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Role of Heparin and Nitric Oxide in the Cardiac and Regional Hemodynamic Properties of Protamine in Conscious Chronically Instrumented Dogs

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Background: **Because protamine is administered to reverse heparin, a drug that might itself affect the pharmacologic properties of protamine, this study was designed to assess the properties of protamine alone and in the presence of heparin in conscious dogs.**

Methods: **Twelve dogs were instrumented to continuously record cardiac and regional hemodynamics. On separate occasions, a dose of protamine (0.5, 1, 3, 5, and 8 mg/kg) was randomly administered either alone or in the presence of heparin (ratio 100 IU/mg). Heparin (300 IU/kg) and protamine (3 mg/kg) were administered in the presence of *N*-methyl-L-arginine, a specific nitric oxide synthase inhibitor. Identical experiments were performed with protamine (8 mg/kg) in the absence of heparin on a separate occasion.**

Results: **Protamine alone produced limited cardiac and regional changes. In the presence of heparin, protamine produced hypotension at 3, 5, and 8 mg/kg, vasodilatation at 3 and 5 mg/kg, and a more pronounced dose-dependent increase in pulmonary pressure at 3, 5, and 8 mg/kg. Simultaneously, transient carotid vasodilatation at 3 and 5 mg/kg, coronary and hepatic vasodilatation at 3, 5, and 8 mg/kg, as well as a decrease in vertebral vascular resistance were recorded at 1, 3, and 8 mg/kg. Protamine produced an immediate increase followed by a secondary decrease in renal vascular resistance. Protamine-induced secondary pulmonary pressor effects were attenuated. In the presence of heparin, nitric oxide synthase blockade selectively attenuated protamine-induced immediate hypotension, systemic vasodilatation, and coronary, mesenteric, and hepatic vasodilations as well as the decrease in portal blood flow and accentuated the renal vasoconstriction.**

Conclusions: **The presence of heparin accentuated the decrease in cardiac function induced by protamine as well as its effects on regional circulation. The data provide evidence that the nitric oxide pathway is involved in the systemic and selective regional heparin–protamine-mediated vasodilatation in conscious dogs.**

DESPITE decades of clinical use, the cardiovascular pharmacology of protamine and how heparin may affect its cardiac and regional hemodynamic responses remained unclear. In dogs, Kien *et al.*[1] reported that protamine elicited profound transient and immediate cardiac depression, whereas Green *et al.*[2] failed to record any significant changes in cardiac function after protamine administration. Furthermore, heparin has been shown to prevent and reduce the cardiac depression produced by protamine on rabbit myocardium.[3] On the other hand, heparin has also been shown to affect protamine-mediated cardiac changes.[1] Thus, the effects of protamine alone and in the presence of heparin on cardiac function remain essentially unknown. Although several studies have reported the effects of protamine on arterial blood pressure, cardiac output, heart rate, or pulmonary arterial pressure alone,[1,2,4,5–7] regional blood flow measurements were not recorded. In addition, most previous *in vivo* studies were conducted in surgically stressed and anesthetized animals.[1,2,4–6,8] We and other investigators have established that basal anesthesia and surgical stress have profound effects on the cardiovascular system.[9,10] We therefore hypothesized that the cardiovascular properties of protamine depend on the presence or absence of heparin and the dose of heparin used, and may vary with time and the circulatory bed. To overcome the limitations of previously published studies on protamine, this study was designed to assess the immediate and secondary cardiac and regional hemodynamic responses to protamine in the absence and presence of heparin in conscious and chronically instrumented dogs that were not acutely surgically stressed.[9,10] However, species-specific differences in terms of the hemodynamic reactions to protamine may exist when compared with humans.

In addition, protamine has been shown *in vitro* to relax vascular smooth muscle *via* an endothelium-dependent mechanisms[11,12]; thus, the present study was also designed to evaluate the role of nitric oxide (NO) on systemic and regional hemodynamic changes induced by protamine and heparin.

Materials and Methods

The protocols were approved by The University of Texas Animal Welfare Committee.

Cardiovascular Instrumentation

A description of the basic model has been previously described.[13] Briefly, 12 mongrel dogs of either sex, heartworm free, weighing 25–34 kg, were instrumented

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Table 1. Cardiac and Regional Hemodynamic Values before Protamine Injection in Dogs Treated with Protamine

Protamine	0.5 mg/kg	1.0 mg/kg	3.0 mg/kg	5.0 mg/kg	8.0 mg/kg
MAP (mm/Hg)	98 ± 4	92 ± 5	87 ± 6	85 ± 4	83 ± 4
CO (l/min)	2.22 ± 0.22	2.57 ± 0.30	2.21 ± 0.12	2.31 ± 0.15	2.17 ± 0.16
SVR (U)	41.1 ± 3.5	37.4 ± 3.5	39.3 ± 1.0	37.4 ± 2.8	39.2 ± 2.9
dP/dt (mmHg/s)	3,095 ± 794	3,012 ± 757	3,102 ± 556	3,177 ± 411	2,694 ± 423
PAP (mmHg)	15 ± 1	15 ± 1	14 ± 1	14 ± 1	15 ± 1
PVR (U)	6.17 ± 0.67	5.40 ± 0.56	5.97 ± 0.19	5.82 ± 0.78	6.44 ± 0.24
HR (beats/min)	78 ± 9	80 ± 10	70 ± 6	74 ± 6	69 ± 6
Car BF (ml/min)	196 ± 9	178 ± 19	169 ± 19	156 ± 22	172 ± 26
Ver BF (ml/min)	13.2 ± 2.5	14.5 ± 2.4	14.5 ± 3.5	15.2 ± 2.4	12.1 ± 1.9
Cor BF (ml/min)	50 ± 13	59 ± 11	49 ± 11	48 ± 9	41 ± 7
Por BF (ml/min)	261 ± 46	314 ± 115	324 ± 60	349 ± 66	277 ± 35
Hep BF (ml/min)	96 ± 21	94 ± 18	111 ± 26	118 ± 31	79 ± 13
T Hep BF (ml/min)	375 ± 46	408 ± 124	437 ± 71	467 ± 79	356 ± 44
Mes BF (ml/min)	126 ± 19	124 ± 21	100 ± 14	110 ± 19	99 ± 11
Ren BF (ml/min)	98 ± 23	94 ± 24	101 ± 23	108 ± 25	89 ± 20
Car VR (U)	0.45 ± 0.02	0.54 ± 0.10	0.55 ± 0.07	0.62 ± 0.11	0.56 ± 0.16
Ver VR (U)	7.26 ± 1.14	6.64 ± 1.00	6.79 ± 1.36	6.01 ± 0.88	7.36 ± 0.99
Cor VR (U)	2.21 ± 0.43	1.86 ± 0.41	2.10 ± 0.29	2.11 ± 0.36	2.29 ± 0.33
Hep VR (U)	1.13 ± 0.26	1.19 ± 0.30	0.95 ± 0.16	0.97 ± 0.22	1.21 ± 0.21
Mes VR (U)	0.79 ± 0.15	0.83 ± 0.13	0.97 ± 0.14	0.91 ± 0.16	0.90 ± 0.12
Ren VR (U)	1.22 ± 0.37	1.38 ± 0.31	1.15 ± 0.31	1.14 ± 0.36	1.23 ± 0.32

n = 6. Data are mean ± SEM.

