Depression of Atrial Contractility by Peptides Isolated from Normal and Shock Plasma[1] (37526)

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Several investigators have reported an increase in the concentration of circulating peptides (1–5) or proteolytic enzymes (2, 6) in plasma obtained from animals during hemorrhagic or endotoxin shock. The pathological significance of these peptides during circulatory shock has not been clearly established (7–9). Lefer *et al.* have characterized a low molecular weight peptide called myocardial depressant factor (MDF) (10). MDF has been identified in several species of animals and in man in various forms of circulatory shock (11). *In vitro,* MDF-induced depression of ventricular contractile force. As a result, this peptide has been postulated to play a primary role in the development of heart failure during circulatory shock (12).

The present study was designed to (i) confirm the presence of cardiotoxic plasma peptides during circulatory shock and (ii) determine the effect of such peptides on atrial contractility.

Methods. Processing of plasma samples. Plasma samples were processed by a procedure similar to that developed by Lefer and associates (13). Each blood sample was placed on ice upon removal from the animal. All subsequent procedures were performed in a cold room maintained at 4°. The blood samples were centrifuged at 2500*g* for 10 min, and the formed elements discarded. Plasma samples were then either frozen for later use or were immediately subjected to ultrafiltration. Thirty milliliter samples were initially filtered through a stirred cell (Amicon Corp., Model 52), pressurized with 40–45 psi of nitrogen and equipped with a PM 30 diaflo membrane (Amicon Corp.). This membrane excluded molecules greater than 30,000 molecular weight. Employing the same stirred cell, the filtrate was passed through a UM2 membrane (Amicon Corp.), which excluded all molecules greater than 1000 molecular weight. The resulting ultrafiltrate thus contained molecules of less than 1000 molecular weight.

Ultrafiltrates were further processed in order to separate components according to their molecular weight. Ten-milliliter samples of ultrafiltrate were lyophilized to dryness. The residue was reconstituted in 2 ml of distilled water and centrifuged at 2500*g* for 15 min. The supernatant was applied to a 1.5 $\times$ 90 cm column packed with Bio-Gel P-2 (200–400 mesh) polyacrylamide gel suspended in dextrose-free Ringer–Locke solution. A flow rate of 0.16–0.23 ml/min was maintained through the column. Four-milliliter samples of eluate were collected by a Gilson Model VFC fraction collector. Each sample of column eluate was read on a photospectrometer (Zeiss, PMG II) at 230 nm, the optimal absorbance for the peptide bond. Optical density was plotted against elution volume (Fig. 1).

Atrial preparation. Rabbits weighing 1.5–2.5 kg were stunned by a blow to the head. Their hearts were immediately removed, and the left atria dissected free. The atria were then sutured to a platinum electrode and suspended in a 10 ml organ bath. The Ringer–Locke solution in the bath had the following composition: NaCl 154 m*M*, KCl 5.4 m*M*, $CaCl_2$ 2.4 m*M*, dextrose 11.0 m*M*, Na

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HCO_3 6.0 m*M* and $NaH_2PO_4 \cdot 18$ m*M* (pH 7.4). Temperature in the organ bath was maintained at 30°, and the solution continuously aerated with 95% O_2–5% CO_2. Tension (1.5–2.0 g) was applied to the atria to produce the maximum degree of contraction. Contractions were measured with a Grass FT 03 force–displacement transducer and recorded on a Sanborn Twin viso recorder. The atria were stimulated by a laboratory stimulator (American Electronic Laboratories, Model No. 194A) at a constant rate of 100/min (20 V, 1 msec duration). Each atrium was allowed to equilibrate for 45 min. The reduction in contractile force was measured as percent change from the original control tension. The viability of isolated rabbit atria bathed for 2 hr in oxygenated normal dog plasma or normal dog plasma ultrafiltrate was also tested.

