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Prothrombotic response to norepinephrine infusion, mimicking norepinephrine stress-reactivity effects, is partly mediated by α-adrenergic mechanisms

Roland von Känel[a,b,1], Nadja Heimgartner[c], Monika Stutz[d], Claudia Zuccarella-Hackl[e], Alexander Hänsel[b], Ulrike Ehlert[c], Petra H. Wirtz[b,e,f,*,1]

[a] Department of Consultation-Liaison Psychiatry and Psychosomatic Medicine, University Hospital, Zurich, Switzerland
[b] Department of BioMedical Research, University of Bern, Switzerland
[c] Clinical Psychology and Psychotherapy, Psychological Institute, University of Zurich, Switzerland
[d] Thrombosis Research Laboratory, Inselspital, Bern University Hospital, Switzerland
[e] Biological and Health Psychology, Department of Psychology, University of Bern, Switzerland
[f] Biological Work and Health Psychology, Department of Psychology, University of Konstanz, Germany

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ABSTRACT

Background: Stress-induced prothrombotic changes are mediated by the sympathetic nervous system and critically involved in mental triggering of acute coronary syndromes, but the underlying psychobiology is not fully understood. We tested the hypothesis that a norepinephrine (NE) infusion to mimic effects of stress-induced NE release on blood coagulation elicits prothrombotic changes and examined to what extent these would be mediated by an alpha-adrenergic mechanism.

Methods and results: In a single-blind placebo-controlled within-subjects design, 24 middle-aged, non-smoking, non-obese and normotensive men participated in three experimental trials with an interval between one and two weeks. Each trial applied two sequential infusions of 1 and 15 min duration with varying substances [i.e., saline as placebo, the non-specific α-blocker phentolamine (2.5 mg/min), and NE (5 μg/min)]: trial 1 = saline + saline; trial 2 = saline + NE, and trial 3 = phentolamine + NE. Plasma levels of clotting factor VIII activity (FVIII:C), fibrinogen, and D-dimer were assessed from blood samples collected immediately before and 1 min and 20 min after infusion procedures. Compared to saline + saline, saline + NE induced increases over time in FVIII:C, fibrinogen, and D-dimer levels. With phentolamine + NE, fibrinogen levels remained increased compared to saline + saline, but changes in FVIII:C and D-dimer levels were no more different. Coagulation changes did not differ between saline + NE and phentolamine + NE.

Conclusions: NE infusion activates blood coagulation. The resulting prothrombotic state could be one psychobiological mechanism underlying mental triggering of acute coronary syndromes. Blockade of α-adrenergic receptors partly attenuated NE effects on coagulation and could be implied to have preventive potential in susceptible individuals.

1. Introduction

Between 10% and 50% of patients with an acute coronary syndrome (ACS) report an emotional trigger like mental stress or intense feelings within 2 h before symptom onset (Tofler et al., 1990; Willich et al., 1991; Tofler et al., 2017) with, for instance, outbursts of anger quadrupling the risk (Mostofsky et al., 2014). With the undeniable role of thrombosis in ACS (Abbate et al., 2012), the literature provides good evidence that stress-induced coagulation activation is a crucial process in emotionally-triggered ACS (Thrall et al., 2007; von Känel, 2015). In an attempt to maintain homeostasis, emotional upset evokes hemodynamic responses with shear forces to the vessel wall, vagal withdrawal, sympathetic and neuroendocrine activation, altogether leading to inflammatory and prothrombotic changes (Steptoe and Brydon, 2009;

* Corresponding author at: Biological Work and Health Psychology, Department of Psychology, University of Konstanz, Universitaetsstrasse 10, 78457, Konstanz, Baden-Wuerttemberg, Germany.
E-mail address: petra.wirtz@uni-konstanz.de (P.H. Wirtz).
[1] RvK and PHW contributed equally to the writing of this manuscript.

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Mittleman and Mostofsky, 2011). In susceptible individuals with atherosclerotic cardiovascular disease (CVD) (Leor et al., 1996), stress-triggered sympathetic, inflammatory and prothrombotic responses are excessive (Wallén et al., 1997; von Känel et al., 2001a; Strike et al., 2004; Kop et al., 2008) and may promote destabilization and rupture of a coronary plaque with thrombotic occlusion leading to ACS (von Känel, 2015; Wirtz and von Känel, 2017). Moreover, acute mental stress-induced platelet activation was shown to be greater and prolonged in patients with versus those without emotional triggering of ACS (Strike et al., 2006).

The prothrombotic stress response is partially mediated by an acute increase in circulating catecholamines. Specifically, norepinephrine (NE) and epinephrine (EPI) bind to adrenergic receptors (AR) whereby stimulating platelets and endothelial cells to release coagulation molecules in the circulation; however the precise mechanisms involved, both in healthy humans and patients with atherothrombotic CVD, are still elusive (Austin et al., 2013). Sympathetic activation includes NE release from sympathetic nerve endings and secretion of EPI and NE from the adrenal medulla into the bloodstream, so mental-stress induced coagulation changes result from combined EPI and NE effects (Austin et al., 2013; von Känel and Dimsdale, 2000). To concur, previous studies in humans showed a positive association between stress-induced increases of NE and D-dimer, a marker of fibrin formation (Wirtz et al., 2006) and of both EPI and NE and thrombin/antithrombin complex, a marker of thrombin generation (von Känel et al., 2002). Regarding the trajectory of these responses, a positive association emerged between delayed post-stress recovery of NE and activated platelets in elderly individuals (Aschbacher et al., 2008).

When EPI is infused, as to mimic this catecholamine's stress effect on blood coagulation, one observes a concomitant increase in plasma NE levels (Wallén et al., 1999). In turn, plasma EPI levels do not increase after NE infusion (Kuebler et al., 2014). Therefore, experimental NE infusion can reveal unique effects of NE (i.e., without confounding EPI effects) on coagulation molecules. With respect to underlying AR mechanisms, study findings on combined effects of catecholamines and AR-blockers on coagulation molecules are not uniform in healthy humans (von Känel and Dimsdale, 2000) and to our knowledge lacking in patients with atherothrombotic CVD. The most reliable findings are that within minutes, EPI infusion induces a dose-response increase in FVIII clotting activity (FVIII:C) mediated by β2-AR, whereas NE and EPI infusion both induce platelet activation through α2-AR (von Känel and Dimsdale, 2000). Based on this literature, a NE infusion paradigm with and without α-adrenergic blockade seems better suited to identify specific NE-related coagulation effects mediated by α-adrenergic mechanisms than a laboratory stressor, as in a stress paradigm the coagulation system will additionally be stimulated by EPI-related α- and β-adrenergic mechanisms.