MAP = mean arterial pressure; CO = cardiac output; SVR = systemic vascular resistance; dP/dt = first derivative of left ventricular pressure; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; HR = heart rate; Car BF = carotid blood flow; Ver BF = vertebral BF; Por BF = portal BF; Hep BF = hepatic BF; T Hep BF = total hepatic BF; Mes BF = mesenteric BF; Car VR = carotid vascular resistance; Ver VR = vertebral VR; Cor VR = coronary VR; Hep VR = hepatic VR; Mes VR = mesenteric VR; Ren VR = renal VR.

with catheters in the iliac artery, left atrium, and pulmonary artery. A precalibrated ultrasonic flow probe was positioned around the pulmonary artery, and a miniature pressure transducer was inserted into the left ventricle. Pulsed Doppler flow probes were positioned around the vertebral, carotid, coronary, mesenteric, hepatic, and renal arteries and the portal vein. All transducer leads and catheters were tunneled subcutaneously to the dorsum of the neck and secured after the thoracotomy was closed. Analgesia and antibiotic prophylaxis were initiated before surgery and were maintained thereafter.

Measurements

A detailed description of the measurement techniques has also been previously published.[13–15] Phasic and mean aortic pressures, pulmonary arterial pressure, first derivative of left ventricular pressure (dP/dt), heart rate, cardiac output, and carotid, vertebral, coronary, hepatic, portal, mesenteric, and renal blood flow were continuously recorded on a 16-channel Gould brush polygraph (Gould, Cleveland, OH). Systemic vascular resistance was calculated as the ratio of mean arterial pressure (MAP) to cardiac output; regional vascular resistance was calculated as the ratio of MAP to regional blood flow.

Experimental Protocols

Dogs were carefully nursed through the first 24 h postoperatively and on subsequent days were trained to lie quietly on the laboratory floor. The dogs were studied no less than 10 days after surgery, when hematocrit was greater than 30% and body temperature, appetite, and general appearance were normal. Body weight, body temperature, arterial blood gases, and hematocrit were routinely measured before each experiment. All experiments were conducted in fasted conscious dogs lying on their right sides. Although either immediate or secondary effects after protamine administration have been previously documented,[16,17] no studies have assessed both changes. Therefore, this study was designed to assess combined immediate and secondary effects of protamine in conscious and chronically instrumented dogs. Dogs were equally distributed to either protocol 1 (n = 6) or protocol 2 (n = 6).

Protocol 1. Each dose of protamine was given on a separate day at least 48 h after the previous dose. On each experimental day, control measurements were obtained in resting conscious animals before the administration of either protamine alone or protamine combined with heparin. The order of administration of the protamine dose alone or in the presence of heparin was randomized.

Protamine sulfate was administered intravenously in doses of 0.5, 1.0, 3.0, 5.0, and 8.0 mg/kg, either alone or in the presence of heparin. The dose of heparin was calculated to maintain a ratio of 100 IU/mg protamine: 50 IU/kg heparin for 0.5 mg/kg protamine; 100 IU/kg heparin for 1 mg/kg protamine; 300 IU/kg heparin for 3 mg/kg protamine; 500 IU/kg heparin for 5 mg/kg protamine, and 800 IU/kg heparin for 8 mg/kg prota-

Table 2. Cardiac and Regional Hemodynamic Values before Protamine Injection in Dogs Treated with Heparin–Protamine

Heparin–Protamine	50 IU/kg + 0.5 mg/kg	100 IU/kg + 1.0 mg/kg	300 IU/kg + 3.0 mg/kg	500 IU/kg + 5.0 mg/kg
MAP (mm/Hg)	90 ± 8	95 ± 4	96 ± 2	90 ± 5
CO (l/min)	2.43 ± 0.27	2.54 ± 0.27	2.06 ± 0.11	2.47 ± 0.25
SVR (U)	38.5 ± 4.4	39.2 ± 3.5	47.6 ± 3.0	38 ± 3.7
dP/dt (mmHg/sec)	3,162 ± 730	3,042 ± 514	3,271 ± 483	2,901 ± 730
PAP (mmHg)	15 ± 2	15 ± 2	15 ± 1	14 ± 1
PVR (U)	5.63 ± 0.98	5.71 ± 0.85	7.11 ± 0.46	5.40 ± 0.57
HR (beats/min)	77 ± 12	85 ± 7	73 ± 5	78 ± 5
Car BF (ml/min)	189 ± 6	204 ± 29	150 ± 25	186 ± 10
Ver BF (ml/min)	17.3 ± 5.6	15.8 ± 13.5	19.2 ± 3.6	14.5 ± 3.2
Cor BF (ml/min)	51 ± 10	47 ± 9	54 ± 10	48 ± 10
Por BF (ml/min)	279 ± 80	336 ± 80	350 ± 36	258 ± 44
Hep BF (ml/min)	98 ± 16	106 ± 29	119 ± 29	90 ± 21
T Hep BF (ml/min)	377 ± 86	442 ± 105	469 ± 56	348 ± 59
Mes BF (ml/min)	92 ± 18	119 ± 19	100 ± 13	90 ± 14
Ren BF (ml/min)	84 ± 22	104 ± 22	112 ± 16	90 ± 27
Car VR (U)	0.46 ± 0.03	0.48 ± 0.05	0.71 ± 0.09	0.47 ± 0.02
Ver VR (U)	6.46 ± 1.59	6.67 ± 1.15	5.80 ± 1.12	6.99 ± 1.26
Cor VR (U)	2.00 ± 0.37	2.35 ± 0.38	2.10 ± 0.38	2.24 ± 0.34
Hep VR (U)	1.01 ± 0.16	1.22 ± 0.38	1.24 ± 0.41	1.29 ± 0.28
Mes V R (U)	1.13 ± 0.21	0.90 ± 0.14	1.04 ± 0.13	1.10 ± 0.14
Ren VR (U)	1.45 ± 0.41	1.22 ± 0.32	0.98 ± 0.17	1.37 ± 0.39

n = 6. Data are mean ± SEM.

