Psychological stress activates interleukin-1β gene expression in human mononuclear cells

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Abstract

The pathophysiological mechanisms underlying the association between psychological stress and cardiovascular disease are unclear. Interleukin-1β (IL-1β) and interleukin-6 (IL-6) are inflammatory cytokines playing a pivotal role in atherosclerosis. IL-1β activates IL-6, and both cytokines are produced by peripheral blood mononuclear cells. One mechanism through which stress could promote atherosclerosis is by regulating mononuclear cell cytokine gene expression. We studied cardiovascular and cytokine responses in 32 healthy men participating in two 5-min mental tasks and in 10 controls. Blood pressure and heart rate, assessed using a Portapres-2, increased significantly following tasks in all participants. Plasma IL-6 levels, determined by ELISA, also increased following tasks, with maximum levels detected 2 h post-stress. Quantitative RT-PCR analysis showed that mononuclear cell IL-1β gene expression rose significantly at 30 min post-stress and remained elevated at 75 and 120 min. Increases in IL-1β gene expression correlated positively with plasma IL-6 responses, cardiovascular responses, subjective stress ratings, and anxiety symptoms. No changes were detected in controls. Stress-induced activation of mononuclear IL-1β is a novel mechanism potentially linking stress and heart disease. This mechanism could also play a role in other inflammatory diseases exacerbated by stress.

Keywords: Stress; Interleukins; Genes; Inflammation; Atherosclerosis

1. Introduction

Evidence from epidemiological and observational studies shows that coronary heart disease (CHD) is more prevalent in humans and animals subject to chronic psychosocial stress. For example, people working in jobs with high demands coupled with low control, caregivers, socially isolated individuals and people with symptoms of anxiety and depression are at a higher risk of developing CHD (Hemingway and Marmot, 1999). Similarly, cynomolgus monkeys housed in unstable, socially stressful conditions display impaired endothelial function and develop more coronary artery lesions compared to unstressed controls (Rozanski et al., 1999; Williams et al., 1991). Heightened cardiovascular reactivity to acute stress predicts future hypertension, left ventricular mass, and progression of carotid atherosclerosis (Treiber et al., 2003). Furthermore, acute mental stress has been shown to cause transient vascular endothelial dysfunction (Ghiadoni et al., 2000) and increases in circulating inflammatory cytokine levels in healthy humans (Steptoe et al., 2001). Elevated plasma levels of IL-1β and IL-6 have been reported in patients with acute coronary syndromes (Ikeda et al., 2001; Simon et al., 2000). IL-1β, produced primarily by monocytes and macrophages, is a central component of the acute phase inflammatory response (Dinarello, 1996). It acts, in collaboration with a network of other cytokines...
including IL-6, to coordinate atherosclerosis through a variety of proinflammatory, prothrombotic processes including migration and/or activation of monocytes, macrophages, T-lymphocytes, and platelets, increased expression of vascular adhesion molecules, fibroblast proliferation, and synthesis of C-reactive protein and fibrinogen (Dinarello, 1996; Young et al., 2002). It also stimulates IL-6 production from a variety of cells. IL-1β acts mainly at the autocrine/paracrine level and is difficult to detect in plasma from healthy individuals. IL-1α and IL-1β lack signal peptides, so are not readily secreted from cells into the systemic circulation. In contrast, IL-6 is an endocrine molecule and can be easily measured in plasma (Yudkin et al., 2000). Therefore, IL-6 is often used as a surrogate marker for biologically active IL-1β (Dinarello, 1996).

Peripheral blood mononuclear cells (PBMC) are a source of both IL-1β and IL-6. A recent study by Bierhaus et al. showed that psychological stress activates the expression of the transcription factor, nuclear factor-kB (NF-kB) in PBMC of healthy subjects (Bierhaus et al., 2003). Notably, NF-kB upregulates the expression of a number of inflammatory proteins including cytokines (Barnes and Karin, 1997). We postulated that one mechanism through which stress could promote atherosclerosis is by regulating cytokine gene expression in these cells. To test this hypothesis we investigated the effect of acute psychological stress on cardiovascular responses, plasma IL-6 levels, and IL-1β and IL-6 gene expression in PBMC of 32 healthy men and a separate group of 10 age-matched controls who underwent the same protocol in the absence of stress.