Here, we designed an experimental study with the overarching goal to better understand both noradrenergic and α-adrenergic mechanisms of sympathetically-mediated prothrombotic changes that could ultimately underlie mental-triggering of ACS. Specifically, the primary aim of our study was to test in a placebo-controlled within-subject design the hypothesis that NE infusion, in a manner and dosage to mimic effects of stress-induced NE release, induces significant increases in plasma levels of FVIII:C, fibrinogen, and the coagulation activation marker D-dimer in healthy men. FVIII, fibrinogen, and D-dimer are clearly stress-responsive coagulation molecules (Thrall et al., 2007; Austin et al., 2013) and also associated with an increased CVD risk in prospective population-based studies (Fibrinogen Studies Collaboration, 2005; Willeit et al., 2013; Lowe and Rumley, 2014). To our best knowledge, reactivity of fibrinogen and D-dimer to NE application have not previously been investigated in healthy subjects, whereas three studies with small sample sizes (n = 5–11) and dated methodology found no change in FVIII:C (von Känel and Dimsdale, 2000). Most similar to our experiment, although not placebo-controlled, one of these studies infused 100–160 μg NE for 10 min (Ingram, 1961). In the two other studies, a bolus of 500–750 μg NE s.c./i.m. (Wachholder et al., 1961) and 210 μg NE i.v. (Gader et al., 1973) were applied, and FVIII:C was measured after a non-specified time interval and 30 min, respectively, likely too late to detect a significant effect.

The secondary aim of our study was to examine the extent to which NE-induced prothrombotic changes would be mediated by an α-adrenergic mechanism. To this end, we infused NE in healthy men, with and without prior application of phentolamine, a non-specific α-AR-blocker, and measured plasma levels of FVIII:C, fibrinogen, and D-dimer before, immediately after, and 20 min after infusion procedures.

2. Methods

2.1. Study participants

The present study is part of a larger project which aims to elucidate effects of a NE infusion to mimic the effects of a stress-induced increase in circulating NE, administered with and without α-adrenergic blockade, on psychobiological processes involved in CVD (Kuebler et al., 2014). As part of this larger study the NE and EPI data have been published before (Kuebler et al., 2014; Beis et al., 2018). The study sample for the coagulation sub-study presented here comprised 24 medication-free, non-smoking and normotensive healthy Caucasian men who completed all three infusion trials (rendering a total of 72 trials) with balanced trial-sequence and assessment of coagulation molecules for at least one trial. Specific coagulation data was missing or incomplete in 1 participant in trial 1, in 2 participants in trial 2, and in 3 participants in trial 3 (cf. below for trial specification). The resulting sample sizes with complete assessments of coagulation molecules were for the comparison between trial 1 and 2 n = 22 for D-dimer, fibrinogen, and FVIII:C; between trial 1 and 3 n = 22 for fibrinogen and FVIII:C and n = 20 for D-dimer; and between trial 2 and 3 n = 21 for fibrinogen and FVIII:C and n = 19 for D-dimer. Complete NE and EPI data was available from 21 participants. Reasons for missing values of biochemical measures were technical problems with blood sampling and/or assays.

Participants were recruited by aid of the Swiss Red Cross of the Canton of Bern and the Clinical Investigation Unit of the Bern University Hospital. Specific exclusion criteria were verified with a structured clinical interview: any regular or current prescribed or non-prescribed medication intake, psychiatric diseases, alcohol abuse and illicit drug use, any heart disease, varicosis, and thrombotic diseases; elevated blood sugar levels and diabetes, elevated cholesterol levels, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, cancer, chronic pain, sleep disturbances, thyroid disease, and current infectious diseases. If the history was not conclusive, we contacted the subjects' primary care physician for clarification. The body mass index was calculated by dividing weight in kilograms by height in meters squared.

The ethics committee of the Canton of Bern, Switzerland, and the Swiss Agency for Therapeutic Products (Swissmedic) formally approved the study protocol. The study was carried out in accordance with the Declaration of Helsinki principles. All participants provided written informed consent before any study procedure took place and were compensated with CHF 120 per study day (CHF 360 for all three days).

2.2. Study design and procedure

The study was performed in the Clinical Investigation Unit of the Bern University Hospital (Inselspital). In a single-blind placebo-controlled within subject design, all participants took part in three different experimental trials varying in terms of the combination of the two sequentially infused substances as previously described (Kuebler et al., 2014); these were saline + saline in trial 1, saline + NE in trial 2, and phentolamine + NE in trial 3. Trial 1 was the placebo condition and performed to control for coagulation effects of the experiment per se,

arising for instance from fear and stress in anticipation of the infusion procedure. Trial 2 was the main experimental trial designed to test for the effects of NE infusion on coagulation molecules. Trial 3 tested whether potential coagulation effects of NE would be modulated by α1- plus α2-AR.

The trial sequence was fully counterbalanced by using a Latin Square design with the sequences 1,2,3 (i.e., infusion day 1 was trial 1, infusion day 2 was trial 2, infusion day 3 was trial 3); 2,3,1 (i.e., infusion day 1 was trial 2, infusion day 2 was trial 3, infusion day 3 was trial 1); and 3,1,2 (i.e., infusion day 1 was trial 3, infusion day 2 was trial 1, and infusion day 3 was trial 2). The trials took place on separate days with inter-trial intervals of at least one week (to allow for phentolamine wash-out) and two weeks. Ethical and safety considerations regarding potential (hemodynamic) side effects of study substances prohibited a double-blind design. Therefore, the participants, but not the experimenters, were blind to trial substances. A board-certified internist performed all infusions.

Participants abstained from physical exercise for 24 h and maintained a regular sleep-wake rhythm the three nights before each trial, with lights out between 22:30 h and 24:00 h and lights on between 07:00 h and 09:00 h. Participants reported to the laboratory at 11:45 h to receive a standardized meal with experimental procedures starting at 13:00 h. Participants were tested in the supine position lying on a bed. Each trial started with a 10-min introduction phase, during which the testing procedure was explained, followed by catheter insertion into the brachial vein of the dominant arm for the infusions. For blood sampling, a second catheter was inserted into the brachial vein of the non-dominant-arm. After a further 45-min interval to acclimatize, the infusion procedure started.

