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Neuropeptide S receptor gene is associated with cortisol responses to social stress in humans

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ABSTRACT

The neuropeptide S (NPS) and its receptor NPSR represent a transmitter system critically involved in the modulation of anxiety and arousal in rodents. Initial human studies indicate that the T-allele of the functional NPSR gene (*NPSR1*) polymorphism (rs324981), which increases NPS potency at NPSR, is associated with anxiety-related phenotypes. Since stress is critically involved in the pathogenesis of anxiety disorders, we tested the association between rs324981 and stress reactivity in 196 healthy males. Participants were exposed to the Trier Social Stress Test for Groups (TSST-G), a standardized laboratory protocol for stress exposure in a group format. Salivary cortisol and subjective stress responses were assessed. A significant genotype by time interaction, and a main effect of genotype were shown, with T-allele carriers displaying larger cortisol and subjective stress responses. This is the first report to show involvement of the NPS system in the regulation of the neuroendocrine stress response in humans.

1. Introduction

Stress is a ubiquitous challenge across human cultures associated with a wide spectrum of diseases, including anxiety disorders. Activation of stress systems is essential for tuning the human organism to demanding circumstances; however, chronic or unpredictable stress can result in dysregulations of stress-responsive physiological systems which can precipitate or sustain stress-related disorders (Chrousos, 2009). Regulation of the hypothalamic–pituitary–adrenal (HPA) axis, the organism's major neuroendocrine stress response system, is governed by hypothalamic circuits which integrate multiple inputs from limbic regions and the brainstem, including neuropeptidergic signals (Ulrich-Lai and Herman, 2009). Recent evidence indicates that neuropeptide S (NPS) and its receptor NPSR represent a transmitter system with a major role in the modulation of anxiety, arousal, and fear in rodent models (Pape et al., 2009). NPS consists of 20 amino acids and is cleaved from a larger precursor peptide. In the rodent, NPS precursor expression is limited to discrete nuclei in the brain stem. In contrast to the limited distribution of NPS precursor, NPSR, a typical member of the G-protein-coupled receptor superfamily, is expressed in various brain regions, with highest densities found in cortex, thalamus, hypothalamus, and amygdala (Xu et al., 2007).

In rodent models, NPS or NPSR agonists have been observed to produce anxiolytic-like effects by acutely reducing fear responses (Xu et al., 2004). Furthermore, long-term aspects of fear memory, such as attenuation of contextual fear or enhancement of fear extinction, have been observed (Jüngling et al., 2008). Specific NPS-mediated modulation of synaptic function in the amygdala seems to underlie these behavioural effects (Pape et al., 2009).

These anxiolytic effects are accompanied by increased arousal as indicated by hyperlocomotion and wakefulness (Xu et al., 2004). Importantly, the NPS system also seems to be critically involved in stress processing. In rats, it was shown to activate the HPA axis (Smith et al., 2006), and stress exposure in mice led to activation of immediate early genes in NPS-producing brain stem nuclei (Liu et al., 2011).

There are no data on the acute effects of NPS administration in humans to date. However, several investigations have used a neurogenetic approach to indirectly assess the role of the NPS system in different anxiety-related phenotypes. The gene coding for NPSR is located on chromosome 7p14 and is encoded by at least 9 exons. The common A/T single nucleotide polymorphism (SNP) rs324981 leads to an amino acid exchange (Asn107Ile) with functional relevance, as the NPSR-Ile107 variant increases NPS potency at NPSR about tenfold (Reinscheid et al., 2005). Several studies in humans have associated this SNP with anxiety-related phenotypes. The T-allele of rs324981 was associated with panic disorder and increased autonomic arousal (Domschke et al., 2011), overinterpretation of

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one's own fear reactions in a fear-conditioning paradigm (Raczka et al., 2011), and increased right amygdala responsiveness to fear-relevant faces (Dannlowski et al., 2011).

Although recent studies highlight the association between *NPSR* and anxiety disorders, we are unaware of any studies that have specifically targeted the role of the NPS system in human stress responsiveness. Since stress is critically involved in the pathogenesis of affective and anxiety disorders, we tested the association between *NPSR1* SNP rs324981 and cortisol as well as subjective responses to acute social stress exposure in humans. In particular, we hypothesized that T-allele carriers show increased adrenal and subjective responses to acute social stress exposure.

