Title: INOTROPIC EFFECTS OF PROTAMINE SULFATE ON ISOLATED MAMMALIAN CARDIAC MUSCLES: MECHANISMS OF ACTION

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Introduction. Intravenous administration of protamine sulfate in humans may result in systemic hypotension, bradycardia, and pulmonary artery hypertension. In view of the controversy (1,2) regarding inotropic effects of protamine, the present study was undertaken 1) to fully document the effects of protamine sulfate on various parameters of cardiac muscle contractility determined in vitro, and 2) to investigate the mechanisms of such effects.

Methods. Papillary muscles were mounted in a temperature-controlled (30°C) muscle chamber (38 ml) that contained a physiological salt solution (mM): Na^+ 135; K^+ 5; Ca^{2+} 2.25; Mg^{2+} 1; Cl^- 103.5; HCO_3^- 24; HPO_4^{2-} 1; SO_4^{2-} 1; acetate^{-1} 20; glucose 10, continuously bubbled with 95% O_2 -5% CO_2. Muscles were held between a force-length servo transducer and a subminiature lucite clip with a built-in platinum punctate stimulation electrode (stimulus interval 4 seconds, 10% above threshold). After recovery, a cumulative dose-response curve for protamine sulfate (X-grade, Sigma) was established, hereby allowing for 30 minutes equilibration at each concentration. Subsequently, the bathing solution was quickly replaced and continuously washed (20 ml/min) for 120 minutes. At each concentration we determined from a preloaded isotonic, a "zero load clamp", and an isometric twitch: peak developed force (F), maximal rate of rise (+dF/dt) and of fall of force (-dF/dt), peak isotonic shortening (D_L), maximal unloaded velocity of shortening (MUVS), maximal isotonic relaxation velocity (-V), and corresponding values for time from stimulation. In further experiments we tested separately for α_1-, α_2-, and β-adrenoceptor agonist effects of protamine sulfate with Phentolamine HCl (10^{-5}M), Prazosin HCl (10^{-6}M), Yohimbine HCl (10^{-7}M), and Bupranolol (3 x 10^{-6}M). Student's t-test for paired data was used to test for significance ($p<0.05$) for each contractile and time parameter within each group, where muscles served as their own control. Between groups, contractile parameters were compared with two-sample Student's t-test. Data are mean ± SD.

Results. At the lower, clinically useful concentrations (0.001-0.003%), protamine exerted a modest positive inotropic effect, which was apparent in parameters of contraction (F, +dF/dt, MUVS, D_L; all $p<0.05$) without changing relaxation (-dF/dt, -V; both $p>0.05$). In addition, protamine caused an earlier onset of myofibrillar activation with tMUVS as its most sensitive indicator ($p<0.05$). All of these changes were blocked by α-adrenoceptor blockade with either phentolamine or prazosin. Similarly, previous β-adrenoceptor blockade blunted the positive inotropic response to protamine sulfate. Protamine concentrations ≥0.01% caused a dose-dependent depression of all contractile parameters, delayed the onset of myofibrillar activation and induced contracture, i.e. a shorter diastolic length or a higher diastolic force.

Discussion. This study demonstrates that in clinically useful concentrations, protamine sulfate exerts a modest positive inotropic effect in cat ventricular myocardium, an effect in which activation of α-adrenoceptors may play a role. These results are not necessarily in conflict with previously reported studies in dog (3) and rabbit (4) myocardium, especially in view of well-known interspecies heterogeneity in adrenoceptor density. Toxic concentrations of protamine sulfate (≥0.01%), such as would occur after very rapid intravenous administration, deteriorate myocardial function, possibly by interfering with excitation-contraction coupling and control of diastolic $[Ca^{2+}]$.

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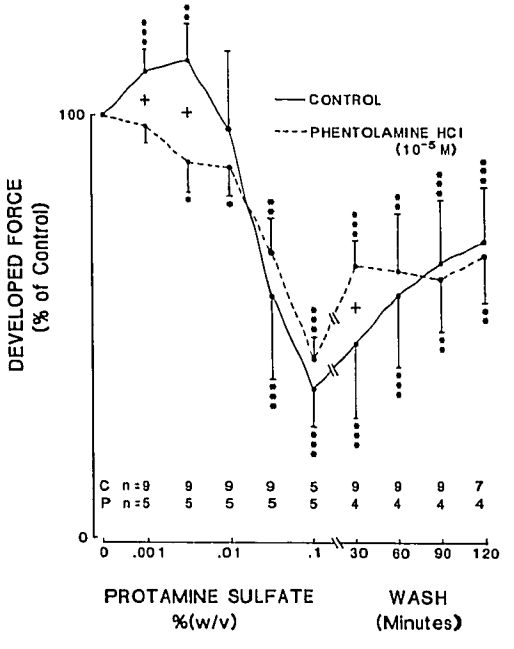

* within a group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$); + between groups (+ $p<0.001$).