2. Methods

2.1. Participants and conditions

Forty-eight healthy men aged 30–59 years were recruited from two London-based Departments of the British civil service. All participants were of white European origin, non-smokers, had no previous history of coronary heart disease or any other relevant physical or mental illness, and were not taking any medication. One participant withdrew after feeling unwell and cytokine data were incomplete in 15 other cases due to blood sampling problems. Thirty-two men were therefore included in the final analyses. A comparative non-stress control group of 10 healthy men in the same age range also took part, in order to determine whether cytokine levels and cardiovascular activity would change over time in the absence of any stress task. Participants were instructed not to drink caffeinated beverages on the morning of the experiment, and not to consume alcohol or exercise on the evening before or the day of testing. They were asked not to take aspirin for 10 days prior to testing, and to avoid a high fat or high protein breakfast. All participants were tested individually in the morning. The study was approved by the UCL/UCLH Committee on the Ethics of Human Research. All participants gave their informed consent before entering the study.

2.2. Physical measures

Before testing, measures of weight, height, waist, and hip circumference were obtained and body fat mass was estimated using a Bodystat 1500 bioelectrical impedance body composition analysis device (Bodystat, Douglas, Isle of Man).

2.3. Psychological measures

Symptoms of anxiety and depression were assessed in the stress group using the Hospital Anxiety and Depression (HAD) Scale, a standard measure of symptoms of anxiety and depression developed for use with medical patients (Zigmond, 1981). Scores could range from 0 to 21, with higher values reflecting greater anxiety and depression. Scores of 11 and above indicate borderline clinical anxiety and depression.

2.4. Mental stress testing procedure

A venous cannula was inserted for periodic collection of blood samples. Participants then rested for 30 min. Blood pressure and heart rate were recorded for the last 5 min of the rest period using a Portapres-2. Participants then rated feelings of stress on a 7-point scale from 1 = low to 7 = high and a baseline blood sample was drawn. Mental stress was induced by two behavioural tasks administered under time pressure (Steptoe et al., 2002). Each task lasted for 5 min. The first was a computerised colour-word interference task, involving the successive presentation of target words, printed in another colour. The second was a hand-eye coordination task involving the tracing of a star with a metal stylus that could only be seen in mirror image. Participants were asked to give accuracy priority over speed on both tasks. Blood pressure and heart rate were recorded throughout tasks. At the end of each task, participants rated task difficulty, involvement, controllability, and feelings of stress on 7-point scales from 1 = low to 7 = high. Participants then rested quietly for the remainder of the experiment. Blood pressure and heart rate were recorded at 25–30, 70–75, and 115–120 min post-stress, and blood samples were drawn at 30, 75, and 120 min post-stress. Control participants followed the exact same procedure as the other participants but simply rested in place of the mental stress tasks. Psychological questionnaires were administered to the stress group during the last hour of the session.
2.5. Measurement of plasma interleukin-6

Blood samples were drawn into EDTA-Vacutainer tubes using 21-gauge Butterfly needles, and centrifuged immediately at 2500 rpm, for 10 min at room temperature. Plasma was removed, aliquoted, and frozen at −80°C until analysis. Plasma IL-6 was measured using a high sensitivity two-site ELISA from R and D Systems (Oxford, UK). The limit of detection of this assay was 0.09 pg/ml, with intra- and interassay coefficients of variation (CVs) of 5.3 and 9.2%.