MAP = mean arterial pressure; CO = cardiac output; SVR = systemic vascular resistance; dP/dt = first derivative of left ventricular pressure; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; HR = heart rate; Car BF = carotid blood flow; Ver BF = vertebral BF; Por BF = portal BF; Hep BF = hepatic BF; T Hep BF = total hepatic BF; Mes BF = mesenteric BF; Car VR = carotid vascular resistance; Ver VR = vertebral VR; Cor VR = coronary VR; Hep VR = hepatic VR; Mes VR = mesenteric VR; Ren VR = renal VR.

mine. Protamine was administered 15 min after heparin administration. Because the magnitude of cardiovascular responses to protamine administration has been shown to depend on the rate of administration,[8] we chose to inject heparin and protamine as a bolus dose over a period of 10 s. Hemodynamic parameters were recorded at steady state before injection of protamine or heparin and for 30 min after protamine injection.

Protocol 2. To study the role of the NO pathway in protamine-associated cardiac changes, *N*-methyl-L-arginine (L-NMA), a specific NO synthase (NOS) inhibitor, was administered at 300 $\mu g \cdot kg^{-1} \cdot min^{-1}$, intravenously, before protamine administration. Protamine (3 mg/kg) was administered after heparin (300 IU/kg) as described in protocol 1 in the presence or absence of L-NMA infused at 300 $\mu g \cdot kg^{-1} \cdot min^{-1}$ on 2 separate occasions. The dose of L-NMA was chosen because it produced maximum pressor effects in conscious dogs (unpublished data). The infusion of L-NMA was started 45 min before the injection of protamine using a 0.2-μm syringe filter (Nalge Company, Rochester, NY). In the non–L-NMA-treated group, the same dose of saline was infused throughout the study. Identical experiments were also performed with protamine (8 mg/kg) in nonheparinized dogs in the absence or presence of L-NMA infusion (300 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The dose of protamine alone and combined with heparin was chosen to obtain a similar decrease in MAP (protocol 1). Hemodynamic parameters were recorded at baseline (before L-NMA infusion), before protamine injection, and for 30 min after protamine injection.

Drugs

Drugs used in all experiments were porcine intestinal mucosal heparin sodium (Elkins-Sinn, Inc., Cherry Hill, NY), protamine sulfate (Fujisawa, USA, Inc., Deerfield, IL), and L-NMA (ALEXIS Corporation, San Diego, CA).

Statistical Analysis

Data obtained from protocol 1 in heparinized and nonheparinized conditions were analyzed using an analysis of variance (ANOVA) for repeated measures in each group. When differences were significant, multiple within-group comparisons to the baseline value obtained before protamine injections were performed using the Dunnett *t* test.[18] In addition, when changes in heparinized and nonheparinized groups were significantly different, the magnitude of change was compared using an unpaired *t* test. Data obtained in protocol 2 were analyzed using an analysis of variance followed by a Dunnett *t* test. An unpaired *t* test was also used to determine statistical differences between control and L-NMA–treated groups in heparinized and nonheparinized conditions. Results in the figures are presented as percent changes *versus* control. A P value < 0.05 was considered significant.

Results

Protocol 1

Cardiac and regional hemodynamics before protamine administration are presented in tables 1 and 2. Values are