Hemorrhagic shock procedure. Healthy male mongrel dogs, weighing 18–22 kg, were used for the shock experiments. The animals (fasted 18 hr prior to hemorrhage) were anesthetized with sodium pentobarbital (30 mg/kg, iv). Heparin (1000 units/kg) was given 20 min before bleeding, and control blood samples were taken just prior to bleedout. All of the dogs were bled from a femoral artery into a closed, siliconized reservoir at a preset hydrostatic pressure. The pressure in the reservoir was set to allow a mean arterial blood pressure (MABP) of 35–40 mm Hg. MABP was continuously monitored on a Grass Model 5D polygraph using a Statham P23Db pressure transducer. MABP was maintained at 35 to 40 mm Hg until 40% of the shed blood was spontaneously taken up by the dog (120–180 min). The remaining 60% of the shed blood was then slowly reinfused into the animal over a 15-min period. MABP approached control level after reinfusion, but deteriorated to 60 mm Hg over a period of 1–3 hr. Blood samples were taken at this time, and the experiment was terminated. Animals whose MABP remained above 60 mm Hg 5 hr after reinfusion were considered survivors, and no blood samples were taken. Eighty percent of the dogs subjected to this method of hemorrhagic shock died within the 5 hr period following reinfusion.

Results. The elution patterns of the control and shock ultrafiltrates are shown in Fig. 1. Each sample produced six principal peaks (peaks I–VI, in order of decreasing molecular weight). There was no appreciable difference in the pattern of elution between control and shock ultrafiltrates. Peaks I-VI of control ultrafiltrate were similar in size to peaks I–VI of shock ultrafiltrates, although peaks III and V were usually larger in shock ultrafiltrates.

The eluant solution (dextrose free Ringer–Locke solution) induced an insignificant depression (8%) of atrial contractile strength, and also the depressant activity induced by peaks I, II, IV, V and VI in both the control and shock ultrafiltrates was negligible (0–20% depression). However, peak III of control and of shock ultrafiltrates produced an appreciable depression. The peptide nature of peak III was confirmed by incubation in trypsin (10 μg/ml; 12 hr at 30°). The incubation abolished the depressant activity of control and shock peak III.

Table I summarizes the effect on atrial

TABLE I. Effect of Peak III Column Eluants of Normal and Shock Plasma on Atrial Contractile Strength.

	n^b	Depression of contractile strength (% of control)[a]
Dextrose free Ringer–Locke solution	12	8.7 ± 1.5
Control plasma peak III (6)[e]	6	39.9 ± 3.8[c]
Shock plasma peak III (6)[e]	6	48.6 ± 2.6[d]

[a] Control systolic contractile strength developed in normal Ringer–Locke solution varied from 1.5 to 2.0 g. Values are mean percent decreases in contractile strength ± SE.

[b] n is number of atria tested.

[c] Significantly greater than ($p < 0.05$) dextrose-free Ringer–Locke solution depression, but not different than shock plasma peak III depression.

[d] Significantly greater than ($p < 0.05$) dextrose-free Ringer–Locke solution depression, but not different than control plasma peak III depression.

[e] Both plasma peaks were tested in dextrose-free Ringer–Locke solution. Numbers in parentheses represent number of dogs used (one plasma sample obtained from each dog).

