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No response of plasma substance P, but delayed increase of interleukin-1 receptor antagonist to acute psychosocial stress

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Abstract

Psychosocial stress has been shown to induce inflammatory reactions, followed by the release of immunosuppressive glucocorticoids. This may be mediated by catecholamines or other stress reactive substances such as neuropeptides or cytokines. We here set out to explore the effects of acute psychosocial stress on plasma levels of substance P (SP), a possible mediator of stress-induced inflammatory reactions, and interleukin-1 receptor antagonist (IL-1ra).

Twelve healthy male subjects (mean age 27 yrs.) were subjected to the psychosocial stress test "Trier Social Stress Test" (TSST) and a resting control condition. Blood and saliva samples were taken before, as well as 1, 20, 45, and 90 min after TSST or rest, respectively. Salivary cortisol and plasma SP and IL-1ra were measured using immunoassays, salivary alpha-amylase (sAA) was measured by an enzyme kinetic method, and plasma epinephrine (E) and norepinephrine (NE) were measured by HPLC.

The TSST induced immediate increases of E, NE, and sAA, and a delayed increase of free cortisol. Plasma IL-1ra showed an even further delayed peak at 90 min after stress. Plasma levels of SP did not respond to stress. No significant associations between changes of stress hormones and IL-1ra or SP were found.

We conclude that substance P, epinephrine, and norepinephrine are probably not involved in mediating peripheral inflammation following psychosocial stress, at least with respect to IL-1ra. Further studies have to reveal the mechanisms involved in the stress-induced up regulation of IL-1ra.

Keywords: Psychological stress; Human; Substance P; Interleukin-1ra; Inflammation; HPA axis; Cortisol; Epinephrine; Norepinephrine; Salivary alpha-amylase

Introduction

It is well established that psychosocial stress induces activation of central and peripheral stress systems with concomitant release of glucocorticoid and catecholamine hormones, which in the short term are regarded as protective for the organism (Sapolsky et al., 2000). However, activation of stress systems is also associated with inflammatory processes, which at least through repeated activation, may foster inflammatory diseases. Repeated or chronic activation of stress systems over the life span has been associated with increased rates of cardiovascular and metabolic diseases, both of which are mediated by inflammatory processes (McEwen and Stellar, 1993). These effects are most likely not mediated by stress hormones alone, but rather by interactions with additional soluble mediators (Black, 2002).

An interesting candidate as a mediator of stress-induced inflammatory reactions is substance P (SP), a member of the neurokinin (tachykinin) family of peptides (Otsuka and Yoshioka, 1993; Pioro et al., 1990). Several studies have demonstrated a role of SP in the acute stress response. In animals, maternal separation of guinea-pig pups led to increased SP release (Kramer et al., 1998), and application of electric foot shocks caused a marked increase in adrenal SP release in rats (Vaupel et al., 1988). In humans, Faulhaber et al. found that SP-

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like immunoreactivity in blood increased after a mental arithmetic test (Faulhaber et al., 1987). Schedlowski et al. investigated plasma SP in unexperienced tandem-parachutists. While no significant increase of SP in response to the jump was found, SP levels were higher in subjects with higher anxiety levels immediately before the jump (Schedlowski et al., 1995). Similarly, Fehder et al. found that SP levels were higher in subjects with high anxiety before a diagnostic medical procedure (Fehder et al., 1997). Weiss et al. measured SP plasma levels in civilians during missile attacks on Israeli cities within the 1991 Persian Gulf War and found SP levels to be significantly elevated (Weiss et al., 1996). For a review of the role of substance P in stress and in affective disorders see Herpfer and Lieb (2005).

While the relationship of SP to inflammatory reactions in humans following acute stress has not been investigated so far, several studies suggest that SP may be a mediator of stress-induced inflammatory reactions. SP has been shown to strongly induce the synthesis of cytokines such as interleukin (IL)-6 by induction of different transcription factors including nuclear factor (NF)-kappaB (Lieb et al., 1997; Lieb et al., 1998). Therefore, SP has been proposed as mediating the stress response of macrophages (Chancellor-Freeland et al., 1995; Lotz et al., 1988), although this has been discussed controversially (Lieb et al., 1996).