Blood samples for coagulation molecules were obtained immediately before the infusion phase (baseline), and 1 min and 20 min after termination of the infusion procedure. The sampling protocol based on findings from our previous stress studies showing that levels of coagulation molecules peak immediately after stress and return to baseline levels within 20 to 45 min of recovery from stress (von Känel et al., 2004; Wirtz et al., 2006). Blood samples for NE and EPI assessments were taken at baseline before the first infusion and 1 min after the second infusion.

2.3. Substance infusion protocol

Two sequential infusions with application of either saline or phentolamine for 1 min (first infusion), and NE or saline for 15 min (second infusion), were applied with an interval of 5 min between infusions. The post-infusion phase began after the second infusion.

NE (Sintetica, SA, Mendrisio, Switzerland) was diluted in saline and the resulting solution of 5 μg/ml was infused with 1 ml/min over 15 min, totaling 75 μg NE, to mimic effects of NE-stress-reactivity. We selected this dosage because earlier studies showed that a dose of 5 μg/ml/min NE, yielding NE plasma levels in excess of 1800 pg/ml, are required to produce hemodynamic and metabolic effects (Silverberg et al., 1978), as are elicited by acute mental stress (Wallén et al., 1999). As reported elsewhere (Kuebler et al., 2014), we previously demonstrated effective NE application in our study protocol by an increase in blood pressure and heart rate. We chose a 15-min infusion time for NE because former protocols typically inflicted laboratory mental stress between 10 and 20 min to elicit significant prothrombotic stress responses (von Känel et al., 2001a, 2004; Strike et al., 2006; Wirtz et al., 2006). The non-selective α-adrenergic antagonist (i.e., α1- and α2-AR-blocker) phentolamine (Regitin®, Novartis Pharma AG, Basel, Switzerland) was diluted in saline and 5 ml of 0.5 mg/ml, totaling 2.5 mg phentolamine, were infused within 1 min. Identical times of 1 min and 15 min were used for saline infusions (Kuebler et al., 2014).

2.4. Biochemical analyses

2.4.1. Coagulation molecules

Venous blood was drawn into polypropylene tubes containing 3.8% sodium citrate (Sarstedt, Numbrecht, Germany). Samples were centrifuged for 20 min at 2000 x *g* at room temperature. Plasma was then aliquoted into polypropylene Eppendorf tubes and stored at -80 °C until assayed in the Thrombosis Research Laboratory, Bern University Hospital. Fibrinogen and FVIII:C were determined using the BCS Coagulation Analyzer (Dade Behring, Liederbach, Germany). FVIII:C was measured by standard coagulometric methods using factor-deficient standard human plasma and reagents (Siemens Healthcare Diagnostics GmbH, Erlangen, Germany). FVIII:C is expressed as percentage relative to normal human plasma with an absolute FVIII:C value of 100% per definition. Fibrinogen levels were measured with a modified Clauss method (Multifibren U, Siemens Healthcare Diagnostics GmbH, Erlangen, Germany), expressed as g/l. D-dimer levels were measured using an enzyme-linked immunosorbent assay (ZYMUTEST DDimer, HYPHEN BioMed, Neuville-sur-Oise, France), expressed as mg/l. Inter- and intra-assay coefficients of variation were < 10% for all coagulation assays.

2.4.2. Catecholamines

For NE and EPI assessment, blood was drawn into EDTA-coated monovettes (ethylenediaminetetraacetic acid; Sarstedt, Numbrecht, Germany), and immediately centrifuged for 10 min at 2000 *g* and 4 °C; plasma was stored at −80 °C until analyzed. Plasma EPI and NE levels were determined by means of high-pressure liquid chromatography (HPLC) using electrochemical detection after liquid-liquid extraction in the Laboratory of Stress Monitoring, Göttingen, Germany (Ehrenreich et al., 1997). The lower limit of detection was 12 pg/ml each for EPI and NE. Undetectable values were replaced by half the detection limit (Hornung and Reed, 1990).

2.5. Statistical analysis

Data was analyzed using SPSS (version 23.0) statistical software package (SPSS Inc., Chicago IL, USA) and presented as mean ± SEM (if not indicated otherwise). The significance level was $p \leq .05$ (two-tailed). Normality of the data distribution was tested with the Kolmogorov-Smirnov test. Values of coagulation molecules were log-transformed before statistical analysis. However, for reasons of clarity, we depict untransformed data in the figure while making adjustments for baseline values. We applied Huynh-Feldt correction for repeated measures to protect against violations of the sphericity assumption.

To test for differences between infusion-trials in baseline levels of NE and EPI concentrations and coagulation molecules, we calculated general linear models with repeated measurements with the baseline levels of each trial as the repeated dependent variables; we report post-hoc tests to explore trial differences (i.e. trial 1 vs. 2, trial 1 vs. 3, and trial 2 vs. 3). We similarly calculated trial differences in NE and EPI changes (calculated as plasma levels at 1 min post infusion minus baseline values before the infusion began).

To test for different effects of the infusion trials on coagulation molecules over time, we compared trials pairwise (i.e., trial 1 vs. 2, trial 1 vs. 3, and trial 2 vs. 3) using general linear modelling with the two repeated factors trial (2 trials) and time (3 time-points). Effect size parameters (*f*) were calculated from partial η^2-values and are reported, where appropriate, according to the following conventions (*f*): .10 = small, .25 = medium, .40 = large. We did not correct for multiple comparisons due to the prespecified hypothesis of significant NE-induced changes in coagulation molecules in the same direction and the fact that these changes are indicative of the same psychobiological process (i.e., the prothrombotic stress response), correlated with each other (r = 0.49–0.64; Zgraggen et al., 2005), and actual observations in nature (Perneger, 1998; Rothman, 1990).

Table 1
Baseline measures in coagulation molecules and catecholamines and infusion-induced changes in catecholamines .