2.6. Isolation of RNA from PBMC and reverse transcription

Whole blood samples, obtained as above, were diluted with an equal volume of RPMI 1640 (Life Technologies) and separated on Ficoll Paque Plus (Amer sham Pharmacia Biotech) by centrifugation at 2000 rpm for 30 min at room temperature. Mononuclear cells were isolated, washed twice with RPMI 1640, then total RNA was extracted using TRIzol reagent (Life Technologies). cDNA was synthesised by reverse transcription using Moloney murine leukaemia virus reverse transcriptase (Life Technologies) at 42°C for 60 min, followed by inactivation of the reverse transcriptase at 99°C for 5 min.

2.7. Analysis of cytokine gene expression in PBMC

Quantitative real-time PCR was used to measure expression of the human IL-1β gene and the human IL-6 gene in cDNA samples, using a LightCycler Instrument (Roche Diagnostics). Ribosomal 18S RNA was amplified as a reference gene. Analysis of this gene demonstrated that it was not affected by mental stress (data not shown). Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi), based on gene sequences published in the GenBank human DNA database (Accession No. M15330 (human IL-1β), NM_000600 (human IL-6), and X03205 (human 18S rRNA)). Sequences of the sense and antisense oligonucleotides and amplification product sizes were: 5′-ACG ATGCACCTGTACGATCA-3′ and 5′-TCTTTCACA CACGACAGCA-3′ and 229 bp for IL-1β, 5′-AAA GAGGACGCTGCAAA-3′ and 5′-AGCTCTGGGT GTAACCTCAC-3′ and 183 bp for IL-6, and 5′-CGGC TACCAATCCAGAAG-3′ and 5′-GCTGGGAATT ACCGCGGCT-3′ and 186 bp for 18S rRNA. DNA standards were prepared by PCR followed by purification and spectrophotometric determination. PCRs consisted of MgCl₂ (2 mM final for 18S rRNA and IL-6 and 4 mM final for IL-1β), cytokine-specific forward and reverse primers (0.5 μM each final), 1× LightCycler- FastStart DNA Master SYBR Green I (Roche Diagnostics), and 2 μl cDNA template including serial dilution of standards and samples, in a final volume of 20 μl. The PCR conditions were as follows: 10 min at 95°C, 45 cycles of 10 s at 95°C, 5 s at 61°C, and 10 s at 72°C. Specificity of the amplification products was assessed by melting curve analysis. Data were analysed with Roche Molecular Biochemicals Light Cycler Software Version 3 using the second derivative maximum method. Standard curves were produced by plotting the logarithm of the concentration of DNA standard against the cycle numbers of the logarithmic linear phase. The amount of target sequence in the samples was extrapolated from the standard curve.

2.8. Statistical analyses

Analyses of changes in cardiovascular, subjective, and gene expression responses were performed using repeated measures analysis of variance with group (stress/control) as the between-subject factor, and trial as the within-subject factor. Multiple regression analyses, product-moment correlations, and t tests were also carried out as appropriate. All analyses were conducted using SPSS v.10.

3. Results

3.1. Effect of psychological stress on cardiovascular measures and subjective stress ratings

Tasks induced substantial increases in blood pressure and heart rate (p < .001) in all participants, with average changes of 28.7 mmHg systolic and 16.6 mmHg diastolic pressure (Fig. 1), and 7.3 bpm in heart rate (Fig. 2A). Blood pressure and heart rate diminished following tasks, but remained above baseline levels throughout the post-stress period. Blood pressure rose slightly over the session in controls while heart rate remained low. Tasks also induced increases in subjective stress, but stress ratings returned to low levels during recovery (Fig. 2B). There were no significant differences between groups in blood pressure or heart rate at baseline, but subjective stress ratings were marginally elevated in the stress group.

3.2. Effect of psychological stress on mononuclear cell cytokine gene expression

To investigate the role of mononuclear cells in an inflammatory response to stress, we studied IL-1β and IL-6 gene expression in PBMC by quantitative RT-PCR. IL-1β gene expression rose significantly at 30 min post-stress (Fig. 3) and remained elevated at 75 and 120 min. No significant changes were detected in controls (Fig. 3). IL-1β gene expression in stress and control groups did not differ at baseline but did at all other time points.
In contrast, IL-6 gene expression was unaltered by stress. The fold changes in IL-6 gene expression were not significant, and averaged $1.30 ± 0.77$, $1.16 ± 0.74$, and $1.15 ± 0.54$ at 30, 75, and 120 min post-stress ($F = 1.50, p = .24$).

