

## Increased Prostacyclin and Adverse Hemodynamic Responses to Protamine Sulfate in an Experimental Canine Model

THOMAS W. WAKEFIELD, M.D.,<sup>1</sup> SHIRLEY K. WROBLESKI, B.S., DOUGLAS J. WIRTHLIN, M.D.,  
THOMAS W. WANG, B.S., AND JAMES C. STANLEY, M.D.

Section of Vascular Surgery, Jobst Vascular Research Laboratories, Department of Surgery, University of Michigan, Ann Arbor, Michigan 48109

Presented at the Annual Meeting of the Association for Academic Surgery, Houston, Texas, November 14-17, 1990

Prostanoid activity was correlated with the hemodynamic effects of protamine sulfate reversal of heparin in 24 dogs undergoing three different pretreatment regimens: Group I ( $n = 8$ ) received saline, Group II ( $n = 8$ ) received the thromboxane synthetase inhibitor U63,557A (30 mg/kg), and Group III ( $n = 8$ ) received indomethacin (10 mg/kg). Pretreatment substances were administered as 5-min intravenous infusions 20 min before anticoagulation with intravenous heparin (150 IU/kg). Protamine sulfate (1.5 mg/kg) was subsequently given as a 10-sec intravenous infusion 30 min after heparin had been administered. Hemodynamic data, as well as prostacyclin ( $\text{PGI}_2$ ) and thromboxane ( $\text{TxA}_2$ ) activity in aortic, venous, and pulmonary artery blood samples, were assessed over a 30-min time period following protamine administration. Group III indomethacin pretreatment provided the most protection from declines in blood pressure, heart rate, cardiac output, venous oxygen saturation, oxygen consumption, and elevations in pulmonary pressures and was accompanied with actual declines in  $\text{PGI}_2$ . Group II U63,557A pretreatment was associated with the most severe hemodynamic changes and the greatest increase in  $\text{PGI}_2$  (+576%). Elevated  $\text{PGI}_2$  correlated with hypotension at 1 and 3 min ( $P < 0.01$ ), as well as pulmonary artery pressure declines at all times following protamine reversal.  $\text{TxA}_2$  changes did not correlate with hemodynamic changes. Protamine's adverse hemodynamic responses were attenuated with cyclooxygenase blockade by indomethacin, but were worsened with selective  $\text{TxA}_2$  blockade with U63,557A. Excess arachidonic acid precursors in the latter setting may increase  $\text{PGI}_2$  production. This study, for the first time, raises the possibility that  $\text{PGI}_2$  contributes to the adverse effects accompanying protamine reversal of heparin anticoagulation. © 1991 Academic Press, Inc.

### INTRODUCTION

Protamine sulfate reversal of heparin anticoagulation in laboratory studies as well as in clinical practice is

<sup>1</sup> To whom reprint requests should be addressed at University Hospital, 2210 THCC, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0329.

known to cause systemic hypotension, bradycardia, pulmonary artery hypotension or hypertension, thrombocytopenia, leukopenia, and declines in oxygen consumption. Increased thromboxane and prostacyclin activity has been reported after protamine administration in a porcine experimental model, and indomethacin has been reported to lessen many of the protamine-related hemodynamic responses [1-3]. The present investigation was designed to assess contributions of the arachidonic acid pathway to the effects of protamine reversal of heparin anticoagulation in an established canine experimental model and to specifically define the role of a thromboxane synthetase inhibitor and a cyclooxygenase inhibitor on usual protamine-related phenomenon.

### METHODS

Twenty-four adult mongrel dogs, mean weight 22.5 kg, were anesthetized with pentobarbital sodium 30 mg/kg, mechanically ventilated to maintain arterial blood gases within physiologic range, and hydrated with lactated Ringers solution as a 20 ml/kg bolus followed by a continuous infusion of 10 ml/kg/hr for the duration of the experiment. Three pretreatment regimens were investigated. Group I control dogs ( $n = 8$ ) received intravenous infusions of saline, Group II dogs ( $n = 8$ ) received the specific thromboxane synthetase inhibitor U63,557A (Upjohn, Kalamazoo, MI) 30 mg/kg intravenously over 5 min, and Group III dogs ( $n = 8$ ) received indomethacin (Spectrum Chemical, Gardena, CA) 10 mg/kg intravenously over 5 min. Twenty minutes following completion of the pretreatment infusions, animals were anticoagulated with intravenous administration of beef-lung heparin, 150 IU/kg. Thirty minutes later protamine sulfate was administered as a bolus, 1.5 mg/kg over 10 sec (Fig. 1).

Oxygen saturations were assessed continuously using Oximetric Swan-Ganz catheters (Oximetric, Mountain View, CA). Measurements included systemic mixed venous saturation ( $S_v\text{O}_2$ ) by a catheter inserted in the pulmonary artery using a femoral vein approach, systemic arterial saturation ( $S_a\text{O}_2$ ) by a catheter inserted into the abdominal aorta using a femoral artery approach, and

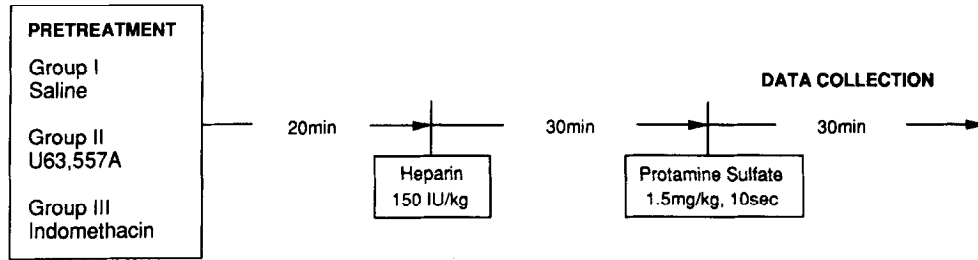


FIG. 1. Experimental protocol.

jugular venous saturation ( $S_{vO_2}$  jugular) by way of a catheter inserted into an external jugular vein.

