Cardiac Actions of a Myocardial Depressant Factor Isolated from Shock Plasma[1] (35263)

ALAN R. THALINGER[2] AND ALLAN M. LEFER[3]

Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

A myocardial depressant factor (MDF) has been isolated from the plasma of cats and dogs in postoligemic (1, 2), endotoxic (3), cardiogenic (4), bowel ischemia (5), and pancreatitis (6) shock. MDF is a peptide having a molecular weight of about 800 to 1000 (7) and appears to result from the hydrolytic action of acid proteases which originate in the lysosomes of the pancreas (8, 9).

MDF depresses the contractility of isolated papillary muscles (2, 9) and constricts superior mesenteric artery strips (10). By virtue of these two actions, MDF triggers a positive feedback reaction. Thus it depresses the heart and further aggravates the splanchnic ischemia resulting in the production of additional MDF which further depresses the heart. The subcellular mechanism of the depressant effect upon the heart is not known, although MDF does not interfere with the electrophysiological properties of isolated papillary muscles (11).

The purpose of this study was to determine the effect of a highly purified preparation of MDF on the isolated perfused cat heart in order to differentiate the coronary vascular, dromotropic, and inotropic effects of MDF under conditions of controlled heart rate, coronary perfusion pressure, and cardiac preload.

Methods. Perfused heart procedures. Cats, 1.9 to 3.8 kg in weight, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and heparinized (1500 units/kg) prior to thoracotomy. The aorta was cannulated, and the heart was excised and transferred to a Langendorff perfusion apparatus. During this procedure, the coronary flow was interrupted for less than 30 sec. The Langendorff preparation used was similar to that described by Anderson and Craver (12) except for the addition of a secondary perfusion system, in parallel to the primary system, to recirculate the cardiac effluent, by means of a roller pump, back to the reservoir above the heart. The two systems could be interchanged by adjusting a three-way stopcock. The perfusion pressure of both systems was maintained at 30 mm Hg by regulating the height of a column of perfusion fluid. The heart was perfused with a modified Krebs–Henseleit (KH) solution (13) maintained at 37° and was gassed with a mixture of 95% O_2 and 5% CO_2.

A ligature from the apex of the heart to a Grass FT-03 force transducer was used to measure cardiac contractile force (CCF). The heart was paced through electrodes sutured to both atria. In a few experiments, electrocardiograms (ECG) were monitored through leads sutured onto the left ventricle and were continuously recorded on a Beckman Type R Dynograph. A length–tension relationship was established for each heart, and the resting tension was set just below the point of maximal developed tension. Heart rate was measured by counting the beats per minute on the CCF tracing. Coronary flow (CF) was periodically determined by collecting and measuring the cardiac effluent during timed intervals.

After the preparation had achieved a stable level of function, perfusion was accomplished by the recirculating perfusion system which contained 25 ml of KH solution. After a 5-min equilibration period, the control peak D_1, in a volume of 1.0 to 3.0 ml, was added

[1] Supported by a Grant-in-Aid from the American Heart Association.

[2] Medical Student Research Fellow.

[3] Established Investigator of the American Heart Association.

to the perfusion fluid. The heart was continuously exposed to the control peak D_1 for 10 min. Perfusion was then switched to the primary perfusion system containing fresh KH solution. The heart was then allowed to stabilize in the absence of MDF. The same procedure was followed for the purified shock peak D_1. Each heart was exposed to only one control and one shock peak D_1.

Preparation of plasma extracts. Arterial blood was drawn from cats in postoligemic shock (15–30 min prior to death) and from sham shock cats 3 to 4 hr after completion of all surgery. The blood was centrifuged at 2200*g* for 20 min at 4° and the plasma was decanted. The plasma was then ultrafiltered for 24–48 hr at 220 mm Hg and 4° in Nojax dialysis tubing. Clear ultrafiltrates were obtained which contained all the MDF activity of whole plasma.

Ultrafiltrates of plasma were applied to a Bio-gel P-2 (200–400 mesh) column and eluted in Krebs–Henseleit solution according to the method of Lefer and Martin (7). The samples were eluted in KH solution and pooled into 6 peaks (O.D. 230 mμ) designated peaks A through F in descending order of molecular weight. Each peak was assayed for MDF activity on cat papillary muscles. In previous studies, angiotensin (7) and brandykinin (6) were found in peak C. Catecholamines and serotonin, if not inactivated by processing, would appear in peak F (7). Therefore, none of the commonly occurring cardioactive substances usually found in plasma would be present in peak D. Samples of peak D (mol wt 800 to 1000) containing all the MDF activity of the original plasma, were applied to a Dowex 50 column and eluted in acetate gradient from pH 3.1 to 5.0 according to the method of Elzinga (14). The first peak eluted from the Dowex 50 column is designated as peak D_1 and contained all the activity of peak D. Two other peaks obtained, D_2 and D_3, were devoid of MDF activity. This highly purified extract, peak D_1, was used in this study on the isolated heart.

