Hemostatic Effects of Stress Hormone Infusion

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Background: Surgery causes changes in hemostasis, leading to a hypercoagulable state. This postoperative increase in hemostatic function is attenuated in patients receiving regional anesthesia compared with those receiving general anesthesia. Regional anesthesia also decreases the neuroendocrine response to surgery compared with general anesthesia, and this effect is hypothesized to be responsible for the differences in hemostasis. To test the hypothesis that neuroendocrine hormones cause changes in hemostasis, we infused stress hormones into normal volunteers and measured hemostatic function.

Methods: After drug screening, 12 normal volunteers were studied. On two admissions, volunteers randomly received either stress hormone (epinephrine, cortisol, or glucagon) or placebo infusion for 24 h. During infusion, patients remained at bed rest and received controlled meals. Blood was obtained from indwelling venous catheters before infusion and 2, 8, and 24 h after the start of infusion. Blood was analyzed for neuroendocrine hormone concentrations, glucose, complete blood count, coagulation proteins, platelet reactivity, and activity of the fibrinolytic system.

Results: In the stress hormone group, concentrations of epinephrine, norepinephrine, cortisol, glucagon, and insulin were increased during the infusion period compared with those in the placebo group. Glucose concentrations and white blood cell counts were increased in the stress hormone group compared with those in the placebo group. Circulating fibrinogen concentrations increased 30% and *ex vivo* collagen-induced platelet reactivity increased 123% (aggregation) and 103% (dense granule release) in the stress hormone infusion group, whereas there was no change in the placebo group. Fibrino-

lytic proteins were similar in both groups, demonstrating a decrease in plasminogen activator inhibitor-1 activity at 8 and 24 h (196% in the hormone group *vs.* 199% in the placebo group).

Conclusions: Infusion of stress hormones to concentrations found during surgery is safely tolerated and causes metabolic changes observed with surgery. Stress hormone infusion increases *ex vivo* platelet reactivity and fibrinogen concentrations that resemble changes seen postoperatively but does not recreate the postoperative decrease in fibrinolytic activity. Differences in neuroendocrine response between types of anesthesia may explain some postoperative changes in platelet function and acute phase reactivity, but additional uncharacterized factors are responsible for the differences in fibrinolysis. (Key words: Blood, coagulation: fibrinolysis; platelet reactivity. Hormones, stress: cortisol; glucagon. Sympathetic nervous system, catecholamines: epinephrine.)

SURGICAL trauma leads to systemic changes in hemostasis. These include (1) increased circulating coagulation proteins,[1,2] (2) increased *ex vivo* platelet reactivity,[3,4] (3) decreased circulating coagulation inhibitors,[5,6] and (4) decreased fibrinolysis.[7,8] The combined effect of these hemostatic changes causes a generalized "hypercoagulable state." Teleologically it may be adaptive for tissue injury to elicit this hemostatic response, and in most surgical patients this is true. However, in patients with stenotic coronary, carotid or peripheral arterial lesions or in those at risk for deep venous thrombosis, this response may lead to vascular thrombotic events.

Regional anesthesia alone,[9,10,11] or combined with general anesthesia[12] has been shown to attenuate the increase in thrombotic diathesis after surgery. Regional anesthesia has also been shown to reduce the incidence of arterial[9,12] and venous thrombotic events postoperatively.[13] The mechanism(s) responsible for the effects of regional anesthesia on hemostasis and postoperative vascular thrombotic events have not been elucidated. One frequently postulated theory is that the neuroendocrine response to surgery (*i.e.,* catecholamines and cortisol) is responsible for increases in hemostatic function;[12,14] and regional anesthesia which limits this response,[15,16] attenuates the hypercoagulable state, thereby decreasing thrombotic events.

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The neuroendocrine hormones cortisol and epinephrine have both been shown to enhance hemostatic function. Steroids inhibit fibrinolysis *in vivo*,[17] and *in vitro* testing demonstrates that steroids increase production of plasminogen activator inhibitor-1 (PAI-1).[18] Epinephrine represents a common agonist for platelet activation *in vitro*[19] and *ex vivo*.[20] We hypothesized that administration of stress hormones at concentrations found during surgery would mimic postoperative changes in hemostatic function. To test this hypothesis, we randomly infused stress hormones and placebo in a crossover study to normal volunteers and measured changes in platelet reactivity, coagulation proteins, and fibrinolysis.

Materials and Methods

Approval for this study was obtained from our Committee on Clinical Investigations and the Clinical Research Center Review Committee. After their informed consent had been obtained, 12 healthy, nonsmoking volunteers, ages 18–22, were admitted to our Clinical Research Center. Two randomly assigned 24-hour infusions with a seven-day washout in between were performed. Volunteers were free of all medications by history (particularly salicylates and nonsteroidal antiinflammatory drugs, which was confirmed by urine toxicology screening on admission). The experimental protocol is shown in figure 1.

