The influence of short-time period of an adaptation to decreased ambient temperature on interleukin-6 and corticosterone levels in female Wistar strain rats in the proestrous phase of the reproductive cycle

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Abstract: To date, there has been little research examining whether short-time changes of external environmental conditions exert any effects on immune responses. The activation of metabolic changes, release of hormones responsive for immunomodulation and the action of interleukins play an important role in interaction with hormones of an anterior pituitary gland in the proestrous phase of the reproductive cycle. The aim of our study was to determine the effects of a short-time change of ambient temperature (30 minutes) on interleukin-6 (IL-6) and corticosterone plasma concentration of female rats in the proestrous phase of the reproductive cycle. The climatic chamber with automatically adjustable and monitored internal environmental parameters (temperature, oxygenation, humidity) was used during the experiment. The estimation of the vaginal lavage using a microscope was done to determine the estrous cycle. On the day of the experiment, animals were divided into 2 groups: the control group (ambient temperature 21°C ± 1°C; normoxia 21% O₂) and the test group (ambient temperature 10°C ± 1°C; normoxia 21% O₂) stayed in the climatic chamber for 30 minutes. The blood samples were collected before the experiment and after 30, 60, 90, 150 and 210 minutes from the beginning of the experiment. The concentrations of IL-6 and corticosterone were measured in blood plasma samples using ELISA method. There was a significant elevation of IL-6 levels after staying in 10°C during the first 150 minutes from the beginning of the experiment, with the highest value occurring after 60 minutes (426.6 pg/ml; SE - 146.1) with comparison to the value at first sampling (108.5 pg/ml; SE - 29.5; p<0.05) and with comparison to the control group at the same time from the beginning of the experiment (87.6 pg/ml; SE - 2.3; p<0.05). The changed level of corticosterone in the test group in comparison to control group was observed but the differences were insignificant. Our observations confirm the proposition, that even short-time changes of ambient conditions can activate adaptation mechanisms in the organism, which in part, is the activation of the immune system.

Key words: Temperature - Interleukin-6 - Acclimation - Climatic chamber - Rat

Introduction

The study was undertaken to determine the short-term effects of a cold environment on interleukin-6 (IL-6) and corticosterone concentration in rats’ plasma. To date, there has been little research examining whether short-term changes in ambient temperature exert effects on the immune system. Cytokines, peptide hormones and neurotransmitters, as well as their receptors/ligands, are ubiquitous within the brain, endocrine and immune systems. These shared ligands and receptors are used as a common chemical language for communication within and between the immune and neuroendocrine systems. Such communication suggests an immunoregulatory role for the brain and sensory function for the immune system. Interplay between the immune, nervous and endocrine systems is most commonly associated with the pronounced effects of stress on immunity. The hypothalamic - pituitary - adrenal (HPA) axis is the key player in stress responses. It is well established that both external and internal stressors activate the HPA axis. Cytokines are chemical messengers that stimulate the HPA axis when the body is under stress or experiencing an infection [1]. These peptides contribute to a chemical signaling language that regulates development, tissue repair, haemopoiesis, inflammation and specific and nonspecific immune
The immune system is regulated in part by the central nervous system (CNS), acting principally via the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system (SNS) [2]. In recent years, our understanding of the interactions between the HPA axis and immune-mediated inflammatory reactions has expanded enormously. Plasma corticosterone as an endogenous factor is a natural inhibitor of cytokine production [3]. Several cytokines are known to affect the release of anterior pituitary hormones by an action on the hypothalamus and/or the pituitary gland. The major cytokines involved are IL-1, IL-2, IL-6, TNF-α, and interferon (IFN) [4]. The predominant effects of these cytokines are to stimulate the HPA axis and to suppress the hypothalamic-pituitary-thyroid (HPT) and gonadal axes and growth hormone (GH) release. The relative importance of systemically and locally produced cytokines in achieving these responses and their precise sites of action have not been fully established [1]. There is accumulating evidence that there are significant interactions between the immune and neuroendocrine systems which may explain, at least in part, some of the effects on growth, thyroid, adrenal and reproductive functions which occur in acute and chronic disease [5-7]. During stimulation of the immune system, peculiar alterations in hormone secretion occur (e.g. hypogonadism, hypercortisolism) [8-10]. A considerable amount of evidence has shown that physical and psychological stress elevates the plasma IL-6 levels [11,12]. Circulating cytokine concentrations are elevated in response to strenuous exercise and other forms of physical stress [13]. Although heat stress is known to accentuate exercise-associated immunomodulation, largely via augmented hormonal fluctuations [14,15], relatively little is known regarding the physiological modulation of the human immune system by cold exposure, either at rest or during sustained exercise [16]. Exposure to cold substantially augments hypothalamic-pituitary-adrenal axis and sympathetic nervous system activation, producing an enhanced secretion of cortisol and catecholamines, respectively [17]. Cold is known to affect leukocyte mobilization [18,19], and can suppress lymphocyte functional activities [18]. Limited evidence suggests that cold exposure may also initiate changes in cytokine expression associated with nonspecific acute phase reaction [16,20]. Because cytokines play a key role in the bidirectional communication between neuroendocrine and immune systems [21], it has been suggested that the interplay between hormones and cytokines during thermal stress may influence immune homeostasis in response to environmental challenge [16]. Adaptation of homeothermic organisms to change of environmental temperature results in the redistribution of the plastic and energetic potentials of the organism.