Ultrafiltrates of plasma from sham shock animals, which had insignificant MDF activity, were processed on the columns in the same manner and used as controls. Activity of peak D and peak D_1 samples was determined by direct bioassay on isolated cat papillary muscles. MDF activity is defined in units; 1 unit equals a 1% decrease in developed tension of the isolated papillary muscle. All shock peak D samples were found to have at least 53 units of activity, and all peak D_1 samples contained at least 68 units of activity.

TABLE I. Initial Control Values for Isolated Perfused Cat Hearts.[a]

Heart rate (beats/min)	124.0 ± 6.8
Coronary flow (ml/min)	15.0 ± 1.2
Cardiac contractile force[b] (g)	31.1 ± 2.4
Normalized contractile force (g/g of heart wt)	2.14 ± 0.15

[a] All values are means ± SEM for 14 hearts.

[b] Measured as the difference between peak developed force and resting force.

Results. The isolated perfused cat heart proved to be a relatively stable preparation yielding reproducible results. After establishment of an active length–tension relationship, all hearts studied exhibited comparable levels of mechanical performance. Table I summarizes the cardiodynamic values for these hearts. These values are comparable to those previously found for isolated perfused cat hearts (15) and remained at a relatively constant level of performance for about 60 min. Performance then gradually declined over the next 120 min. Therefore, measurements were made during the initial 60-min period of stable cardiac function.

Table II summarizes the cardiac effects of samples of peak D_1 from control and from shock cats in perfused hearts paced at constant heart rates. The final concentration of MDF in the peak D_1 was equivalent to the plasma MDF concentration of cats during the late stages of postoligemic shock. The major effects observed were a marked reduction in cardiac contractile force ($p<0.001$), and a significant increase in coronary flow ($p<0.01$). Control peak D_1 samples did not exert significant effects on these variables. Cardiac arrest occurred in seven of the eight hearts within 1 to 2 min after addition of peak D_1 to the perfusion fluid. Following a 15 to 90-sec period of total cardiac arrest, the

TABLE II. Cardiac Effects of Control Peak D_1 and Purified Shock Peak D_1.[a]

	Control peak D_1	Shock peak D_1	Significance of difference
Cardiac contractile force (% change)	-11.7 ± 7.2 (9)	-82.5 ± 3.1 (8)	$p < 0.001$
Coronary flow (% change)	$+3.9 \pm 5.0$ (9)	$+34.5 \pm 6.3$ (5)	$p < 0.01$
Incidence of cardiac arrest	0/9	7/8	
Incidence of postarrest A-V conduction block	0/9	8/8	

[a] All values are means $\pm$ SEM. Number of hearts used is given in parentheses. See text for definition of shock peak D_1.

atria started beating at the paced rate but the ventricles remained quiescent. This was termed "post-arrest A-V conduction block," and occurred in all eight of the hearts perfused with shock peak D_1.

Figure 1 graphically depicts a representative response of a perfused cat heart to a shock peak D_1. This heart exhibited a decrease in contractile force of 85%, 50 sec after addition of the extract. During this negative inotropic response, no escape from the paced heart rate occurred. At this time, two strong ventricular contractions occurred followed by a 15-sec period of total ventricular arrest despite continued electrical stimulation. During this period of ventricular arrest, atrial beats occurred which were not recorded on the oscillograph. Twenty-eight sec after the onset of cardiac arrest, weak ventricular contractions reappeared. These weak contractions became progessively stronger, so that 55 sec after the onset of the first detectable beat, contractile force was 47% of the pre-MDF value. The tracing shows that the post-arrest ventricular rate was slower than the controlled paced rate, indicating the continuing A–V conduction block.