After admission, volunteers were given a controlled snack (all meals excluded caffeine, xanthines, or theobromine), and vital signs were recorded. Bed rest began at 8:00 PM, and within 1–2 h a 16-G antecubital intravenous catheter was placed for subsequent blood sampling and a 20-G intravenous catheter was placed in the opposite arm for hormone or placebo administration. Physiologic saline was infused through the 16-G catheter at 40 ml/h and an armboard secured the elbow at full extension to maintain patency. The next morning, preinfusion (time point 1 = 8:00 AM) blood samples were obtained while the volunteers remained in a darkened room, supine and at bed rest. Samples were obtained through the indwelling 16-G antecubital catheter without a tourniquet, after stopping the normal saline infusion for 5 min. The first 10 ml of blood initially drawn through the catheter was discarded before sampling.

After baseline samples were obtained, the experimental infusion was begun. Volunteers were randomized by the investigational pharmacy to receive either
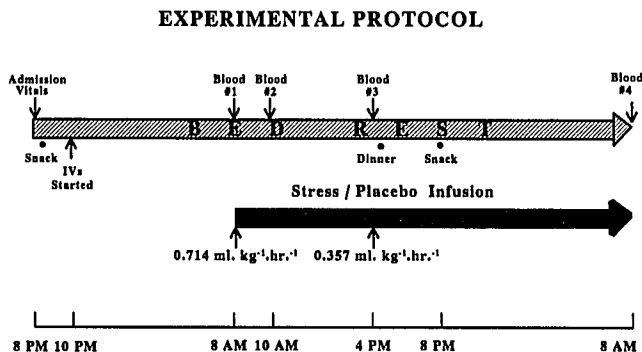
EXPERIMENTAL PROTOCOL

Fig. 1. The experimental protocol. References to time in figures 2–8 correspond to the times indicated here.

stress hormone or placebo. The stress hormone infusion contained epinephrine (1.25 mg), sodium hydrocortisone (98 mg), glucagon (0.125 mg), ascorbic acid (500 mg), and albumin (1.5 g) in 500 ml 0.9% saline.[21] The placebo infusion contained only the ascorbic acid and human albumin in 500 ml 0.9% saline. Ascorbic acid is necessary to inhibit the hydrolysis of epinephrine and human albumin is used as a carrier for glucagon. Each 500-ml container of infusate was prepared within 6 h before use and stored refrigerated at 4°C. Infusions were administered at $0.714 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 8 h and then $0.357 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 16 h (fig. 1). Additional blood samples were obtained at 2 h (time point 2 = 10:00 AM), 8 h (time point 3 = 4:00 PM), and 24 h (time point 4 = the following morning at 8:00 AM) of the infusion (fig. 1) in conditions similar to those used for preinfusion samples.

Blood samples were analyzed for complete blood count, epinephrine, norepinephrine, cortisol, glucagon, insulin, glucose, fibrinogen, plasminogen, α_2-antiplasmin, PAI-1 activity and antigen, tissue plasminogen activator (tPA) activity and antigen, platelet aggregation, platelet dense granule release and platelet cytoplasmic tyrosine kinase (TK) activity. Volunteers remained at bed rest in a darkened room throughout the study period and were fed controlled meals after the 4:00 PM blood sample and in the evening. Voided urine (obtained supine) was collected in sanitized plastic containers throughout the 24-hour infusion.

Sample Preparation and Assay

Plasma Proteins. Blood for fibrinogen, α_2-antiplasmin, and plasminogen analysis was anticoagulated in 3.8% sodium citrated tubes (9:1), placed on ice, and immediately centrifuged at $2,000g$ for 20 min at 4°C.

The plasma supernatant was removed and stored frozen at $-70°C$ until assay. Fibrinogen was assayed by the Clauss method[22] and reported in grams per liter. α_2-Antiplasmin was assayed by chromogenic substrate method (Diagnostic Stago, Asnieres-Sur-Seine, France) and reported as a percentage of normal.[23] Plasminogen was assayed using a radial immunodiffusion method (Helena Laboratories, Beaumont, TX) and reported in milligrams per deciliter.[24]

Fibrinolytic Proteins. Blood for PAI-1 activity and antigen and tPA activity and antigen was anticoagulated in tubes containing 3.8% sodium citrate (9:1). After homogeneous mixing, an aliquot dedicated to tPA activity was removed (1 ml) and immediately acidified (1:1) with acetate buffer (0.2 M, *p*H 3.9). All samples were placed on ice and centrifuged at 2,000*g* for 20 min at $4°C$. After centrifugation, plasma supernatant was removed and stored frozen at $-70°C$. Hydrochloric acid (1 M) was added (1:15) to the tPA activity plasma before freezing at $-70°C$. PAI-1 activity, antigen, and tPA antigen were measured by immunoradiometric technique,[25] and tPA activity was measured with a chromogenic assay (CoA-set tPA Kabi Diagnostica, Beaumont, TX).[26] Antigen results are reported in nanograms per milliliter and activity in activity units.