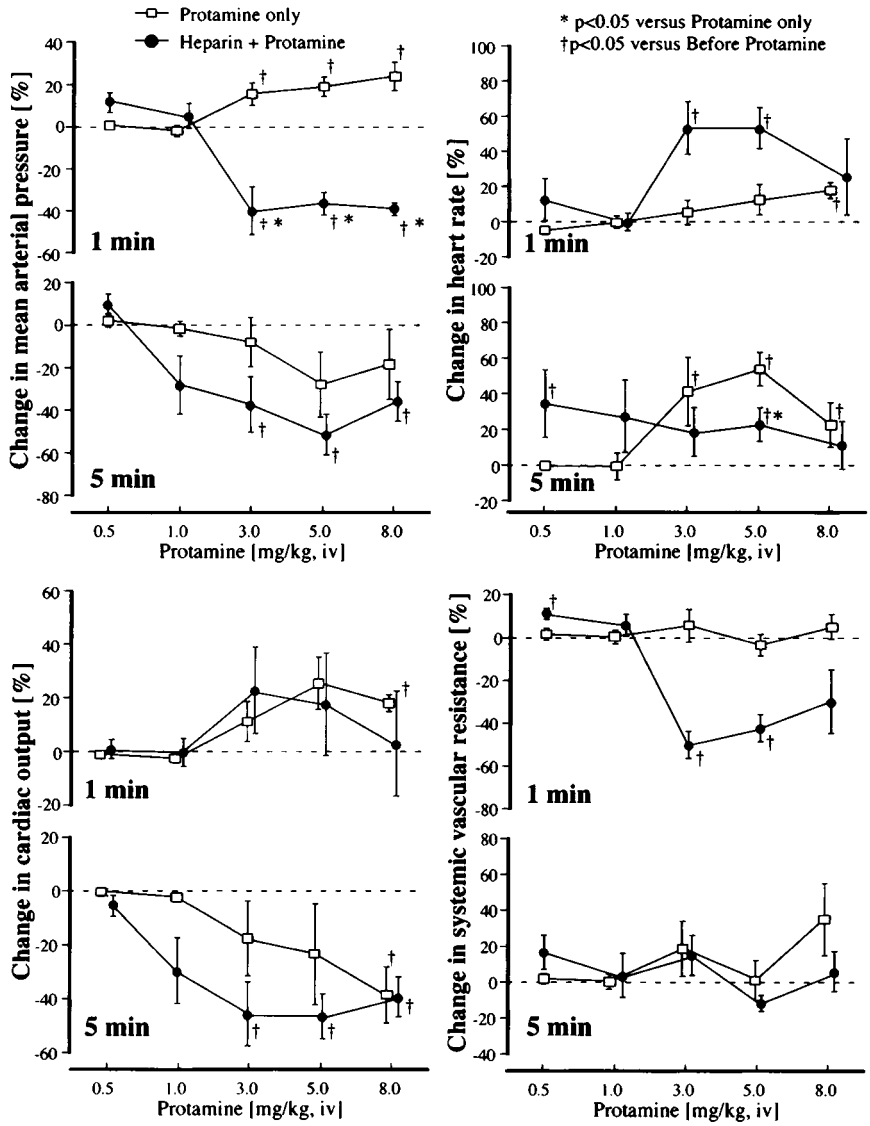

Fig. 1. Dose–response relation of protamine-induced percent changes in mean arterial pressure, heart rate, cardiac output, and systemic vascular resistance at 1 and 5 min after protamine injection in six conscious dogs (open squares) and the effects of heparin on the protamine-induced changes (closed circles). Data are presented as mean ± SEM. *P < 0.05 compared with the value obtained in the experiments without heparin at each concentration. †P < 0.05 compared with the control value within each group. iv = intravenous.

consistent with those previously reported for trained and unstressed animals.[9,10] Changes in cardiac and regional hemodynamic parameters after protamine alone and in the presence of heparin are presented in figures 1–4. Hemodynamic changes after protamine alone and in the presence of heparin varied according to the parameter, the time course (whether immediate or secondary responses), and the dose under investigation. By itself, heparin produced no cardiac and regional hemodynamic changes (table 2).

Responses at 1 Minute. Protamine alone produced an initial and short-lasting increase in cardiac function and an increase in MAP followed by a secondary decrease in cardiac function and hypotension. Its effects on regional circulation were limited. Thus, we recorded immediate and transient increases in MAP at 3, 5, and 8 mg/kg, pulmonary arterial pressure and dP/dt at 5.0 and 8.0 mg/kg, and cardiac output and heart rate at 8 mg/kg, as well as mesenteric vasoconstriction and vertebral vasodilatation at 5 and 8 mg/kg.

In the presence of heparin, the protamine-induced pressor effect converted into hypotension at 3.0, 5.0, and 8.0 mg/kg. Consequently, systemic vasodilatation was also recorded. Furthermore, protamine also induced pulmonary vasoconstriction at 3.0, 5.0, and 8.0 mg/kg, tachycardia at 3.0 and 5.0 mg/kg, and an increase in dP/dt at 5 mg/kg. Regional circulation in peripheral vascular beds was also affected after protamine administration. Vasodilatation was recorded in the carotid circulation at 3 and 5 mg/kg and in the coronary and hepatic circulation at 3, 5, and 8 mg/kg. Mesenteric vascular resistance initially increased at 1 mg/kg, and vertebral vasodilatation was recorded at 1, 3, and 8 mg/kg. A decrease in portal blood flow was observed at 3 mg/kg. Finally, renal vasoconstriction was detected after protamine was administered at 8 mg/kg.

Responses at 5 Minutes. The effects of protamine alone recorded at 5 min were limited to cardiac output, heart rate, dP/dt, pulmonary and renal vascular resistance, and portal blood flow. At the highest dose, cardiac

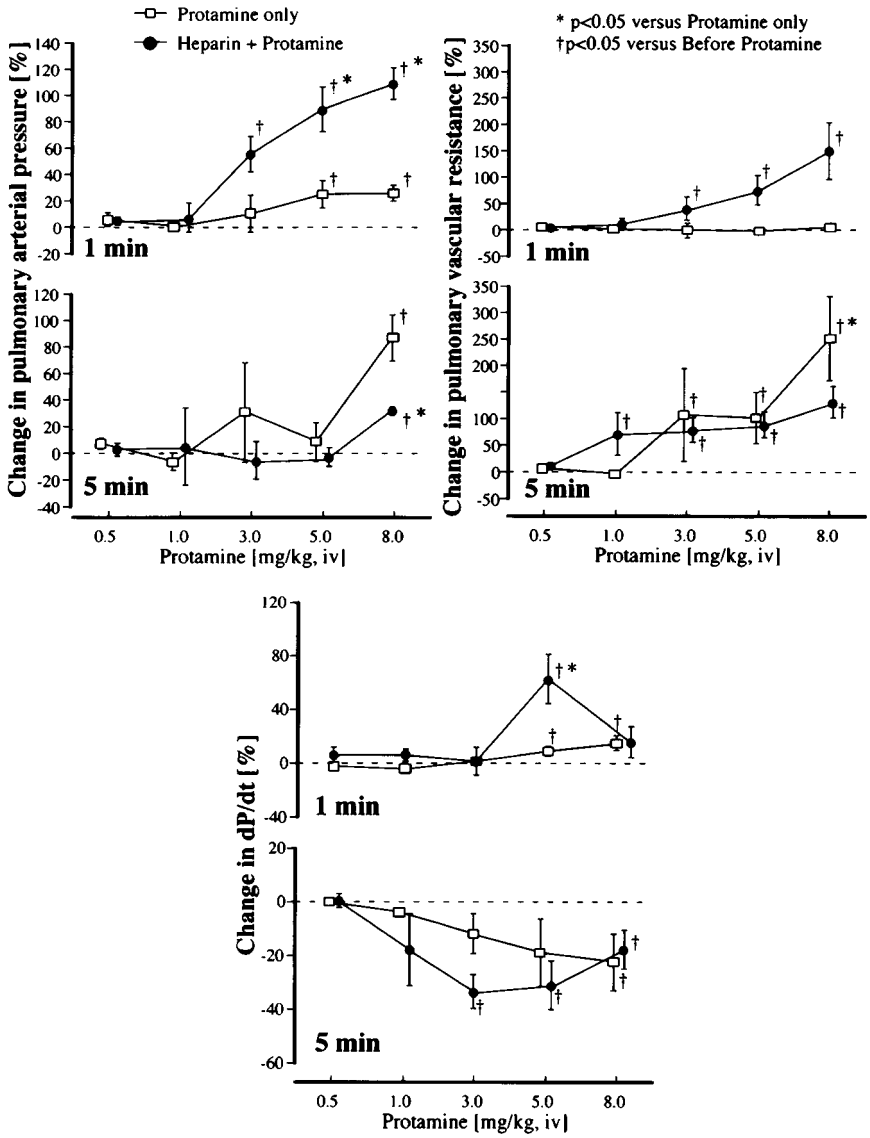

Fig. 2. Dose–response relation of protamine-induced percent changes in pulmonary arterial pressure, pulmonary vascular resistance, and first derivative of left ventricular pressure (dP/dt) at 1 and 5 min after protamine injection in six conscious dogs (open squares) and the effects of heparin on the protamine-induced changes (closed circles). Data are presented as mean ± SEM. *$P < 0.05$ compared with the value obtained in the experiments without heparin at each concentration. †$P < 0.05$ compared with the control value within each group. iv = intravenous.

output, dP/dt, and renal vascular resistance decreased at 8 mg/kg, whereas pulmonary vascular resistance increased at 3, 5, and 8 mg/kg. In addition, a decrease in portal blood flow was recorded at 5 and 8 mg/kg. Finally, heart rate increased at 3, 5, and 8 mg/kg.

