Differentiation of a Myocardial Depressant Factor Present in Shock Plasma from Known Plasma Peptides and Salts[1] (37039)

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A variety of substances including several biologically active peptides are released into the blood during circulatory shock. One such substance is a myocardial depressant factor (MDF) which appears to be a peptide with a molecular weight of 500–1000 (1, 2) and is produced primarily by the ischemic pancreas (3, 4) during several types of circulatory shock (5). The primary action of MDF is a marked negative inotropic effect on isolated cardiac tissue (6, 7) as well as on the intact heart (4). In addition, MDF exerts a selective vasoconstrictor effect on splanchnic vascular strips (8), depresses contractility of isolated intestinal strips (9), and perhaps contributes to the reticuloendothelial depression observed in shock (10).

In purifying MDF for studies of its chemical properties, we found that MDF activity could be eluted in a single peak from Bio-gel P-2 columns. The elution volume of the MDF peak was 80–100 ml (3, 11), not 120–150 ml as previously reported (2). This latter elution volume contains principally salts, some amino acids, and little MDF activity. Since improper calibration of the gel filtration column could result in elution of salt rather than MDF, the present study seeks to determine (a) whether salts could interfere with MDF activity, and (b) determine the relative inotropic activities of peptides which are released into plasma during shock to ascertain whether these peptides could account for MDF activity.

Methods. Cat papillary muscle preparation. Papillary muscles were removed from the right ventricle of pentobarbital anesthetized cats (35 mg/kg iv or ip) according to the method of Lefer, Manwaring and Verrier (12). Each muscle was electrically stimulated at an intensity of 2 V above threshold for a duration of 16.7 msec at frequency of 1 per sec and maintained at 37° in Krebs–Henseleit solution in which the bicarbonate concentration was reduced to achieve a pH 7.3 (12). The solution was continuously gassed with 95% O_2 and 5% CO_2. A length–tension relationship was established for each muscle, and the resting tension was set at a level just below that which yielded a maximum developed tension. Developed tensions were calculated in grams of force developed per square millimeter maximal cross-sectional area of the muscles.

Cat jejunal strip preparation. Jejunal segments were isolated from cats and washed free of internal contents by flushing with cold Krebs–Henseleit solution. The segments (6–12 cm long) were stored at 4° for 24 hr. Strips of jejunum (2 to 3 cm long by 5 to 7 mm wide) were dissected and placed in oxygenated (*i.e.*, 95% O_2 and 5% CO_2) Krebs–Henseleit solution at 37°. A resting tension of 1.0 to 1.4 was applied to the strips, and when a regular spontaneous rhythm occurred, drugs were added to the bath.

Drugs and chemicals. All drugs or substances were added directly to the bath in which the muscles were contracting. All drugs except bradykinin were freshly prepared from crystalline material for each experiment and were directly dissolved in Krebs–Henseleit solution. In the case of bradykinin, appropriate vehicle controls were used, although the diluent exerted no significant effect alone.

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All responses to drugs or chemicals were expressed in percent change in developed isometric tension from the pre-drug control values in both preparations.

Column chromatography. Samples of cat plasma [*e.g.*, from normal controls and from cats subjected to splanchnic artery occlusion (SAO) shock according to the method of Glenn and Lefer (11)] were deproteinized in 5% TCA according to the technique of Lefer and Barenholz (13). The TCA was removed from the samples by four to five extractions in ether, and the ether removed by bubbling with nitrogen. Ten milliliter aliquots of these clear deproteinized samples were lyophilized to dryness and reconstituted to 2 ml with distilled water. These 2 ml samples were then applied to Bio-gel P-2 columns according to the method of Lefer and Martin (2). Five milliliter fractions were eluted from the column with either glucose-free Krebs–Henseleit solution or distilled water. Peaks were determined by reading OD_{230} (for peptide bonds), by Ninhydrin analysis at OD_{570} (for free amino nitrogen groups), and for sodium ion concentration by flame photometry.