Platelet Aggregation and Dense Granule Release. Blood for platelet reactivity was anticoagulated in 3.8% sodium citrate tubes (9:1) and kept at room temperature. Within 1 h of sample removal, agonist induced whole-blood impedance platelet aggregation and dense granule secretion were simultaneously measured using a lumi-aggregometer (Chronolog, Havertown, PA) as previously described.[4] Agonists used for platelet activation were 2 μg/ml collagen, 5 μM adenosine diphosphate, and 0.37 mg/ml ristocetin.

Tyrosine Kinase Activity. TK activity was measured as previously described.[27] Briefly, platelets were washed by differential centrifugation and washed platelets, nonstimulated or activated with 0.25 units/ml of thrombin, were lysed in ice-cold lysing buffer 1 min after agonist addition. Platelet extracts normalized for total protein content were immunoprecipitated with saturating amounts of monoclonal antiphosphotyrosine immunoglobulin G covalently linked to Sepharose beads (Upstate Biotechnology, Lake Placid, NY). After washing in phosphate buffered saline three times, the beads were boiled in sodium dodecyl sulfate buffer. Supernatants were analyzed on Western blots using a mouse monoclonal antibody against phosphotyrosine (Upstate Biotechnology) diluted 1:1,000, followed by a goat antimouse immunoglobulin G antibody labeled with horseradish peroxidase (Hyclone, Logan, UT). Western blots were developed using the enhanced chemiluminescence technique (Amersham Life Sciences, Arlington Heights, IL)[28] and analyzed by densitometry. Chemilumigrams were analyzed by an investigator blinded to the volunteers' treatment, and after integrating the densities of individual bands for each lane, TK activity was expressed in arbitrary units of density.

Catecholamines. Blood for epinephrine and norepinephrine was collected in tubes containing ethylenediaminetetraacetic acid, placed on ice and immediately centrifuged at 2,000*g* for 20 min at $-4°C$. Plasma was removed and stored frozen at $-70°C$. Analysis was performed by high-pressure liquid chromatography.[29] Epinephrine sensitivity was 15 pg/ml (coefficient of variation [CV] 5%) and norepinephrine sensitivity 15 pg/ml (CV 6.5%).

Cortisol, Glucagon, and Insulin. Blood for cortisol, glucagon, and insulin was collected in heparinized tubes centrifuged at 2,000*g* for 15 min at $4°C$ and stored frozen at $-70°C$. Cortisol was assayed by fluorescent polarization[30] (sensitivity 0.6 μg/dl, CV 6.5%), and glucagon[31] (sensitivity 20 pg/ml, CV 11%) and insulin by radioimmunoassay[32] (sensitivity 5 μU/ml, CV 8%).

Urine Urea Nitrogen, Complete Blood Count, and Glucose. Urine was collected throughout the 24-h study period and refrigerated at $4°C$. Total volume was measured and an aliquot was analyzed for urea nitrogen by a urease method[33] (sensitivity 0, CV 4.2%). Blood for complete blood count was collected in tubes containing ethylenediaminetetraacetic acid and measured in a Coulter (Hialeah, FL) counter. Glucose was measured in blood from tubes without additives by the hexokinase method[34] (sensitivity 0, CV 2.5%).

Statistical Analysis

Two-way analysis of variance for repeated measures (Crunch statistical package) was used to evaluate the main effect of treatment over time and the differences between groups. One-way analysis of variance, with Newman-Keuls *post hoc* test, was then performed on measured values within groups over time. Data are presented as mean ± standard error of the mean and considered significant at $P < 0.05$.

Results

All 12 volunteers were men aged 18–22 yr and in good health. Toxicology testing performed on admis-

Table 1. Neuroendocrine Hormones

		(1)	(2)	(3)	(4)
Epinephrine (pg/ml)	Stress*	25 ± 7.0	471 ± 27†	471 ± 45†	236 ± 30†
	Placebo	36 ± 8.0	34 ± 6.0	24 ± 4.0	24 ± 4.0
Norepinephrine (pg/ml)	Stress*	106 ± 24	281 ± 40†	278 ± 43†	184 ± 28
	Placebo	118 ± 28	113 ± 25	121 ± 25	121 ± 31
Cortisol (μg/dl)	Stress*	14.0 ± 1.2	32.0 ± 0.9†	41.0 ± 1.2†	24.0 ± 0.9†
	Placebo	13.8 ± 1.6	11.6 ± 1.1	8.4 ± 1.0‡	15.5 ± 1.2
Glucagon (pg/ml)	Stress*	98 ± 12	161 ± 18†	151 ± 15†	100 ± 7.0
	Placebo	85 ± 6.0	85 ± 7.0	73 ± 7.0	89 ± 6.0
Insulin (μU/ml)†	Stress*	8.9 ± 1.0	18.6 ± 2.0†	23.0 ± 3.3†	21.3 ± 2.6†
	Placebo	8.2 ± 1.0	8.0 ± 0.9	7.1 ± 0.9	8.4 ± 1.3

Time points: (1) = preinfusion, (2) = 2 h, (3) = 8 h, (4) = 24 h.