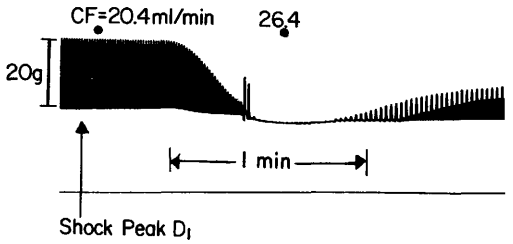

FIG. 1. Representative tracing depicting the cardiac contractile force of an isolated perfused cat heart in response to a plasma extract which contained MDF (shock peak D_1). Calibration marks indicate 20 g of force (ordinate) and 1 min of time (abscissa). The horizontal line under the tracing represents 0 g of force. Coronary flow (CF) values (ml/min), prior to additions of MDF and at the peak of the negative inotropic effect, are given. The heart was paced at a rate of 150 beats/min, maintained at coronary perfusion pressure of 30 mm Hg and set at a resting tension of 28 g. A marked negative inotropic effect occurred within 1 min of addition of the extract to the perfusion solution. Most of this latent period represented transit time from the injection site to the heart. Cardiac contractile force started to decline within 5 sec after the extract reached the heart. Coronary flow increased as cardiac contractile force decreased. Cardiac arrest occurred 20 sec after the onset of the negative inotropic effect. No detectable arrhythmias resulted until after the recovery from cardiac arrest, however, a complete A–V conduction block occurred after cardiac arrest.

In four additional experiments, peak D_1 from shock cats was added to the perfusion fluid in unpaced hearts with direct ECG recording electrodes on the right atrium and left ventricle. These hearts exhibited very similar responses to active peak D_1 comparable to those of the paced hearts. In spite of the dramatic decreases in cardiac contractile force and the moderate increases in coronary flow, no arrhythmias or rate changes occurred until after the complete negative inotropic effect (cardiac arrest). After cardiac arrest, several types of arrhythmias occurred, predominately premature ventricular contractions and A–V conduction block. During the occurrence of these arrhythmias, cardiac contractile force gradually increased toward control values. However, within the 10-min observation period, cardiac contractile force did not completely return to control values.

Thus, similar responses to MDF were obtained in unpaced hearts in which continuous ECG records were obtained.

Discussion. Impairment of myocardial contractility leading to cardiac failure has been implicated in the pathogenesis of postoligemic shock (16–18). The mechanism for this cardiac depression is not well understood. One hypothesis which has been advanced by several investigators (19, 20) is that a toxic substance released during oligemia and/or postoligemia acts to depress the myocardium. We have evidence that a myocardial depressant factor is elaborated during hemorrhagic (2, 9) as well as other forms of shock (3–6, 9). In postoligemic shock, hemodialysis resulted in the removal of MDF from the blood as well as in the significant improvement of myocardial performance (21).

The present data indicate that MDF exerts a direct negative inotropic effect in the isolated perfused heart. The isolated heart appears to be more sensitive to the effects of MDF than the *in situ* heart, perhaps because the isolated heart received the entire dose of MDF and no means of inactivating or excreting the MDF were available to it. The cardiodepressant effect occurred in spite of an increase in coronary blood flow and prior to the onset of cardiac arrhythmias. The increase in coronary flow is apparently a coronary vasodilation, possibly in response to an increase in myocardial metabolic activity brought about by the MDF. Cardiac arrest was a frequent consequence of the negative inotropic effects of MDF, reflecting a progressive decrease in contractile force rather than a sudden conduction block. These effects occurred in the presence of concentrations of MDF comparable to that measured in the plasma of cats in late postoligemic shock. Addition of a comparable plasma extract from sham shock cats exerted insignificant effects on the isolated perfused cat heart. Since heart rate, coronary perfusion pressure, and resting cardiac tension were maintained constant in each heart, the negative inotropic effect observed with the extract from shock cat plasma can be considered to be a valid manifestation of a decrease in myocardial contractility.

Previous investigation has revealed that MDF does not alter the magnitude of the transmembrane resting or action potential in isolated cat papillary muscles determined by the use of intracellular microelectrodes (11). These experiments indicate that MDF exerted an effect directly on the contractile machinery of the myocardial cell or on the process of excitation–contractile coupling (11). The present results do not alter this conclusion. However, the results eliminate the possibilities that MDF acts in the whole heart primarily on the coronary vasculature or on the special conduction system of the heart. These data lend further support to the contention that the myocardial depression which occurs in postoligemic shock can be explained largely on the basis of a humoral cardiodepressant factor.

