Acute Stress Elicited by Bungee Jumping Suppresses Human Innate Immunity

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Although a relation between diminished human immunity and stress is well recognized both within the general public and the scientific literature, the molecular mechanisms by which stress alters immunity remain poorly understood. We explored a novel model for acute human stress involving volunteers performing a first-time bungee jump from an altitude of 60 m and exploited this model to characterize the effects of acute stress in the peripheral blood compartment. Twenty volunteers were included in the study; half of this group was pretreated for 3 d with the β-receptor blocking agent propranolol. Blood was drawn 2 h before, right before, immediately after and 2 h after the jump. Plasma catecholamine and cortisol levels increased significantly during jumping, which was accompanied by significantly reduced *ex vivo* inducibility of proinflammatory cytokines as well as activation of coagulation and vascular endothelium. Kinome profiles obtained from the peripheral blood leukocyte fraction contained a strong noncanonical glucocorticoid receptor signal transduction signature after jumping. In apparent agreement, jumping downregulated Lck/Fyn and cellular innate immune effector function (phagocytosis). Pretreatment of volunteers with propranolol abolished the effects of jumping on coagulation and endothelial activation but left the inhibitory effects on innate immune function intact. Taken together, these results indicate that bungee jumping leads to a catecholamine-independent immune suppressive phenotype and implicate noncanonical glucocorticoid receptor signal transduction as a major pathway linking human stress to impaired functioning of the human innate immune system.

INTRODUCTION

It is well recognized that cognitive perception of the environment is a major determinant of immune function, and conditions loosely grouped under the common denominator of stress are perceived as a significant risk factor for infection and autoimmunity (1–3). The activation of the stress response system influences the close relationship between the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system and the immune system (4). Therefore, stress presumably interacts with immunity owing to the release of stress hormones such as catecholamines and cortisol. Epinephrine, norepinephrine and cortisol in general may result in antiinflammatory effects. These antiinflammatory properties have been reproduced experimentally in humans using the model of intravenous injection of lipopolysaccharide (LPS) (5–8).

We postulated that acute stress-induced release of stress hormones causes an immune suppressive phenotype and aimed to investigate this by using a novel human model of acute stress. For investigating the molecular basis of stress-dependent changes in immunity, use of human models seems inevitable. Practical and ethical considerations hamper investigations on the effects of severe stress on human immunity, and there is a need for an ethically acceptable human model that produces severe stress with moderate intraindividual variation. High-altitude jumping seems to be almost universally associated with substantial induction of flight/fright responses. A recent study showed that during height exposure in all participants, fear, dizziness and body

sway were increased, indicating that exposure to substantial heights induces a universal stress response (9). We decided to examine the usefulness of this phenomenon for investigating the effect of human stress on immune physiology. To this end, healthy male volunteers naive to bungee jumping were exposed to a jump from an altitude of 60 m. To study the role of catecholamines in the responses observed, half of the volunteers were pretreated with the β-receptor antagonist propranolol.

MATERIALS AND METHODS

Subjects

A prospective clinical trial was conducted in a single center. Twenty healthy male volunteers, naive to bungee jumping or skydiving and aged between 18 and 35 years, were included in the study. Mean age was 27 years (range 18–35, n = 10) in the control group and 31 years (range 23–35, n = 10) in the propranolol group. Subjects were randomized between the use of propranolol, 40 mg 3× a day for 3 d, or no pretreatment (control). The study was reviewed and approved by the local medical ethics committee. Written informed consent was obtained from all subjects.

Bungee Jump Protocol

The study site was located at the hospital grounds, where a crane was placed. Bungee jumps took place from an altitude of 60 m, under supervision and guidance from an experienced commercial bungee jump crew. On the morning of the study day, an intravenous access catheter was placed in the cubital vein. Exactly 2 h before the jump, the first blood sample was drawn (in total 20 mL blood). Subsequent samples (20 mL) were drawn directly before the jump (while elevated at jump level), immediately after the jump and 2 h after jumping. Blood was drawn in Vacutainer tubes (Becton-Dickinson, Breda, the Netherlands) containing EDTA-K3, for leukocyte and differential counts; in sodium citrate–containing tubes for measurement of coagulation and en-

dothelial cell activation markers; in sodium heparin–containing tubes for *ex vivo* stimulations, phagocytosis and cortisol measurements; or in tubes containing reduced glutathione-EGTA buffer for measurement of epinephrine and norepinephrine. Plasma was separated and stored at –80°C until assays were performed. Measurements of blood pressure and heart rate were performed 30 min before, directly before, directly after and 2 h after the bungee jump using an automated device (Omron HEM-705CP, Omron Healthcare, Kyoto, Japan) (10).