Hemodynamic measurements included systemic mean arterial blood pressure, pulmonary artery systolic and diastolic pressure, and heart rate. Carotid artery flow was measured by an electromagnetic square-wave flow meter (Carolina Instruments, King, NC). Cardiac output (CO) was determined by thermodilution technique (Abbott Critical Care, Abbott Park, IL). Systemic oxygen consumption ( $\dot{V}O_2$ ) was calculated using the Fick equation  $\dot{V}O_2 = CO \times \text{hemoglobin} \times 1.34 (S_aO_2 - S_vO_2)$ . Cerebral oxygen consumption was similarly approximated as  $\dot{V}O_2 \text{ cerebral} = \text{carotid flow} \times \text{hemoglobin} \times 1.34 (S_aO_2 - S_{vO_2} \text{ jugular})$ , with recognition that  $S_{vO_2}$  jugular also includes cranial-facial venous return. All animals underwent a left lateral thoracotomy and placement of a left atrial catheter for blood sampling.

Hemodynamic measurements were made at 15-sec intervals for 1 min prior to protamine reversal of heparin anticoagulation, at 15-sec intervals for the first 3 postreversal min, and subsequently at 5, 10, 20, and 30 min postreversal. Cardiac outputs were measured as rapidly as possible, such that one measurement was made approximately every 30 sec during the first 3 postreversal min and thereafter at 5, 10, 20, and 30 min. Platelet counts and white blood cell counts, using a hand hemocytometer, were determined at baseline, 3 min after pretreatment, immediately prior to protamine administration, and thereafter at 3, 5, and 30 min postreversal. Adequate anticoagulation and its reversal were documented by measuring activated clotting times (ACT, Hemochron, Edison, NJ).

Prostacyclin was measured as its stable metabolic degradation product, 6-keto-PGF<sub>1 $\alpha$</sub> , and thromboxane was measured as its stable degradation product, TxB<sub>2</sub>. These prostanoids were measured at baseline, 3 min following completion of pretreatment, immediately prior to protamine administration, and at 1, 3, and 30 min postreversal. Assays were undertaken in aortic, femoral venous, and left atrial blood samples, the latter reflecting pulmonary blood. Prostacyclin was determined by RIA with a tritiated-labeled tracer (New England Nuclear, Boston, MA) having limited cross-reactivity with other prostaglandins (PGE<sub>2</sub>, 2.5%; PGF<sub>1 $\alpha$</sub> , 0.3%; PGE<sub>1</sub>, 0.2%; PGF<sub>2 $\alpha$</sub> , 0.05%; and TxB<sub>2</sub>, <0.3%). Thromboxane was

also measured with RIA using a tritiated tracer, having little cross-reactivity with other prostaglandins (PGD<sub>2</sub>, 1.0%; PGD<sub>1</sub>, 0.32%; PGF<sub>1 $\alpha$</sub> , 0.04%; and PGF<sub>2 $\alpha$</sub> , 0.04%). Prostanoid assays were performed in duplicate.

To determine if elevations in prostacyclin levels after protamine observed in the present study resulted from the protamine administration alone, or from the accompanying hypotensive response, another group of 8 animals was studied. They were monitored in the exact same fashion as the previous 24 dogs with the administration of nitroprusside at an infusion rate producing a hypotensive response parallel to that produced by protamine reversal of heparin. Prostacyclin and thromboxane levels were measured in these dogs. These animals were not given heparin or protamine, only nitroprusside.

Platelet aggregation studies were conducted in all study groups before and after pretreatment. Platelet-rich plasma (PRP) was prepared by centrifugation of venous blood at 200g for 10 min, and platelet-poor plasma (PPP) was prepared by centrifugation of the supernatant at 1000g for an additional 10 min. PRP (450  $\mu$ l) was then stimulated by addition of  $2 \times 10^{-5}$  M ADP, and aggregation was observed after a 3-min period in a platelet aggregometer (Sienco, Turkey Creek, CO), with PRP considered as 0% transmission and PPP as 100% transmission. Platelet aggregation measurements were made in duplicate.

Animal care complied with the *Principles of Laboratory Animal Care* and the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23, revised 1985). Statistical analyses included ANOVA and unpaired two-tailed *t* tests for parametric data, Kruskal-Wallis and Wilcoxon's rank sum for nonparametric data, and correlation statistics when appropriate.

## RESULTS

Baseline values for blood pressure, heart rate, and pulmonary artery pressures were statistically similar between the three groups indicative of steady-state conditions at the time of the protamine administration with no adverse influence by the saline, U63,557A, or indomethacin. Significant differences in blood pressure between the three groups were noted from 75 sec to 10 min following protamine administration (Fig. 2). Group III