Therapeutic management of shock should take into account the presence of MDF in shock. However, caution must be used in extrapolation to clinical shock since cardiotonic agents may exert deleterious effects in shock in addition to the primary stimulation of the heart. For example, cardiac glycosides such as ouabain constrict the splanchnic vasculature (22) in addition to stimulating myocardial contractility. Since MDF originates in the ischemic splanchnic region, this secondary action would have the undesired effect of enhancing the further production of a substance which depresses the heart. This, in fact, may explain the lack of success of digitalis preparations in the therapy of shock.

Summary. A myocardial depressant factor (MDF) which appears in the plasma during a variety of forms of shock was tested on the isolated perfused cat heart (Langendorff preparation) for cardiotoxic properties. MDF exerted a marked negative inotropic effect (83% decrease in cardiac contractile force) under conditions of controlled heart rate, coronary perfusion pressure, and resting tension. Coronary flow increased an average of 35% during this rapid decline in contractile force. Cardiac arrest occurred in 88% of the experiments within 1 to 2 min after addition of the plasma extract containing MDF to the perfusate. As contractile activity reappeared following cardiac arrest, A–V blocks frequently occurred. These results indicate that

MDF has a direct negative inotropic effect on the isolated cat heart. This cardiotoxic effect is not a result of restriction of coronary flow, since coronary flow increased during the negative inotropic effect, nor of the induction of arrhythmias, since the arrhythmias (*e.g.*, A–V block and premature ventricular contractions) occurred after the negative inotropic effect had occurred.

The authors acknowledge the expert technical assistance of Thomas F. Inge, Jr., and thank Dr. Marshall Elzinga of the Retina Foundation, Boston, Mass. for his preparation of the purified D_1 peaks.

1. Brand, E. D., and Lefer, A. M., Proc. Soc. Exp. Biol. Med. **122**, 200 (1966).

2. Lefer, A. M., Cowgill, R., Marshall, F. F., Hall, L. M., and Brand, E. D., Amer. J. Physiol. **213**, 492 (1967).

3. Wangensteen, S. L., Geissinger, W. T., Lovett, W. L., Glenn, T. M., and Lefer, A. M., Surgery (1971) in press.

4. Glenn, T. M., Lefer, A. M., Morris, J. N., Jr., Martin, J. B., Lovett, W. L., and Wangensteen, S. L., Amer. Heart J. in press.

5. Lefer, A. M., and Glenn, T. M., *in* "Vascular Aspects of Gastrointestinal Disease" (S. Boley, ed.). Appleton-Century-Crofts, New York (1971) in press.

6. Lefer, A. M., Glenn, T. M., O'Neill, T. J., Lovett, W. L., Geissinger, W. T., and Wangensteen, S. L., Surgery (1971) in press.

7. Lefer, A. M., and Martin, J., Circ. Res. **26**, 59 (1970).

8. Lefer, A. M., and Martin, J., Amer. J. Physiol. **218**, 1423 (1970).

9. Lefer, A. M., Fed. Proc. **29**, 1836 (1970).

10. Glucksman, E. E., and Lefer, A. M., Amer. J. Physiol. (1971) in press.

11. Lefer, A. M., and Rovetto, M. J., Proc. Soc. Exp. Biol. Med. **134**, 269 (1970).

12. Anderson, F. F., and Craver, B. M., J. Pharmacol. Exp. Ther. **93**, 135 (1948).

13. Lefer, A. M., J. Pharmacol. Exp. Ther. **151**, 294 (1966).

14. Elzinga, M., Biochemistry **9**, 1365 (1970).

15. Lefer, A. M., and Sayers, G., Amer. J. Physiol. **208**, 649 (1965).

16. Gomez, O. A., and Hamilton, W. F., Circ. Res. **14**, 327 (1964).

17. Siegel, H. W., and Downing, S. E., Amer. J. Physiol. **218**, 722 (1970).

18. Crowell, J. W., and Guyton, A. C., Amer. J. Physiol. **208**, 248 (1962).

19. Rothe, C. F., and Selkurt, E. E., Amer. J. Physiol. **207**, 203 (1964).

20. Fukuda, T., Nature (London) **205**, 392 (1965).

21. Wangensteen, S. L., deHoll, J. D., Kiechel, S. F., Martin, J., and Lefer, A. M., Surgery **67**, 935 (1970).

22. Harrison, L. A., Blascke, J., Phillips, R. S., Price, W. E., deV. Cotten, M., and Jacobsen, E. D., J. Pharmacol. Exp. Ther. **169**, 321 (1969).

Received Sept. 8, 1970. P.S.E.B.M., 1971, Vol. 136.