Effects of nonspecific β-adrenergic stimulation and blockade on blood coagulation in hypertension

Roland von Känel,1,2 Joel E. Dimsdale,1 Karen A. Adler,1 Elaine Dillon,1 Christy J. Perez,1 and Paul J. Mills1
1Department of Psychiatry, University of California, San Diego, California 92093; and 2Institute for Behavioral Sciences, Swiss Federal Institute of Technology, 8092 Zurich, Switzerland

Submitted 26 September 2002; accepted in final form 3 December 2002

A hypercoagulable state may contribute to atherosclerosis development and complicate atherothrombotic events in hypertension (15). The sympathetic nervous system (SNS) is felt to be hyperactive, particularly in mild hypertension (6, 8); given that the SNS and catecholamines affect hemostatic function (32, 34), increased clotting diathesis in hypertensive subjects might relate to altered SNS activity. Indeed, it has been shown that, in response to acute mental stress, platelet activation was higher (28) and fibrinolytic activity was lower (23) in hypertensive subjects than in normotensive controls. Similarly, after epinephrine infusion, platelet activation was higher in hypertensive individuals than in normotensive controls (11, 12). One needs to be aware that these previous findings on procoagulant stress responses in hypertension were observed in relatively small sample sizes (11, 12, 23, 28); thus they may not necessarily apply to a general hypertensive population.

The β-adrenergic receptor has long been implicated in regulation of hypertension (19), and there is much evidence that catecholamines may alter hemostatic activity via a β-adrenergic mechanism (32). Adrenergic infusion and blockade studies on healthy individuals suggest that hemostatically active von Willebrand factor (vWF), clotting factor VIII, and tissue-type plasminogen activator, a fibrinolytic enzyme, are all released from their extravascular storage pools into the circulation via stimulation of endothelial β2-adrenoreceptors (32). Moreover, our laboratory has previously shown that the sensitivity of the β-adrenergic receptor and norepinephrine surge together explained more than one-half of the variance in thrombin formation in response to acute mental stress (35).

In this study, we investigated plasma levels of soluble tissue factor (TF), vWF antigen, and dimerized fibrin fragment D (D-dimer) in mildly hypertensive and normotensive volunteers after nonspecific β-adrenergic stimulation with isoproterenol and blockade with propranolol. In contrast to the natural stress hormones epinephrine and norepinephrine, isoproterenol has solely β-adrenergic properties that allow one to test for the unique effect of β-adrenergic stimulation on hemostasis in vivo (32). In brief, TF is the main physiological initiator of the coagulation cascade (25), whereas vWF mediates platelet adhesion to subendothelial structures and platelet aggregation (18). Increased plasma vWF antigen levels (i.e., the concentration of the vWF molecule in plasma) is viewed as an indicator of vascular injury and endothelial cell damage in cardiovascular disease (14). D-dimer is a hypercoagulability marker that indicates fibrin turnover comprising both fibrin formation on conversion of fibrinogen to fibrin by thrombin and fibrin degradation by plasmin (16). Several studies have shown that hypertensive subjects have greater TF procoagulant activity (20) and higher

Address for reprint requests and other correspondence: P. J. Mills, UCSD Medical Center, 200 West Arbor Dr., San Diego, CA 92103-0804 (E-mail: pmills@ucsd.edu).
plasma levels of vWF (1) and D-dimer (30) than normotensive subjects.

The three hemostasis molecules were measured before and after a 30-min infusion of isoproterenol and with pretreatment of either placebo or propranolol. We speculated that isoproterenol would elicit exaggerated increases in soluble TF and vWF, which would give rise to downstream fibrin formation in hypertensive individuals. We further hypothesized that propranolol would blunt these procoagulant changes.

MATERIALS AND METHODS

Subjects. Volunteers were recruited from the local community and financially compensated for participation in the study. Subjects were 15 mildly hypertensive and 21 normotensive men and women (mean age ± SD, 40 ± 5 yr) who provided written, informed consent in the study protocol approved by the University of California San Diego (UCSD) Institutional Review Board. Blood pressure (BP) diagnoses were made on the basis of three BP measurements taken on two separate screening occasions 1 wk apart. Subjects who had systolic and/or diastolic BP of ≥140/90 mmHg were categorized as being hypertensive. Five subjects who were on an antihypertensive drug regimen had their medication tapered and were then followed during a 3-wk washout period. As per definition, hypertensive individuals. We further hypothesized that propranolol would blunt these procoagulant changes.