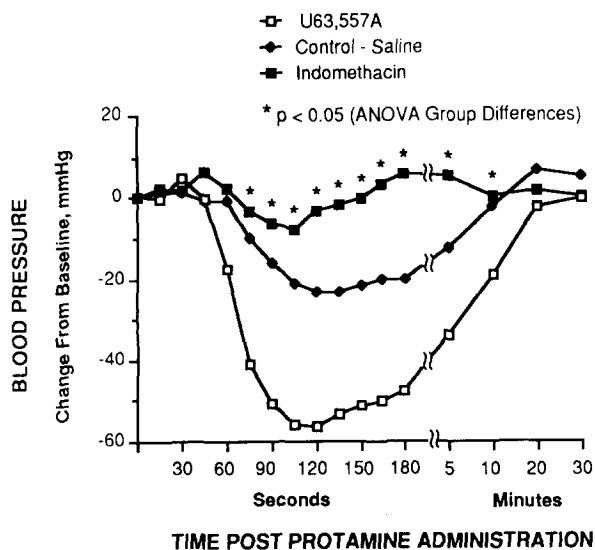


FIG. 2. Changes in blood pressure in three groups.

indomethacin-pretreated dogs attenuated the hypotensive response, when compared to Group II U63,557A pretreated dogs ( $P \leq 0.01$ ), with maximum declines of  $-8 \pm 27$  and  $-56 \pm 24$  mm Hg occurring in these two groups, respectively, and a  $-23 \pm 33$  mm Hg decline occurring in Group I control dogs. Heart rate differences were noted at 90, 120, and 150 to 165 sec and at 5 and 10 min postreversal (Fig. 3), with maximum declines being significantly less in Group III indomethacin-pretreated animals ( $-9 \pm 17$  beats/min) compared to Group II U63,557A-pretreated dogs ( $-30 \pm 29$  beats/min). Group differences in pulmonary artery systolic pressure occurred 165 sec to 10 min postreversal, and differences in pulmonary artery diastolic pressure occurred 75 sec to 10 min postreversal. Although increases in pulmonary artery diastolic pressures affected Group I control dogs,

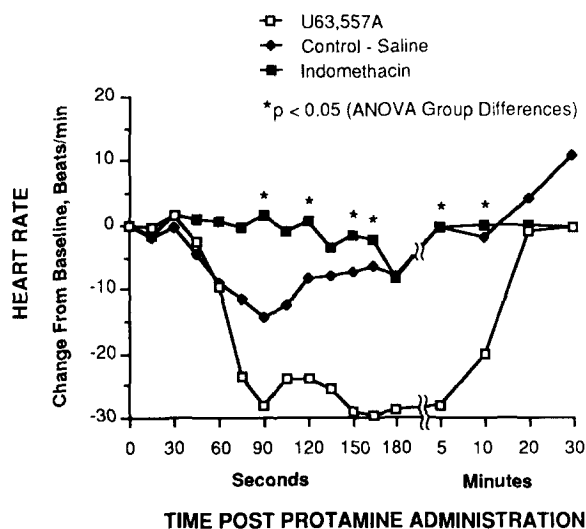


FIG. 3. Changes in heart rate in three groups.

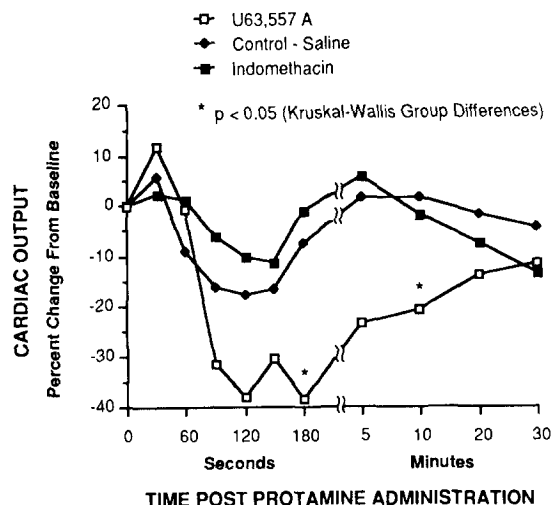


FIG. 4. Changes in cardiac output in three groups.

changes in pulmonary artery diastolic pressures were minimal in Group III indomethacin-pretreated dogs with actual declines in Group II U63,557A-pretreated dogs. Maximum declines in cardiac output (Fig. 4) were less pronounced in indomethacin pretreated Group III dogs ( $-12 \pm 20\%$ ) compared to Group I control dogs ( $-18 \pm 24\%$ ) or U63,557A-pretreated Group II dogs ( $-39 \pm 32\%$ ). Differences between groups, by Kruskal-Wallis analysis, occurred 165 to 180 sec and 10 min postreversal. Similarly, more severe declines in carotid artery flow occurred with Group II U63,557A pretreatment ( $-26 \pm 27\%$ ) compared to Group I controls ( $-14 \pm 26\%$ ) or Group III indomethacin pretreatment ( $-14 \pm 28\%$ ).

Systemic mixed venous oxygen saturations differed at 30, 120, and 150 sec to 10 min postreversal, being statistically different between Groups I and II ( $P < 0.05$ ) and Groups II and III ( $P < 0.05$ ). Maximal declines were  $-3 \pm 8\%$  in Group III indomethacin-pretreated dogs,  $-4 \pm 6\%$  in Group I control dogs, and  $-18 \pm 19\%$  in Group II U63,557A-pretreated dogs. Jugular venous oxygen saturations were also different between groups at 120 sec and at 10 and 20 min postreversal. No differences in systemic arterial oxygen saturations were observed between these groups.

Systemic oxygen consumption, although not statistically different among pretreatment groups (Fig. 5), exhibited greater declines in Group II animals receiving U63,557A ( $-36 \pm 29\%$ , 75 to 90 sec postreversal) than in Group I control dogs ( $-17 \pm 24\%$ , 105 to 120 sec postreversal) or Group III dogs pretreated with indomethacin ( $-15 \pm 22\%$ , 75 to 90 sec postreversal). On the contrary, although not statistically different, maximum declines in cerebral oxygen consumption (Fig. 5) in Group I animals of  $-32 \pm 40\%$  and Group III dogs of  $-25 \pm 24\%$  were not matched by similar declines in Group II dogs receiving U63,557A, where actual increases were observed.