	Trial 1 (Sal/Sal)	Trial 2 (Sal/NE)	Trial 3 (Ph/NE)	*Trial differences*		
				$p^{(1vs.2)}$	$p^{(2vs.3)}$	$p^{(1vs.3)}$
Fibrinogen baseline (g/l)	2.47 ± .13 (1.70–4.79)	2.35 ± .09 (1.70–3.63)	2.34 ± .10 (1.52–3.61)	.23	.30	.10
FVIII:C baseline (%)	108.88 ± 6.17 (63–181)	105.26 ± 5.96 (63–188)	107.58 ± 6.88 (58–200)	.13	.66	.37
D-dimer baseline (mg/l)	272.50 ± 42.50 (72–837)	264.65 ± 41.08 (80–772)	266.67 ± 37.68 (65–696)	.69	.70	.92
NE baseline (pg/ml)	413.43 ± 46.40 (190.20–976.51)	378.02 ± 44.97 (145.61–1097.49)	375.96 ± 36.00 (126.10–794.86)	.44	.62	.19
EPI baseline (pg/ml)	29.98 ± 3.81 (6.00–66.01)	30.45 ± 3.22 (6.00–58.65)	27.96 ± 2.69 (6.00–61.84)	1.00	.64	.70
NE change (pg/ml)	4.01 ± 18.18 (−183.35–281.97)	831.87 ± 89.94 (314.53–1858.21)	769.90 ± 72.81 (187.79–1661.87)	**< .001**	.70	**< .001**
EPI change (pg/ml)	2.59 ± 2.36 (−20.14–37.51)	−5.67 ± 1.34 (−16.41–11.20)	0.42 ± 2.00 (−10.06–36.42)	**.003**	**.007**	.52

Values are given as means ± SEM (range). Changes are calculated as post- minus pre-infusion measurements. Post-hoc tests of general linear models with repeated baseline or change measures were conducted to test for trial differences in baseline values and change scores. Bold values indicate significance. EPI, epinephrine; FVIII:C, clotting factor VIII activity; NE, norepinephrine; Ph, phentolamine; Sal, saline (placebo).

3. Results

3.1. Participant characteristics

The 24 study participants were middle-aged to older (52.1 ± 2.1, range: 29–64 years), non-obese (BMI: 24.2 ± 0.5, range 20.7–29.0) men, with blood pressure values in the normotensive range (mean systolic: 114.0 ± 1.5 mmHg, range: 100.5–130.0; mean diastolic: 70.9 ± 1.1 mmHg, range: 61.8–82.3).

3.2. Trial comparisons in baseline measures and infusion-induced catecholamine changes

As shown in Table 1, there were no baseline (i.e. pre-infusion) differences between the 3 trial conditions in plasma levels of catecholamines (p´s ≥ .19) and prothrombotic molecules (p´s ≥ .10). Both saline + NE and phentolamine + NE led to increased NE levels compared with saline + saline (p´s < .001); whereas NE changes did not differ between saline + NE and phentolamine + NE (p = .70). Saline + NE led to decreased EPI levels compared with saline + saline (p = .003) and phentolamine + NE (p = .007); whereas EPI changes were similar between saline + saline and phentolamine + NE (p = .52). Of note, the catecholamine data presented in Table 1 have already been published (Kuebler et al., 2014; Beis et al., 2018).

3.3. Trial comparisons in infusion-induced coagulation molecule reactivity

Fig. 1, depicts changes over time in coagulation molecules in response to substance infusions across the three trials. Compared to saline + saline, saline + NE induced higher levels of FVIII:C (interaction trials (1 vs. 2)-by-time: F(1.62/34.08) = 3.61, p = .047, η^2 = .15, f = .42), fibrinogen (interaction trials (1 vs. 2) -by-time: F(1.42/29.81) = 7.24, p = .004, η^2 = .27, f = .61), and D-dimer (interaction trials (1 vs. 2) -by-time: F(2.0/42.0) = 5.52, p = .007, η^2 = .21, f = .51). Compared to saline + saline, phentolamine + NE reduced the effects of saline + NE on FVIII:C (p = .22) and D-dimer (p = .25) to non-significance. In contrast, the fibrinogen response to phentolamine + NE remained significantly different from that seen with saline + saline (interaction trials (3 vs. 1)-by-time: F(2.0/42.0) = 3.72, p = .032, η^2 = .15, f = .42). Saline + NE and phentolamine + NE did not induce significantly different changes over time in any coagulation molecule (FVIII:C: p = .47; fibrinogen: p = .17; D-dimer: p = .80).

4. Discussion

4.1. Study purpose

We investigated whether FVIII:C, fibrinogen, and D-dimer, three coagulation molecules showing robust stress responses and with relevance for CVD risk, are reactive to NE infusion, and whether this reactivity is possibly mediated by α-adrenergic mechanisms. The results from our study shed more light on the complex and still poorly understood interplay between the sympathetic nervous system and the hemostatic system with its numerous molecules and activating and inhibiting steps involved (von Känel et al., 2001b; Austin et al., 2013).

4.2. Principal findings

We found that a 15-min NE infusion induced significantly greater increases over time in plasma levels of FVIII:C, fibrinogen and D-dimer compared to placebo (saline infusion), suggesting overall that NE caused a prothrombotic state. Although the absolute changes in coagulation molecules from rest were quite small, the calculated effect sizes were large by convention. Therefore, the observed prothrombotic changes could be of clinical relevance, particularly so with regard to a possible role in mental triggering of ACS. Whether stress-induced coagulation changes measured in a laboratory setting, including the unique contribution of an increase in circulating NE, predict the risk of atherothrombotic CVD has not previously been investigated. However, stress-induced changes in fibrinogen, similar in magnitude to our NE infusion study, have been shown to predict systolic ambulatory blood pressure at 3-year follow-up, adjusting for demographic factors, health behaviors and baseline blood pressure (Brydon and Steptoe, 2005). The infusion procedure did not result in increased levels of EPI and, as reported elsewhere, of cortisol (Kuebler et al., 2014). In contrast, laboratory stress protocols do provoke EPI and cortisol increases, which moreover have been shown to be associated with stress-induced prothrombotic changes (von Känel et al., 2002; Wirtz et al., 2006). These observations allow us to interpret that NE effects on the coagulation system are sufficient to induce a prothrombotic state in response to acute mental stress. We further found that the non-specific α-AR-blocker phentolamine attenuated the NE-induced increase in FVIII:C and D-dimer, but not in fibrinogen, to a level that was not significantly different from the placebo condition. This suggests that α-adrenergic mechanisms may partly underlie NE effects on the prothrombotic stress response.