Assays

Plasma epinephrine and norepinephrine were assayed, as described previously, by reversed phase high-performance liquid chromatography (RP-HPLC) with fluorimetric detection after solvent extraction and derivatization with 1,2-diphenylethylenediamine (11). Cortisol was measured using a luminescence enzyme immunoassay (Immulite, Siemens Healthcare Diagnostics, Deerfield, IL, USA). Leukocyte counts, differentials, and CD4/CD8 numbers were assessed by using standard methods at the institutional clinical laboratory. Levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and IL-10 were determined by cytometric bead array (CBA; BD Biosciences, Breda, the Netherlands). Factor VIII levels were measured using an automated coagulation analyzer (Behring Coagulation System) with reagents and protocols from the manufacturer (Dade Behring, Marburg, Germany). Measurements of prothrombin fragment F1 + 2 (Dade Behring), thrombin-antithrombin (TAT) complexes (Dade Behring), tissue-type plasminogen activator (tPA; Innotest, Innogenetics, Gent, Belgium) and von Willebrand factor antigen (antibodies from Dako, Glostrup, Denmark) were performed by enzyme-linked immunosorbent assay.

Ex Vivo Blood Stimulations

Whole blood was mixed with an equal volume of plain RPMI 1640 (Invitrogen, Breda, the Netherlands) or with RPMI

1640 containing LPS (from Escherichia coli 0111:B4, ultra pure; InVivoGen, San Diego, CA, USA; 100 ng/mL). Blood was incubated at 37°C in 5% CO_2 for 24 h for cytokine measurement.

Phosphoproteome Assays

Kinome array analysis was done as described earlier (12,13). In short, the peptide arrays (Pepscan Presto, Lelystad, the Netherlands) were incubated with cell lysates, containing ^{33}P-γ-ATP or ^{33}P-α-ATP (Perkin Elmer, Waltham, MA, USA) for 2 h at 37°C. Subsequently, the arrays were washed, dried and exposed to a phospho-imaging screen for 72 h and scanned on a phospho-imager (Storm 860, Molecular Dynamics, Stanford, CA, USA). For each peptide, the average and standard deviation of phosphorylation were determined and plotted in an amplitude-based hierarchical fashion. Peptides of which the average phosphorylation minus 1.96 times the standard deviation was higher than the value expected from describing the background distribution were considered to represent true phosphorylation events. Phospho-Fyn determinations were performed in blood taken 2 h before the jump and directly after jumping in four volunteers from the control group, as described earlier (14,15). In short, blood was lysed and the remaining leukocyte fraction was solubilized and subsequently subjected to immunoprecipitation using a Fyn antibody and blotted with a pan phospho-Src family antibody (Cell Signaling Technology, Bioké, Leiden, the Netherlands).

Phagocytosis

Phagocytosis of *E. coli* by blood granulocytes and monocytes was determined as described (16). Briefly, 100 μL blood was added to 20 μL fluorescein isothiocyanate–labeled opsonized *E. coli* and incubated at 37°C for 10 min. Cells were quenched and washed. Thereafter, erythrocytes were lyzed and DNA staining solution was added. Cells were analyzed within 1 h on a FACScan flow cytometer.

Statistical Analysis

All values are means ± SEM. Changes related to time to jump were analyzed by one-way analysis of variance (repeated measures). Differences between groups were analyzed by two-way analysis of variance. A *P* value <0.05 was considered statistically significant. With regard to kinome profiles, statistical significance was tested by determining individual-term binomial distribution probability on paired samples from the same volunteer before and after jumping.

All supplementary materials are available online at www.molmed.org.

RESULTS

Blood Pressure and Heart Rate

Mean arterial blood pressure (MAP) increased directly before the bungee jump relative to 2 h earlier in both groups (both *P* < 0.05, Figure 1). Directly after the jump, mean arterial blood pressure had decreased again, which was followed by a further decrease until the end of the observation period. Heart rate increased in a similar fashion in volunteers not pretreated with propranolol, but this change was absent in volunteers who were pretreated with propranolol (see Figure 1).

Catecholamine and Cortisol Levels

Bungee jumping resulted in a time-dependent increase in the plasma concentrations of both epinephrine and norepinephrine directly before the jump in both groups (*P* < 0.05 for both catecholamines for both groups, Figure 2A). A sharp decrease in epinephrine levels was observed directly after the bungee jump, whereas norepinephrine concentrations remained elevated longer. Cortisol levels remained unaltered before the jump but increased significantly during the jump (Figure 2B). Propranolol did not influence the release of catecholamines or cortisol.

Leukocyte Counts and Differentials

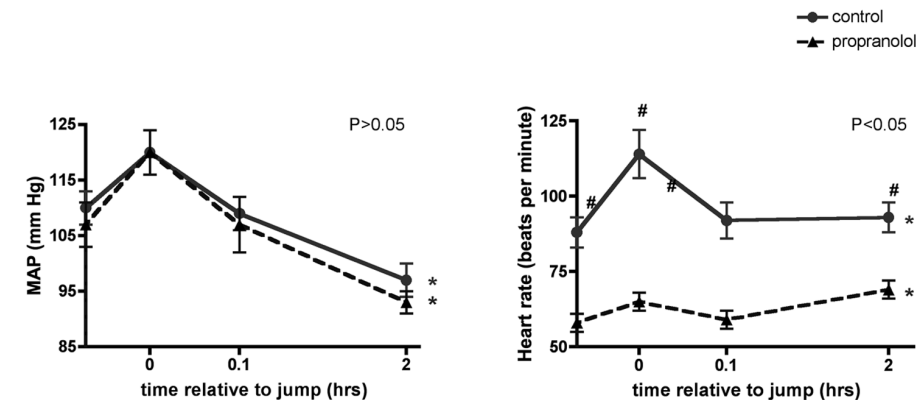The aggravation induced by the jump clearly was echoed in the systemic com-