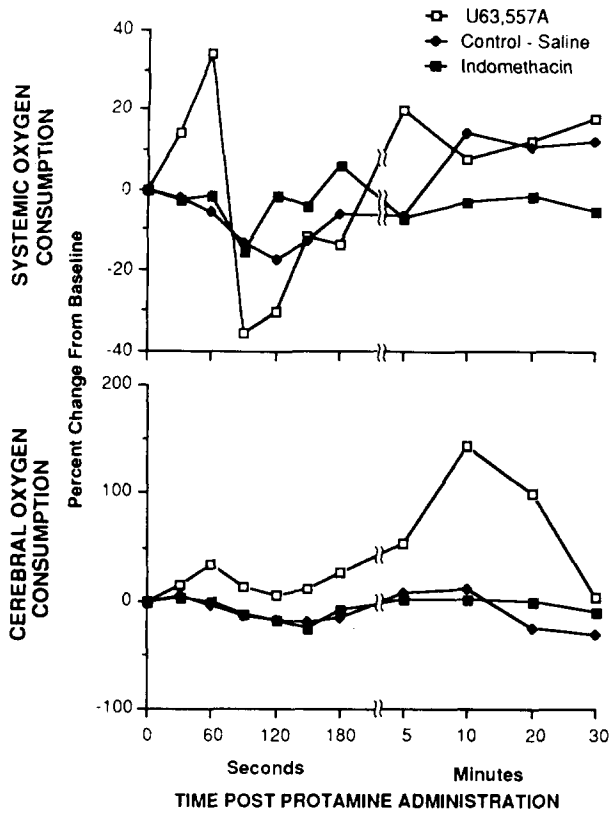


FIG. 5. Changes in systemic oxygen consumption and cerebral oxygen consumption in three groups.

Platelet count declines were pronounced in all groups, with maximum declines of  $-54 \pm 21\%$  in Group I control dogs,  $-55 \pm 10\%$  in Group II U63,557A-pretreated animals, and  $-52 \pm 18\%$  in Group III indomethacin-pretreated dogs. White blood cell declines were more marked with Group II U63,557A pretreatment ( $-40 \pm 15\%$ ) than in Group I controls ( $-7 \pm 27\%$ ) or with Group III indomethacin pretreatment ( $-27 \pm 18\%$ ).

Prostacyclin (Fig. 6) and thromboxane (Fig. 7) levels were significantly different in the study groups. Three minutes following pretreatment, prostacyclin levels fell most with Group III indomethacin pretreatment ( $-35 \pm 24$ ,  $-45 \pm 18$ , and  $-41 \pm 18\%$  within aortic, venous, and pulmonary samples, respectively), compared to similar measurements in Group I controls ( $-9 \pm 10$ ,  $-7 \pm 23$ , and  $-13 \pm 15\%$ ) and with Group II U63,557A pretreatment ( $+48 \pm 20$ ,  $+46 \pm 31$ , and  $+46 \pm 45\%$ ). One minute after protamine reversal, Group III indomethacin pretreated dogs exhibited less prostacyclin elevations in aortic and pulmonary samples than in Group I or II animals ( $P < 0.05$ ) with actual declines observed in Group III. Maximal elevation in prostacyclin of  $+576 \pm 1264\%$  was found in Group II aortic blood. Group III indomethacin pretreatment, in comparison to Group I or II, resulted in significantly less elevation in prostacyclin 3 min after protamine reversal within venous and pulmonary samples ( $P < 0.05$ ), whereas at 30 min, differences

occurred in all samples (aortic, venous, and pulmonary) between Group III indomethacin-pretreated dogs and Group I and II dogs ( $P < 0.05$ ), with declines in prostacyclin in Group III animals.

Thromboxane levels 3 min after pretreatment declined most with Group III indomethacin pretreatment, were slightly less in Group II U63,557A-pretreated animals, and were elevated in Group I controls. Significant group differences in  $TxB_2$  were noted only in venous samples at 1, 3, and 30 min postreversal ( $P < 0.05$ ), with maximal increases in thromboxane of  $157 \pm 152\%$  occur-