4.3. Possible explanations

How could NE-infusion and AR function possibly affect molecular processes of the coagulation cascade and result in prothrombotic changes relevant to mental triggering of ACS? Catecholamine infusion and acute mental stress both induce fibrinolysis and platelet activation (von Känel and Dimsdale, 2000; Austin et al., 2013). Here, we studied effects of non-specific α-AR-blockade. However, it has become clear that α2-AR, which have high affinity for both EPI and NE, mediate platelet activation by catecholamines (von Känel and Dimsdale, 2000). Specifically, NE infusion has been shown to induce platelet factor 3

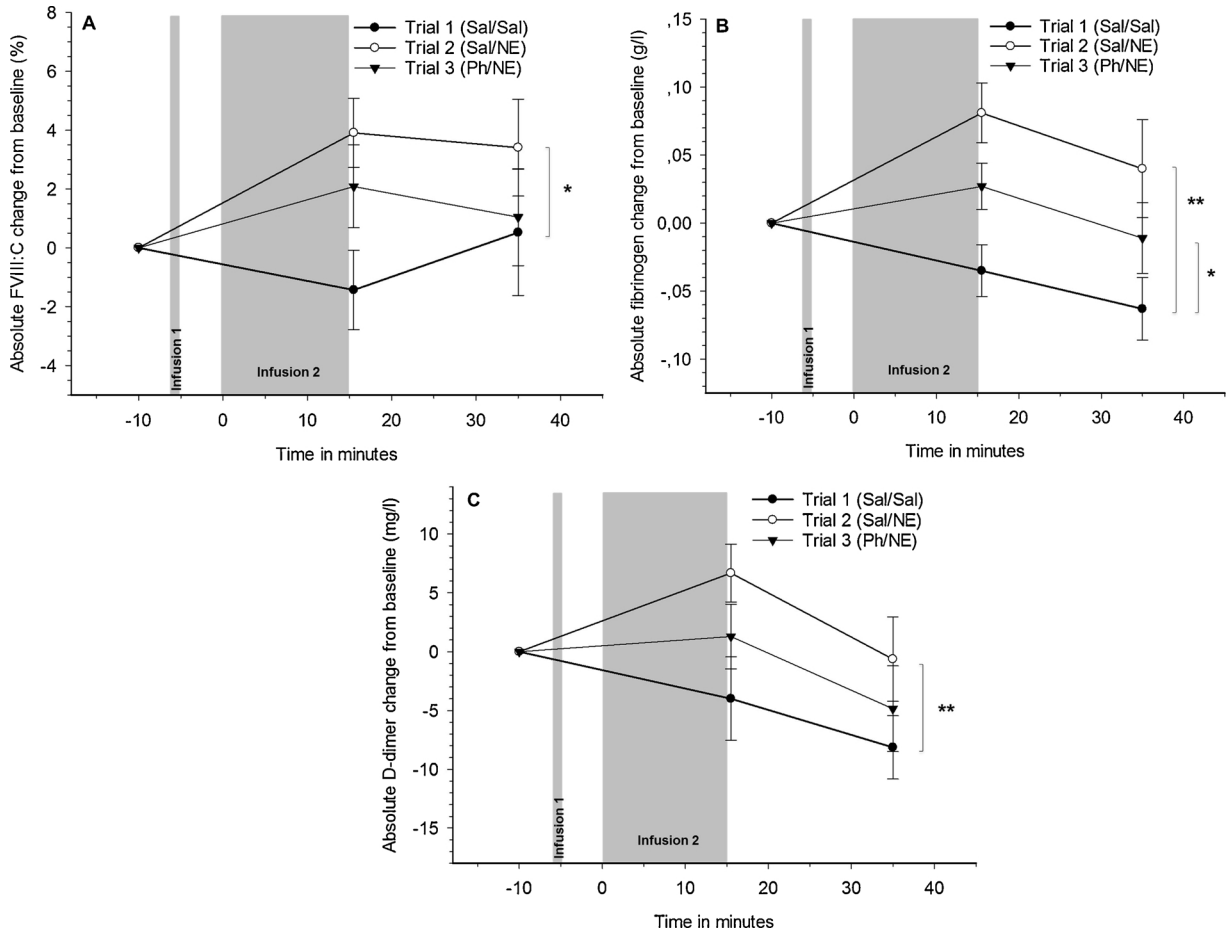

Fig. 1. Changes over time in coagulation molecules in response to substance infusion.
FVIII:C (A), fibrinogen (B), and D-Dimer (C) reactivity to substance infusion (Sal, saline; NE, norepinephrine; Ph, phentolamine). Values are means ± SEM. General linear models with repeated measures revealed that infusion reactivity of FVIII:C (p = .047), fibrinogen (p = .004), and D-dimer (p = .007) plasma levels differed between Sal + NE and Sal + Sal. Whereas fibrinogen reactivity also differed between Sal + Sal and Ph + NE (p = .032), no other trial comparisons were significant (p's ≥ .17).*, $p < .05$;**, $p < .01$;***, $p < .001$.

activity (Nordły et al., 1975), expression of platelet fibrinogen receptors (Lam et al., 2002), platelet release reaction (plasma β-thromboglobulin) (Larsson et al., 1992), and ultimately platelet aggregation (Larsson et al., 1992). Of importance to the present study, activated platelets also release FVIII from their alpha granules into plasma (Yarovoi et al., 2003). In brief, platelet factor 3, the source of procoagulant phospholipids exposed on the surface of activated platelets, converts prothrombin to thrombin, which in turn potentiates FVIII:C and converts fibrinogen to crosslinked fibrin. Fibrin degradation by the fibrinolytic system results in increased D-dimer plasma levels indicating activated coagulation (Lowe and Rumley, 2014), for instance as part of the fight-or-flight response (Austin et al., 2013). A direct association between changes in plasma levels of NE and D-dimer has been shown in healthy subjects (Wirtz et al., 2006) and exaggerated platelet activation was found in patients with mental triggering of ACS (Strike et al., 2006) over a period of one and two hours, respectively. Of clinical importance, this corresponds to the time interval during which ACS risk is increased after emotional upset (Mostofsky et al., 2014).

