norepinephrine from peripheral and central noradrenergic neurons, as well as inhibit the firing of locus coeruleus neurons via activity at presynaptic NPY $Y_2$ receptors (Colmers and Bleakman 1994; Illes and Regenold 1990). This finding is also consistent with work by Corder et al (1992) demonstrating an enhancement of acute footshock-induced increases in plasma norepinephrine in chronically stressed rats with concomitant decreases in plasma NPY.

In contrast, yohimbine-stimulated changes in plasma NPY and MHPG were positively correlated in the PTSD and control groups considered together and in the PTSD group alone. This is likely because of the co-release of these colocalized compounds under conditions of high sympathetic nervous system stimulation. The lack of correlation between yohimbine-induced changes in plasma NPY and MHPG in the control group alone may reflect the presence of two opposing forces at work. Whereas yohimbine effectively induced the release of NPY in this group (Figure 1), the higher plasma levels of NPY achieved in the healthy group may have more effectively braked the further release of NPY and MHPG.

### *The Relationship between Plasma NPY and Psychiatric Symptoms in Subjects with PTSD*

There are numerous studies relating "dose" of trauma exposure to the etiology of PTSD (Kaylor et al 1987; Kulka et al 1990). In turn, this study found a negative relationship between CES scores and baseline plasma NPY levels, as well as between baseline plasma NPY levels and baseline PTSD and panic symptoms in the PTSD group. Although it must be noted that the CES scores were obtained retrospectively and thus may have been subject to recall bias (Roemer et al 1998; Southwick et al 1997b), these correlations suggest that stress-induced alterations in NPY physiology may be one biologic mechanism that contributes to the development of PTSD. Indeed, given the role of NPY in restraining noradrenergic system reactivity and the role of the noradrenergic system in mediating arousal, combat-related decreases in baseline plasma NPY would be expected to lower the set point for arousal and activation of the sympathetically mediated fight or flight response. In an active combat setting, this response would be expected to facilitate survival; however, the persistence of this decrease in set point in veterans with PTSD may mediate the expression of inappropriate or exaggerated alarm reactions, anxiety reactions, or both within the relatively safe civilian surroundings to which they return after war.

In contrast to the negative relationship between baseline plasma NPY levels and baseline PTSD and anxiety symptoms in the PTSD group, there was a positive correlation between maximum NPY levels after yohimbine and increases in panic and PTSD symptoms. This correlation may reflect the ultimate degree to which the sympathetic nervous system was activated by yohimbine and is consistent with the trend for a positive correlation between peak effects of yohimbine on MHPG levels and PTSD symptoms scale scores observed by Southwick et al (1993).

It has been suggested that increases in brain norepinephrine release may be responsible for increases in anxiety in response to yohimbine (Bremner et al 1997a; Charney et al 1982, 1984; Peskind et al 1989; Southwick et al 1993). The present work suggests the possibility that stimulated increases in brain NPY release also may contribute to PTSD and anxiety symptoms. Thus, it is notable that a low intracerebroventricular dose of NPY has been shown to increase anxiety in animals via activity at central $Y_2$ receptors (Nakajima et al 1998), whereas higher doses of NPY have been shown to exert anxiolytic effects via central $Y_1$ receptors (Britton et al 1997b; Heilig 1995; Heilig et al 1989, 1993; Kask et al 1996, 1997; Nakajima et al 1998; Wahlestedt et al 1993). This suggests that a dose-dependent balance between activation of central $Y_1$ and $Y_2$ receptors may determine how much anxiety is experienced in response to increases in brain synaptic NPY levels. Therefore, in considering the marked differences in anxiety experienced by PTSD and control subjects at baseline and after yohimbine (Southwick et al 1993, 1997a), it may be important that the yohimbine-stimulated NPY levels of the PTSD group were not significantly different from the placebo day NPY levels of the healthy group. This suggests that recruitment of possible dose-dependent anxiolytic effects of NPY may not have occurred in the PTSD subjects. This possibility is supported by observations of Morgan et al (in press) wherein plasma NPY levels achieved after interrogation stress correlated negatively with dissociative symptoms in healthy active duty military personnel participating in an intense training exercise.

