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EFFECTS OF ACUTE TIME-LIMITED STRESSORS ON IMMUNE MODULATION

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Abstract: Psychoneuroimmunology is the science that links psychological processes and the immune system. Many studies showed that psychological stress has immune modulatory activity. This article describes a typical psycho-neuro-endocrino-immune network in response to acute stress. Finally, it has been shown that acute time limited stressors have immune stimulatory activities which are unnecessary and harmful to one's homeostasis.

Keywords: Psychoneuroimmunology, stress, HPA axis, glucocorticoids, β-endorphin and Met-enkephalin

Introduction

The modern medical science is highly influenced by mechanistic view, which describes mind and body as separate entities. The reductionism of the past also led us into the trap of supposing that the autonomic nervous system, the endocrine and immune systems are independent and autonomous self-regulating structures (Rossi, 1993; Sternberg, 2000). However, this narrow focus has also tended to obscure the importance of the interactions between mind and body and to overshadow the possible importance of the mind in producing and alleviating disease. But during the past three decades, there has been a powerful scientific movement to explore the mind's capacity to affect the body and has been shown that autonomic nervous system, the endocrine and immune systems constitute a cybernetic whole (Felton *et al.*1991; Sternberg, 2000; Glaser and Kiecolt-Glaser, 2005); any division is merely the hallucination of somewhat blinkered theorists. Psychoneuroimmunology (PNI) is the broad interdisciplinary research field that describes some aspects of joined-up-ness of the nervous, endocrine and immune systems (Biondi and Zannino, 1997; Rabin, 1999; Ader, 2000; Ader *et al,* 2001).

Studies undertaken the past three decades have provided evidence that immune alterations are stimulated by stressful events. Stress is a complex process involving social, psychological and physiological elements. According to psychobiological theories stress is determined by "the balance between the perceived demands from the environment and the individual's resources to meet those demands" (Frankenhaeuser, 1986; Lundberg, 1995). More broadly, psychobiological stress ensues when events or environmental demands exceed an individual's perceived ability to cope (Cohen *et al,* 1998). Some researchers have been categorized stressors on two important

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dimensions: duration and course (Elliot and Eisdorfer, 1982). According to them acute time-limited stressors ranging from 5 to 100 minutes that involves laboratory challenges such as public speaking or mental arithmetic (Segerstrom and Miller, 2004). And some studies supported that acute time limited stressor showed immune stimulatory or immune modulatory activity (Dopp *et al.,* 2000; Sapolsky, 1998).

On the basis of these findings in this study a typical psycho-neuro-endocrino-immune network has been shown (Fig. 1). From this network Corticotropin Releasing Factor (CRF), Adrenocorticotropic Hormone (ACTH), Glucocorticoids (GC), β-endorphin and Met-enkephalin are found as important endocrine components, and T cells, B cells, monocytes/macrophages, Natural Killer(NK) cells and their cytokines that is Tumor Necrosis Factor-α (TNF-α), Interferon Gamma (IFN-γ) and interleukins such as IL-1, IL-2, IL-4, IL-6, IL-10, IL-12 etc. are found as important immune components. Finally, it has been shown that, acute stressors have immune modulatory activities which are harmful to one's homeostasis.

Methods and Materials

The classical markers for acute stress are cortisol, epinephrine, heart rate and blood pressure, which will increase rapidly to stress exposure. There is a disadvantage with epinephrine, heart rate and blood pressure is that these measures will not reflect the emotional value of the stressor and are rather nonspecific. Also activation associated with positive mood and pleasant activities will increase the levels of these measures. For this it has been proposed by some experts (Lundberg and Frankenhaeuser, 1980), that cortisol is a better marker if the emotional value is of interest. Hence, in our study we have taken cortisol as the marker of stress.

Results

Psychoneuroendocrine network: All organisms, from bacteria to humans, have evolved mechanisms to deal with significant changes in their external or internal environments, that is, stressors. First the environmental and psychological stresses are perceived and processed in the cerebral cortex of the forebrain. Then through cortical and limbic forebrain structure hypothalamus is stimulated. As a result hypothalamus secretes stress hormone CRH (Corticotropin Releasing Hormone) (Chrousos, 1995; Straub *et al.*, 1998). CRH of the hypothalamus peptide plays the major role in regulation of pituitary corticotrope tropic activity (Gertz *et al.*, 1987) and POMC (proopiomelanocortin) gene transcription (Autelitano *et al.*, 1990). CRH is the major physiologic regulator of the basal and stress-induced release of POMC-derived peptides from the anterior pituitary (Dunn and Berridge, 1990; Owens and Nemeroff, 1991).