The short length of the estrous cycle of rats makes it useful for investigation of changes occurring during the reproductive cycle. The estrous cycle lasts some days and is composed of: proestrus, estrus, metestrus and diestrus phases. The ovulation occurs from the beginning of proestrus to the end of estrus [22].

Our specific aims were to study the effect of 30 minutes of acclimation to novel ambient conditions on rat plasma levels of IL-6 and corticosterone to determine if cold stress is associated with alterations of profile of circulating IL-6 and corticosterone and to examine whether changes in these mediators are associated with each other.

Materials and methods
Female Wistar rats (Rattus norvegicus), three months old, weighing 200 to 300 g were used. The animals were housed in standard cages, six per cage, in a controlled temperature room (21°C ± 1°C), with a 12 h light: 12 h dark cycle, lights were turned on at 6:00 a.m. Standard laboratory chow and tap water were available ad libitum. The experimental protocol was approved by the Ethical Committee on Human and Animal Experimentation of The Medical University of Lublin.

One month prior to the experiment, every morning between 8:00 and 9:00 a.m. each animal cage was carried to the experimental room. The estimation of the vaginal lavage using a microscope was done to determine the phase of the estrous cycle. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40 × objective lenses. Three types of cells could be recognized: round and nucleated ones were epithelial cells; irregular ones without nucleus were the cornified cells; and little round ones were the leukocytes. The proportion among them was used for the determination of the estrous cycle phases [22,23].
On the day of the experiment, animals (in proestrus phase of estrous cycle) were divided into 2 groups: A. Control group - (CG - 21°C) (n = 6), acclimation in climatic chamber in normal environmental conditions (ambient temperature - 21°C ± 1°C; normoxia - 21% O₂; and relative humidity - 60%) during 30 minutes. B. Test group - (TG - 10°C) (n = 6), acclimation in climatic chamber in low temperature conditions (ambient temperature - 10°C ± 1°C; normoxia - 21% O₂; and relative humidity - 60%) during 30 minutes. The animals were tested once at a time in the climatic chamber. The experiment started at 12 a.m. The climatic chamber (Multiserv model KBI - 100, Lublin, Poland) is a box with plexiglas walls of dimensions 50×40×40 cm. Internal environmental parameters (oxygenation, temperature, humidity) are automatically adjustable and monitored. The blood samples were collected in tubes containing EDTA (using catheter implanted to the external jugular vein before the experiment [24], before and 30, 60, 90, 150 and 210 minutes from the beginning of the experiment. The collected samples were centrifuged and the plasma was stored at -20°C until assayed for IL-6 and corticosterone. Concentration of IL-6 and corticosterone in blood plasma were determined using commercially available ELISA kits (R&D Systems, Minneapolis; IBL Hamburg). The ELISAs were performed according to the instructions of the manufacturer. Statistic data obtained in this study are expressed as mean values with a 95% confidence interval. Data was analyzed by ANOVA with Tukey post-hoc analysis to determine differences. The level of significance was set at 0.05.