At this point though, we do not yet know whether yohimbine-induced changes in plasma NPY levels are paralleled in the brain or impact on the central mediation of anxiety or fear; however, yohimbine is known to cross the blood-brain barrier and has been shown to increase the central release of norepinephrine (Peskind et al 1989), suggesting that NPY colocalized with norepinephrine in central noradrenergic neuronal terminals also may be released by the peripheral administration of yohimbine. In addition, peripherally circulating NPY may have direct access to brain areas that regulate visceral somatic responses

associated with anxiety and monitored in this study by the PASS. For example, NPY-responsive $Y_4$ receptors have been identified in the blood-brain barrier-free area postrema and subpostremal area (Larsen and Kristensen 1997). These nuclei interact with the immediately adjacent nuclei of the dorsal vagal complex and nucleus tractus solitarius that, in turn, integrate afferent and efferent neural and humoral signals regulating the respiratory, cardiovascular, and gastrointestinal systems.

### *The Relationship between Plasma NPY and Weight in PTSD*

Consistent with a recent report documenting increases in plasma NPY in obese women (Baranowska et al 1997), a positive relationship between weight and baseline NPY was observed in the PTSD group. To the extent that NPY levels in brain regions relevant to appetite control correlate with plasma NPY levels in the subjects with PTSD, this finding is consistent with research demonstrating the appetitive effects of centrally administered NPY and the effects of peripheral NPY in reducing energy metabolism and increasing fat storage (Bennet et al 1996; Moltz and McDonald 1985; Wettstein et al 1995; White 1993). This finding also may relate to increases in serum total triiodothyronine (T3) levels and noradrenergic hyperreactivity observed in veterans with PTSD (Mason et al 1994; Wang et al 1997). Thus, in the current study, the PTSD subjects with the lowest plasma NPY levels would be expected to have the highest noradrenergic reactivity, the highest serum T3 levels, the highest metabolic rates, and the greatest capacity for stress-induced weight loss.

### *Cardiovascular Effects of NPY*

**HEART RATE.** It is well known that the heart rate response to sympathetic nervous system activation reflects a balance between parasympathetically mediated negative chronotropic inputs and sympathetically mediated positive chronotropic inputs to the heart (Korner 1977). NPY appears to play a role in the mediation of both. For instance, NPY exerts a $Y_2$ receptor-mediated bradycardic effect in the nucleus tractus solitarius (Ergine et al 1993; Shih et al 1992) but inhibits vagal action at the heart via presynaptic $Y_2$ receptors during sympathetic stimulation (Potter and Ulman 1994; Shine et al 1994; Ulman et al 1997). The latter phenomenon is consistent with the observed correlation between yohimbine-induced increases in plasma NPY and maximum heart rate in the control group. The lack of a correlation between yohimbine-induced increases in plasma NPY and maximum heart rate in the PTSD group may be because of the blunted NPY response in this group. In addition, the finding of an increase in baseline heart rate in the PTSD group could reflect deficient activation of $Y_2$ receptors in the nucleus tractus solitarius under conditions of mild stress while subjects anticipated the possible administration of an anxiogenic agent (Orr et al 1995; Paige et al 1990; Prins et al 1995; Shalev et al 1992, 1998). Future studies will be needed to investigate such hypotheses.

**BLOOD PRESSURE.** The data in Table 2a show a negative correlation between pre-yohimbine and post-yohimbine plasma NPY levels and yohimbine-stimulated peak changes in sitting systolic blood pressure in both the PTSD and healthy control groups and suggest that circulating plasma NPY inhibits stimulated changes in systolic blood pressure in humans. This is consistent with activity of NPY at presynaptic NPY ($Y_2$) receptors that inhibit the firing of locus coeruleus neurons and the terminal release of norepinephrine centrally and peripherally (Colmers and Bleakman 1994; Illes and Regenold 1990).

Relationships between absolute levels of MHPG and NPY and absolute BP measures, however, diverged in the PTSD and control groups. The healthy controls demonstrated a strong positive correlation between absolute circulating plasma MHPG levels and blood pressure. This is consistent with the assertion that catecholamines rather than endogenously released NPY normally provide the greatest neurogenic influence on vascular tone under baseline conditions (Zukowska-Grojec 1998). Data from this study further suggest that catecholamines may provide the greatest neurogenic influence on vascular tone after sympathetic activation in healthy nontraumatized individuals but not in traumatized individuals with PTSD. Because NPY has been shown to enhance postsynaptic receptor responses to neurotransmitters with which it is colocalized (Colmers and Bleakman 1994), low NPY levels in the PTSD group may render norepinephrine less effective in influencing blood pressure in this group.