POMC gets glycosylated and then cleaved to give a number of neurohormones. The anterior pituitary hormones ACTH (adrenocorticotrophic hormone) and LPH (lipotropin hormone) are derived from POMC in the pars distalis of the anterior pituitary. In the pars intermedia area of the anterior pituitary, the ACTH derived from cleavage of POMC is cleaved further to produce γ-MSH (melanocyte-stimulating hormone). Thus, POMC is processed differently in different areas of the pituitary. We can also get β-end (endorphin) out of POMC cleavage, which means POMC is a source of an endogenous opioid peptide. In fact, POMC is the precursor to ACTH, α-, β -LPH (lipotropin hormone), α-, β -, γ-MSH (melanocyte-stimulating hormone), α-, β-, γ-end, and met-enk (enkephalin) (Foye *et al.*, 1995).

ACTH the processed product of POMC stimulates the secretion of glucocorticoids (Felton *et al.*, 1991; Chrousos, 1995), corticosterone in rodents, and cortisol in primates by the cells of the adrenal cortex (Munck *et al.* 1984). Glucocorticoids help maintain blood pressure and form an essential component of the body's response to stress. Cortisol feeds back to the pituitary and hypothalamus to suppress levels of ACTH and CRH. Under basal (non-stress) conditions, cortisol is secreted with a pronounced circadian rhythm, with higher levels early in the morning and low levels late in the evening. Under stressful conditions, the circadian variation is blunted (Foye *et al.* 1995).

Regulatory activity of endocrine components on immune system: CRH is a mediator of endocrine, autonomic and immune responses to stress (Dunn and Berridge, 1990; De Souza, 1995). CRH plays a significant role in integrating the stress-related responses to immunological agents through its coordinated actions in the nervous, endocrine and immune systems (Blalock, 1989; Owens and Nemeroff, 1991; Webster, *et al.*, 1991). CRH has direct effects on immune function and inflammatory processes. CRH induces the secretion of POMC derived peptides such as ACTH and β-endorphin in human peripheral blood and mouse splenic leukocytes. Furthermore, CRH stimulates the secretion of IL (interleukin)-1 and IL-2, as well as lymphocyte proliferation and IL-2 receptor expression in peripheral blood leukocytes (De Souza, 1995). CRH also stimulates B cell proliferation and NK cell activity and IL-6 production (Leu and Singh, 1992). Receptors for CRH have been found on immune cells (Webster, *et al.*, 1990), providing a mechanism for these effects.