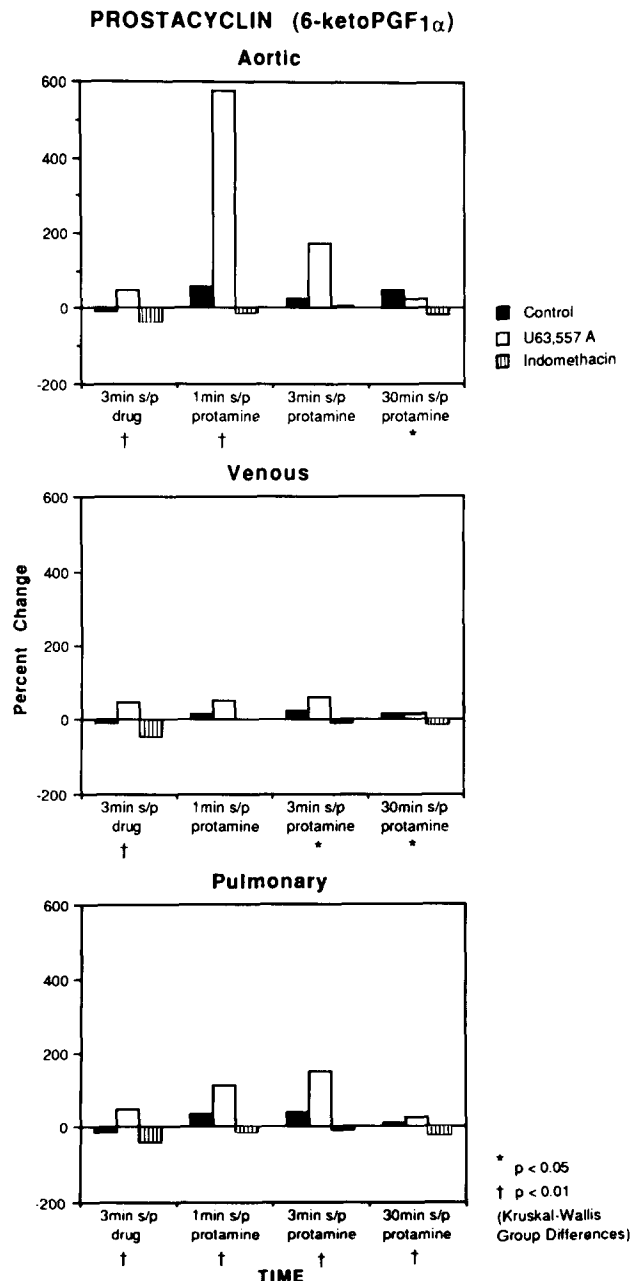
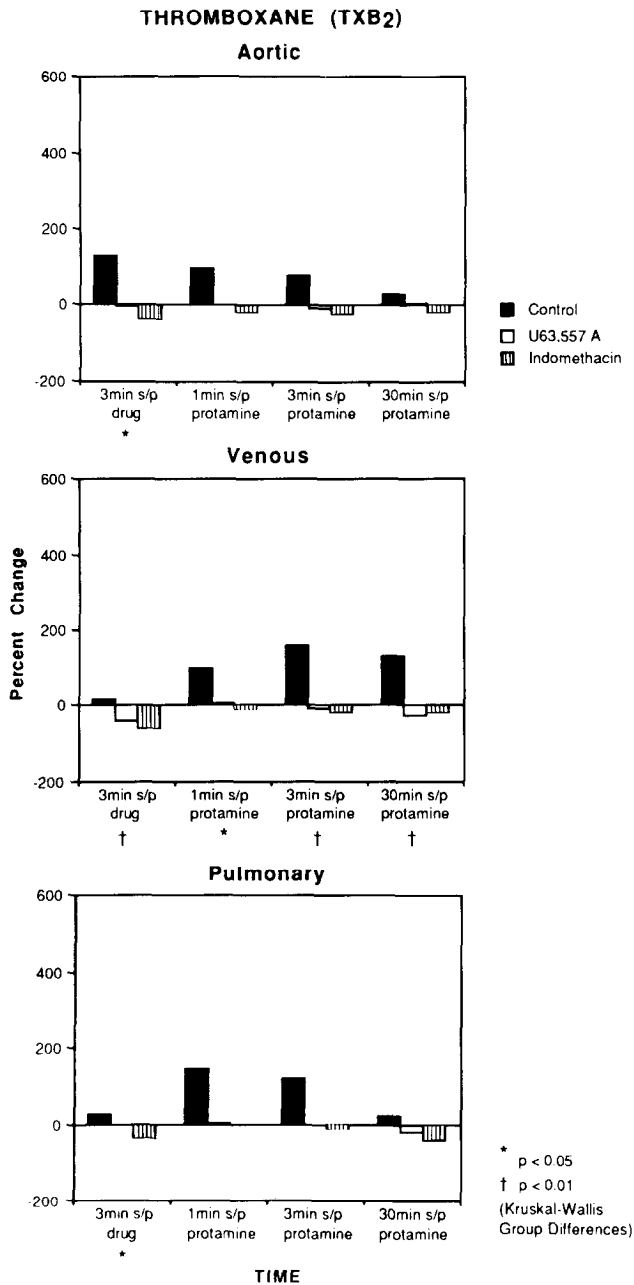


FIG. 6. Prostacyclin levels in aortic, venous, and pulmonary blood.



**FIG. 7.** Thromboxane levels in aortic, venous, and pulmonary blood.

ring in Group I controls 3 min postreversal, in contrast to a  $-11 \pm 19\%$  decline in Group II U63,557A-pretreated dogs ( $P < 0.01$ ) and a  $-20 \pm 16\%$  decline in Group III indomethacin-pretreated dogs ( $P < 0.01$ ).

Elevations of prostacyclin in aortic, venous, and pulmonary samples correlated well with declines in blood pressure at 1 min after protamine administration ( $r = 0.53, 0.52, \text{ and } 0.53$ , respectively;  $P \leq 0.01$ ), as well as at 3 min postreversal ( $r = 0.53, 0.70, 0.60$ , respectively;  $P < 0.01$ ). No significant correlations were found 30 min postreversal. No correlation existed between thromboxane elevations and declines in blood pressure. Correla-

tions between prostacyclin elevations and oxygen consumption declines were found to be significant only in aortic and venous samples 3 min postreversal ( $r = 0.46, 0.43$ ;  $P < 0.05$ ). No significant correlations existed between thromboxane elevations and oxygen consumption. Changes in thromboxane and prostacyclin activity were not correlated with cerebral oxygen consumption changes. Finally, correlations between prostacyclin elevations and reductions in pulmonary artery systolic or diastolic pressures occurred at all time periods, whereas only one isolated correlation between thromboxane and elevations in pulmonary artery diastolic pressure occurred in venous blood 3 min postreversal ( $r = 0.48$ ;  $P < 0.05$ ).