Values are mean ± SEM.

* $P < 0.01$ *versus* placebo group.

† $P < 0.01$ *versus* time point 1 within hormone group.

‡ $P < 0.01$ *versus* time points 1 and 4 within placebo group.

sion confirmed the volunteers were free of medications, caffeine and nicotine. Volunteers tolerated the 24-h infusions without detectable clinical or electrocardiographic alteration from normal. Concentrations of stress hormones are shown in table 1. Epinephrine, norepinephrine, cortisol, glucagon and insulin concentrations were all increased in the hormone group compared with those in the placebo group. Within the hormone group, epinephrine, cortisol and insulin concentrations were increased at time points 2, 3 and 4 compared with baseline, while norepinephrine and glucagon were increased at time points 2 and 3 compared with baseline. Within the placebo group cortisol concentrations demonstrated normal circadian changes (morning peaks).[35] As expected within the placebo group, epinephrine, norepinephrine, and glucagon did not show any circadian variation. Insulin concentrations normally fluctuate in response to serum glucose and demonstrate a circadian variation.[36] However, with this paradigm, there was no statistical difference in the placebo insulin concentrations.

Hemodynamic data are shown in table 2. These demonstrate an increase in heart rate and decrease in diastolic pressure in the hormone group compared with the placebo group. Within the hormone group there was an increase in heart rate and decrease in diastolic pressure at time points 2, 3, and 4 compared with baseline. There was no difference in systolic pressure between or within groups. Metabolic and hematologic changes are shown in table 3. There was an increase in glucose in the hormone group compared with placebo. Within the hormone group, there was an increase in glucose concentration at time points 2, 3, and 4 compared with baseline. There was also a decrease in glucose at time point 3 in the placebo group compared with time points 1 and 2. There was a trend toward

Table 2. Hemodynamics

		(1)	(2)	(3)	(4)
Systolic pressure (mmHg)	Stress	120 ± 4.0	127 ± 5.0	132 ± 5.0	131 ± 6.0
	Placebo	121 ± 3.0	117 ± 3.0	121 ± 3.0	117 ± 3.0
Diastolic pressure (mmHg)	Stress*	79 ± 1.8	70 ± 2.4†	66 ± 2.3†	67 ± 2.3†
	Placebo	79 ± 3.1	75 ± 1.9	76 ± 2.6	77 ± 2.4
Pulse (beats/min)	Stress*	60 ± 2.7	76 ± 4.1†	88 ± 4.9†	80 ± 3.4†
	Placebo	60 ± 2.9	63 ± 4.6	64 ± 3.9	59 ± 1.7

See table 1 for explanation of time points. Values are mean ± SEM.

* $P \leq 0.01$ *versus* placebo group.

† $P \leq 0.01$ *versus* time point 1 within hormone group.

increased 24-h urine urea nitrogen excretion in the hormone group, but this did not reach statistical significance ($P = 0.06$). Stress hormone infusion caused an increase in white blood cell count compared with placebo. Within the hormone group there was an increase at time points 2, 3, and 4 compared with baseline. Platelet counts and hematocrit values were not different between placebo and hormone infusion groups. There was an increase in platelet count at time point 3 in the group receiving hormone infusion compared with baseline.

Platelet aggregation is shown in figure 2, platelet dense granule release (adenosine triphosphate) in figure 3, and platelet TK activity in figures 4 and 5. Platelet aggregation increased in response to collagen at time points 2, 3 and 4 in the stress group with no change in the placebo group to any agonist. Platelet dense granule release increased in response to collagen at time points 2, 3, and 4 in the stress group, with no change in the placebo group to any agonist. Platelet TK activity increased in the stress group at time points 2 and 3 returning to baseline at time point 4. There was no change in placebo group at any time point. Figure 5 is a representative chemilumigram from a patient, depicting the changes in TK activity.