Figure 1. Heart rate and blood pressure relative to jump. Heart rate and MAP relative to jump are shown. Both values changed significantly during the study, indicating that bungee jumping affected blood pressure and heart rate (*P* < 0.05). Heart rate response in time differed significantly between both groups (*P* < 0.05). Asterisks at the end of each curve indicate a significant (*P* < 0.05) change of the parameter during the experiment. *P* values indicate curve comparison. #Significant posttests between groups at the indicated time point.

partment, since in anticipation of the bungee jump, leukocyte counts increased significantly in both groups (Figure 3). During the jump, total leukocyte counts increased further, which was caused by an increase in numbers of monocytes, T cells (CD4+ and CD8+ cells) and neu-

trophils (see Figure 3, all *P* < 0.05). None of these changes were influenced by propranolol.

Inducibility of Cytokine Release

To determine the impact of acute stress on the cytokine production capacity of
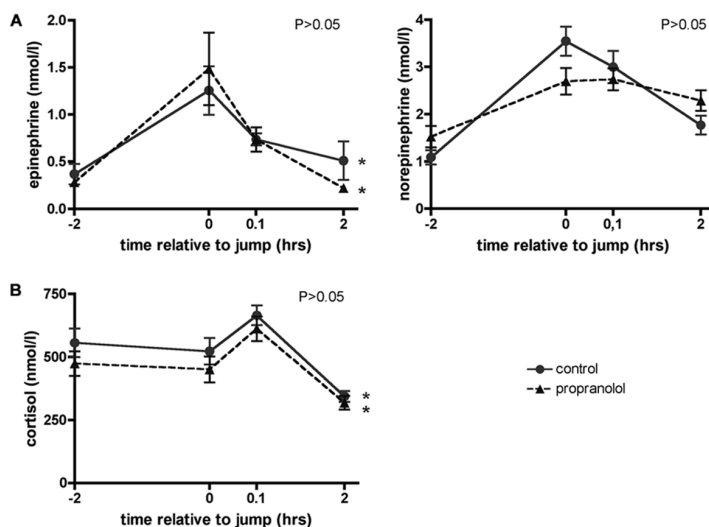

Figure 2. Catecholamine and cortisol levels relative to jump. Levels of epinephrine and norepinephrine (A) and cortisol (B) in blood are shown. All values changed significantly during the study, indicating that bungee jumping affected catecholamine and cortisol levels (*P* < 0.05). No difference in catecholamines or cortisol levels was observed between both groups (*P* > 0.05). Asterisks at the end of each curve indicate a significant (*P* < 0.05) change of the parameter during the experiment. *P* values indicate curve comparison.
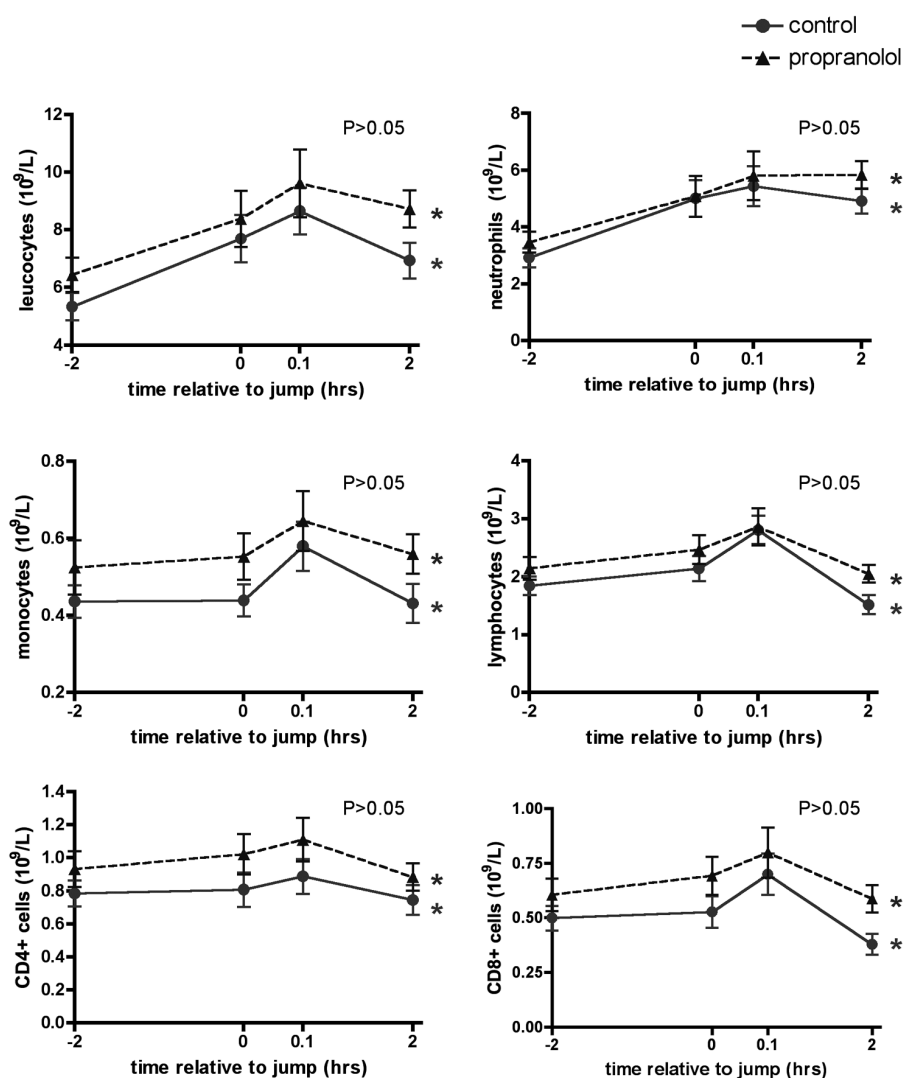
Figure 3. Blood leukocytes relative to jump. Numbers of leukocytes and differentiation relative to jump are shown. All values changed significantly during the study in both groups, indicating that bungee jumping affected leukocyte levels and differentiation ($P < 0.05$). Leukocyte numbers and differentiation did not differ between groups ($P > 0.05$). Asterisks at the end of each curve indicate a significant ($P < 0.05$) change of the parameter during the experiment. P values indicate curve comparison.