In the presence of heparin, protamine induced more pronounced cardiac and regional hemodynamic changes. Protamine at 3, 5, and 8 mg/kg produced decreases in MAP, cardiac output, and dP/dt with a renal vasodilatation and a decrease in portal blood flow. In addition, hepatic vasodilatation was recorded at 8 mg/kg, and mesenteric vasodilatation was recorded at 5 mg/kg after protamine administration. In contrast, the secondary pulmonary vasoconstriction produced by protamine in the presence of heparin was attenuated.

Protocol 2

Cardiac and regional hemodynamics in control conditions and before L-NMA are presented in tables 3 and 4. In the saline groups, each measurement remained essentially unchanged. L-NMA infused at 300 $\mu g \cdot kg^{-1} \cdot min^{-1}$ induced changes consistent with those previously observed using L-NMA administered intravenously at 20 mg/kg in conscious dogs.[13] Hemodynamic changes after protamine alone or in the presence of heparin varied according to the presence of L-NMA, the hemodynamic parameters, the time course (whether immediate or secondary response), and the dose under investigation.

Immediate Responses. Nitric oxide synthase blockade resulted in an inhibition of protamine-induced immediate increase in dP/dt and an accentuation of the renal vasoconstriction. However, administration of L-NMA did not significantly prevent other hemodynamic changes caused by protamine.

In the presence of heparin, NOS blockade resulted in an inhibition of protamine-induced immediate decreases in MAP, portal blood flow, and systemic, coronary, mes-

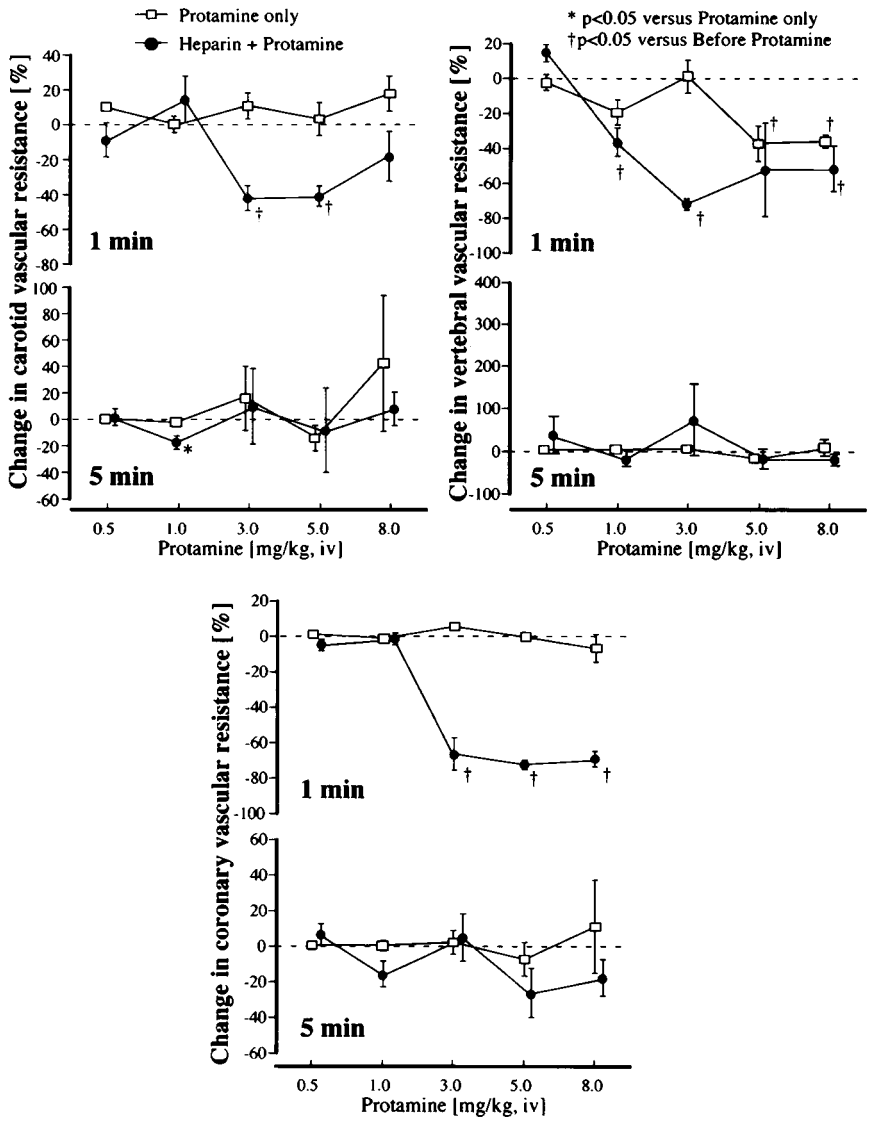

Fig. 3. Dose–response relation of protamine-induced percent changes in carotid, vertebral, and coronary vascular resistance at 1 and 5 min after protamine injection in six conscious dogs (open squares) and the effects of heparin on the protamine-induced changes (closed circles). Data are presented as mean ± SEM. *P < 0.05 compared with the value obtained in the experiments without heparin at each concentration. †P < 0.05 compared with the control value within each group. iv = intravenous.

enteric, and hepatic vascular resistance. Accentuation of the renal vasoconstriction was also detected.

Secondary Responses. Nitric oxide synthase blockade resulted in an accentuation of protamine-induced renal vasodilatation. In the presence of heparin, NOS blockade did not affect the sustained hypotension observed at 5 min.