Circulating concentrations of fibrinogen, α_2 antiplasmin and plasminogen were measured. Figure 6 demonstrates the increase above baseline in fibrinogen concentration at 24 h in the stress group with no change in placebo group (only measured at time points 1 and 4). There were no changes in α_2-AP or plasminogen
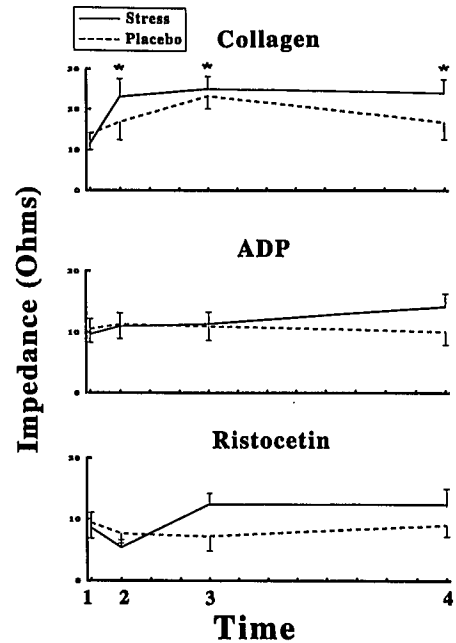

Fig. 2. Platelet aggregation in ohms of impedance to three agonists (collagen 2 μg/ml, adenosine diphosphate 5 μM, and ristocetin 0.37 mg/ml). *$P < 0.01$ *versus* time point 1.

within or between groups (data not shown). Fibrinolytic protein data are shown in figures 7 and 8 and demonstrate similar changes over time. PAI-1 antigen and activity concentrations (fig. 7) decreased at time point 3 and 4 in both groups. Figure 8 demonstrates the changes in tPA antigen and activity concentrations.

Table 3. Metabolic and Hematologic Variables

		(1)	(2)	(3)	(4)
Glucose (mg/dl)	Stress*	90 ± 1.8	159 ± 5.8†	161 ± 4.4†	124 ± 4.2†
	Placebo	92 ± 2.2	93 ± 1.9	81 ± 1.4‡	87 ± 2.2
UUN (g/24 h)	Stress				15.2 ± 1.0
	Placebo				12.0 ± 1.1
Leukocytes (1,000/mm³)	Stress*	6.6 ± 0.6	9.6 ± 1.4†	14.0 ± 1.0†	12.8 ± 1.1†
	Placebo	6.9 ± 0.4	6.9 ± 0.4	7.6 ± 0.5†	6.9 ± 0.4
Platelets (1,000/mm³)	Stress	260 ± 20	285 ± 21	311 ± 21§	289 ± 25
	Placebo	254 ± 21	254 ± 22	259 ± 23	257 ± 20
Hematocrit (%)	Stress	40 ± 0.8	41 ± 0.7	40 ± 0.7	39 ± 0.5
	Placebo	41 ± 0.7	41 ± 0.7	40 ± 0.6	40 ± 0.5

See table 1 for explanation of time points. Values are mean ± SEM.

UUN = urine urea nitrogen.

* $P < 0.01$ *versus* placebo group.

† $P < 0.01$ *versus* time points 1 and 2 within hormone group.

‡ $P < 0.05$ *versus* time points 1 and 2 within placebo group.
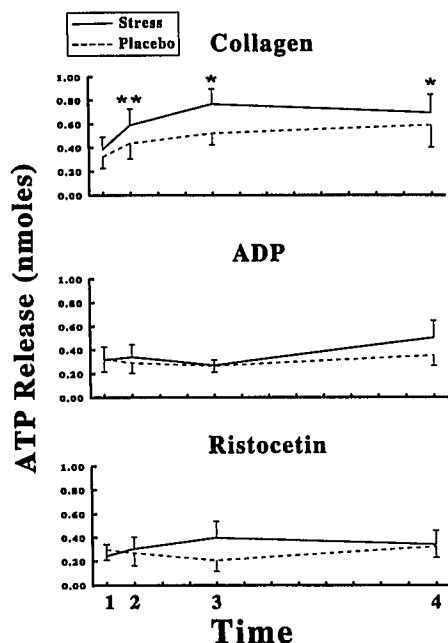
§ $P < 0.01$ *versus* time points 1, 2 and 4 within hormone group.

Fig. 3. Platelet dense granule release (adenosine triphosphate) in nanomoles to three agonists (collagen 2 μg/ml, adenosine diphosphate 5 μM, and ristocetin 0.37 mg/ml). *$P < 0.01$ *versus* time point 1; **$P < 0.05$ *versus* time point 1.

Fig. 5. This gel demonstrates a representative platelet tyrosine kinase response (antiphosphotyrosine immune complexes) to stress hormone infusion. Thrombin-activated platelets (lanes 1, 3, 5, and 7) or thrombin-inactivated platelets (lanes 2, 4, 6 and 8), corresponding to serial time points 1 (lanes 1 and 2), 2 (lanes 3 and 4), 3 (lanes 5 and 6), and 4 (lanes 7 and 8). There is increased tyrosine phosphorylation of several 50–130-kDa bands at time points 2 and 3 with near-return to baseline phosphorylation concentrations the next morning at time point 4. Tyrosine phosphorylation at all time points was submaximal: additional tyrosine phosphorylation could be obtained by *ex vivo* activation of the platelets with thrombin.