Figure 4. Cytokine levels after *ex vivo* LPS stimulation relative to jump. Levels of TNF-α, IL-8 and IL-10 after whole blood stimulation with endotoxin, as described in the *Materials and Methods*, are shown. TNF-α and IL-8, but not IL-10, values changed significantly ($P < 0.05$) during the study in both groups, indicating that bungee jumping affected the *ex vivo* release of proinflammatory cytokines to LPS ($P < 0.05$). No difference in cytokine levels were observed between both groups. Asterisks at the end of each curve indicate a significant ($P < 0.05$) change of the parameter during the experiment. P values indicate curve comparison.

peripheral blood leukocytes, we performed *ex vivo* stimulation of whole blood with LPS and measured the levels of TNF-α (a proinflammatory cytokine), IL-8 (a chemokine) and IL-10 (an anti-inflammatory cytokine) in stimulated blood samples. Remarkably, bungee jumping was associated with a decreased capacity of whole blood leukocytes to release TNF-α and IL-8, which was already apparent directly before the jump (Figure 4, $P < 0.05$). However, levels of

IL-10 induction remained unaltered during the study. The reduced capacity to release TNF-α and IL-8 was not inhibited by pretreatment with propranolol, indicating that this hyporesponsiveness was not mediated by β-adrenergic stimulation (see Figure 4).

Kinome Assays

Since we established that inhibition of *ex vivo* release of cytokines after bungee jumping was not related to the release
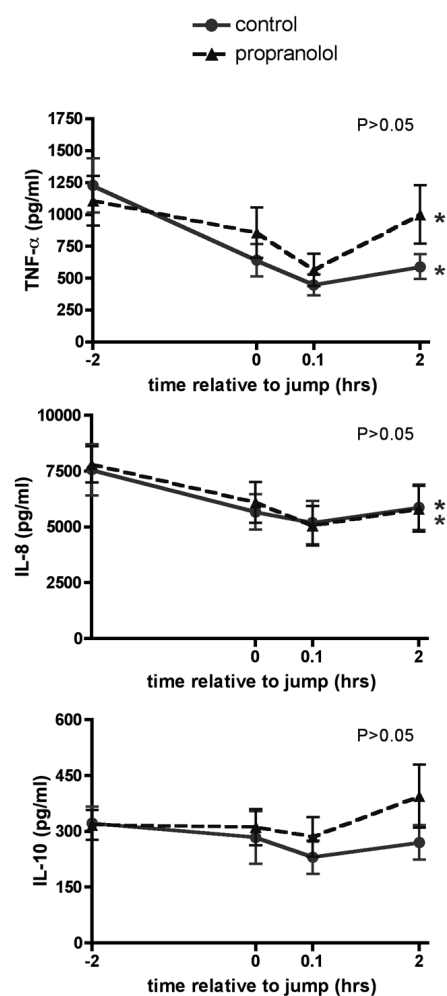
of catecholamines, we further investigated the molecular basis of suppressed immunity after bungee jumping. For this, a kinome profile was constructed before and after bungee jumping in untreated volunteers (control group).