2. Methods

2.1. Participants

We studied 196 healthy male university students with mean age 23.7 years (±2.9 SD) of German (92%) and eastern European (8%) descent, to participate in a study about "job interviews". Exclusion criteria were history of psychiatric disorder, chronic or acute illness, smoking, medication or substance abuse, and studying psychology. All participants gave informed consent and were paid 25 euro for participation. The study was approved by the Ethics Committee of the University of Freiburg.

2.2. Experimental protocol

Experimental sessions were all conducted in the late afternoon to control for diurnal variations in cortisol secretion. Participants arrived at the laboratory in groups of four to six and were instructed not to communicate with one another for the duration of the study. The Trier Social Stress Test for Groups (TSST-G; von Dawans et al., 2011) was used for induction of psychosocial stress. The TSST-G, a standardized 20-min laboratory protocol for controlled simultaneous social stress exposure in a group format, consists of public speaking and mental arithmetic tasks performed in front of a panel of two evaluators and two cameras. The TSST-G combines high levels of socio-evaluative threat and uncontrollability and leads to significant cortisol and adrenocorticotropic hormone (ACTH) responses.

Before the stress task, participants were given 10 min to prepare for the interview (anticipatory phase) in a waiting area. After the anticipatory phase, the group of participants was led to the stress room. During stress exposure, participants were separated by dividing walls that prevented eye contact and interaction with the other participants. Each participant was called upon in random order to deliver a free speech for 2 min. In the remaining 8 min, each participant was required to perform a mental arithmetic task for 80 s. After the task, participants were led back to the waiting area and rested there for 60 min.

2.3. Stress response measures

Saliva samples for the assessment of cortisol were collected with Salivettes (Sarstedt, Nümbrecht, Germany) at 1 min before and 1, 10, 20, 30, and 60 min after cessation of the TSST-G. A subjective stress questionnaire was given immediately before stress exposure (i.e., at the end of the 10 min preparation phase, immediately after the first saliva sample was taken), and 20 min after the stress task. Participants indicated their desire to leave the situation, their level of anxiety, and their emotional arousal on visual analogue scales ranging from 0 (not at all) to 10 (maximum). Subjective stress was operationalized as the mean value of these three items. Preliminary analyses showed acceptable internal consistency, with Cronbach's alpha of .85 for the pre-stress and .80 for the post-stress measurement occasion.

2.4. Biochemical analyses

Cortisol concentrations were determined by a commercially available chemiluminescence immunoassay (CLIA; IBL Hamburg, Germany). Inter- and intrassay coefficients of variation were both under 8%. DNA was extracted from mouthwashes by standard desalting procedure. Genotyping of the *NSPR1* rs324981 SNP was performed by KBiosciences (Hoddesdon, UK) using a system of fluorescence-based competitive allele-specific PCR.

2.5. Statistical analyses

General Linear Models (GLMs) were computed to assess the repeated measures effect time, the between-subjects effect genotype as well as the interaction time × genotype for endocrine and subjective responses to the TSST exposure. All genotype groups were included in the model to test the genotypic model. Given previous reports of a dominant T-allele effect (Dannlowski et al., 2011; Domschke et al., 2011; Raczka et al., 2011), A/A homozygotes were compared to T-allele carriers (A/T, T/T) in a second step. The potential confounding effect of group size (four, five or six participants per group) was controlled by including group size as a covariate in our models. Greenhouse–Geisser corrections were applied where appropriate, and only adjusted results are reported. η^2 values are given as an effect size measure. Post-hoc comparisons following significant GLM results were performed with the LSD test.

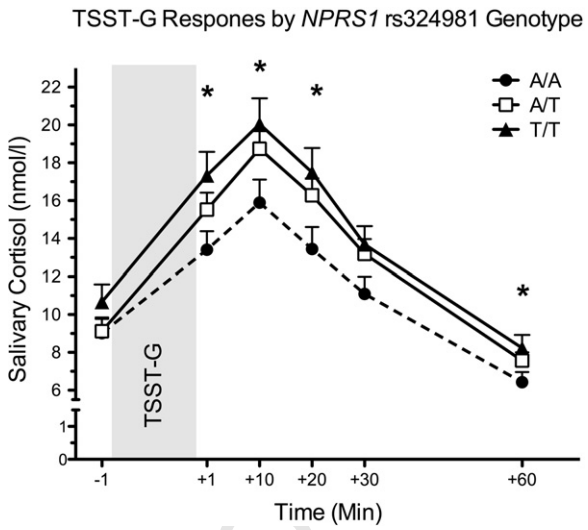

Fig. 1. Salivary cortisol responses to social stress in *NPSR1* rs324981 genotype groups. Post-hoc tests showed significant differences between T/T and A/A carriers (all *ps* < .03, indicated by *). Stress was induced by a standardized social laboratory stressor in a group format (Trier Social Stress Test for Groups, TSST-G; shaded area). Error bars are s.e.m.