There was a decrease in tPA antigen concentrations in both groups at time point 3 which remain decreased at time point 4. Tissue PA activity concentrations increased in the stress group at time points 3 and 4, while there was no change in placebo tPA activity.

tions,[1] increased platelet reactivity,[4] and decreased fibrinolysis.[7] The effects on hemostasis caused by stress hormone infusion were increased circulating fibrinogen concentration, minimally increased platelet reactivity, and increased fibrinolysis. Therefore, stress hormone infusion for 24 h does not recreate fully the postoperative hypercoagulable state.

Discussion

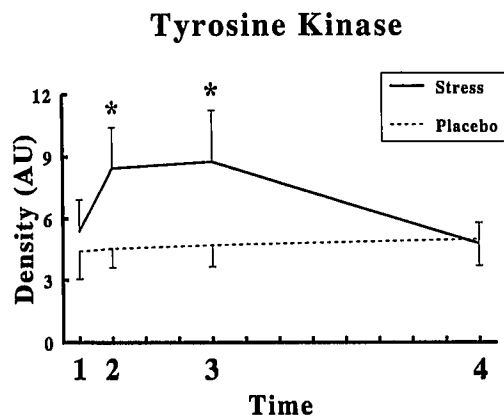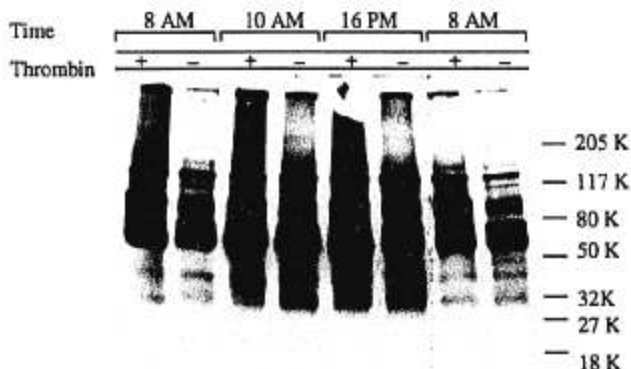Hemostatic changes at 24 h after surgery with general anesthesia include increased fibrinogen concentra-

Concentrations of epinephrine, cortisol and glucagon in volunteers receiving hormone infusion were comparable to those measured postoperatively with major surgery.[37] Hormone infusion also caused endogenous
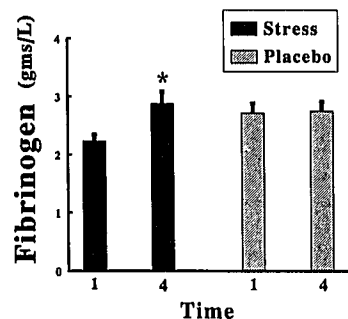

Fig. 4. Platelet tyrosine kinase activity in arbitrary units of density. *$P < 0.01$ *versus* time points 1 and 4.

Fig. 6. Circulating fibrinogen concentrations in grams per liter. *$P < 0.05$ *versus* time point 1.
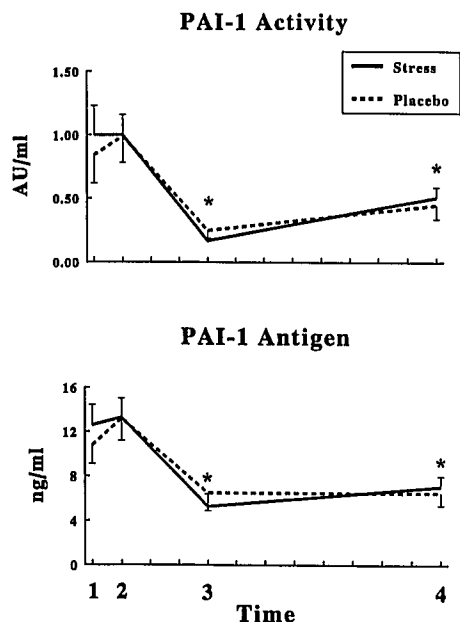
PAI-1 Activity

PAI-1 Antigen

Fig. 7. (*Top*) Plasminogen activator inhibitor-1 (PAI-1) activity (activity units [AU] per milliliter) concentrations over time. *$P < 0.05$ *versus* time points 1 and 2 within both hormone and placebo groups. (*Bottom*) PAI-1 antigen (nanograms per milliliter) concentrations over time. *$P < 0.01$ *versus* time points 1 and 2 within both hormone and placebo groups.