Discussion

Our data obtained from the first protocol demonstrate that the cardiac and regional vascular properties of protamine in the tissue or organ examined are altered by the presence of heparin. They also confirm that the time course and the dose used are important determinants of the cardiac and regional hemodynamic properties of protamine. Protamine alone at doses of 3 mg/kg or greater appears to produce an immediate and mostly dose-independent increase in MAP as well as cardiac stimulation that is followed by a cardiac depression. Protamine induced immediate mesenteric vasoconstriction and vertebral vasodilatation and secondary renal vasodilatation, especially at doses of 5 and 8 mg/kg. In the presence of heparin, the changes previously documented with protamine were either accentuated, attenuated, or unchanged. Thus, the secondary changes in MAP, cardiac output and dP/dt, pulmonary vascular tone, heart rate, and vertebral and renal vasodilatations produced by protamine were accentuated in the presence of heparin.

In contrast, the immediate systemic and mesenteric vasoconstriction produced by protamine was changed into hypotension and systemic and mesenteric vasodilatations. In the presence of heparin, protamine failed to produce cardiac stimulation, and the magnitude of the secondary increase in pulmonary arterial pressure was attenuated. In addition, protamine administered in the presence of heparin led to immediate and dose-indepen-

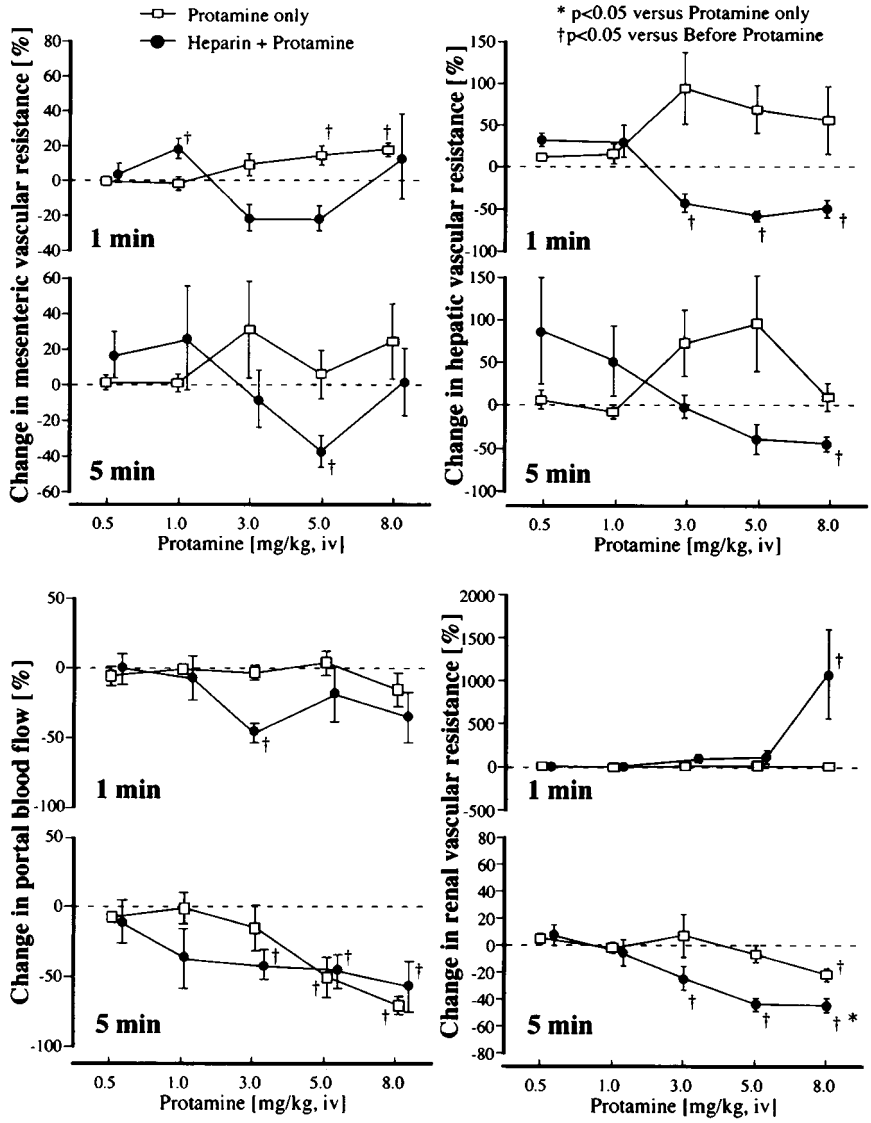

Fig. 4. Dose–response relation of protamine-induced percent changes in mesenteric, hepatic, and renal vascular resistance and portal blood flow at 1 and 5 min after protamine injection in six conscious dogs (open squares) and the effects of heparin on the protamine-induced changes (closed circles). Data are presented as mean ± SEM. *$P < 0.05$ compared with the value obtained in the experiments without heparin at each concentration. †$P < 0.05$ compared with the control value within each group. iv = intravenous.

dent carotid, coronary, and hepatic vasodilatations, territories that were not affected by protamine alone.

Our data obtained from the second protocol indicate that the NO pathway was involved in the immediate hemodynamic changes associated with protamine in the presence of heparin. Thus, the immediate increase in dP/dt after the administration of protamine alone was inhibited by NOS blockade, and renal vasoconstriction was accentuated. In the presence of a NOS inhibitor, the combination of heparin and protamine failed to induce immediate hypotension, portal hypoperfusion, or coronary, mesenteric, or hepatic vasodilatation, and the magnitude of the systemic vasodilatation was attenuated. In contrast, our data do not support the role of NO in the secondary hemodynamic changes produced by protamine.