In contrast, data in Table 2b indicate that circulating levels of NPY correlate positively with sitting systolic blood pressure in the PTSD group but not the healthy control group. This may be explained by direct effects of NPY at postsynaptic $Y_1$ receptors that mediate vasoconstriction (Han et al 1998b). The greater strength of this relationship in the PTSD group is consistent with observations of vasoconstrictor hyperresponsivity to NPY in other hyperadrenergic states (Zukowska-Grojec 1998); this has been attributed to β-adrenergic receptor-mediated priming of vascular smooth muscle cell responses. It is also possible that peripheral $Y_1$ receptors are upregulated in PTSD in response to low plasma NPY levels, although this possibility has yet to be examined experimentally. Alternatively, NPY activation of central $Y_1$ receptors,

which mediate reductions in blood pressure, may be greater in the healthy controls (Klemfuss et al 1998).

**POSSIBLE HEALTH RISKS.** Because the half-life of plasma NPY is much greater than that of norepinephrine (Pernow et al 1986) and because systolic blood pressure in PTSD subjects appears to be hyperresponsive to circulating plasma NPY levels, stress-induced increases in plasma NPY may result in more sustained elevations of blood pressure in this group. Under conditions of combat or other prolonged exposure to physical stress, this may be adaptive. However, stress-induced increases in plasma NPY also may result in more persistent ischemic risk to end organs, such as the heart and brain, and could account for increased rates of stroke and other circulatory diseases observed in persons with histories of trauma (Bao et al 1997; Boscarino 1997; Brass and Page 1996). In addition, Zukowska-Grojec (1995) suggest that the NPY fragment, $NPY_{3-36}$, which has mitogenic effects on vascular smooth muscle cells, may mediate stress-related long-term increases in blood pressure; this may be relevant to the increases in baseline and post-yohimbine diastolic blood pressure observed in the PTSD subjects compared with the control subjects in this study.

### *Future Directions*

Given the small number of subjects in the control group, the mismatch in age between the control and PTSD groups, and the presence of comorbid psychiatric disorders in a substantial number of PTSD subjects, the results of this study must be considered preliminary. Future investigations of NPY physiology in PTSD must be designed to address these issues. Also, it will be important to know whether psychiatrically healthy combat veterans show persistent alterations in baseline and stimulated plasma NPY levels as animal studies have demonstrated chronic stress-mediated decreases in plasma NPY in normal animals; future studies also should determine whether psychological stress or trauma-conditioned stimuli differentially induce the release of NPY in PTSD subjects compared with trauma controls. In addition, it will be important to know whether changes in NPY physiology occur in noncombat-related PTSD and whether there are gender differences in the effect of trauma on NPY physiology (Sato et al 1995; Zukowska-Grojec et al 1991).

This study also suggests that targeting NPY physiology may be useful in the treatment of PTSD. Thus, studies should begin to explore whether current medications used to treat PTSD affect plasma NPY levels (Friedman 1998) and whether medications known to influence plasma NPY levels are effective in treating PTSD and comorbid stress-related conditions (Koide et al 1995). In addition, it will be important to investigate whether alterations in NPY physiology are related to other clinical phenomena observed in PTSD, including decreases in hippocampal volume (Greber et al 1994; Stein et al 1998), memory dysfunction (Cleary et al 1994; Flood et al 1987; Wolfe and Schlesinger 1998), poor exercise tolerance (Shalev et al 1990), sleep disturbances (Biello et al 1997; Mellman et al 1995), blunting of positive emotions (Josselyn and Beninger 1992, Macey et al 1997), and chronic pain (Beckham et al 1997; Walker et al 1988). Finally, it may be fruitful to determine to what extent trauma-induced allostatic alterations in NPY physiology may play a role in the pathophysiology of common stress-related medical disorders, such as hypertension, heart disease, stroke, and diabetes (McEwen 1998).

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