release of insulin and norepinephrine. Increased insulin concentrations are probably in response to increased serum glucose concentrations.[38] Volunteers receiving the hormone infusion exhibited mild anxiety which may have been responsible for the increased norepinephrine concentrations. Additionally, or alternatively, increased concentrations of cortisol have been shown to attenuate norepinephrine O-methylation[39] and this may have increased plasma norepinephrine concentrations. These circulating norepinephrine concentrations (240–320 pg/ml) are comparable to concentrations measured with mild surgical stress.[37] Norepinephrine was not included in the experimental hormone infusion, because circulating concentrations represent only the norepinephrine that has diffused away from the neuronal synaptic cleft and would not adequately recreate endogenous noradrenergic stimulation.

Hemodynamic changes caused by the hormone infusion indicate a predominant epinephrine effect on the cardiovascular system. Increased heart rate and decreased diastolic pressure with a trend toward increased systolic pressure represents primarily beta-adrenergic stimulation from continuous epinephrine infusion.[39] Metabolic changes seen with hormone infusion were

hyperglycemia and increased protein catabolism. These effects have been demonstrated by other authors and closely mimic postoperative changes.[40] Similarly, hormone-induced leukocytosis, which is thought to be caused by demargination[41] is routinely seen after surgery.[42] Increased platelet count in the hormone infusion group probably represents the response to epinephrine,[43] which differs from the postoperative period where there is an initial thrombocytopenia followed by thrombocytosis.[44]

Fibrinogen, plasminogen and α_2-AP were examined because of their effects on hemostasis. Fibrinogen, in addition to being the precursor of fibrin, is the major plasma adhesive protein involved in platelet aggregation[45] and viscosity.[46] Plasminogen is converted by circulating plasminogen activators to plasmin, which is the major fibrinolytic protein,[47] and α_2-antiplasmin rapidly binds and inactivates circulating free plasmin.[48] Hormone infusion caused an increase in circulating fibrinogen at 24 h and no change in α_2-AP or plasminogen. There appears to be some difference (not statistically significant) between the baseline values in the hormone group (2.2 g/l) and placebo group (2.7 g/l). We believe that this was caused by the six vol-
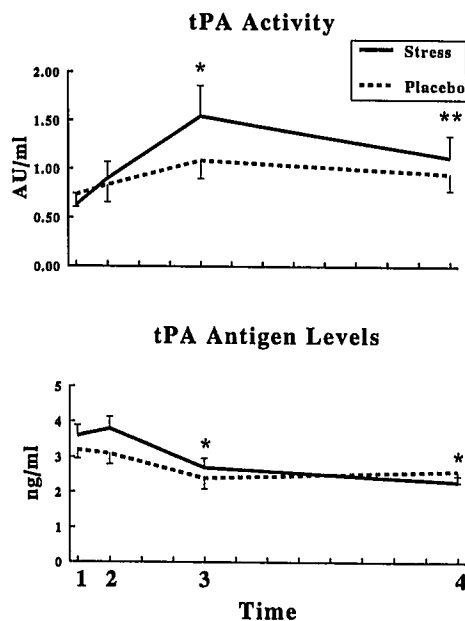
tPA Activity

tPA Antigen Levels

Fig. 8. (*Top*) Tissue plasminogen activator (tPA) activity (activity units [AU] per milliliter) concentrations over time. *$P < 0.01$ *versus* time points 1 and 2 within the hormone group; **$P < 0.05$ *versus* time point 1 within the hormone group. (*Bottom*) tPA antigen (ng/ml) concentrations over time. *$P < 0.01$ *versus* time points 1 and 2 within both hormone and placebo groups.

unteers who received the hormone infusion first; since the half-life of fibrinogen is 72–120 h,[49] seven days may not have been sufficient time for fibrinogen concentrations to return to baseline. However, the numbers are too small (n = 6) to demonstrate a statistical difference between these two groups. The concentration of fibrinogen, an acute phase reactant, normally increases after surgery, peaking at 72–96 h postoperatively.[1,4] In animals, circulating fibrinogen concentrations increase after stress hormone administration[50] and increased gene transcription for fibrinogen synthesis has been demonstrated immediately after surgery.[51] Plasminogen has not been shown to increase after surgery[11] and is probably not an acute phase protein.[52] Alpha$_2$-AP does not increase after surgery in some studies,[53] but rises in others.[54] However, the increase does not occur until one week postoperatively exceeding the time course of this study. Our data suggest that in human volunteers, neuroendocrine stimulation leads to increased hepatic acute phase response.

