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Determinants of the NF-κB response to acute psychosocial stress in humans

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ABSTRACT

Previous research has shown that psychosocial stress is associated with an increased activity of the transcription factor nuclear factor-kappaB (NF-κB), a major inducer of inflammatory genes. While considerable individual variation has been noted, factors contributing to this variation have not been described so far.

Therefore, 29 healthy participants (35.8 ± 12 yrs) were exposed to the Trier Social Stress Test. Blood was collected before and repeatedly afterward for determination of NF-κB activity, leukocyte subset numbers, cortisol, norepinephrine, and *in vitro*-stimulated IL-6 production. Additionally, age, sex, and ratings of perceived chronic and acute stress were assessed.

Regression analyses revealed that older participants showed a lower NF-κB stress response compared to younger adults ($\beta = -.42$, $p = .026$). Higher NF-κB stress responses were associated with lower cortisol stress responses ($\beta = -.37$, $p = .05$), higher pre-stress IL-6 production ($\beta = .38$, $p = .043$), and high chronic in combination with low acute stress, or vice versa ($\beta = -.61$, $p = .06$). Norepinephrine and sex were not associated with NF-κB stress responses (all $p \geqslant .13$).

In summary, the present study shows for the first time in human psychosocial stress the negative association of cortisol and NF-κB. This parallels results from *in vitro* studies. Our finding of lower NF-κB stress responses in older age and in people with high chronic and acute stress might be interpreted as an adaptive dampening of NF-κB activity. In the absence of longitudinal data, however, this interpretation remains speculative.

1. Introduction

Stress is known to activate major biological systems, including the hypothalamus-pituitary adrenal (HPA) axis and the sympatho-adrenal-medullary (SAM) system. (Dickerson and Kemeny, 2004; Goldstein, 2000; Kvetnansky and McCarty, 2000). Stress-related changes in cortisol and catecholamine levels follow a specific pattern, such that catecholamines are released within seconds after stressor onset and return to baseline levels shortly after stressor termination, while the release of cortisol shows a time-lag of approximately 10 min and takes up to 1 h after stress cessation to return to baseline levels (Sapolsky et al., 2000). These phased responses are thought to be adaptive and beneficial for the organism. This concerns especially their effects on the immune system, where cortisol is thought to terminate immune processes—activated among others by stress-induced increases in catecholamines (DeRijk et al., 1994)—in order to protect the organism from an overactive immune system (Besedovsky and del Rey, 1996, 2000; Sapolsky et al., 2000). Consequently, deviations from these expected response patterns have been associated with different disease processes or susceptibilities towards different diseases (Chrousos, 1998), such that, for example, a decreased HPA axis activity is found in individuals with fibromyalgia (Chikanza et al., 1992) and a blunted cortisol response in combination with an elevated catecholamine response in atopic diseases (Buske-Kirschbaum et al., 2002).

Cortisol and catecholamines exert their immune effects via specific signal transduction pathways. In detail, cortisol binds in the cytoplasm to its glucocorticoid receptor (GR) and this hormone–receptor complex then translocates to the nucleus, where it binds to glucocorticoid bindings elements (GRE) or negative GREs (nGRE), thereby activating or inhibiting the transcription of responsive genes. Catecholamines exert their effects by binding to adrenoceptors (AR), which can be classified into three major groups, α1-, α2-, and β-AR types (Hasko and Szabo, 1998). Norepinephrine predominantly activates α-ARs and β1-AR and is a weak stimulator of β2-AR, whereas epinephrine is a strong stimulator of β-ARs (Motulsky and Insel, 1982). ARs directly activate G-proteins, with different types of ARs coupling to different G-proteins and thus initiating different signal transduction pathways. For example, β-ARs couple to G_s proteins, which activates adenylate cyclase

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(AC). AC in turn increases intracellular cyclic adenosine monophosphate (cAMP), which activates protein kinase A (PKA). Contrary, coupling of α2-AR to G_i proteins inhibits AC and thus subsequently the formation of cAMP. The α1-AR coupling to G_q proteins activates another intracellular effector, namely phospholipase C (PLC), which increases inositol triphosphate (IP_3) and diacylglycerol (DAG). DAG then activates protein kinase C (PKC) and IP_3 mobilizes Ca^{2+} from intracellular stores. The latter is further linked to the Ca^{2+}/calmodulin (Ca^{2+}/CaM) pathway, which—like PKA—subsequently transfers signals to the nucleus (Elenkov et al., 2000).