Discussion

Several studies have established that different types of stress can alter immune functions, cytokine levels and hormone levels [25] but only a few investigations have focused on the impact of heat or cold exposure on immunological changes. We have shown that exposure to cold environmental conditions modulates cytokine expression. Our findings provide evidence that changes in sympathoadrenal activation are linked to exertional- and cold-induced modification of this cytokine production profile. Unfortunately, our study measure only integrated accumulation of secreted cytokines, reflecting the net outcome of produced, absorbed, and degraded molecules within biological fluids. The numerous sources of cytokines have been identified in vitro and only a few studies have attempted to identify the origin of cytokines in vivo [26,27]. Some studies demonstrated that blood monocytes can be a source of circulating inflammatory cytokine production during exercise [15]. Blood monocytes are a first line of defense against invading pathogens and a major source of immuno-inflammatory mediators [28]. When activated by various noninfectious and infectious agents, such as bacteria-derived lipopolysaccharide (LPS), monocytes sequentially release a cascade of cytokines, including TNF-α, followed by IL-1β and IL-6 [28]. The prolonged cold exposure substantially magnified the extent of monocytosis [15]. Such cold-enhanced recruitment of monocytes has been previously documented in humans [16,19] and is presumably mediated by pronounced SNS activation accompanying prolonged cold stress. This activation may influence cell mobilization through indirect adjustments in hemodynamics or via direct receptor- mediated alterations in cellular adhesive properties, thereby affecting cell mobilization [29]. Exposure of humans, rats and mice to cold ambient temperature results in elevated blood pressure and heart rate. It appears that the tachycardia and hypertension are indirect results of SNS activation of thermoregulatory mechanisms, because elevated plasma norepinephrine levels corre-
late with elevated blood pressure in the cold [30,31]. There is a highly sensitive and linear effect of raising or lowering ambient temperature within the range 18-30°C on cardiovascular function [32]. Short duration, moderate cold-air exposure (2 h, 5°C) in a climatic chamber elicits significant plasma elevations of IL-6 in resting subjects [16]. Others have found that short-term exposure to cold air (1 h, 11°C) or cold water (1 h, 14°C) has no effect on systemic IL-1β or IL-6 release [33,34]. The different mechanisms of cytokine induction may be operative in moderate vs. severe cold exposure. The mechanism underlying the abovementioned differences is not clear. The cold-associated modulation of cytokine production may be related to induction of systemic endotoxemia, provoked by alterations in central hemodynamics and stress hormone release associated with enhanced thermoregulatory demands. The moderate cold exposure leads to a sharp reduction in splanchic blood flow and ischemia [35] that promotes translocation of LPS into the systemic circulation [36]. Swoap et al. demonstrated that even a modest change in ambient temperature can influence the autonomic nervous system in such a way as to significantly influence arterial blood pressure, heart rate and metabolic rate [32]. Some investigators speculate that noncirculating cells, including vascular endothelium, hepatocytes and fibroblasts, may be chiefly responsible for the enhanced secretion of these cytokines with exercise [37,38]. There is an important role for reciprocal interactions between neuroendocrine and immune systems in the maintenance of homeostatic balance between pro- and anti-inflammatory cytokine responses [21,39]. Exercise and cold-induced catecholamine secretion is closely related to systemic IL-6 release [16]. IL-6 activates the HPA axis and induces the upregulation of cortisol and IL-1ra that in turn suppresses the synthesis of monocyte IL-1β, TNF-α and IL-6 thereby controlling the extent of local and systemic inflammatory responses [39]. The administration of IL-6 activates the HPA axis by increased activity of corticotrophin releasing hormone (CRH), elevated plasma ACTH and corticosterone levels [40,41]. Cytokine production may be differentially regulated by circulating catecholamines during exercise and cold exposure. Rhind et al. demonstrated that cold exposure differentially modulates cytokine production, upregulating the expression of IL-6 and IL-1ra but downregulating that of IL-1β and TNF-α. Secretion of sympathoadrenal hormones was significantly associated with changes in both circulating and intracellular cytokine profiles [15]. The molecular signaling pathways involved in exercise and/or thermal stress-induced cytokine alterations remain largely unknown. A change in ambient temperature by only a few degrees Celsius is enough to significantly impact metabolic rate, heart rate, and the mean blood pressure in mice and rats. Our findings are consistent with studies indicating that adrenergic and nonadrenergic mechanisms are involved in the regulation of cytokine production under various forms of physical stress [42]. We observed the insignificant changes of levels of corticosterone. Studies in rats, mice and humans have shown that the acute exposure to stressors is characterized by an increase in corticotrophin releasing hormone (CRH), adrenocorticotropin (ACTH) and corticosterone in rodents and cortisol in humans [43]. Chronic stress causes a decrease in hypothalamic CRH content, an increase in plasma levels of ACTH and glucocorticoids [43]. The final evidence for such cold-evoked, HPA axis associated modulation of cytokine expression must await future studies that interdict specific steps in the signaling pathways leading to cytokine induction.

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References


[12] Besedovsky HO, del Rey A, Klusman I, Furukawa H, Monge AG, Kabiersch A. Cytokines as modulators of the hypothalam-
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