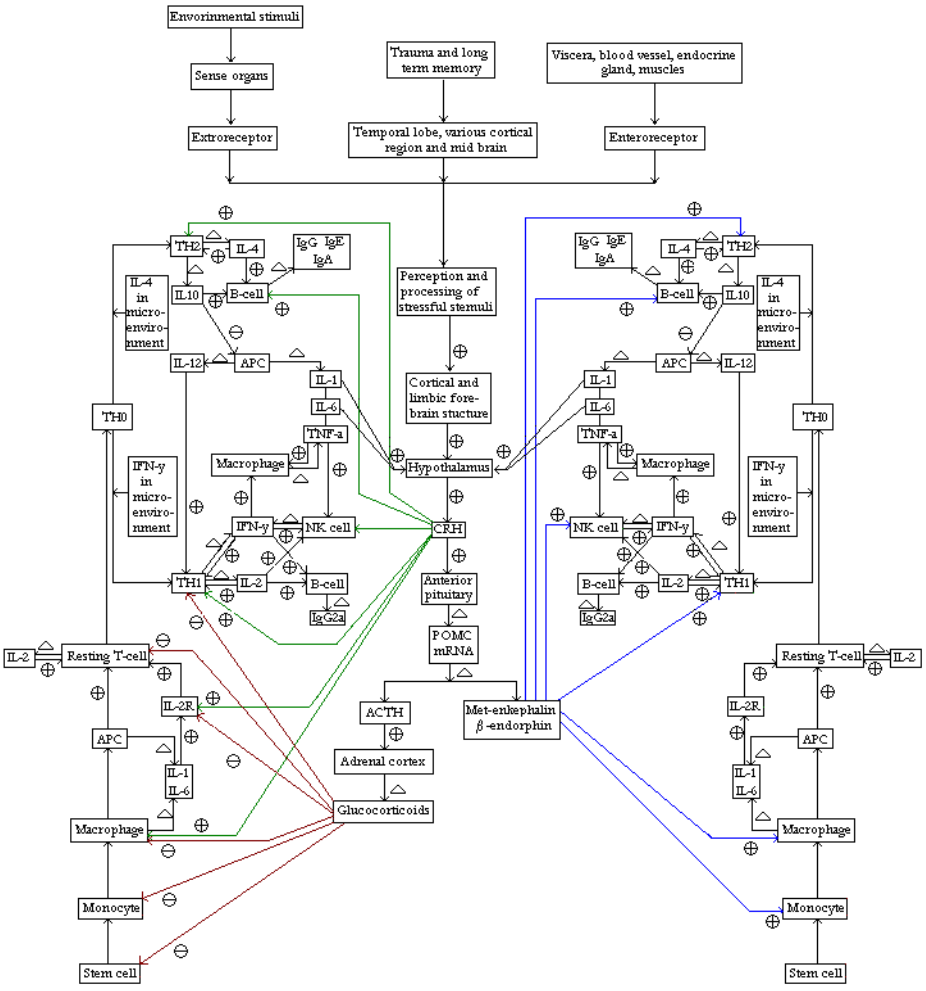

Fig. 1. A typical psycho-neuro-endocrine-immune network (⊕ : activating reaction; Θ: inhibiting reaction; Δ: releasing activity)

It was observed that the increase in β-end actually causes the increase in the count of NK cells and also increases cytotoxicity in a dose dependent manner and that the effects seem to be mediated by μ and δ opioid receptors (Jonsdottir, *et al.*, 1997). Thus an increase in endogenous opioids leads to an increase in NK cells which may enhance one's immunity. On the other hand the Met-enk stimulates B- and T-cell proliferation (Kowalski, 1998) and also able to stimulate the migration of monocytes, lymphocytes and neutrophils *in vitro* towards the site of injection (Stefano *et al.* 1996; Weigent and Blalock, 1997). Met-enk stimulates the release of proinflammatory cytokines such as IL-6 (Goumon, *et al.*, 1998). Moreover, pro-enk mRNA levels in peripheral human blood monocytes are increased in response to IL-6 (Goumon *et al.* 1998; Kamphuis *et al.* 1998). Met-enk also influences intracellular signal transduction with T-cells, as the $Ca2^{+}$ levels were lower in T-cells incubated with met-enk, as compared with controls (Sorensen and Claesson, 1998; Li, 1998). Prepro-enk mRNA and Met-enk are present at higher levels in TH2 cultures compared with TH1 cultures (Hook *et al.* 1999). They concluded that prepro-enk has a role in enhancing TH2 cell function (Hook, *et al.*, 2000). Met-enk is now considered as a new cytokine (Plotnikoff, *et al.*, 1997), probably implicated as a pro-inflammatory signal in the immune response (Stefano, *et al.*, 1998; Salzet *et al.* 2000; Tasiemski, *et al.*, 2000). However, high concentration of Met-enk inhibits the inflammatory response like do the POMC derived peptides such as ACTH and MSH (Lipton and Catania, 1997).