Protocol. After a light standardized noncaffeine lunch, subjects were studied between 1:00 and 2:30 PM at the UCSD General Clinical Research Center. All subjects refrained from caffeinated beverages and smoking for 12 h before being studied. On arrival at the laboratory, subjects had placed an indwelling 22-gauge venous forearm catheter. After a 10-min rest in the supine position, propranolol was infused in two sequential fixed-order doses of 20 and then 40 ng · kg⁻¹ · min⁻¹ for 15 min without a wait period between each dose. Blood samples were obtained immediately before the isoproterenol infusion and immediately at the end of each dosage. The half-life of isoproterenol in the body is ~2 to 3 min. A nested subgroup of 13 subjects, of whom 3 were hypertensive, was studied twice, double blind, once after 5 days of placebo treatment and once after 5 days of 100 mg/day of the nonspecific β-blocker propranolol (Inderal LA).

Hemostasis assays. After discarding the first 2 ml, venous blood was drawn in EDTA tubes and immediately placed on ice to minimize artificial platelet activation. Plasma samples were obtained within 3 h by centrifugation at 3,000 g at 4°C for 10 min and immediately stored in plastic tubes at ~80°C until assayed. Commercially available ELISA kits were used to measure plasma levels of D-dimer (Asserachrom D-dimer, Diagnostica Stago, Asnières, France) and soluble TF antigen (Imubind TF, American Diagnostica, Greenwich, CT). The TF ELISA measures antigenic TF and TF complexed to inactivated and activated clotting factor VII in plasma. The ELISA for plasma vWF antigen followed a previous method (27), with reference to a standard derived from pooled normal human plasma (kindly provided by Drung T. Le; director of Coagulation Laboratory, UCSD), that used commercial antibodies (Dako, Carpinteria, CA) and substrates (BioRad, Hercules, CA). Intra-assay coefficients of variation for soluble TF antigen, vWF antigen, and D-dimer were 7.3, 2.6, and 3.5%, respectively. The respective interassay coefficients of variation were 6.5 and 8.9% for vWF antigen and D-dimer, respectively (no interassay coefficients of variation for soluble TF because the kit provides no control).

Statistical analyses. Data were analyzed by using SPSS (9.0) statistical software package (Chicago, IL). Results were considered statistically significant at the P ≤ 0.05 level; all tests were two tailed. To approximate a normal distribution, values for soluble TF and D-dimer were log₁₀ transformed.

RESULTS

Demographic variables. As per definition, hypertensive individuals had higher systolic BP (145 ± 11 vs. 116 ± 6 mmHg, P < 0.001) and higher diastolic BP (98 ± 6 vs. 71 ± 9 mmHg, P < 0.001) than normotensive subjects. Although not significantly, hypertensive subjects were slightly older (42 ± 6 vs. 39 ± 5 yr, P = 0.182) and they also had higher body mass index (30 ± 5 vs. 27 ± 4 kg/m², P = 0.132) than normotensive subjects. The gender distribution was not significantly different between the two groups (hypertensive: 4 women, 11 men; normotensive: 10 women, 11 men; P = 0.302).

Placebo condition. Although resting levels of vWF (131 ± 85 vs. 127 ± 74%, P = 0.865) and D-dimer (335 ± 221 vs. 358 ± 235 ng/ml, P = 0.699) were not different between hypertensive and normotensive subjects, there was a trend toward higher soluble TF in the hypertensive group (358 ± 451 vs. 200 ± 83 pg/ml, P = 0.071). In addition, neither systolic nor diastolic BP correlated significantly with any of the three hemostasis variables at rest. Of the potential relationships between age and body mass index with hemostasis variables, only the one between soluble TF and age turned out to be significant (r = 0.349, P = 0.037).