Another macrophage product of essential role in this context is interleukin-1ra (IL-1ra) (Re et al., 1993). IL-1ra binds to human IL-1 receptors without cellular activation, and thus functions as a potent inhibitor of IL-1 effects (Arend et al., 1989). Due to this properties and the fact that IL-1ra is induced by inflammatory stimuli, it is considered a natural anti-inflammatory protein (Gabay et al., 1997). Plasma levels of IL-1ra can be experimentally increased by injections of IL-1 or IL-6 (Bargetzi et al., 1993; Steensberg et al., 2003; Tilg et al., 1994), which is mediated by activation of toll-like receptors (TLR; Carl et al., 2002), and NF-kappaB further downstream (Smith et al., 1998).

Some studies have shown induction of IL-1ra by physical and psychosocial stress. Marathon running for example increased plasma levels of IL-1ra (Suzuki et al., 2000), and also shorter periods of exercise led to increased expression of IL-1ra together with pro-inflammatory genes in peripheral blood mononuclear cells (PBMC; Connolly et al., 2004). Less clear-cut results have been obtained for psychosocial stress. IL-1ra production in cell cultures, as well as levels in crevicular fluid were not altered due to academic stress (Uchakin et al., 2001; Waschul et al., 2003). Three recent studies reported responses of plasma IL-1ra to mild laboratory stress, which however, was not able to increase cortisol (Kunz-Ebrecht et al., 2003; Steptoe et al., 2002; Steptoe et al., 2001). This is of special importance, given that cortisol suppresses IL-1ra secretion in PBMCs in vitro (Sauer et al., 1996). While in vivo effects of cortisol are unknown so far, epinephrine infusion has been shown to increase plasma IL-1ra (Sondergaard et al., 2000).

To investigate the role of SP as being a possible mediator linking psychosocial stress with peripheral inflammatory processes, we set out to use the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) to induce acute psychosocial stress and pronounced increases of the stress hormones cortisol, epinephrine, and norepinephrine, and the stress sensitive enzyme salivary alpha-amylase and relate these to plasma responses of substance P and interleukin-1-receptor antagonist (IL-1ra), which we chose as an index for peripheral inflammation.

Materials and methods

Subjects

We investigated twelve healthy male subjects with a mean age of 27.0±1.2 years (range: 21 to 33 yrs.) and a mean body mass index (BMI) of 22.5±0.6 kg/m^2; range: 20 to 25 kg/m^2). Subjects were recruited at the University of Düsseldorf. All subjects underwent a comprehensive medical examination for past or current health problems. Exclusion criteria were any psychiatric, endocrine, cardiovascular, or other chronic diseases, medication with psychoactive drugs, beta-blockers, or glucocorticoids, and a BMI <20 or >25 kg/m^2, and age <20 or >35 yrs, respectively. Subjects' chronic stress levels were tested with the Trier Inventory for Assessment of Chronic Stress (TICS; Schulz and Schlotz, 1999) and stress susceptibility was tested with the Stress Reactivity Scale (SRS; Schulz et al., 2005). Mean values on all scales were within the normal ranges provided with the TICS and SRS, respectively.

Experimental protocol

After a telephone interview regarding basic inclusion criteria (e.g. age, BMI, or medication), subjects were scheduled for two laboratory sessions on consecutive weekdays, always beginning between 15:00 and 17:00 h. The first lab session served as a control condition, whereas the second lab session served as a stress condition. The sequence was not randomized, because during the first lab session subjects should get used to the laboratory situation. On the first lab session, the medical examination was done and depressive symptoms were checked with the ADS-L (Allgemeine Depressions-Skala). The ADS-L is a German version of the (CES-D; Center for Epidemiological Studies Depression Scale) (Hautzinger and Bailer, 1992). If inclusion criteria were met, a written explanation of the study goals and protocol was handed to the subject. Upon agreement to the study protocol, all subjects signed a written informed consent letter. The study protocol was approved by the ethics committee of the German Psychological Association (DGPs). Subjects were paid EUR 50 for their participation.

Subjects received 300 ml of a high glucose drink (regular grape juice) to raise blood glucose levels, since low glucose levels have been shown to blunt stress-induced increases of cortisol (Kirschbaum et al., 1997). A catheter (Vasofix Braunüle, Braun, Melsungen, Germany) was inserted into an antecubital vein, followed by a resting period of 45 min to reduce the impact of the possibly stressful process of reporting to an unknown laboratory and catheter insertion. A first blood and saliva sample was taken, followed by a resting condition on lab