3. Results

Genotype frequencies for rs324981 were 28.6% A/A ($n = 56$), 50.0% A/T ($n = 98$), and 21.4% T/T ($n = 42$). No deviation from Hardy–Weinberg equilibrium was observed ($X^2 < .01$, $p = .94$). GLM for repeated measures showed that the TSST-G led to significant increases in cortisol (main effect time: $F_{2.70, 497.33} = 186.39$; $p < .0001$).

Results of the GLM assessing the genotypic model revealed a significant time by genotype interaction ($F_{2.26, 493.11} = 3.01$; $p = .045$, $\eta^2 = .02$; main effect genotype: $F_{2, 183} = 2.28$; $p = .065$, $\eta^2 = .03$), indicating differential endocrine response patterns between the genotype groups. As shown in Fig. 1, rs324981 T/T carriers showed the largest cortisol increases in response to stress, the A/T genotype displayed intermediate levels and A/A the lowest levels. Post-hoc comparisons showed significant differences for time points +1, +10, +20, +60 min post stress. A/A carriers differed significantly from the T/T genotype for all these time points (all *ps* < .030). Furthermore, there was a trend for significant differences between A/A and A/T genotypes for time points +10, +20, +30 post stress (corresponding *p* values: .062, .053, .080, respectively). There were no differences between the A/T and T/T genotypes at any time point (all *ps* > .210). Results of the post-hoc analyses as well as graphical inspection of the data support the assumption of a dominant T allele effect. This is further supported by results of the GLM assessing the dominant T-allele model, which revealed a significant time by genotype interaction ($F_{2.70, 497.33} = 3.73$; $p = .014$, $\eta^2 = .02$) and a significant main effect of genotype: $F_{1, 184} = 4.50$; $p = .035$, $\eta^2 = .02$).

Analyses of the subjective stress measure revealed no significant differences (time by genotype interaction: $F_{2, 188} = 1.46$; $p = .235$, $\eta^2 = .01$; main effect genotype: $F_{2, 188} = 2.19$; $p = .115$, $\eta^2 = .02$). Descriptively, as shown in Fig. 2, T/T and A/T carriers showed higher anticipatory stress levels. Results of the dominant T-model revealed a significant main effect of genotype ($F_{1, 189} = 4.33$; $p = .039$, $\eta^2 = .02$; time by genotype interaction: $F_{1, 189} = .53$; $p = .466$, $\eta^2 < .01$). All reported results were stable when controlling for group size.

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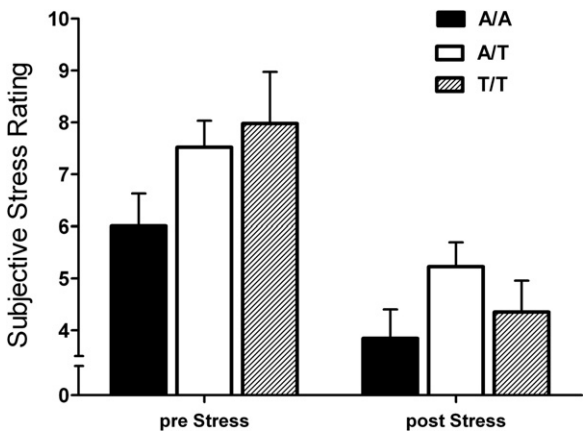

Fig. 2. Subjective stress levels in *NPSR1* rs324981 genotype groups in the anticipation of stress and 20 min following the TSST-G. Error bars are s.e.m.

4. Discussion

This is the first report to show involvement of the NPS system in the regulation of the neuroendocrine stress response in humans. Our results suggest an activating function of NPS on the HPA axis, as the gain-of-function T-allele was associated with larger salivary cortisol responses to acute psychosocial stress and with increased subjective stress levels in the stress anticipation phase. The results are consistent with rodent models showing a stimulating effect of NPS on the HPA axis (Smith et al., 2006) and a stress-induced expression of immediate early genes in NPS-producing neurons in the brainstem (Liu et al., 2011).