We measured platelet reactivity using whole blood impedance aggregometry, dense granule release, and platelet TK activity, and observed increased platelet reactivity in the stress hormone group. Platelet TKs such as the abundant members of the pp60$^{c\text{-src}}$ family, are involved in the activation of glycoprotein IIb–IIIa receptors through intracellular protein phosphorylation.[55] Activated glycoprotein IIb–IIIa receptors bind fibrinogen, which is necessary for platelet aggregation.[56] TK activity was increased at time points 2 and 3 and then returned to baseline at time point 4. The return to baseline at time point 4 may have been caused by decreasing the hormone infusion by 50% at time point 3. Alternatively, there may be an intracellular feedback inhibition mechanism involving tyrosine phosphatase activation.[57] Epinephrine has previously been shown to increase platelet fibrinogen binding[58] and intracellular platelet TK elevation may represent the mechanism causing this phenomenon. The combination of increased platelet TK activity and increased circulating fibrinogen concentrations in the hormone infusion group provides a mechanism to explain increased platelet reactivity after surgery. However, the agonist-induced increase in platelet function in this study is much less than we have previously demonstrated at 24 h postoperatively.[4] Whether higher norepinephrine concentrations (more pronounced α_2 effects) or other mediators (e.g., arginine vasopressin and cytokines) are responsible for the postoperative increase in platelet reactivity warrants further study.

Tissue PA activity increased at time points 3 and 4 within the stress hormone group, with no change in the placebo group, but tPA activity was similar between groups over time. Both groups also demonstrate a similar decrease in PAI-1 antigen, PAI-1 activity and tPA antigen concentrations at time point 3 that remain low the following morning (time point 4). Previous studies in volunteers have demonstrated that PAI-1 activity concentrations decrease at 4:00 PM from early morning concentrations, but rise again the following morning.[59,60] PAI-1 activity is thought to be the controlling factor in systemic fibrinolysis, with tPA activity fluctuating in response to PAI-1 concentrations.[61] This has led to the belief that there is a daily harmonic oscillation for fibrinolysis, with decreased activity in the early morning, coinciding with cardiovascular thrombotic events.[62] However, this harmonic oscillation has only been demonstrated in volunteers and patients pursuing normal daily activity. Our volunteers remained at continuous bed rest for over 24 h and do not demonstrate the early morning increase in PAI-1 activity on day 2 (time point 4). This tendency toward increased fibrinolysis was not altered by hormone infusion. Whether this represents an endogenous protective mechanism to offset the thrombotic effects of bed rest requires further study. The increase in tPA activity within the hormone group is probably epinephrine mediated,[63] but the difference between groups was not significant.

We did not measure any marker of coagulation system activation (thrombin-antithrombin complex, prothrombin fragment F1.2, soluble fibrin), nor did we measure concentrations of circulating coagulation inhibitors (antithrombin III, protein C). Instead, we concentrated on the two hemostatic systems (platelet reactivity and fibrinolysis) which have recently been shown to differ according to the type of anesthesia performed.[9,12] Our data suggests that stress hormones, which are modulated by different types of anesthesia, may explain, in part, some differences in hemostatic function seen in postoperative patients. Specifically, decreased concentrations of stress hormones with regional anesthesia may decrease platelet-fibrinogen reactivity, but do not explain differences in fibrinolysis. These results are consistent with a clinical study by Tuman et al.,[12] which showed a decrease in postoperative platelet–fibrinogen interaction in patients who received combined epidural–general anesthesia, compared with those who received general anesthesia alone. However, neuroendocrine indicators of the stress

response were not made by Tuman's group, precluding a mechanistic explanation for the observed differences in outcome between anesthetic regimens. We have previously demonstrated that epidural anesthesia and analgesia attenuates both the increase in catecholamines[64] and PAI-1 concentrations seen postoperatively,[9] and that lower PAI-1 concentrations are associated with fewer thrombotic complications. The results of the present study suggest that attenuation of postoperative PAI-1 concentrations with regional anesthesia is not explained by decreased circulating catecholamines, cortisol or glucagon. Rem *et al.* have also reported that postoperative changes in fibrinolysis are independent of neurohumoral stimuli.[65] Alternative explanations for the effects of regional anesthesia on fibrinolysis are (1) sympathectomy-induced changes in blood flow, altering endothelial cell release of fibrinolytic proteins, or (2) a direct action of local anesthetics on endothelial cells, altering the release and circulating balance of PAI-1 and tPA.

In summary, epinephrine, cortisol and glucagon stimulation caused increased circulating concentrations of fibrinogen and increased platelet reactivity, with no procoagulant effect on fibrinolysis. Therefore, the stress hormone infusion used in this protocol does not mimic the overall postoperative hypercoagulable response previously described, suggesting that there are other perioperative factors (*e.g.,* cytokines and tissue thromboplastins) necessary for this to occur. Further research is necessary to delineate the mechanisms responsible for postoperative changes in fibrinolysis and the effects of anesthetics.

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