Glucocorticoids also have immune modulatory activity. Macrophage functions are relatively sensitive to glucocorticoids inhibitory action (Parrillo and Fauci, 1979). Glucocorticoids alter macrophage functions that effect cell function. Glucocorticoids induce a monocytopenia, suppressed committed marrow monocyte forming stem cells, and block the differentiation of monocytes into macrophages (Bar-Shavit, *et al.*, 1984). By blocking the production of IFN-γ, glucocorticoids can also decrease the levels of Fc receptors on monocyte and macrophages (Larsen and Henson, 1983); these receptors facilitate the phagocytosis of particular antigens and other functions of the cells in the inflammatory responses (Swanson and Hoppe, 2004). Thus, glucocorticoids block the ability of the monocytes to bind to antibody coated cells, elicit bactericidal activity and cytotoxicity (Parrillo and Fauci, 1979). Glucocorticoids suppress macrophage production of IL-1, which is involved in T cell mitogenesis and of chemokines that prevent the exit of macrophages from inflammatory sites (Fahey *et al.* 1981). Glucocorticoids block the production of IFNγ and TNF-α by T cells and their actions on macrophages (Munck *et al.* 1984). They suppress T lymphocyte production of IFN-γ and IL-2 and they inhibit NK cell activity (Fahey *et al.* 1981; Munck *et al.* 1984). Glucocorticoids do not suppress ADCC (antibody dependent cellular cytotoxicity) of human cells (Parrillo and Fauci, 1979). Antibody production by B cells results from a series of steps involving early activation, later, B cell growth factor mediated proliferation, and final differentiation to the antibody producing state. These steps are affected by suppressor T cell and helper T cell functions and can be suppressed by glucocorticoids (Claman, 1975; Parrillo and Fauci, 1979; Fahey *et al.* 1981). In vitro studies suggested that glucocorticoids affect substantially the early activation, have a lesser effect on the B cell growth factor response, and do not affect the stage of maturation (Cupps, *et al.*, 1985). Thus, glucocorticoid's effects on B cell functions are very modest. Cortisol and other glucocorticoids increase glucose production, inhibit protein synthesis and increase protein breakdown, stimulate lipolysis, and affect immunological and inflammatory responses (Foye *et al.* 1995).

Effects of acute time limited stress: Data from a number of studies have shown that various stressors can adversely affect immune function (Graham *et al.*2006; Felton *et al.*1991). Stress has long been suspected of playing a role in the etiology of many diseases, and numerous studies have shown that stress can be immunosuppressive and hence may be detrimental to health

(Khansari *et al.,* 1990; Herbert and Cohen, 1993; Maier *et al.* 1994; Kiecolt-Glaser *et al.,* 1996; Marucha *et al.,* 1998). Both meta-analytic (Herbert and Cohen, 1993) and enumerative (Kiecolt-Glaser et al., 1992; Kiecolt-Glaser and Glaser, 1995) reviews clearly showed that chronic stress can lead to fewer circulating B cells, T cells, and large granular lymphocytes, to decreased proliferative responses of lymphocytes to several mitogens as well as to decreased natural killer cell activity (NKCA). In the case of chronic stress, the downregulation of components of the immune system may lead to increased susceptibility to infections (colds, herpes, HIV) and worsen existing disease processes, such as cancer (Rosen et al., 1984; Kiecolt-Glaser and Glaser, 1995). A reduced functioning of the immune system could possibly contribute to increased physical morbidity and mortality (Shekelle and Raynor, 1981; Whiteside et al., 1990; Weisse, 1992). On the other hand it has been found that Brief naturalistic stressors cause decrease of TH1 type cytokine IFN-γ and increases TH2 type cytokine IL-6 (Maes et al., 1999). These also increase the production of IL-10 (Kiecolt-Glaser et al., 2002) which inhibits TH1 cytokine production. At that time production of IL-12 also decreased (Elenkov et al., 2006), as a result NK-cell activity inhibited. Brief naturalistic stressors thus cause shift of TH1 to TH2 cytokine response or cellular to humoral immunity (Mahbub-E-Sobhani et al., 2008). In this article we briefly review some of the immunological changes that have been associated with acute time limited stress as well as evidence for the efficacy of various interventions.

Acute stressors elicit various patterns of immune change across a wide spectrum of durations ranging from 5 to 100 minutes and irrespective of whether they involved social (e.g., public speaking), cognitive (e.g., mental arithmetic), or experiential (e.g., parachute jumping) forms of stressful experience (Segerstrom and Miller, 2004). Secretory immunoglobulin A (sIgA), measured in saliva, is a convenient and much used indicator of immune status. Measurement of this parameter is thought to be indicative of the functional status of the entire mucosal immune system (Mestecky, 1993). Numerous studies have shown that salivary sIgA is sensitive to psychological variables. Recent it was suggested that the acute response to a psychological challenge is a rise in sIgA, albeit transient (Evans *et al.,* 1997). This mobilisation of sIgA has been reported in response to acute laboratory psychophysiological stress tests, such as public speaking (Bristow *et al.,* 1997), computer game challenge (Carroll *et al.,* 1996), cold pressure task and mental arithmetic (Willemsen *et al.,* 1998). However, the study of Carroll *et al.* (1996) revealed that the sIgA response only characterised novice players. Following an acute stressor, increase in both sIgA and cortisol were found in several findings (Evans *et al.,* 1994).