In *in vivo* studies, Kien *et al.*[1] reported that in chronically instrumented dogs during halothane anesthesia, the cardiac depression induced by protamine was not affected by heparin. Our data obtained in the absence of anesthesia and surgical stress indicate that, in dogs, protamine alone secondarily decreased myocardial function in doses of 3 mg/kg and higher. Thus, cardiac output and dP/dt decreased in the absence of changes in systemic vascular resistance and in the presence of increased pulmonary vascular resistance and heart rate.[19] Although in our experimental conditions heparin did not affect the threshold and maximum cardiac changes, it accentuated the myocardial depression produced by protamine, especially at 3 and 5 mg/kg. Kien *et al.*[1] previously reported that, in anesthetized dogs, protamine produced similar effects on cardiac function. However, in the latter studies the cardiac depression appears to be of greater magnitude when given at 3 and 5 mg/kg in anesthetized animals as compared with our awake model. It is most likely that the presence of halothane was a major contributor to the accentuation of cardiac depression with protamine. We previously demonstrated that halothane may accentuate the cardiac depression induced by cal-

Table 3. Cardiac and Regional Hemodynamic Values before Saline, L-NMA Infusion, and Protamine Administration in Dogs Treated with Protamine

	Control Protamine		L-NMA Protamine	
	Before Saline	Before Protamine	Before L-NMA	Before Protamine
MAP (mm/Hg)	83 ± 3	83 ± 4	81 ± 3	102 ± 3*
HR (beats/min)	71 ± 7	69 ± 6	76 ± 9	61 ± 7*
CO (l/min)	2.17 ± 0.16	2.17 ± 0.16	2.24 ± 0.27	1.95 ± 0.29*
SVR (U)	39.3 ± 2.5	39.2 ± 2.9	38.7 ± 5.2	56.5 ± 7.8*
PAP (mmHg)	14 ± 1	15 ± 1	14 ± 1	16 ± 1
PVR (U)	6.01 ± 0.24	6.44 ± 0.24	5.85 ± 0.50	7.49 ± 0.77*
dP/dt (mmHg/s)	2,699 ± 453	2,694 ± 423	2,890 ± 568	2,932 ± 589
Car BF (ml/min)	177 ± 24	172 ± 26	186 ± 9	131 ± 19*
Ver BF (ml/min)	12.7 ± 1.5	12.1 ± 1.9	12.5 ± 3.2	12.3 ± 2.3
Cor BF (ml/min)	42.8 ± 7.9	41.1 ± 7.1	40.3 ± 8.9	40.8 ± 10.3
Por BF (ml/min)	271 ± 42	277 ± 35	246 ± 66	281 ± 34
Hep BF (ml/min)	78.6 ± 13.6	79.3 ± 12.9	113.5 ± 26.7	70.9 ± 18.4*
T Hep BF (ml/min)	350 ± 52	356 ± 44	360 ± 62	352 ± 35
Mes BF (ml/min)	97.1 ± 11.2	98.9 ± 11.3	100.6 ± 24.7	82.2 ± 12.2*
Ren BF (ml/min)	89.1 ± 20.9	89.0 ± 19.9	82.4 ± 22.9	66.6 ± 20.2*
Car VR (U)	0.52 ± 0.12	0.56 ± 0.16	0.44 ± 0.03	0.82 ± 0.12*
Ver VR (U)	6.79 ± 0.67	7.36 ± 0.99	7.71 ± 1.50	8.98 ± 1.59
Cor VR (U)	2.21 ± 0.32	2.29 ± 0.33	2.43 ± 0.48	3.22 ± 0.77
Hep VR (U)	1.24 ± 0.23	1.21 ± 0.21	0.90 ± 0.20	1.83 ± 0.38*
Mes VR (U)	0.92 ± 0.12	0.90 ± 0.12	0.98 ± 0.18	1.36 ± 0.20*
Ren VR (U)	1.23 ± 0.29	1.23 ± 0.32	1.35 ± 0.38	2.17 ± 0.61*

n = 6. Data are mean ± SEM.

* $P < 0.05$ *versus* before *N*-methyl-L arginine (L-NMA).

MAP = mean arterial pressure; CO = cardiac output; SVR = systemic vascular resistance; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; dP/dt = first derivative of left ventricular pressure; Car BF = carotid blood flow; Ver BF = vertebral BF; Cor BF = coronary BF; Por BF = portal BF; Hep BF = hepatic BF; T Hep BF = total hepatic BF; Mes BF = mesenteric BF; Ren BF = renal BF; Car VR = carotid vascular resistance; Ver VR = vertebral VR; Cor VR = coronary VR; Hep VR = hepatic VR; Mes VR = mesenteric VR; Ren VR = renal VR.

cium channel blockers.[20] This is of special interest because protamine properties have also been demonstrated to depend on calcium movement.[11]

In *in vitro*, Akata *et al.*[11] demonstrated that protamine produced a dose-dependent vasodilatation in isolated mesenteric arteries precontracted with potassium and norepinephrine. This protamine-induced relaxation appears to be calcium-dependent and endothelium-dependent and -independent. More recently, Akata *et al.*[21] demonstrated that pretreatment with heparin blocked this response. Further studies from the same group also revealed that the ratio of heparin to protamine is an important determinant of the magnitude of the interaction. The investigators reported that in an *in vitro* experiment, heparin did not affect protamine-induced relaxation for ratios less than 1.45 IU/μg, whereas heparin completely blocked protamine responses for ratios greater than 4.7 IU/μg.[22] Our data indicate that protamine in doses of 5 and 8 mg/kg produced an immediate and transient mesenteric vasoconstriction. In the presence of heparin at a ratio of 100 IU/mg, mesenteric vasoconstriction was observed at the lowest dose (1 mg/kg), whereas protamine administered at 3 and 5 mg/kg produced an immediate and sustained vasodilatation. This apparent discrepancy between *in vitro* and *in vivo* studies may result from alterations in local circulation caused by elimination of exogenous neural control as well as alterations in blood flow–mediated changes in endothelial function. In the absence of heparin, protamine at 1.0, 3.0, and 8 mg/kg produced an immediate and brief increase in MAP combined with a modest, transient increase followed by a more pronounced decrease in cardiac output at 8 mg/kg. Furthermore, in *in vitro* studies, protamine-mediated mesenteric vasodilatory properties required a precontracted vessel with either potassium or norepinephrine. Therefore, it is likely that in the absence of high levels of norepinephrine or potassium, protamine may have failed to induce relaxation in *in vivo* studies.