3.3. Relationship between mononuclear IL-1β gene expression and cardiovascular responses, subjective stress ratings, and anxiety symptoms

There was a significant association between stress-induced increases in IL-1β gene expression and cardiovascular measures. The fold increase in IL-1β gene expression from baseline to 30 min post-stress correlated positively with heart rate responses to tasks ($r = .32, p = .05$). Similarly, the fold change in gene expression from baseline to 75 min correlated with heart rate at 75 min ($r = .44, p = .005$) and the fold change at 2 h correlated with heart rate at 2 h ($r = .40, p = .015$). Systolic blood pressure responses to tasks correlated positively with the fold change in gene expression at 2 h ($r = .37, p = .021$). All correlations were independent of age, body mass index (BMI), and body fat.

Increases in IL-1β gene expression were also associated with psychological measures. Subjective stress ratings obtained immediately after tasks correlated positively with the fold change in gene expression at 30 min ($r = .34, p = .034$). Furthermore, stress-induced increases in IL-1β gene expression were greater in participants who reported more anxiety symptoms on the Hospital Anxiety and Depression Scale. Scores of anxiety and depression on this scale averaged $5.06 ± 3.2$ and $3.22 ± 2.3$, respectively. Although all scores were in the normal range, there was a significant relationship between increases in IL-1β gene expression and anxiety symptoms ($r = .38, p = .036$), after controlling for age and BMI.

3.4. Effect of psychological stress on plasma IL-6 and association with mononuclear IL-1β gene expression

Plasma IL-6 increased in response to stress ($F = 32.3, p < .001$), with maximum levels detected 2 h post-stress (Fig. 4). No changes were observed in controls ($F = 1.71,$...
**4. Discussion**

Our results show that an acute psychological stressor increases mononuclear cell IL-1β gene expression and plasma IL-6, in a group of healthy volunteers. The sensitivity of inflammatory cytokines to psychological stress is supported by other studies in animal models and humans. For example, restraint stress was previously shown to alter kinetics of IL-6 responses and IL-1β gene expression. The fold increase in IL-1β gene expression from baseline to 30 min post-stress correlated positively with the increase in IL-6 from baseline to 75 min ($r = .40$, $p = .013$) and the increase in IL-6 from baseline to 2 h post-stress ($r = .36$, $p = .026$), independently of age and BMI.

This, together with the rapidity of changes in IL-1β, suggests that this is a sympathetically driven response. However, the long duration of cytokine responses suggests that alternative pathways may also be important. IL-1β gene expression is upregulated by the transcription factor, NF-κB (Barnes and Karin, 1997). A recent study showed that stress-induced upregulation of NF-κB protein in PBMC from healthy volunteers paralleled increases in catecholamines and cortisol (Bierhaus et al., 2003). In contrast, other studies have reported that catecholamines and corticosteroids inhibit the production of inflammatory cytokines from PBMC (Agarwal and Marshall, 1998; Mohamed-Ali et al., 2001). It is possible that other stress-responsive neuroendocrine mediators are involved, such as substance P and angiotensin II, which stimulate cytokine production from mononuclear cells (Brasier et al., 2002; Cuesta et al., 2002).

