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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 on 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 (Meis et al., 2008; 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). Importantly, stress exposure led to an increase in NPS levels in the amygdala (Ebner et al., 2011), and NPS in the amygdala prevented stress-impaired fear extinction in mice (Chauveau et al., 2012).

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

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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 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 recruited 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.

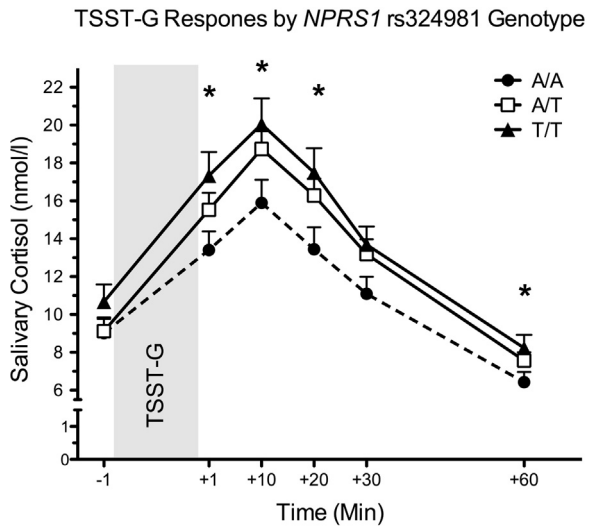

Fig. 1. Salivary cortisol responses to social stress in *NPSR1* rs324981 genotype groups. Post-hoc tests showed significant differences between T/T and A/A carriers (all *ps* < .03, indicated by *). Stress was induced by a standardized social laboratory stressor in a group format (Trier Social Stress 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