Assay of MDF. All peaks were assayed on cat papillary muscles for MDF activity according to previously standardized techniques (6) which are essentially the same as described in the papillary muscle section of the Methods above. One MDF unit is equal to a 1% decrease in developed tension of the papillary muscle under these standardized conditions.

Results. The inotropic effects of the major naturally occurring peptides used in this study (*i.e.*, angiotensin II, arginine vasopressin, bradykinin and eledoisin) are summarized in Fig. 1. The concentrations employed covered a range of 1000, and in all cases the maximal effect was obtained within this range. As shown in Fig. 1, angiotensin exerted a marked positive inotropic effect, vasopressin and bradykinin only a very small positive inotropic effect, and eledoisin exerted a marked negative inotropic effect. Since eledoisin is not present in mammals, all of the major peptides which are known to be released into the blood of mammals during hemorrhage or other forms of shock exert a positive inotropic effect.

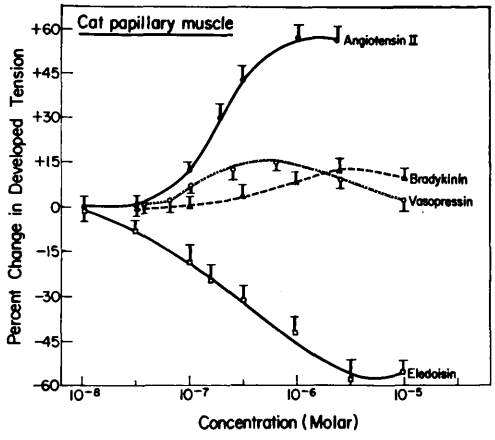

FIG. 1. Concentration–response relationships for angiotensin II, bradykinin, vasopressin, and eledoisin on isolated cat papillary muscle preparations. Each point is a mean response expressed as percentage change in developed tension for six separate muscles. Molar concentrations refer to the final concentration of each peptide in the 10 ml muscle bath. Brackets indicate ± SEM.

Figure 2 summarizes the concentration–response relationship of the same four peptides on intestinal smooth muscle. In this case, a concentration range of almost 10,000 was necessary to bracket the entire response to some of these peptides. Eledoisin and bradykinin stimulated the jejunal strips considerably, whereas angiotensin exerted a moderate stimulatory effect. In contrast, arginine vasopressin exerted no detectable effect on the jejunal strips in the concentration range employed in this study. Since eledoisin is found only in invertebrate species, none of the peptides which are released into the plasma during shock depress jejunal strips.

A series of seven synthetic bradykinin and kallidin derivatives were also tested for cardiac and jejunal activity. These compounds included glycyl^1-bradykinin, L-thr^6-bradykinin, L-lys^1-bradykinin, 1-desarg-bradykinin, *P*-fluoro-L-phe^8-bradykinin, des-pro^7-bradykinin, and L-lys^2-kallidin. All these derivatives stimulated the jejunal strips but always less than that obtained with bradykinin. Moreover, none of these compounds significantly altered developed tension of the papillary muscles, although a few occasionally stim-

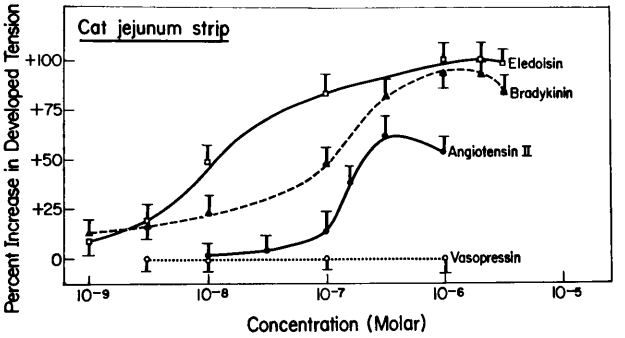

FIG. 2. Concentration–response relationships for eledoisin, bradykinin, angiotensin II and vasopressin on isolated cat jejunal strips. Each point is a mean response expressed as percentage increase in developed tension for six separate preparations. Molar concentrations refer to the final concentration of each peptide in the 20 ml muscle bath. Brackets indicate ± SEM.

ulated a papillary muscle by 5 to 10%. The concentrations used were identical to those employed for the other peptides.