Platelet aggregation revealed a 10% decline in Group I control animals between the baseline sample and the sample obtained following pretreatment (15.9 to 14.3%), a 40% decline in Group II dogs receiving U63,557A (13.9 to 8.4%), and a 32% decrease in Group III animals receiving indomethacin (14.7 to 10%). Baseline aggregation values were low, perhaps being an effect of the sodium pentobarbital anesthetic [4].

The 8 animals given nitroprusside exhibited a  $-65$  mm Hg drop in blood pressure 3 min after infusion of this agent began. Declines in prostacyclin were observed at 1, 3, and 30 min in aortic, venous, and pulmonary samples, but no significant correlations between declines in blood pressure and changes in prostacyclin levels were evident. This suggests that hypotension itself cannot explain the prostacyclin elevations. In these same animals, thromboxane levels were elevated after nitroprusside administration, but did not correlate with blood pressure changes.

## DISCUSSION

Protamine reversal of heparin anticoagulation typically causes potentially adverse hemodynamic and hematologic responses, including vasodilation and hypotension, bradycardia, pulmonary artery hypertension or hypotension, thrombocytopenia, and leukopenia. Clinically, significant systemic arterial hypotension and pulmonary artery hypertension have been noted in 4% of cases and nearly 100 deaths have been attributed to the use of protamine [3, 5, 6]. Initial vasodilation occurring after protamine is manifested by a fall in systemic vascular resistance [7]. During this early period of vasodilation, cardiac output increases in a compensatory fashion. However, approximately 60 sec after vasodilation begins, cardiac output and oxygen consumption fall, suggesting a more direct central effect of protamine [8]. Several investigations, including recent work from our laboratory using an isolated rabbit heart model, suggest that protamine may produce direct dose-dependent depression of myocardial contraction [9-14]. However, few studies have addressed the mechanism for protamine-induced vasodilation and hypotension. The present in-

vestigation strongly supports the tenet that prostacyclin generation is associated with the hypotensive and vasodilatory effects of protamine administration.

The nature of changes in arachidonic acid pathway metabolites during protamine reversal of heparin has been examined by others. In a porcine experimental model, at the conclusion of a 3-min protamine infusion (2 mg/kg), there was a 35 mm Hg drop in mean arterial blood pressure, an increase in mean pulmonary artery pressure of 17 mm Hg, a decrease in left ventricular end diastolic pressure, and a concomitant decrease in arterial and mixed venous  $PO_2$  [1]. At the same time, thromboxane levels increased 460%, prostacyclin levels increased 150%, and prostaglandin  $F_{2\alpha}$  levels increased 220%, with its major degradation product 13-14-dihydro-15-keto prostaglandin  $F_{2\alpha}$  ( $KH_2$   $PGF_{2\alpha}$ ) increasing 690%. There was little difference in arterial and mixed venous plasma prostanoid activities. In this same study, indomethacin (10 mg/kg), given intravenously before administration of heparin and protamine, blocked these changes with no evidence of hypotension, pulmonary artery hypertension, blood gas changes, or prostanoid elevation. These former investigators concluded that activation of the arachidonic acid cascade occurs in the lungs, that the increased pulmonary vascular resistance can best be explained by liberation of the vasoconstricting prostanoids  $TxA_2$  and  $PGF_{2\alpha}$ , and that prostacyclin may be partially responsible for the low systemic vascular resistance noted.

Conzen and associates studied the  $TxA_2$  receptor antagonist BM 13.177 (10 mg/kg) infused 5 min before protamine administration and indomethacin (10 mg/kg) given before heparin and protamine [2]. In the group given BM 13.177, levels of  $TxB_2$  and  $PGF_{2\alpha}$  remained similarly elevated after protamine as in control pigs, but significant hemodynamic alterations were not observed. In fact, a 21% decline in pulmonary vascular resistance occurred 5 min after the completion of the protamine infusion. No hemodynamic changes or alterations in plasma prostanoid levels occurred after protamine administration in animals pretreated with indomethacin. These authors concluded that most of the adverse hemodynamic effects associated with protamine were mediated by  $TxA_2$ . However, the model utilized produced little hypotension with at most an 11% decrease in mean arterial blood pressure 5 min after the termination of protamine infusion. This would not have represented significant hypotension as reflected in our study by maximum changes of -45% in Group II U63,557A-treated dogs and -21% in control animals. Such differences most likely relate to species differences. The slight decreases in pulmonary artery pressure and pulmonary vascular resistance in these pigs pretreated with BM 13.177 were thought likely to be related to increased production of prostacyclin, as evident by the 102% increase in 6-keto- $PGF_{1\alpha}$  in this group compared to the 63% in-

crease in control animals 2 min after protamine administration.

Pulmonary artery vasoconstriction and hypertension have been associated with thromboxane elevations in pigs, awake sheep, and patients during protamine reversal of heparin anticoagulation [3, 15, 16]. Although platelet sequestration with release of vasoactive agents may play a role in the generation of thromboxane [17], platelets are not necessary for the pulmonary responses. This has been documented in studies involving a rodent pulmonary vascular injury model using protamine in cell- and plasma-free solutions [18], in pigs using protamine in dextran perfusions [19], in isolated dextran-perfused cat lung preparations [20], and in sheep having platelet depletion by antibody administration [21]. In the current study the lessening of pulmonary systolic and diastolic hypertension that followed U63,557A pretreatment supports the importance of thromboxane in the pulmonary response. In addition, the fact that all three groups of animals in our study exhibited similar profound declines in platelet counts suggests that platelet sequestration occurs from a direct effect of protamine rather than through an arachidonic acid-mediated mechanism, as has been suggested previously [22].