Table 1. Association of observed effects on peptide phosphorylation with reported effects of glucorticoid signaling.[a]

Kinase	Relevant peptides (see supplemental data)	Effect observed	Reported effect of glucorticoid signaling (reference)
Insulin receptor	33, 269	Downregulated	Downregulated (18)
Protein kinase B	205, 725, 941	Downregulated	Downregulated (18,20)
Lck/Fyn	265, 996	Downregulated	Downregulated (18–20)
c-Jun N-terminal kinases	160	Downregulated	Downregulated (18)
Mitogen-activated protein kinase	248, 644	Downregulated	Downregulated (18)
Glycogen synthase kinase 3 β	204	Upregulated	Upregulated (18)
IκB kinase	405	Downregulated	Downregulated (22)
Rho-associated kinase	615, 842	Upregulated	Upregulated (21)
p70S6 kinase	720	Downregulated	Downregulated (18)
Focal adhesion kinase	539	Downregulated	Downregulated (18)
Sarcoma	44, 76, 112, 332, 550, 848	Downregulated	Downregulated (20)
Protein kinase G	165	Downregulated	Downregulated (18,20)
Signal transducer and activator of transcription 3	72	Upregulated	Upregulated (21)

[a]Selected kinases displaying differential significant up- or downregulation are shown and compared with previously reported effects of glucorticoid signaling.

Kinome profiles were generated by incubating lysates of samples obtained from volunteer peripheral blood 2 h before jumping with those obtained directly after the jump, using arrays exhibiting 976 different kinase substrates and ^{33}P-γ-ATP (13). Arrays incorporated substantial amounts of radioactivity, and representative phosphoimager scans are shown (Supplementary Figure A). Radioactivity incorporation involved the covalent transfer of the terminal phosphate group of the ATP to peptide substrates, since incubation of arrays with cell lysates and ^{33}P-α-ATP did not produce meaningful incorporation of radioactivity into arrays (not shown). It appeared that phosphorylation of 104 peptide substrates was shared when lysates obtained from blood collected 2 h before the jump were compared with lysates obtained from blood collected directly after jumping, but that phosphorylation of 96 substrates was unique to the lysates obtained before jumping (that is, kinase reactions whose enzymatic activity is downregulated as a consequence of the jump) and that phosphorylation of 94 substrates was induced in lysates obtained from blood directly after the jump (see Supplementary Data for specific peptides). The kinome profile thus obtained was compared with evidence from previous studies, by our group and others, with regard to kinases involved in glucocorticoid signaling (Table 1) (17–23). This comparison revealed that the effects of stress on the immune system are characterized by a noncanonical (also termed nongenomic) glucocorticoid signature (17–23). The information provided was combined with data from the bibliome and used to construct a provisional signal transduction diagram detailing the effects of the stress stimulus on signaling in the leukocyte compartment (Figure 5). Because downregulation of Fyn signaling is a hallmark of noncanonical glucocorticoid receptor signaling (17), we quantified Fyn activation in the leukocyte compartment before and after bungee jumping. As judged by immunoprecipitation from leukocyte lysates followed by conventional Western blotting, depressed Fyn activation was evident in four volunteers, from whom blood was drawn 2 h before the jump and directly after jumping (Figure 6A).

Phagocytosis

To test the functional consequences of activation of noncanonical glucocorticoid signaling in our volunteers, we isolated phagocytes from volunteer blood and assessed their effectiveness in the phagocytosis of bacteria—maybe the most important functionality in the innate immune system and a known target for noncanonical glucocorticoid signal transduction (24). Strikingly, in both granulocytes and especially in monocytes, exposure to the jump more or less instantaneously strongly reduced the capacity for bacterial phagocytosis (Figure 6B). Phagocytosis was impaired in peripheral blood for at least 2 h after jumping (the latest time point analyzed).