Unlike the above discussed role of α2-AR in NE-induced coagulation activation, the role of α1-AR, which have high affinity for NE, but lower affinity for EPI (Scanzano and Cosentino, 2015), seems far less clear. Stimulation of α1-AR releases vasopressin in the brain (Sladek and Song, 2008). Desmopressin - a synthetic analogue of vasopressin - rapidly releases FVIII from regulated storage pools into plasma (Haberichter et al., 2006); in turn, α1-AR-blockade could attenuate NE-induced vasopressin release and thus FVIII:C increase too, but this is speculative. Moreover, to our knowledge, it is unknown, whether NE-induced vasoconstriction, via activation of α1- and α2-AR, both expressed in vascular smooth muscles (Gordan et al., 2015), affects coagulation activity.

Other than FVIII:C and D-dimer responses to phentolamine + NE, that of fibrinogen was significant when compared with placebo, implying that neither α1-AR nor α2-AR are evidently involved in the stress response of fibrinogen. This should not discount a potential role of acute elevation in fibrinogen in mental triggering of ACS due to other mechanisms, as infusion of human fibrinogen hastened thrombotic occlusion of carotid arteries in a mouse model (Lowe and Rumley, 2014). Although not studied so far (Austin et al., 2013), an alternative could be involvement of β1-AR, which have affinity for NE, and increase heart rate and cardiac contractility in fight-or-flight (Gordan et al., 2015). However, non-specific β-AR-blockade did not previously reduce mental stress-induced changes in fibrinogen (von Känel et al., 2008). Taken together, NE surge could play an important role in prothrombotic mechanisms underlying mental triggering of ACS, and α2-adrenergic mechanisms, arguably more than α1-adrenergic mechanisms, could mediate part of this risk.

4.4. Potential clinical implications

The possible mediation of the prothrombotic stress response by α-AR may have clinical implications in preventive terms. Regular treatment with platelet-aggregation inhibiting aspirin and β-AR-blockers may

partially break the link between outbursts of anger and ACS onset (Mostofsky et al., 2014), reducing the risk by about half (Mittleman et al., 1995). Aspirin was shown to partially inhibit NE-induced platelet activation (Larsson et al., 1994) and to attenuate mental stress-induced platelet fibrinogen and P-selectin expression (Aschbacher et al., 2009). Cardio-selective β1-AR-blockers may cut off the peak of a sudden rise in blood pressure, thereby reducing the risk of plaque rupture (Möller et al., 1999). The non-selective β-AR-blocker propranolol in combination with aspirin, but not aspirin alone, diminished the acute stress response of FVIII:C (von Känel et al., 2008). The latter finding concurs with research showing that preformed FVIII is released in the circulation from extravascular storage pools, like endothelial cells and the liver (Haberichter et al., 2006), via β2-AR stimulation upon sympathetic activation (Austin et al., 2013). Thus, α- and β-adrenergic mechanisms could both mediate a role of FVIII in mental triggering of ACS. Future studies could be valuable as to whether modulation of adrenergic effects on the coagulation system at times of emotional upset may sever the risk of ACS onset in susceptible individuals.

4.5. Strengths and limitations

Major strengths of our study were the placebo-controlled experimental within-subject design with a NE-infusion protocol to mimic the effects of a stress-induced increase in circulating NE and prevention of confounding EPI effects on coagulation activation. Our study has its limitations, too. The total dose of the infused NE was selected based on the rationale that it would provoke cardiovascular changes comparable to acute stress (Kuebler et al., 2014). The resulting NE concentrations were about 3- to 5-fold higher than plasma concentrations that are usually reached in response to acute mental stress (von Känel et al., 2002; Beis et al., 2018). We speculate that the high i.v. dosage is required to compensate for stress-induced NE co-secreted from sympathetic nerve endings in addition to secretion from the adrenal medulla. Nevertheless, we cannot ultimately settle the question to what extent the higher NE plasma concentrations may account for the observed coagulation effects in the present study, respectively, whether stress-induced NE concentrations alone are sufficiently high to induce changes in FVIIIC, fibrinogen and D-dimer. We did not include a saline + phentolamine trial to rule out possible sole effects of phentolamine on blood coagulation. However, phentolamine is not known to have side effects in terms of an increased risk of either thrombotic or hemorrhagic complications. Moreover, a previous study found that phentolamine did not affect platelet aggregability in vivo at rest, but abolished EPI-induced platelet activation (Larsson et al., 1992). We investigated healthy male subjects, so our findings cannot be transferred to women and patients with CVD, the latter showing exaggerated prothrombotic stress responses (Wallén et al., 1997; von Känel et al., 2001a; Strike et al., 2004; Kop et al., 2008), partly due to impaired antithrombotic properties of a dysfunctional endothelium (Austin et al., 2013). However, experimental catecholamine infusions in patients with atherothrombotic CVD might pose safety concerns, a possible reason for why we could not find any previous such studies in the literature. We did not account for potential hemoconcentration effects, as NE-infusion and acute mental stress were shown to increase hematocrit by about 6% (Uehlinger et al., 1987) and 3% (Austin et al., 2012), respectively. However, in a previous study, stress-induced FVIII:C levels, although not fibrinogen and D-dimer levels, remained significantly elevated after laboratory correction for stress-induced plasma volume contraction (Austin et al., 2012). We did not assess polymorphisms of AR subtypes, which may variously affect organ physiology during the fight-or-flight-response (Ahles and Engelhardt, 2014). Although we infused the same NE-dosage in all subjects, genetic polymorphisms, but also sensitivity of AR (von Känel et al., 2002) are two examples of unmeasured factors that may increase inter-individual variability in coagulation responses and thus the risk for non-significant findings.

4.6. Conclusions

To sum up, the findings from this placebo-controlled experimental study suggest that a NE infusion to mimic effects of NE released during acute stress directly induces increases in plasma levels of stress-responsive coagulation molecules and that α-AR mediate part of this effect. Although demonstrated in healthy subjects here, such mechanisms may help to dissect the underlying psychobiology of mentally-triggered ACS. The clinical relevance of our findings, including for preventive interventions, remains to be established.