Central regulation of the HPA axis involves integration of multiple excitatory and inhibitory inputs at the hypothalamic paraventricular nucleus (PVN; (Ulrich-Lai and Herman, 2009). In the rat brain, NPS-producing neurons are restricted to three distinct brainstem structures (Xu et al., 2007). Investigation of transmitter systems that control NPS release, and the functional characterization of NPS signalling in stress circuitry, is in its early stages. However, it has been shown in the mouse model that both forced swim and restraint stress caused expression of c-fos in both NPS producing brain stem nuclei (Liu et al., 2011). Furthermore, in the rat model, intra-PVN injection of NPS significantly increased plasma ACTH levels. NPS also caused significant increases in corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) release from hypothalamic explants, whereas other hypothalamic hormones such as thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and neuropeptide Y (NPY) were not affected. Finally, there was no effect of NPS on ACTH release from pituitary explants. These findings suggest a direct and specific effect of NPS on HPA axis reactivity via the hypothalamus through release of CRH and AVP (Smith et al., 2006).

Our findings extend recent experimental studies in humans on amygdala reactivity (Dannlowski et al., 2011) and autonomic arousal (Domschke et al., 2011; Raczka et al., 2011), and add to the picture of increased physiological reactivity to psychosocial challenge in rs324981 T-allele carriers. However, results observed in humans seem to contradict the observation of anxiolytic-like effects of NPS challenge in rodents. Several possible explanations may resolve this paradox. First, pharmacological administration in the mature animal does not readily mimic genetically based differences in receptor efficacy acting throughout development. Similar to findings concerning the serotonin transporter (Ansorge et al., 2004), increased neuropeptide S signalling during specific maturational phases in early development might have long-term effects on neuronal circuits involved in anxiety and stress processing. Second, association of the more active T-allele with cortisol responses and anxiety-relevant phenotypes could be due to increased levels of arousal driven by NPS. Third, genetically based differences in NPSR efficacy may affect turn-over rate and expression of the receptors (Reinscheid et al., 2005; Xu et al., 2004).

As a limitation, it has to be noted that the observed effect sizes were small, with 2–3% of variance explained. This not surprising for a candidate association study, since only one gene studied was studied, and several genes are involved in the complex regulation of the HPA axis (e.g. Kumsta et al., 2007). Nevertheless, despite small effect sizes, the study shows for the first time involvement of NPS in stress regulation. As a further limitation, it should be noted that only men were studied. Thus, the results cannot be directly generalized to women. Replication of this study with a female sample is necessary, also to test for a possible sex by genotype interaction. Furthermore, in addition to the current measures, a validated instrument for subjective stress, and data on autonomic responses such as heart rate and/or blood pressure should be incorporated in future studies.

In summary, we studied the association between a common *NPSR1* SNP and hormonal as well as subjective stress responses in humans. The T-allele, previously associated with anxiety-related phenotypes, was associated with increased cortisol and emotional stress reactivity, which represents an important endophenotype for stress-related mental disorders. As the majority of individuals carry at least one T-allele, it likely does not constitute a risk factor per se; rather, it may represent one vulnerability factor for stress- and anxiety-related disorders, only manifesting in disorder in combination with chronic stress or traumatic events. Future studies incorporating data on early environmental factors, chronic stress, or traumatic events are warranted to test for specific gene–environment interactions.

In addition to existing studies involving patients with panic disorder and agoraphobia (Domschke et al., 2011), it would be worthwhile to investigate patient samples with other anxiety disorders (e.g. social phobia or specific phobias) in order to test whether *NPSR1* variation is related to anxiety symptoms in patients. With regard to diagnosis and treatment, the utility of biomarkers in the identification of biologically homogenous disorder subtypes has been discussed (Kapur et al., 2012). Biomarker panels, including information on genetic variation (Schmidt et al., 2011), might stratify broad-illness phenotypes into a finite number of subgroups, potentially informing treatment choices. Biologically informed diagnosis and treatment choices are still far down the road and will require validation using longitudinal designs. However, first results showing that treatment response to psychotherapeutical intervention is conditional on genotype have been reported (Eley et al., 2012). It is possible that information on genetic variability of *NPSR1* and other stress- or anxiety-related genes will be useful for designating specific subgroups of anxiety patients and possibly guiding treatment regimes. Our findings on the role of NPS in human stress regulation might stimulate the development of novel neuropharmacological approaches targeting NPSR and/or NPS release to provide new avenues for a better treatment of stress-related disorders.

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