Coagulation, Fibrinolysis and Endothelial Activation

To obtain insight into the impact of bungee jumping on activation of the coagulation system, we sequentially measured the plasma levels of markers of thrombin generation, that is, the prothrombin activation fragment F1 + 2 and TAT complexes. Bungee jumping resulted in an increase in plasma F1 + 2 concentrations and TAT complexes ($P < 0.05$, Figure 7). Of considerable interest, propranolol treatment completely prevented the changes in F1 + 2 and TAT complexes ($P < 0.05$, see Figure 7). To determine the effect of bungee jumping on the vascular endothelium, we sequentially measured the plasma concentrations of factor VIII, von Willebrand factor and tPA. All these
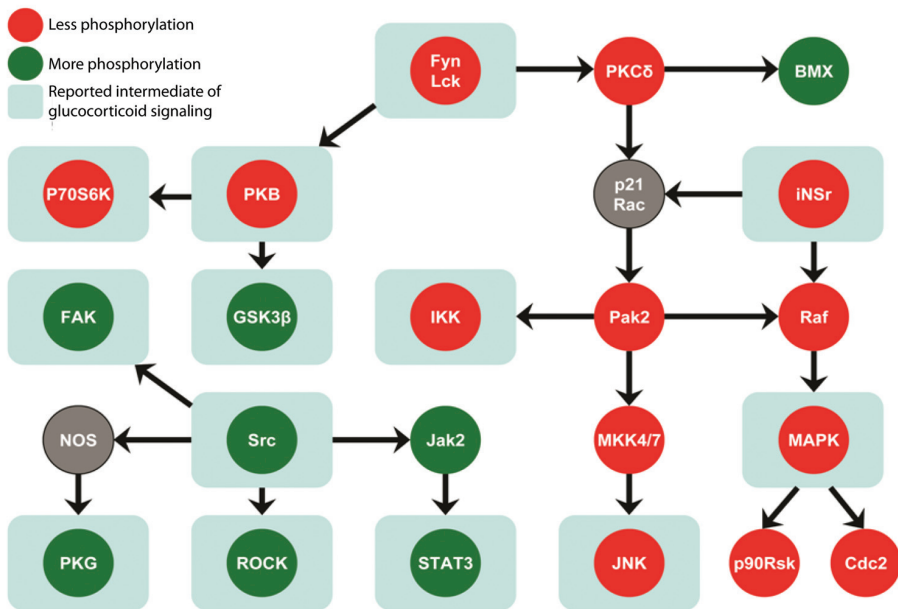
Figure 5. Kinome profiling of human peripheral blood reveals acute stress-induced activation of noncanonical glucocorticoid receptor signal transduction. Peptides displaying differential significant phosphorylation by leukocyte lysates prepared from volunteer blood obtained 2 h before and directly after jumping were used to construct provisional signal transduction schemes, detailing the effects of acute stress on signaling biochemistry. Inconsistent peptides (for example, from the protein kinase C (PKC)α-responsive peptides, six peptides showed upregulation before jumping, whereas five other peptides were upregulated after jumping) were discarded from the provisional signal transduction scheme. From the profiles obtained, a clear noncanonical glucocorticoid receptor signaling signature emerged. Arrows represent biochemical connection (bibliome). Green circles represent upregulation after the jump; red circles represent downregulation after the jump. Blue boxes depict kinases that are reported intermediates of noncanonical glucocorticoid signaling.

markers of endothelial cell activation increased during bungee jumping; the largest rises were detected directly after the jump (see Figure 7, all *P* < 0.05). Importantly, propranolol treatment prevented the rises in factor VIII, von Willebrand factor and tPA (see Figure 7, all *P* < 0.05 versus control).

DISCUSSION

In this study, we evaluated whether an acute severe stress response induced by bungee jumping suppresses key inflammatory responses involved in the innate immune response to infection (that is, the capacity of leukocytes to release cytokines and to phagocytose). We evaluated the potential role of catecholamines herein by pretreating half of our study population with the β-receptor antago-

nist propranolol. We specifically investigated the effects of catecholamines, since these hormones are universally released during stress and β-adrenergic stimulation has been shown to dramatically influence cytokine secretion in a variety of *in vitro* and *in vivo* models (5,7,25,26). In these studies, both exogenously administered and endogenously released catecholamines were reported to decrease TNF-α secretion in humans and mice challenged with LPS *in vivo* (7,25,26), whereas the ability of epinephrine to enhance the release of IL-10 has been demonstrated in a human study in which this catecholamine was intravenously infused (5). In this study, bungee jumping was associated with an immune suppressive phenotype, since we observed a reduction in the release of

the proinflammatory mediators TNF-α and IL-8 in whole blood stimulated with LPS as well as a reduction in the ability of leukocytes to phagocytose bacteria after jumping. The capacity to release the antiinflammatory cytokine IL-10 was not affected by bungee jumping, indicating a preferential effect on proinflammatory cytokines. In contrast to our expectations, propranolol treatment did not influence the effect of bungee jumping on cytokine release. Along the same line, the impaired phagocytic response observed after bungee jumping was not influenced by propranolol.