The pulmonary effects and the systemic hypotensive response are often separate and distinct, as has been noted by the blocking of the pulmonary hypertensive response but not the systemic fall in blood pressure with acetylsalicylic acid and iloprost [23, 24]. Data from the current investigation indicate that systemic hypotension and vasodilation relate to prostacyclin generation and not to thromboxane production. Dogs pretreated with the thromboxane synthetase inhibitor U63,557A exhibited the most pronounced hemodynamic changes, suggesting that  $PGG_2/PGH_2$  may shunt toward production of more prostacyclin or  $PGD_2$ , which is another potent vasodilator and platelet inhibitor [25-31]. It is believed that this process occurs in contiguous tissues, by the isomerase activity of plasma albumin, or from leukocyte production, respectively, leading to an increased load of vasodilator prostaglandins. In fact, 3 min after infusion of U63,557A, prostacyclin levels were increased 48, 46, and 46% in artery, vein, and pulmonary blood samples, respectively, in contrast to decreases of -35, -45, and -41% in the same blood samples after indomethacin pretreatment. The strong statistical correlations between prostacyclin elevations and blood pressure declines at 1 and 3 min after protamine administration support the probable existence of prostanoid shunting in this setting.

During thromboxane synthetase inhibition, the endoperoxide precursors  $PGG_2$  and  $PGH_2$  may occupy and activate the thromboxane receptor [32]. Using the thromboxane receptor antagonist SQ 29,548 (Squibb, Princeton, NJ), we observed no inhibition in the magni-

tude of protamine sulfate-induced adverse hemodynamic responses in the same canine model used in the current investigation (unpublished observations). This lends further credence to our conclusion that thromboxane is not the major mediator of protamine-induced hypotension and vasodilation.

Protamine causes declines in oxygen consumption both *in vivo* [8] and *in vitro* [33], most likely because of its polycationic character [18]. One would not expect either U63,557A or indomethacin pretreatment to completely block these metabolic changes. Indomethacin pretreatment did attenuate to some degree the decline in systemic oxygen consumption, suggesting that a portion of the decline in oxygen consumption is related to its hemodynamic effect rather than to its metabolic effect, as has been shown previously [24]. However, it is clear that the decline in oxygen consumption relates in part to direct metabolic toxic effects of the drug on cellular elements and is unrelated to systemic hypotension, vasodilation, or pulmonary artery hypertension.