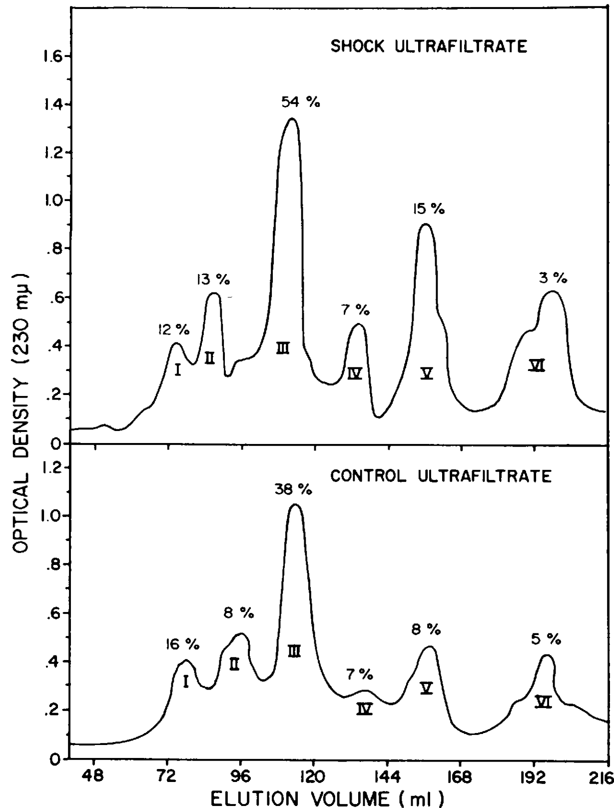

FIG. 1. Bio-Gel P-2 column elution pattern of plasma ultrafiltrates. The top figure is a column separation of a shock plasma ultrafiltrate. At the bottom is the elution pattern of a normal plasma ultrafiltrate. The values over each peak represent the percent of atrial contractile depression produced by that sample.

contractility induced by peak III from the control and shock ultrafiltrates. Control peak III and shock peak III both significantly depressed contractility. Although atrial depression produced by shock peak III was greater than the depression induced by control peak III, the difference in depression between the two was not significant.

The onset of depression with peak III was rapid, reaching maximum depression within two minutes. Contractility gradually returned to normal over a 30-min interval. Thus, the depression produced by the peak III samples was readily and completely reversible. Onset and duration of depression were similar with both shock and control peak III samples. Prolonged (30–45 min) and/or repeated exposure (5 times) to the depressant peak produced no further augmentation of the initial depression and did not effect the reversibility of the depression. Also there was no attenuation of control contractile strength after incubation of atria for 2 hr in oxygenated normal dog plasma or normal plasma ultrafiltrate.

Discussion. The existence of circulating peptides in both animals and man is well documented (1–5). Also, Lefer and associates have extensively investigated a peptide (myocardial depressant factor) which appears to increase in various forms of circulatory shock (11). These workers have demonstrated a marked depression of cat papillary muscle contractility and of isolated whole cat hearts (10, 14). Hinshaw did not observe a depression of myocardial contractility in isolated hearts perfused with blood from dogs subjected to endotoxin shock (15, 16). Ureschel *et al.* also failed to demonstrate a blood-borne cardiotoxic substance in

dogs in hemorrhagic shock (17).

The present experiments demonstrated the presence of a peptide (peak III) in shock dog plasma that induced depression of contractile strength of isolated atria. Although the depression produced by this peptide was significant, it was not significantly different from the depression of atrial contractility produced by the same peptide (peak III) isolated from control plasma. The atrial depression was spontaneously and completely reversible in the presence of the peptide (peak III) isolated from normal or shock plasma.

There is an obvious disparity between our results and those of Lefer *et al.* Discounting any variation in isolation techniques, this disparity may partially reflect differences in species and/or cardiac tissue response. Lefer has primarily assayed the cardiotoxic activity of MDF on isolated cat papillary muscle and isolated cat hearts. In contrast, the present study was performed with isolated rabbit atria. In spite of possible experimental differences, it is still difficult to account for the lack of difference between the depression produced by control plasma peak III and that atrial contractile depression seen with peak III isolated from shock plasma. Therefore, from the present study it is unreasonable to assume that this peptide *per se* could produce irreversible heart failure during circulatory shock. However, the fact that an atrial depressant peptide does exist in plasma suggests a possible interaction with other substances. Elevated plasma level of metabolic end products or abnormal electrolyte concentrations could potentiate the cardiotoxic action of the peptide *in vivo*. Also an interaction of the peptide with substances known to be released during circulatory shock, *viz.*, bradykinin (5), prostaglandins (18) and proteases (19), may contribute significantly to the heart failure of circulatory shock.

Summary. The presence of peptides in normal dog plasma and in plasma of dogs subjected to hemorrhagic shock has been investigated. The column chromatographic elution pattern for both normal and hemorrhagic plasma samples were similar. Of the six peptide peaks in the column elution pattern, only one (peak III) produced significant depression of atrial contractile strength. The depression produced by the shock peak III sample was not significantly greater than the contractile depression produced by control peak III. The results indicate that the atrial contractile depression produced by this peptide could only be significant during circulatory shock if it in some way interacted with other abnormal conditions existing during the shock state.

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