

TITLE: EFFECTS OF PROTAMINE AND HEPARIN ON SERUM IONIZED CALCIUM IN VITRO AND IN VIVO

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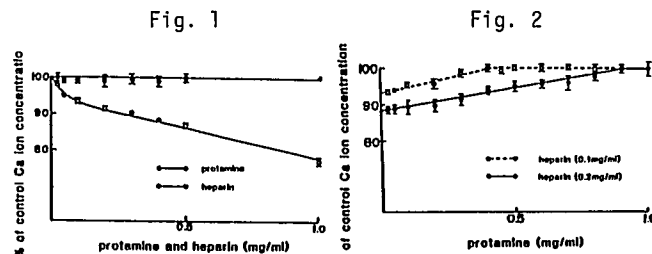
**Introduction:** Despite long-standing clinical experience, much controversy still exists concerning the exact mechanisms of the hypotensive effects of protamine sulfate and sodium heparin. It has been postulated that protamine induced hypotension is due in part to the reduction of ionized calcium ( $\text{Ca}^{++}$ ). Unlike protamine, heparin is mostly negatively charged, therefore it might be the heparin that decreases  $\text{Ca}^{++}$ . The purpose of this study was to examine the interaction of serum  $\text{Ca}^{++}$  with protamine and heparin, in vitro and in vivo.

**Methods:** In vitro study, Part 1: Serum was obtained from mongrel dogs. Solutions of distilled deionized water (DDW) which contained eight different concentrations of either protamine or heparin (Upjohn Co.) were prepared. 0.1 ml of each solution was added to 0.9 ml of serum, making a total volume of 1.0 ml. Final concentrations of protamine and heparin ranged from 0.025 mg/ml to 1.0 mg/ml. In vitro study, part 2: Protamine in DDW (total volume 0.1 ml) and heparin in DDW (total volume 0.1 ml) were added to 0.8 ml of serum, making a total volume of 1.0 ml. Heparin concentration was fixed at either 0.1 mg/ml or 0.2 mg/ml. Protamine concentration was varied as in Part 1. Serum  $\text{Ca}^{++}$  was measured five times for each sample using an ion specific electrode and percent changes of  $\text{Ca}^{++}$  levels were plotted against the protamine and heparin concentrations. In vivo study: Twelve mongrel dogs were anesthetized, intubated, paralyzed and ventilated with 100%  $\text{O}_2$  and enflurane 0.5-1.0%. A femoral arterial line was placed to allow sampling and to measure blood pressure continuously. After a steady state was achieved, six dogs received protamine 4 mg/kg i.v. over 30 seconds, and six other dogs received heparin 3 mg/kg i.v. over 30 seconds. Arterial blood was sampled for arterial gas tensions and pH, and  $\text{Ca}^{++}$  measurements. These were performed 5 min before and 1, 5 and 10 min after administration of each drug. Student's t test was used for statistical analysis.

**Results:** In vitro study: Even in high concentrations, protamine did not affect serum  $\text{Ca}^{++}$  levels (Fig. 1). Heparin, however, did decrease the  $\text{Ca}^{++}$  in a dose-dependent fashion. Initially decreased  $\text{Ca}^{++}$  levels by either 0.1 mg/ml or 0.2 mg/ml of heparin were restored toward the control level by increasing the concentration of protamine (Fig. 2). In vivo study: mean arterial pressure and heart rate decreased significantly at 1 min after protamine administration (Table 1). However, no change in  $\text{Ca}^{++}$  levels, blood gas tensions and pH were observed. After heparin administration, none of the parameters changed significantly (Table 2).

**Discussion:** In vitro part 1 study indicates that  $\text{Ca}^{++}$  does not bind to protamine molecules.

Protamine is in positively charged form in the serum, thus, there will be no electrostatic attraction between  $\text{Ca}^{++}$  and protamine molecules. On the other hand,  $\text{Ca}^{++}$  binds to negatively charged heparin, resulting in the dose-dependent reduction of  $\text{Ca}^{++}$ . However, in vivo, heparin 3 mg/kg, did not decrease  $\text{Ca}^{++}$  level. It is thought that in vivo,  $\text{Ca}^{++}$  levels decreased by heparin can be restored, since muscles and skeletal tissue might release  $\text{Ca}^{++}$ . In vitro part 2 study indicates that the electrostatic attraction between protamine and heparin is stronger than that between  $\text{Ca}^{++}$  and heparin. This may suggest that protamine administered for heparin reversal releases  $\text{Ca}^{++}$  that has been bound to heparin molecules. Our results of the in vivo protamine study agree with a study by others,<sup>1</sup> in which there was no significant change in  $\text{Ca}^{++}$  levels after protamine administration in man after extracorporeal circulation. In conclusion, results of this study indicate that the hypotension occasionally observed after protamine or heparin administration is not likely to be related to the ionized calcium.



Circles and vertical bars indicate mean  $\pm$  SE.

	$\text{Ca}^{++}$ (mM)	ABP	HR	$\text{PaO}_2$	$\text{PaCO}_2$	pH
Control	1.25 $\pm$ 0.05	100.0 $\pm$ 4.5	122.0 $\pm$ 3.3	505.8 $\pm$ 22.6	36.8 $\pm$ 4.0	7.34 $\pm$ 0.02
1 min	1.24 $\pm$ 0.03	51.0 $\pm$ 11.6*	113.6 $\pm$ 3.1*	505.2 $\pm$ 24.1	32.0 $\pm$ 3.6	7.36 $\pm$ 0.02
5 min	1.22 $\pm$ 0.04	89.0 $\pm$ 18.9	117.2 $\pm$ 4.5	475.2 $\pm$ 32.1	35.6 $\pm$ 3.3	7.32 $\pm$ 0.03
10 min	1.22 $\pm$ 0.06	116.3 $\pm$ 11.1	119.6 $\pm$ 4.8	485.2 $\pm$ 26.0	37.9 $\pm$ 4.1	7.29 $\pm$ 0.05

	$\text{Ca}^{++}$ (mM)	ABP	HR	$\text{PaO}_2$	$\text{PaCO}_2$	pH
Control	1.14 $\pm$ 0.10	99.0 $\pm$ 5.0	113.2 $\pm$ 5.2	508.2 $\pm$ 17.0	26.1 $\pm$ 1.3	7.42 $\pm$ 0.03
1 min	1.18 $\pm$ 0.08	102.6 $\pm$ 7.1	113.7 $\pm$ 5.2	508.3 $\pm$ 19.4	27.7 $\pm$ 3.3	7.42 $\pm$ 0.03
5 min	1.17 $\pm$ 0.08	103.7 $\pm$ 7.2	116.4 $\pm$ 4.4	497.8 $\pm$ 9.7	26.4 $\pm$ 2.9	7.43 $\pm$ 0.03
10 min	1.16 $\pm$ 0.07	111.3 $\pm$ 8.3	113.6 $\pm$ 6.5	512.7 $\pm$ 8.9	27.8 $\pm$ 3.8	7.44 $\pm$ 0.03

#### Reference:

1. Conahan TJ III, Andrews RW, MacVaugh H III: Cardiovascular effects of protamine sulfate in man. *Anesth Analg* 60:33-36, 1981