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Conflicts of interest

none

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References

Abbate, R., Cioni, G., Ricci, I., Miranda, M., Gori, A.M., 2012. Thrombosis and acute coronary syndrome. Thromb. Res. 129, 235–240.
Ahles, A., Engelhardt, S., 2014. Polymorphic variants of adrenoceptors: pharmacology, physiology, and role in disease. Pharmacol. Rev. 66, 598–637.
Aschbacher, K., Mills, P.J., von Känel, R., Hong, S., Mausbach, B.T., Roepke, S.K., Dimsdale, J.E., Patterson, T.L., Ziegler, M.G., Ancoli-Israel, S., Grant, I., 2008. Effects of depressive and anxious symptoms on norepinephrine and platelet P-selectin responses to acute psychological stress among elderly caregivers. Brain Behav. Immun. 22, 493–502.
Aschbacher, K., von Känel, R., Mills, P.J., Roepke, S.K., Hong, S., Dimsdale, J.E., Mausbach, B.T., Patterson, T.L., Ziegler, M.G., Ancoli-Israel, S., Grant, I., 2009. Longitudinal platelet reactivity to acute psychological stress among older men and women. Stress 12, 426–433.
Austin, A.W., Wirtz, P.H., Patterson, S.M., Stutz, M., von Känel, R., 2012. Stress-induced alterations in coagulation: assessment of a new hemoconcentration correction technique. Psychosom. Med. 74, 288–295.
Austin, A.W., Wissmann, T., von Kanel, R., 2013. Stress and hemostasis: an update. Semin. Thromb. Hemost. 39, 902–912.
Beis, D., von Känel, R., Heimgartner, N., Zuccarella-Hackl, C., Bürkle, A., Ehlert, U., Wirtz, P.H., 2018. The role of norepinephrine and alpha-adrenergic receptors in acute stress-induced changes in granulocytes and monocytes. Psychosom. Med. 80 (7), 649–658.
Brydon, L., Steptoe, A., 2005. Stress-induced increases in interleukin-6 and fibrinogen predict ambulatory blood pressure at 3-year follow-up. J. Hypertens. 23, 1001–1007.
Ehrenreich, H., Schuck, J., Stender, N., Pilz, J., Gefeller, O., Schilling, L., Poser, W., Kaw, S., 1997. Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. Alcohol. Clin. Exp. Res. 21, 1285–1293.
Fibrinogen Studies Collaboration, 2005. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 294, 1799–1809.
Gader, A.M., Clarkson, A.R., Cash, J.D., 1973. The plasminogen activator and coagulation factor VIII responses to adrenaline, noradrenaline, isoprenaline and salbutamol in man. Thromb. Res. 2, 9–15.
Gordan, R., Gwathmey, J.K., Xie, L.H., 2015. Autonomic and endocrine control of cardiovascular function. World J. Cardiol. 7, 204–214.
Haberichter, S.L., Shi, Q., Montgomery, R.R., 2006. Regulated release of VWF and FVIII and the biologic implications. Pediatr. Blood Cancer 46, 547–553.
Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46–51.
Ingram, G.I., 1961. Increase in antihaemophilic globulin activity following infusion of adrenaline. J. Physiol. 156, 217–224.
Kop, W.J., Weissman, N.J., Zhu, J., Bonsall, R.W., Doyle, M., Stretch, M.R., Glaes, S.B., Krantz, D.S., Gottdiener, J.S., Tracy, R.P., 2008. Effects of acute mental stress and