Figure 3 illustrates a typical elution pattern of shock cat plasma. Four major peaks were observed using both a measure of peptide bonds (*i.e.*, OD_{230}) and amino nitrogen (*i.e.*, measured by Ninhydrin) to scan the column eluates. There are also two minor peaks, but these are probably portions of the major peaks. The major peaks were eluted at 45–55, 60–70, 85–95, and 120–135 ml. All these peaks were assayed for MDF activity and for Na^+ concentration. Most of the MDF activity could be recovered in the peak eluted at 85–95 ml. No other peak demonstrated significant MDF activity. Moreover, the peak containing the high MDF activity had a very low sodium ion concentration (*i.e.*, 12–15 mEq/liter). Indeed, most of the salt was found in the last major peak, which was eluted at 120–135 ml. Thus, there is a good separation between the active peptide material (as evidenced by peptide bond and by amino N activity) and the salt peak. Furthermore, significant amounts of MDF activity were found in only one of the peaks, indicating a good separation of MDF from all the other peaks. The low concentration of sodium (and thus of the other ions as well) indicate that the peak containing the MDF activity was effectively desalted by the column.

The amount of salt present in a 10 ml aliquot of deproteinized plasma sample is not enough to exert any significant inotropic effect on cat papillary muscles. Figure 4 illustrates the effect of Krebs–Henseleit solution on developed tension of papillary muscles. Increasing the salt concentration to twice that present in cat plasma (*i.e.*, to about 260 mEq/liter of sodium) increases developed tension by 10%. This modest increase in tension represents a balance between the

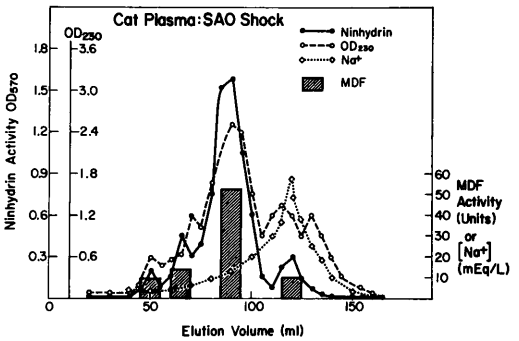

FIG. 3. Column elution pattern for a deproteinized plasma sample from a cat in splanchnic artery occlusion (SAO) shock. Ten milliliter of deproteinized plasma were added to the Bio-gel P-2 column, eluted in distilled water and collected in 5 ml fractions. (—) The plasma amino N activity pattern as determined by the ninhydrin reaction measured at 570 nm; (- -) the optical density readings at 230 nm which is optimal for peptide bonds; (• • • •) Na^+ measurements via flame photometry and expressed in mEq/liter. The bars indicate actual MDF bioassay results expressed in MDF units. The major MDF peak was eluted at 85 to 95 ml and the salt peak at 120–135 ml.