Because we observed a clear suppression of innate immunity induced by bungee jumping unrelated to the release of catecholamines, we subsequently investigated other potential molecular mechanisms for bungee jumping–induced immunosuppression with a special emphasis on glucorticoid receptor signaling. Elucidating the specific effects of stress on immune cell physiology is hampered by a lack of *a priori* knowledge as to the signaling pathways that possibly mediate such effects. To investigate which signaling pathways were involved in bungee jump–induced immune suppression, we obtained kinome profiles derived from the leukocyte compartment before and after bungee jumping. Comprehensive descriptions of mammalian kinomes have become possible through the sequential spotting of kinase substrates, encompassing the entire human kinome, on a carrier. When such peptide arrays are incubated with cellular lysates and radioactive ATP, kinases active in the lysate will phosphorylate their respective substrates, and upon determining substrate phosphorylation using a phosphoimager, comprehensive descriptions of cellular signaling may be generated (27,28). From the kinome profiles obtained before and after bungee jumping, a noncanonical (also termed nongenomic) glucocorticoid signature stands out. Classically, glucocorticoid effects are explained from their effects on gene transcription via receptor translocation to the nucleus and altered
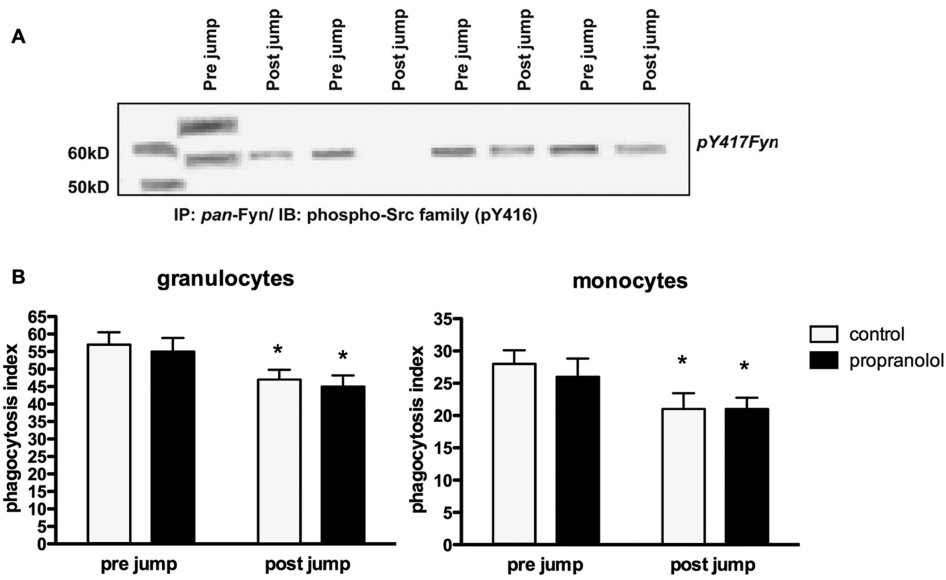
Figure 6. Phagocytosis and c-Fyn depression after bungee jumping. (A) Blood from four volunteers, taken 2 h before the jump and directly after jumping, was lysed, and the remaining leukocyte fraction was solubilized and subjected to immunoprecipitation using an anti-Fyn antibody and blotted with a pan phospho-Src family antibody. The results show that after jumping, Fyn activation in the leukocyte compartment is depressed. The upper band in the first lane is an artifact. (B) Phagocytosis index (number of phagocytosed fluorescein isothiocyanate–positive *E. coli* per cell) before and after a bungee jump was assessed. Phagocytosis index decreased after bungee jump in both groups ($P < 0.05$); no significant difference between control and propranolol group was observed. Asterisks indicate a significant ($P < 0.05$) change of the parameter respective to jump.
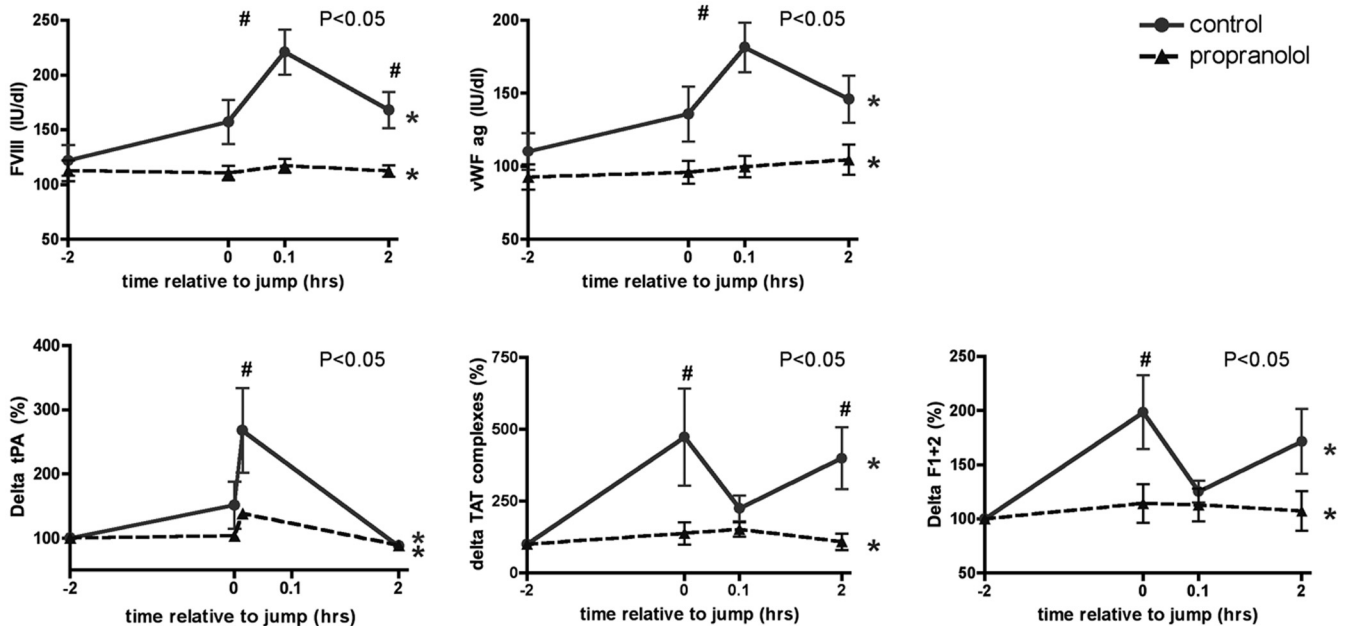