While activation of the above signal transduction pathways can have a wide range of effects, they also converge at one point. Eventually they all influence the activity of the transcription factor nuclear factor-kappaB (NF-κB). The designation NF-κB refers to the most frequently occurring and ubiquitously expressed heterodimeric complex between two members of the NF-κB/Rel family of proteins, the p50 and the p65 (Rel A) subunit (Caamano and Hunter, 2002; Wulczyn et al., 1996). NF-κB is found in virtually every cell of the immune system and regulates a great number of genes, including genes of growth factors (e.g., granulocyte/macrophage colony stimulating factor), pro-inflammatory cytokines (especially interleukin-1 (IL-1), IL-2, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α)), or cell adhesion molecules (e.g., vascular cell adhesion molecule; for review see McKay and Cidlowski, 1999). It is thought that many of the immune-inhibitory functions of cortisol are due to cortisol interfering with NF-κB activity, either by inducing expression of the NF-κB inhibitory protein IκB-α (Auphan et al., 1995; Scheinman et al., 1995), by GRs interacting with the p65 subunit and thereby repressing NF-κB DNA binding activity (De Bosscher et al., 1997, 2000; Ray and Prefontaine, 1994), or by competing for limited amounts of essential coactivators (Lee et al., 1998; Sheppard et al., 1998). In parallel to glucocorticoids, several lines of evidence exist of catecholamine signal transduction pathways interfering with activity of NF-κB as well. Haraguchi et al. suggested that all three pathways, i.e., the cAMP/PKA, the PKC, and the Ca^{2+}/CaM pathway, modulate NF-κB activity by regulating its phosphorylation status (Haraguchi et al., 1995). Furthermore, elevated levels of cAMP are known to inhibit NF-κB activity by inhibiting the binding of NF-κB to the NF-κB DNA binding site (Chen and Rothenberg, 1994; Neumann et al., 1995; Tsuruta et al., 1995). The cAMP/PKA pathway also induces impaired nuclear translocation and DNA binding of p65 (Neumann et al., 1995; Paliogianni et al., 1993). Alternatively, this pathway may inhibit NF-κB transcription by phosphorylating the transcription factor CREB (cAMP response element-binding protein), which then competes with NF-κB p65 for limited amounts of CREB-binding protein (CBP) (Parry and Mackman, 1997). Contrary, NF-κB activity can be stimulated via the Ca^{2+}/CaM pathway enhancing inactivation of the inhibitory protein IκB-α (Frantz et al., 1994).

Given the tight interaction between stress hormone and NF-κB signaling pathways, we hypothesized that NF-κB activity should vary in response to stress. We were able to show that NF-κB activity indeed increased in response to the Trier Social Stress Test (TSST) in healthy young men (Bierhaus et al., 2003). These findings have been replicated in patients with major depression (Pace et al., 2006). As we further showed in subsequent animal and *in vitro* studies, norepinephrine in physiological concentrations (but not epinephrine) was able to increase NF-κB activity (Bierhaus et al., 2003). Although these findings showed that psychosocial stress impacts a biological pathway with major health implications, little is still known about the determinants of this inflammatory stress response. No study has yet tested *in vivo* the hypotheses that NF-κB activity, and thus, peripheral inflammation, is activated by catecholamines, and negatively controlled by glucocorticoids. Although these might remain impossible to test in humans, not even correlational studies are available that tested associations compatible with these hypotheses. Little is also known about the role of demographic variables, i.e., sex and age differences in NF-κB stress responses as well as the role of perceived acute and chronic stress.

The current study therefore aimed at testing potential determinants of the NF-κB stress response and recovery. Specifically, we hypothesized that the NF-κB stress response would be higher in older participants and in women, based on the findings of age-related increases of inflammatory activity (Ershler and Keller, 2000) and on documented sex differences in inflammatory responses (O'Connor et al., 2007). We further hypothesized that perceived stress, both acutely and chronically, would be associated with higher NF-κB stress responses, the former based on findings from acute stress studies (e.g., Bierhaus et al., 2003), the latter based on findings of altered inflammatory regulation in chronic stress (Miller et al., 2002). In both cases, we also aimed at testing the effect of the interaction between age and sex as well as the interaction between acute and chronic stress on NF-κB stress responses, since various reports exists on, for example, endocrine stress responses being influenced by the interaction between age and sex (Kudielka et al., 2004), and on, for example, GR and β2-AR gene expression being influenced by the interaction of acute and chronic stress (Miller and Chen, 2006). Furthermore, we set out to test the hypothesis that norepinephrine responses would be positively and cortisol responses inversely associated with the NF-κB stress response, as outlined above. Since reports exist that changes measured in NF-κB activity may be due to changes in the cell type composition of the assessed sample (Richlin et al., 2004), we additionally aimed at testing whether the NF-κB stress response is associated with stress-induced changes in leukocyte subsets. Lastly, we also assessed *in vitro*-stimulated IL-6 production. As pointed out above, NF-κB is centrally involved in many inflammatory processes, among others by inducing one of the most important pro-inflammatory cytokines, namely IL-6. *In vitro*-stimulated IL-6 production, in turn, is a parameter which is frequently used to assess inflammatory activity (e.g., Miller et al., 2002). Given the tight connection between the two parameters and both parameters being indicators of inflammatory activity, we aimed at comparing the results of *in vivo* stimulation of the relevant pathways by psychosocial stress via assessing changes in NF-κB activity and the *in vitro* stimulation of the relevant pathways by assessing one important outcome, i.e., the stimulated production of IL-6. Lastly, we aimed at testing whether NF-κB recovery, instead of the NF-κB stress response, is predicted by any of the above proposed determinants.

2. Study design and methods

2.1. Subjects

A total of 29 adults were recruited through advertisements in newspapers and by flyers. All subjects underwent a comprehensive medical examination for past or current health problems. Exclusion criteria were any psychiatric, endocrine, cardiovascular, or other chronic disease, as well as medication with psychoactive drugs, β-blockers, or glucocorticoids. The sample consisted of 17 women and 12 men with a mean age of 35.8 yrs (SD = 12.2; range = 20–59 yrs) and a mean BMI of 23.3 kg/m^2 (SD = 3.84; range = 17.8–37.1). Men and women did not significantly differ with regard to BMI and age ($t = 0.35$; $p = 0.73$ and $t = -0.045$; $p = 0.96$, respectively). Three female participants reported to be habitual smokers. All participants were Caucasians.

2.2. Experimental protocol

Participants reported to the laboratory between 13:00 h and 15:00 h and were examined by a physician for past and current health problems. A venous blood catheter was inserted and partic-