Across all subjects, isoproterenol infusion led to an increase in plasma vWF [129 ± 77% (0 ng · kg⁻¹ · min⁻¹), 138 ± 74% (20 ng · kg⁻¹ · min⁻¹), 160 ± 82% (40 ng · kg⁻¹ · min⁻¹); P(1,35) = 7.98, P = 0.008]. In post hoc analyses, vWF levels with the 40 ng · kg⁻¹ · min⁻¹ dose were significantly higher than those with the 20 ng · kg⁻¹ · min⁻¹ dose (P = 0.050). However, differences in vWF levels between rest and after the 20 ng · kg⁻¹ · min⁻¹ dose did not reach statistical significance (P = 0.140). In addition, whereas soluble TF remained unchanged across dose increments [266 ± 303 (0 ng · kg⁻¹ · min⁻¹), 265 ± 300 pg/ml (20 ng · kg⁻¹ · min⁻¹), 237 ± 172 pg/ml (40 ng · kg⁻¹ · min⁻¹); P(1,35) = 0.902, P = 0.345], there was a trend for
D-dimer to show a quadratic time effect \( F(1,35) = 3.06, P = 0.089 \). Values of D-dimer were lower with the lower isoproterenol dose (314 ± 172 ng/ml) than with the higher isoproterenol dose (334 ± 191 ng/ml) and the resting D-dimer values (349 ± 226 ng/ml).

There was an interaction between isoproterenol and hypertension in terms of vWF \( F(2,34) = 5.02, P = 0.032; \text{Fig. 1B} \) and in terms of D-dimer \( F(2,34) = 4.57, P = 0.040; \text{Fig. 1C} \), but not in terms of soluble TF \( F(2,34) = 2.65, P = 0.113; \text{Fig. 1A} \). Figure 1, B and C, shows that isoproterenol infusion resulted in a significantly greater vWF increase and a significantly greater D-dimer decrease in hypertensive than in normotensive subjects. When age and body mass index were controlled, the findings for D-dimer became insignificant \( F(4,32) = 2.59, P = 0.117 \) but maintained significance for vWF \( F(4,32) = 4.21, P = 0.048 \).

Propranolol condition. In the 13 subjects who received placebo and propranolol in a double-blind crossover design, resting levels between the placebo and propranolol condition were not different in any of the three hemostasis factors (Fig. 2). As was found for the entire study population, isoproterenol elicited a significant increase in plasma vWF antigen \( F(1,12) = 9.87, P = 0.009 \), which, moreover, was significantly blocked by propranolol \( F(1,12) = 10.25, P = 0.008; \text{Fig. 2B} \). This result held significance when controlled for hypertension status \( F(2,11) = 5.00, P = 0.047 \). However, because only 3 of 13 subjects had hypertension, it was inappropriate to compute three-way interaction across isoproterenol infusion, drug condition, and hypertension status on vWF. On the other hand, there were no interactions between isoproterenol and the drug condition on soluble TF (Fig. 2A) and on D-dimer (Fig. 2C) with and without controlling for hypertension status.

**DISCUSSION**

Consistent with a previous study performed in healthy subjects (13), we found that short-term β-adrenergic stimulation significantly increases levels of vWF antigen in plasma in a dose-response relationship. We further showed that vWF increase was significantly greater in hypertensive than in normotensive individuals. This finding adds to the hypothesis that, in response to sympathetic stressors, hypertensive subjects show enhanced procoagulant changes (11, 12, 23, 28). However, to strengthen the assumption of such a prothrombotic mechanism, further investigations on the cellular level are clearly needed.

Our study was not prospective, and we did not assess markers of SNS activity. Therefore, we can only speculate whether a hyperactive clotting diathesis related to sympathetic activation may have relevant implications in terms of the known atherothrombotic risk in