Figure 7. Coagulation parameters relative to jump. Levels of parameters of endothelial activation and coagulation relative to jump are shown. All values changed significantly during the study, indicating that bungee jumping affected endothelial activation and coagulation systems ($P < 0.05$). Factor 1 + 2, TAT complexes and tPA are shown as delta (%) from baseline. Asterisks at the end of each curve indicate a significant ($P < 0.05$) change of the parameter during the experiment. *P* values indicate curve comparison. [#]Significant posttests between groups at the indicated time point. FVIII, factor VIII.

transactivation of glucocorticoid response elements containing promoters (18,22,23,29). The acute clinical effects of steroid treatment, however, suggest that nongenomic mechanisms are important as well (for example, the immediate relief that glucocorticoids bring to allergic patients). Accordingly, many fast nontranscriptional effects after glucocorticoid application have been described (18,22,23,29), including alterations in the cytoskeleton architecture, increased nitric oxide production, downregulation of insulin receptor signaling and protein kinase B signaling, inhibition of the JNK and p42/p44 mean arterial blood pressure kinase signaling cassette and, probably upstream of these effects, the glucocorticoid receptor ligation-dependent disassembly of protein complex containing Hsp90, Fyn, and Lck (17–23). All these events were retrieved from the kinome profiles we obtained. Secondary evidence for the involvement of glucocorticoid signaling was obtained by measuring the activity of Fyn signaling. Because downregulation of Fyn signaling is a hallmark of noncanonical glucocorticoid receptor signaling (17), we quantified Fyn activation in the leukocyte compartment before and after bungee jumping. Downregulation of Fyn signaling as judged by immunoprecipitation from leukocyte lysates followed by conventional Western blotting was evident in volunteers in the control group, from which blood was drawn 2 h before the jump and directly after jumping. It is tempting to speculate on a causative connection between diminished Fyn activation after the bungee jump and the coincidential block in phagocytosis. Upon stimulation with opsonized particles, macrophages are known to activate this Src-like tyrosine kinase Fyn, and it becomes redistributed to actin-rich phagocytic cups and the phagosomal membrane. PP1, a tyrosine kinase inhibitor that is relatively specific for Fyn, blocks both this redistribution as well as phagocytosis *per se*, at least in murine macrophages (30). Also, because of the absence of other clear candidates from

our kinome profiles as mediators of the phagocytic block, the most straightforward interpretation of our data thus is that Fyn inhibition by noncanonical glucocorticoid signaling mediates the inhibition of innate immunity observed after the jump, but definitive proof of this notion would require stress experiments in Fyn-deficient animals. Taken together, activation of noncanonical glucocorticoid signaling in the leukocyte compartment is a hallmark of the response to acute stress in humans.

A weakness of our study is the lack of direct evidence of glucorticoid receptor signaling, which would require a subsequent study using *in vivo* glucocorticoid receptor blocking. Nonetheless, our results strongly suggest that noncanonical glucocorticoid receptor signal transduction is a major pathway linking stress to impaired functioning of the human innate immune system. Stress in humans is associated with higher vulnerability to infection as well as the development of autoimmune disorders such as allergy (31) or inflammatory bowel disease (32). It is becoming increasingly clear that development of such disorders is associated with reduced innate immunity rather than with excessive activation of this system (33,34); thus, our results may have importance for linking stress to the risk of infection as well as autoimmunity, although this would require further experimentation. Irrespective of the exact implications for human disease, our results now further define the mechanism by which the brain-immune axis is deregulated under conditions of acute aggravation.

Although bungee jumping attenuated leukocyte functions (cytokine release and phagocytosis), it enhanced activation of the coagulation system and the vascular endothelium. These data are in-line with previous studies that have suggested that acute stress results in profound changes in coagulation, resulting in a procoagulant phenotype (35,36). In evolutionary terms, such a response could be explained by a protective mechanism to stop bleeding, which

might occur during stressful fight-or-flight circumstances (37). Notably, the procoagulant phenotype during stress was virtually prevented by β-adrenergic receptor blockade, pointing to an important role for catecholamines. It was suggested that β-adrenergic agents can inhibit the expression of tissue factor, which is considered the main initiator of coagulation activation, by increasing intracellular cyclic AMP levels (38). In line with this, intravenous infusion of epinephrine attenuated systemic coagulation activation upon intravenous injection of LPS in healthy humans; moreover, epinephrine attenuated endothelial cell activation in this model (39). Although the effect of β-adrenergic inhibition on systemic coagulation activation has not been reported previously, our group recently found that inhalation of propranolol enhanced activation of coagulation in the bronchoalveolar space of mice challenged with LPS via the airways (40). In light of these earlier studies, our current finding that propranolol strongly inhibited these responses comes as a surprise and suggests that the systemic effects of endogenously released catecholamines are dissimilar from exogenous infusion of catecholamines.

In conclusion, our results indicate that an acute stress response leads to a catecholamine-independent immune suppressive phenotype, and we identify noncanonical glucocorticoid receptor signal transduction as the major pathway linking human stress to impaired functioning of the human innate immune system.

DISCLOSURE

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