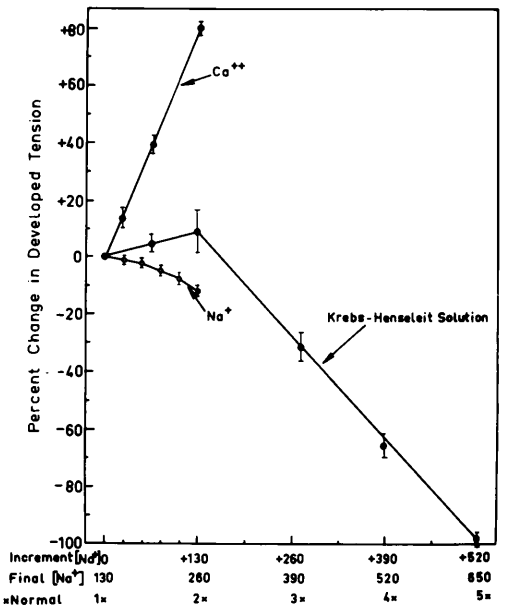

FIG. 4. Influence of salts present in Krebs–Henseleit solution on developed tension of isolated cat papillary muscles. Each point is the mean of 6 to 8 muscles; brackets indicate standard errors of the mean. Note that the Krebs–Henseleit curve is a summation of the calcium (Ca^{2+}) and sodium (Na^{+}) curves. Moreover, myocardial depressant activities comparable to that seen in samples containing MDF were not observed with salts until a sodium concentration of about 520 mEq/liter.

marked positive inotropic effect of calcium ions and the slight negative inotropic effect of sodium ions. Of course, both ions are present in plasma samples as well as in Krebs–Henseleit solution. Only when total sodium approaches 520 mEq/liter does the salt effect approximate the negative inotropic effect of MDF. The value of 520 mEq/liter represents about a fourfold concentration of plasma sodium. Such concentration is extremely unlikely to occur spontaneously. Indeed, proper preparation of the samples and elution of the gel filtration columns yield sodium concentrations of about 120 to 150 mEq/liter.

Discussion. Four naturally occurring and seven synthetic peptides were studied in isolated cardiac and intestinal smooth muscle preparations and their activities were compared to the known activity of a myocardial depressant factor which accumulates in plasma during shock. None of these peptides exhibited a biological profile comparable to MDF, although bradykinin, angiotensin and vasopressin are significantly elevated in shock plasma (4–6). Of the four naturally occurring peptides, only eledoisin was cardiodepressant, but it markedly stimulated intestinal smooth muscle in contrast to the inhibition of smooth muscle contractility induced by MDF. Clearly, MDF is not identical with any of these peptides. The fact that the known peptides which are released into the plasma during shock (*i.e.*, bradykinin, vasopressin and angiotensin) all exert moderate cardiotonic effects could be of positive survival value. However, each of these peptides exerts other actions which would be of no value or could be deleterious during shock. Thus, angiotensin and vasopressin are potent vasoconstrictor agents which constrict the splanchnic, renal and coronary vascular beds. Despite the fact that these peptides tend to increase arterial blood pressure in shock, they could cause ischemia and cellular damage in the constricted areas. In the case of bradykinin, vasodilation would result, but bradykinin is also known to increase capillary permeability and cause damage to the microcirculation. These latter actions would be deleterious in shock.

Plasma ions, particularly the cations, exert a profound effect on myocardial contractility. However, most cations are so well regulated that even in severe acute disease states such as shock their plasma concentration changes only slightly (6, 17). During circulatory shock, the plasma concentration of sodium decreases slightly (17), calcium remains unchanged (6) and potassium increases slightly (6). Moreover, MDF has previously been shown not to be associated with elevated potassium or with decreased calcium or hydrogen ion concentration (6, 18). The present studies clearly show that elevated sodium or total plasma osmolality could not account for MDF activity. Only by concentrating the plasma fourfold or greater could a salt effect approximate the MDF effect, and then only if the incorrect peak is taken. Thus, it is highly unlikely that salt could interfere with the assay of

MDF in column eluates.

Summary. Several potent biologically active peptides, some of which are found in mammalian plasma during circulatory shock, were studied for their cardiac and intestinal smooth muscle actions. None of the peptides studied over a concentration range of 10^{-9} to 10^{-5} *M* exhibited a biological profile similar to MDF. Moreover, MDF activity could not be accounted for by sodium or by total salts (*i.e.*, osmolality) since a concentration of at least four times normal total salts was required to exert a negative inotropic effect of a comparable magnitude to that induced by plasma concentrations of MDF in shock. Thus, MDF appears to be a small peptide not identifiable with common plasma peptides nor with salts.

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