Reliable effects of acute time limited stressors on the immune system include increase in immune parameters, especially natural immunity. The most robust effect of this kind of experience was a marked increase in the number of NK cells and large granular lymphocytes in peripheral blood (Segerstrom and Miller, 2004). This effect is consistent with the view that acute stressors cause immune cells to redistribute into the compartments in which they will be most effective (Dhabhar and McEwen, 1997). However, other types of lymphocytes do not show robust redistribution effects: whereas, B cells and T-helper cells show a very little change. T-cytotoxic lymphocytes tend to increase reliably in peripheral blood, though to a lesser degree than their natural immunity counterparts; this increase drives a reliable decline in the T-helper:T-cytotoxic ratio (Segerstrom and Miller, 2004). Other indicators of upregulated natural immunity include increased neutrophil numbers in peripheral blood, increased production of a proinflammatory cytokine (IL-6) (Maes *et al.,* 1995; Dentino *et al.,* 1999; Lutgendorf *et al.,* 1999), and increased production of a cytokine (IFN-γ) that potently stimulates macrophages and NK cells as well as T cells (Segerstrom and Miller, 2004). The only exception to this pattern is the increased secretion of IgA antibody, which is a product of the specific immune response (Segerstrom and Miller,

2004). The data for acute stressors, therefore, support an upregulation of natural immunity (Dhabhar and McEwen, 1997; Dhabhar and McEwen, 1999; Dopp *et al.,* 2000), as reflected by increased number of NK cells in peripheral blood, and potential down regulation of specific immunity, as reflected by decreased proliferative responses (Segerstrom and Miller, 2004).

Discussion

Acute time limited stressors increase the number of natural killer cells and large granular lymphocytes in peripheral blood (Segerstrom and Miller, 2004). Moreover, it increases the production of a proinflammatory cytokine IL-6 (Dentino *et al.* 1999; Lutgendorf *et al.* 1999; Maes *et al.* 1999), and cytokine IFN-γ that potently stimulates macrophages and natural killer cells as well as T cells (Segerstrom and Miller, 2004). That means, acute stressor upregulate natural immunity and some components of humoral immunity. This upregulation may be occurred due to the action of CRH and CRH induced secretion of POMC-derived peptides such as met-enk and β-end.

Just after the perception of stress, CRH level in the blood becomes high (Claes, 2004). Immunoreactive CRH and CRH mRNA are expressed in human peripheral blood leukocytes. The CRH has direct effects on immune function and inflammatory processes (De Souza and Grigoriadis, 1995) and receptors for CRH have been found on immune cells (Webster *et al.* 1990) which may facilitate the mechanism of inflammatory responses by the immune cells (Kane *et al.* 2006). Furthermore, CRH stimulates the secretion of IL-1, IL-2 and IL-6 as well as lymphocyte proliferation and IL-2 receptor expression in peripheral blood leukocytes (De Souza and Grigoriadis, 1995). So by increasing the secretion of ILs CRH may activate the immune cells and facilitate their proliferation.

On the other hand POMC derived Met-enk is also found to be able to stimulate the migration of monocytes, lymphocytes and neutrophils in vitro towards the site of injection (Padros *et al.* 1989; Stefano *et al.* 1996) and also stimulate the release of proinflammatory cytokines such as IL-6 (Goumon *et al.* 1998). Met-enk is probably implicated as a pro-inflammatory signal in the immune response (Stefano *et al.* 1998; Salzet *et al.* 2000; Tasiemski *et al.* 2000).

The only exception to this pattern is the increased secretion of IgA antibody, which is a product of the specific immune response (Evans *et al.* 1994, 1997; Carroll *et al.* 1996; Bristow *et al.* 1997; Willemsen *et al.* 1998 ; Segerstrom and Miller, 2004;). But the time frame of these acute stressors is too short for the a significant mount of new antibody; therefore, this increase is probably due to the release of already synthesized antibody from plasma cells and increased translocation of antibody across the epithelium and into saliva (Bosch *et al.,* 2002). This effect therefore represents relocation, albeit of an immune protein rather than immune cells.

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