---

**Fig. 1.** In response to the two 15-min infusions with increasing doses of isoproterenol, there was no difference in plasma levels of soluble tissue factor antigen between hypertensive and normotensive subjects \( P = 0.113; \text{A} \). B: on the other hand, hypertensive subjects showed significantly greater increase in plasma von Willebrand factor (vWF) antigen levels than normotensive individuals \( P = 0.032 \). Post hoc analyses revealed a trend toward higher vWF with the 40 ng · kg⁻¹ · min⁻¹ dose in hypertensive subjects compared with normotensive subjects \( 191 ± 88 \text{ vs. } 138 ± 71 \text{, } P = 0.055 \). C: dimerized fibrin fragment D (D-dimer) significantly decreased in hypertensive subjects compared with normotensive controls \( P = 0.040 \). Post hoc testing showed that, with the higher isoproterenol dose, hypertensive subjects tended to have lower D-dimer than normotensive subjects \( 278 ± 161 \text{ vs. } 375 ± 203 \text{ ng/ml, } P = 0.076 \). Values are means ± SE.
hypertension (15). Nonetheless, hemodynamic hyperactivity with stress (4, 26) is viewed as a risk factor for atherosclerosis progression in hypertension (7, 10). In terms of this link, increased clotting might be of similar importance.

The observation that propranolol but not $\alpha_1$- or $\alpha_2$-adrenergic blockade blunted epinephrine mediated release of vWF from human umbilical venous endothelial cells ex vivo (31, 36) suggests $\beta$-adrenergic mechanism involvement. The findings from the present study are the first to directly support such a hypothesis in vivo, given that the $\beta$-adrenergically mediated vWF increase was completely abolished with propranolol but not with placebo. Because of the small sample size, we are unable to state whether $\beta$-blockade might inhibit vWF increase more effectively in hypertensive or in normotensive individuals.

Although speculative, adrenergic blockade of stress-triggered vWF increase might provide cardiovascular benefit. Parallel lines of investigation have shown that patients who are taking $\beta$-blocking drugs after an acute myocardial infarction may reduce their risk for a subsequent acute coronary event (9). In addition, in socially distressed monkeys, propranolol may decelerate atherosclerosis progression (17), in which procoagulant changes are critically involved (3).

Sympathetic activation by acute mental stress elicits an increase in plasma D-dimer levels (33, 35). Therefore, unchanged D-dimer across all subjects and its decrease even in hypertensive subjects in response to isoproterenol infusion were contrary to expectations. We offer three plausible explanations for these observations. First, $\beta$-adrenergic stimulation alone may not suffice to elicit fibrin formation downstream. Activation of the whole clotting cascade also requires $\alpha_2$-adrenergic stimulation of platelets (32), which, moreover, are inhibited by isoproterenol (13). Second, impaired fibrinolytic activation after stress in hypertension (23) might underlie reduced fibrin degradation and production of D-dimer, respectively. Third, the SNS regulates hepatic clearance of tissue-type plasminogen activator (2), and D-dimer is also cleared by the liver (24). Perhaps unique $\beta$-adrenergic stimulation may have augmented clearance of D-dimer from the circulation by an adrenergic mechanism. In hypertension, such a mechanism might be more relevant because of altered $\beta$-adrenergic responsiveness (8).

Negative results in terms of TF might be a consequence of our small sample size and inclusion of mildly instead of severely hypertensive subjects. Moreover, adrenergic responsiveness of soluble TF may not necessarily reflect procoagulant activity of TF expressed on monocytes and endothelial cells (22, 25). Whether adrenergic stimulation of monocytes and endothelial cells in vivo may elicit TF procoagulant activity is ambiguous (5, 29). Interestingly, it has been shown that epinephrine infusion led to expression of P-selectin on the platelet surface and release of platelet factor 4 from platelets (32), which are both known to induce TF procoagulant activity in monocytes (21).

In conclusion, nonselective $\beta$-adrenergic stimulation elicits a dose-dependent increase in plasma vWF in vivo, which is more pronounced in hypertensive than in normotensive subjects and appears to be blunted by nonselective $\beta$-blockade. As opposed to general activation of the SNS, such as with acute mental stress,
isolated β-adrenergic stimulation does not result in fibrin formation. Prospective studies need to demonstrate whether adrenergic blockade of stress procoagulant activity may decrease cardiovascular events in apparently healthy individuals and in subjects with cardiovascular disease and hypertension in particular.

This work was supported by National Institutes of Health Grants MO1 RR-00827, HL-57265, and AG-13332.

REFERENCES