#### REFERENCES

- Hobbhahn, J., Conzen, P. F., Zenker, B., Goetz, A. E., Peter, K., and Brendel, W. Beneficial effect of cyclooxygenase inhibition on adverse hemodynamic responses after protamine. *Anesth. Analg.* **67**: 253, 1988.
- Conzen, P. F., Habazettl, H., Gutmann, R., Hobbhahn, J., Goetz, A. E., Peter, K., and Brendel, W. Thromboxane mediation of pulmonary hemodynamic responses after neutralization of heparin by protamine in pigs. *Anesth. Analg.* **68**: 25, 1988.
- Morel, D. R., Zapol, W. M., Thomas, S. J., Kitain, E. M., Robinson, D. R., Moss, J., Chenoweth, D. E., and Lowenstein, E. C5a and thromboxane generation associated with pulmonary vaso- and broncho-constriction during protamine reversal of heparin. *Anesthesiology* **66**: 597, 1987.
- O'Rourke, S. T., Folts, J. D., and Albrecht, R. M. Inhibition of canine platelet aggregation by barbiturates. *J. Lab. Clin. Med.* **108**: 206, 1986.
- Gupta, S. K., Veith, F. J., Wengert, K. R., Franco, C., Amar, D., El-Gawet, E.-S., and Gupta, A. Anaphylactic reactions to protamine: An often lethal complication in insulin-dependent diabetes undergoing vascular surgery. *J. Vasc. Surg.* **9**: 342, 1989.
- Linblad, B. Protamine sulphate: A review of its effects—Hypersensitivity and toxicity. *Eur. J. Vasc. Surg.* **3**: 195, 1989.
- Wakefield, T. W., Whitehouse, W. M., Jr., and Stanley, J. C. Depressed cardiovascular function and altered platelet kinetics following protamine sulfate reversal of heparin activity. *J. Vasc. Surg.* **1**: 346, 1984.
- Wakefield, T. W., Ucross, I., Kresowik, T. F., Hinshaw, D. B., and Stanley, J. C. Decreased oxygen consumption as a toxic manifestation of protamine sulfate reversal of heparin anticoagulation. *J. Vasc. Surg.* **9**: 772, 1989.
- Housmans, P. R., and Ferguson, D. M. Inotropic effects of protamine sulfate on isolated mammalian cardiac muscles: Mechanisms of action. *Anesthesiology* **67**: A24, 1987.
- Caplan, R. A., and Su, J. Y. Differences in threshold for protamine toxicity in isolated atrial and ventricular tissue. *Anesth. Analg.* **63**: 1111, 1984.
- Hendry, P. J., Taichman, G. C., and Keon, W. J. The myocardial contractile responses to protamine sulfate and heparin. *Ann. Thorac. Surg.* **44**: 263, 1987.
- Iwatsuki, N., Matasukawa, S., and Iwatsuki, K. A weak negative inotropic effect of protamine sulfate upon the isolated canine heart muscle. *Anesth. Analg.* **59**: 100, 1980.
- Lin, C.-I., Luk, H.-N., Wei, J., and Tsao, S.-J. Electromechanical effects of protamine in isolated human atrial and canine ventricular tissue. *Anesth. Analg.* **68**: 479, 1989.
- Wakefield, T. W., Bies, L. E., Wroblewski, S. K., Bolling, S. F., Stanley, J. C., and Kirsh, M. M. Impaired myocardial function and oxygen utilization due to protamine sulfate in an isolated rabbit heart preparation. *Ann. Surg.* **212**: 387, 1990.
- McIntyre, R. W., Flezani, P., Knopes, K. D., Reves, J. G., and Watkins, W. D. Pulmonary hypertension and prostaglandins after protamine. *Am. J. Cardiol.* **58**: 857, 1986.
- Morel, D. R., Lowenstein, E., Nguyenduy, T., Robinson, D. R., Repine, J. E., Chenoweth, D. E., and Zapol, W. M. Acute pulmonary vasoconstriction and thromboxane release during protamine reversal of heparin anticoagulation in awake sheep. *Circ. Res.* **62**: 905, 1988.
- Wakefield, T. W., Bouffard, J. A., Spaulding, S. A., Petry, N. A., Gross, M. D., Lindblad, B., and Stanley, J. C. Sequestration of platelets in the pulmonary circulation as a consequence of protamine reversal of the anticoagulant effects of heparin. *J. Vasc. Surg.* **5**: 187, 1987.
- Chang, S.-W., Westcott, J. Y., Henson, J. E., and Voelkel, N. F. Pulmonary vascular injury by polycations in perfused rat lungs. *J. Appl. Physiol.* **62**: 1932, 1987.
- Degges, R. D., Foster, M. E., Dang, A. Q., and Read, R. C. Pulmonary hypertensive effect of heparin and protamine interaction: Evidence for thromboxane B<sub>2</sub> release from the lung. *Am. J. Surg.* **154**: 696, 1987.
- Jastrzebski, J., Hilgard, P., and Sykes, M. K. Pulmonary vasoconstriction produced by protamine and protamine-heparin complex in the isolated cat lung perfused with blood or dextran. *Cardiovasc. Res.* **9**: 691, 1975.
- Montalescot, G., Kreil, E., Lynch, K., Greene, E.-M., Torres, A., Carvalho, A., Fitzgibbon, C., Robinson, D. R., Lowenstein, E., and Zapol, W. M. Effect of platelet depletion on lung vasoconstriction in heparin-protamine reactions. *J. Appl. Physiol.* **66**: 2344, 1989.
- Eika, C. On the mechanism of platelet aggregation induced by heparin, protamine and polybrene. *Scand. J. Haematol.* **9**: 248, 1972.
- Radegran, K., and McAslan, C. Circulatory and ventilatory effects of induced platelet aggregation and their inhibition by acetylsalicylic acid. *Acta Anaesthesiol. Scand.* **16**: 76, 1972.
- Wakefield, T. W., Wroblewski, S. K., and Stanley, J. C. Reversal of depressed oxygen consumption accompanying *in vivo* protamine sulfate-heparin interactions by the prostacyclin analogue, iloprost. *Eur. J. Vasc. Surg.* **4**: 25, 1990.
- Gorman, R. R., Johnson, R. A., Spilman, C. H., and Aiken, J. W. Inhibition of platelet thromboxane A<sub>2</sub> synthase activity by sodium 5-(3'-pyridinylmethyl)benzofuran-2-carboxylate. *Prostaglandins* **26**: 325, 1983.
- Bertele, V., Falanga, A., Tomasiak, M., Chiabrando, C., Cerletti, C., and de Gaetano, G. Pharmacologic inhibition of thromboxane synthetase and platelet aggregation: Modulatory role of cyclooxygenase products. *Blood* **63**: 1460, 1984.
- Patscheke, H. Thromboxane synthase inhibition potentiates

- washed platelet activation by endogenous and exogenous arachidonic acid. *Biochem. Pharmacol.* **34**: 1151, 1985.
28. Mehta, J., Metha, P., Lawson, D. L., Ostrowski, N., and Brigrman, L. Influence of selective thromboxane synthetase blocker CGS-13080 on thromboxane and prostacyclin biosynthesis in whole blood: Evidence for synthesis of prostacyclin by leukocytes from platelet-derived endoperoxides. *J. Lab. Clin. Med.* **106**: 246, 1985.
  29. Simpson, P. J., Smith, C. B., Rosenthal, G., and Lucchesi, B. R. Reduction in the incidence of thrombosis by the thromboxane synthetase inhibitor CGS 13080 in a canine model of coronary artery injury. *J. Pharmacol. Exp. Ther.* **238**: 497, 1986.
  30. Gresele, P., Arnout, J., Deckmyn, H., Huybrechts, E., Pieters, G., and Vermeylen, J. Role of proaggregatory and antiaggregatory prostaglandins in hemostasis. Studies with combined thromboxane synthase inhibition and thromboxane receptor antagonism. *J. Clin. Invest.* **80**: 1435, 1987.
  31. Fitzgerald, D. J., Fragetta, J., and Fitzgerald, G. A. Prostaglandin endoperoxides modulate the response to thromboxane synthase inhibition during coronary thrombosis. *J. Clin. Invest.* **82**: 1708, 1988.
  32. Bech, F. R., Cronenwett, J. L., McDaniel, M. D., Ogletree, M. L., and Freeman, D. H., Jr. The effect of thromboxane receptor blockade versus thromboxane synthase inhibition on canine arterial graft patency. *J. Vasc. Surg.* **12**: 119, 1990.
  33. Wakefield, T. W., Hinshaw, D. B., Burger, J. M., Burkel, W. E., and Stanley, J. C. Protamine induced reductions of endothelial cell ATP. *Surgery* **106**: 378, 1989.