exercise on inflammatory markers in patients with coronary artery disease and healthy controls. Am. J. Cardiol. 101, 767–773.
Kuebler, U., von Känel, R., Heimgartner, N., Zuccarella-Hackl, C., Stirnimann, G., Ehlert, U., Wirtz, P.H., 2014. Norepinephrine infusion with and without alpha-adrenergic blockade by phentolamine increases salivary alpha amylase in healthy men. Psychoneuroendocrinology 49, 290–298.
Lam, N.Y., Rainer, T.H., Ng, M.H., Leung, Y., Cocks, R.A., 2002. Effect of stress hormones on the expression of fibrinogen-binding receptors in platelets. Resuscitation 55, 277–283.
Larsson, P.T., Wallén, N.H., Egberg, N., Hjemdahl, P., 1992. Alpha-adrenoceptor blockade by phentolamine inhibits adrenaline-induced platelet activation in vivo without affecting resting measurements. Clin. Sci. (Lond.) 82, 369–376.
Larsson, P.T., Wallén, N.H., Hjemdahl, P., 1994. Norepinephrine-induced human platelet activation in vivo is only partly counteracted by aspirin. Circulation 89, 1951–1957.
Leor, J., Poole, W.K., Kloner, R.A., 1996. Sudden cardiac death triggered by an earthquake. N. Engl. J. Med. 334, 413–419.
Lowe, G., Rumley, A., 2014. The relevance of coagulation in cardiovascular disease: what do the biomarkers tell us? Thromb. Haemost. 112, 860–867.
Mittleman, M.A., Mostofsky, E., 2011. Physical, psychological and chemical triggers of acute cardiovascular events: preventive strategies. Circulation 124, 346–354.
Mittleman, M.A., Maclure, M., Sherwood, J.B., Mulry, R.P., Tofler, G.H., Jacobs, S.C., Friedman, R., Benson, H., Muller, J.E., 1995. Triggering of acute myocardial infarction onset by episodes of anger. Determinants of myocardial infarction onset study investigators. Circulation 92, 1720–1725.
Möller, J., Hallqvist, J., Diderichsen, F., Theorell, T., Reuterwall, C., Ahlbom, A., 1999. Do episodes of anger trigger myocardial infarction? A case-crossover analysis in the Stockholm Heart Epidemiology Program (SHEEP). Psychosom. Med. 61, 842–849.
Mostofsky, E., Penner, E.A., Mittleman, M.A., 2014. Outbursts of anger as a trigger of acute cardiovascular events: a systematic review and meta-analysis. Eur. Heart J. 35, 1404–1410.
Nordły, A., Gjesdal, K., Jaeger, S., Berntsen, H., 1975. The effect of noradrenalin infusion on plasma and platelet lipids and platelet function in man. Thromb. Diath. Haemorrh. 33, 328–334.
Perneger, T.V., 1998. What's wrong with Bonferroni adjustments. BMJ 316, 1236–1238.
Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1, 43–46.
Scanzano, A., Cosentino, M., 2015. Adrenergic regulation of innate immunity: a review. Front. Pharmacol. 6, 171.
Silverberg, A.B., Shah, S.D., Haymond, M.W., Cryer, P.E., 1978. Norepinephrine: hormone and neurotransmitter in man. Am. J. Physiol. 234, E252–E256.
Sladek, C.D., Song, Z., 2008. Regulation of vasopressin release by co-released neurotransmitters: mechanisms of purinergic and adrenergic synergism. Prog. Brain. Res. 170, 93–107.
Steptoe, A., Brydon, L., 2009. Emotional triggering of cardiac events. Neurosci. Biobehav. Rev. 33, 63–70.
Strike, P.C., Magid, K., Brydon, L., Edwards, S., McEwan, J.R., Steptoe, A., 2004. Exaggerated platelet and hemodynamic reactivity to mental stress in men with coronary artery disease. Psychosom. Med. 66, 492–500.
Strike, P.C., Magid, K., Whitehead, D.L., Brydon, L., Bhattacharyya, M.R., Steptoe, A., 2006. Pathophysiological processes underlying emotional triggering of acute cardiac events. Proc. Natl. Acad. Sci. U. S. A. 103, 4322–4327.
Thrall, G., Lane, D., Carroll, D., Lip, G.Y., 2007. A systematic review of the effects of acute psychological stress and physical activity on haemorheology, coagulation, fibrinolysis and platelet reactivity: implications for the pathogenesis of acute coronary syndromes. Thromb. Res. 120, 819–847.
Tofler, G.H., Stone, P.H., Maclure, M., Edelman, E., Davis, V.G., Robertson, T., Antman, E.M., Muller, J.E., 1990. Analysis of possible triggers of acute myocardial infarction (the MILIS study). Am. J. Cardiol. 66, 22–27.
Tofler, G.H., Kopel, E., Klempfner, R., Eldar, M., Buckley, T., Goldenberg, I., National Israel Survey of Acute Coronary Syndrome Investigators, 2017. Triggers and timing of acute coronary syndromes. Am. J. Cardiol. 119, 1560–1565.
Uehlinger, D.E., Zaman, T., Weidmann, P., Shaw, S., Gnädinger, M.P., 1987. Pressure dependence of atrial natriuretic peptide during norepinephrine infusion in humans. Hypertension 10, 249–253.
von Känel, R., 2015. Acute mental stress and hemostasis: when physiology becomes vascular harm. Thromb. Res. 135 (Suppl. 1), S52–S55.
von Känel, R., Dimsdale, J.E., 2000. Effects of sympathetic activation by adrenergic infusions on hemostasis in vivo. Eur. J. Haematol. 65, 357–369.
von Känel, R., Dimsdale, J.E., Ziegler, M.G., Mills, P.J., Patterson, T.L., Lee, S.K., Grant, I., 2001a. Effect of acute psychological stress on the hypercoagulable state in subjects (spousal caregivers of patients with Alzheimer's disease) with coronary or cerebrovascular disease and/or systemic hypertension. Am. J. Cardiol. 87, 1405–1408.
von Känel, R., Mills, P.J., Fainman, C., Dimsdale, J.E., 2001b. Effects of psychological stress and psychiatric disorders on blood coagulation and fibrinolysis: a biobehavioral pathway to coronary artery disease? Psychosom. Med. 63, 531–544.
von Känel, R., Mills, P.J., Ziegler, M.G., Dimsdale, J.E., 2002. Effect of beta2-adrenergic receptor functioning and increased norepinephrine on the hypercoagulable state with mental stress. Am. Heart. J. 144, 68–72.
von Känel, R., Preckel, D., Zgraggen, L., Mischler, K., Kudielka, B.M., Haeberli, A., Fischer, J.E., 2004. The effect of natural habituation on coagulation responses to acute mental stress and recovery in men. Thromb. Haemost. 92, 1327–1335.
von Känel, R., Kudielka, B.M., Helfricht, S., Metzenthin, P., Preckel, D., Haeberli, A., Cung, T., Fischer, J.E., 2008. The effects of aspirin and nonselective beta blockade on the acute prothrombotic response to psychosocial stress in apparently healthy subjects. J. Cardiovasc. Pharmacol. 51, 231–238.
Wachholder, K., Egli, H., Kesseler, K., Buscha, H., Felderhoff, B., 1961. Der Einfluss von Adrenalin und Noradrenalin auf die Blutgerinnung. Med. Exp. Int. J. Exp. Med. 4, 151–162.
Wallén, N.H., Held, C., Rehnqvist, N., Hjemdahl, P., 1997. Effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls. Eur. Heart J. 18, 807–815.
Wallén, N.H., Goodall, A.H., Li, N., Hjemdahl, P., 1999. Activation of haemostasis by exercise, mental stress and adrenaline: effects on platelet sensitivity to thrombin and thrombin generation. Clin. Sci. (Lond.) 97, 27–35.
Willeit, P., Thompson, A., Aspelund, T., Rumley, A., Eiriksdottir, G., Lowe, G., Gudnason, V., Di Angelantonio, E., 2013. Hemostatic factors and risk of coronary heart disease in general populations: new prospective study and updated meta-analyses. PLoS One 8 e55175.
Willich, S.N., Löwel, H., Lewis, M., Arntz, R., Baur, R., Winther, K., Keil, U., Schröder, R., 1991. Association of wake time and the onset of myocardial infarction. Triggers and mechanisms of myocardial infarction (TRIMM) pilot study. TRIMM Study Group Circ. 84 (6 Suppl), VI62–V167.
Wirtz, P.H., von Känel, R., 2017. Psychological stress, inflammation, and coronary heart disease. Curr. Cardiol. Rep. 19, 111.
Wirtz, P.H., Ehlert, U., Emini, L., Rüdisüli, K., Groessbauer, S., Mausbach, B.T., von Känel, R., 2006. The role of stress hormones in the relationship between resting blood pressure and coagulation activity. J. Hypertens. 24, 2409–2416.
Yarovoi, H.V., Kufrin, D., Eslin, D.E., Thornton, M.A., Haberichter, S.L., Shi, Q., Zhu, H., Camire, R., Fakharzadeh, S.S., Kowalska, M.A., Wilcox, D.A., Sachais, B.S., Montgomery, R.R., Poncz, M., 2003. Factor VIII ectopically expressed in platelets: efficacy in hemophilia a treatment. Blood 102, 4006–4013.
Zgraggen, L., Fischer, J.E., Mischler, K., Preckel, D., Kudielka, B.M., von Känel, R., 2005. Relationship between hemoconcentration and blood coagulation responses to acute mental stress. Thromb. Res. 115, 175–183.