Pearson *et al.*[12] demonstrated that *in vitro*, protamine produced a dose-related relaxation of canine coronary and renal arteries precontracted with prostaglandin F_2 α, and that heparin did not interfere with protamine properties. Our findings also contrasted with those obtained in the portal circulation, in which protamine had minimal effects in the absence of heparin or produced modest changes in the presence of heparin. As previously discussed, autoregulatory mechanisms play a major role in the control of vascular tone in the carotid, vertebral, and hepatic circulation. In contrast, the coronary circulation is mostly metabolically regulated. However, the profound coronary vasodilatation produced by protamine in the presence of heparin and the consequences of NOS inhibition suggest that these effects are most likely related to surface active properties of protamine

Table 4. Cardiac and Regional Hemodynamic Values before Saline, L-NMA Infusion, and Protamine Administration in Dogs Treated with Heparin–Protamine

	Control (Heparin–Protamine)		L-NMA (Heparin–Protamine)	
	Before Saline	Before Protamine	Before L-NMA	Before Protamine
MAP (mm/Hg)	93 ± 3	96 ± 2	88 ± 5	111 ± 3*
HR (beats/min)	72 ± 5	73 ± 5	79 ± 4	67 ± 5*
CO (l/min)	2.08 ± 0.11	2.06 ± 0.11	2.18 ± 0.16	1.86 ± 0.22*
SVR (U)	45.4 ± 3.4	47.6 ± 3.0	40.9 ± 2.6	62.4 ± 6.4*
PAP (mmHg)	14 ± 2	15 ± 1	16 ± 1	16 ± 1
PVR (U)	6.37 ± 0.57	7.11 ± 0.46	6.73 ± 0.50	8.38 ± 1.12
dP/dt (mmHg/s)	3,198 ± 451	3,271 ± 483	3,207 ± 458	3,371 ± 566
Car BF (ml/min)	157 ± 27	150 ± 25	149 ± 21	102 ± 10*
VerBF (ml/min)	17 ± 3	19 ± 4	16 ± 4	17 ± 4
Cor BF (ml/min)	56 ± 11	54 ± 10	50 ± 8	60 ± 14
Por BF (ml/min)	334 ± 46	350 ± 36	376 ± 75	315 ± 56
Hep BF (ml/min)	114 ± 22	119 ± 29	130 ± 30	94 ± 29
T Hep BF (ml/min)	448 ± 66	469 ± 56	506 ± 74	409 ± 48*
Mes BF (ml/min)	107 ± 15	100 ± 13	107 ± 19	85 ± 16*
Ren BF (ml/min)	107 ± 17	112 ± 16	117 ± 22	101 ± 19*
Car VR (U)	0.66 ± 0.08	0.71 ± 0.09	0.66 ± 0.11	1.13 ± 0.09*
Ver VR (U)	6.13 ± 0.95	5.80 ± 1.12	6.90 ± 1.44	8.65 ± 2.39
Cor VR (U)	2.01 ± 0.38	2.10 ± 0.38	2.09 ± 0.32	2.41 ± 0.49
Hep VR (U)	1.01 ± 0.21	1.24 ± 0.41	1.02 ± 0.32	2.09 ± 0.61
Mes VR (U)	0.94 ± 0.12	1.04 ± 0.13	0.92 ± 0.12	1.50 ± 0.21*
Ren VR (U)	0.97 ± 0.16	0.98 ± 0.17	.92 ± 0.20	1.45 ± 0.41*

n = 6. Data are mean ± SEM.

* $P < 0.05$ *versus* before *N*-methyl-L arginine (L-NMA).

MAP = mean arterial pressure; CO = cardiac output; SVR = systemic vascular resistance; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; dP/dt = first derivative of left ventricular pressure; Car BF = carotid blood flow; Ver BF = vertebral BF; Cor BF = coronary BF; Por BF = portal BF; Hep BF = hepatic BF; T Hep BF = total hepatic BF; Mes BF = mesenteric BF; Ren BF = renal BF; Car VR = carotid vascular resistance; Ver VR = vertebral VR; Cor VR = coronary VR; Hep VR = hepatic VR; Mes VR = mesenteric VR; Ren VR = renal VR.

through the release of NO. However, in our second protocol, a NOS inhibitor prevented coronary vasodilatation in the presence of heparin–protamine, whereas dP/dt was still depressed. Because the coronary artery was precontracted with prostaglandin $F_2 \alpha$ in the study by Pearson *et al.*,[12] the lack of a high concentration of prostaglandin $F_2 \alpha$ in *in vivo* studies may be the reason for the discrepancy between *in vivo* and *in vitro* studies.

In several *in vivo* studies,[6,8,23–25] protamine administered either alone or in the presence of heparin has been shown to produce pulmonary vasoconstriction. However, in these studies, the properties of protamine alone and in the presence of heparin have been documented in separate experimental designs. Therefore, the role played by heparin in the pulmonary vasoconstriction induced by protamine was not established. Our data indicate that protamine administered alone increased pulmonary arterial pressure, a change usually observed immediately after protamine administration. However, at the highest dose, a secondary pulmonary vasoconstriction was recorded. In the presence of heparin, the immediate increase in pulmonary arterial pressure was accentuated, whereas heparin attenuated the secondary increase in the pulmonary vascular tone.

Protamine is rich in the basic amino acid arginine, which is the precursor of endothelial cell synthesis of NO. Although *in vitro* protamine has been shown to relax vascular smooth muscle through an endothelium-dependent mechanism,[11] Hakim *et al.*[6] indicated that the NO system was not involved in the heparin–protamine response in anesthetized pigs, in which local blood flows could not be obtained. Our data from the second protocol would explain these discrepancies between *in vitro* and *in vivo* experiments. Therefore, it seems that the importance of the NO system as the mechanism of protamine-induced vasodilatation depends on the local circulation. Furthermore, heparin potentiated the protamine-induced systemic and selective vascular NO-dependent vasodilatations in the coronary, mesenteric, portal, and hepatic circulations at 1 min. However, our data do not support the role of NO in the decrease in cardiac function and pulmonary, carotid, and vertebral vascular changes induced by protamine-heparin complex.

In conclusion, our data provide evidence that protamine itself causes biphasic changes in arterial blood pressure and cardiac function, pulmonary vasoconstriction, and limited and selective regional vasodilatation and/or vasoconstriction in conscious dogs. The presence of heparin had multiple consequences on the cardiac and hemodynamic properties of protamine; it abolished the pressor and cardiac stimulation and accentuated hypotension and cardiac depression as well as the initial pulmonary pressor response of

protamine. In the presence of heparin, protamine also produced several regional hemodynamic consequences: (1) carotid, coronary, and hepatic circulation appeared to be selectively affected by the combination of heparin plus protamine and not by protamine alone; (2) the effects of protamine on renal and mesenteric circulations are dependent on the time course and the dose, respectively; and (3) the secondary pulmonary pressor effect of protamine was attenuated in the presence of heparin. Finally, our findings also indicate that NO-dependent mechanisms are involved in the systemic and selective regional protamine-mediated vasodilatation, especially in the coronary, mesenteric, and hepatic circulations in heparinized dogs.

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