Baseline stress ratings and plasma IL-6 were marginally higher in the stress group compared to controls. These differences may have been due to an anticipatory response in the stress group, who knew that they would be performing challenging tasks. However, we do not believe that this is a serious confound, since our results clearly show that there was a significant stress-induced increase in plasma IL-6 in the stress group only. Plasma IL-6 responses to stress were positively correlated with increases in IL-1β gene expression. This association was delayed inasmuch as the change in IL-1β gene expression at 30 min post-stress correlated with the increase in IL-6 at 75 min and 2 h post-stress. This suggests that stress-induced activation of IL-1β stimulates the IL-6 response. Since IL-6 gene expression was unaltered by stress, it is unlikely that the plasma IL-6 response is due to increased IL-6 production by PBMC. Stress might activate translation of pre-formed stocks of IL-6 mRNA in these cells or release of stored IL-6 protein. However, the delayed plasma IL-6 response suggests this cytokine is being synthesised de novo. Instead, we propose that stress-induced upregulation of PBMC IL-1β leads to increased intercellular levels of this cytokine which stimulate IL-6 production from a different cell source. Endothelial cells, smooth muscle cells, and adipocytes all produce IL-6 and interact with PBMC (Flower et al., 2003; Norioka et al., 1990; Sironi et al., 1989). Furthermore, IL-1β has been shown to stimulate IL-6 production from these cells in vitro (Flower et al., 2003; Norioka et al., 1990; Sironi et al., 1989).

PBMC are a mixed population of different immune cell subsets, including monocytes, neutrophils, and lymphocytes. Previous studies have shown that psychological stress induces leukocytosis and a re-distribution of lymphocyte subsets (Benshop et al., 1996). We therefore needed to consider whether a change in the relative composition of immune cells in the PBMC fraction would affect our measurements. In agreement with previous findings, total blood cell counts measured in samples...
taken at baseline, immediately post-stress and at 30 and 75 min post-stress in a subset of 14 of the stress group participants, showed that stress induced a significant increase in total leukocytes (data not shown). This was due to an increase in lymphocyte numbers, and stress had no effect on the number of monocytes or neutrophils in participants’ blood. The quantitative RT-PCR method used in this study means that differences in total leukocyte numbers would not affect gene expression measurements. Since IL-1β is produced primarily by monocytes, it is likely that increases in IL-1β gene expression are occurring predominately in these cells (Toda et al., 2002). However, it is conceivable that lymphocytes could contribute to this effect, since they also express IL-1β. Importantly, we found no correlation between IL1β gene expression and the numbers of lymphocytes, monocytes or neutrophils in participants’ blood, suggesting that changes in relative immune cell numbers in the PBMC fraction do not account for our findings. However, lymphocytes may be further subdivided into natural killer (NK) cells, T cells, and B cells and psychological stress has been shown to specifically increase the prevalence of NK cells and CD8+ T lymphocytes in the PBMC pool (Benschop et al., 1996). Relevant to this, Richlin et al. recently reported that an increase in NF-κB DNA-binding activity in human PBMC following acute SNS activation during exercise stress could be mainly accounted for by an increase in the relative numbers of NK cells, which constitutively express higher levels of this transcription factor than other leukocyte subsets, as opposed to a change in NF-κB expression within specific cells per se (Richlin et al., 2004). As mentioned previously, NF-κB activates the expression of a number of inflammatory proteins, including IL-1β and IL-6. Since we did not quantify lymphocyte subsets, we cannot rule out a stimulatory effect of psychological stress on the prevalence of NK cells (or indeed other lymphocyte phenotypes) as a potential confounding factor in our study. However, the fact that stress did not alter mononuclear cell IL-6 gene expression indicates that a constitutive effect alone is unlikely to account for our findings. Future studies will be aimed at further delineating the signalling pathways and mononuclear cell phenotypes involved in the IL-1 response.

This investigation was carried out in a sample of healthy, white, middle-aged men, and results may not generalise to other groups. The magnitude of stress-induced elevations in blood pressure, heart rate, IL-1β gene expression, and plasma IL-6 was relatively small. However, the challenges imposed were relatively mild compared with the difficulties many people experience on a day-to-day basis, and may therefore be under-representative of the true stress response. Furthermore, although these responses may not be clinically relevant in themselves, they could well be relevant when repeated frequently on a long-term basis.

Taken together, our results indicate that psychological stress could promote the inflammatory process underlying atherosclerosis through activation of mononuclear cell IL-1β and increases in plasma IL-6. This mechanism may also play a role in other inflammatory diseases exacerbated by stress.

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References


