Influence of a Myocardial Depressant Factor on Physiologic Properties of Cardiac Muscle[1] (34774)

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A myocardial depressant factor (MDF) has been found in the plasma of cats in postoligemic shock (1, 2). MDF has been shown to be a peptide having a molecular weight of 800–1000 (3, 4), which is produced primarily by the ischemic pancreas (5). The nature of the precise trigger mechanism for the release of MDF is unknown. MDF is known to depress the developed tension of isolated cardiac muscle (2) and may depress the function of the reticuloendothelial system (6). The mechanism by which MDF exerts a negative inotropic effect is not known. The purposes of the present investigation were to determine the electrophysiologic effects of MDF in isolated cardiac tissue, and to correlate them with the mechanical effects in order to determine if MDF depresses cardiac tissue by altering membrane properties or by some other mechanism.

Methods. Papillary muscles from the right ventricles of cats were removed as previously described (2). The muscles were then placed in a 15-ml bath containing modified Kreb's Henseleit (KH) solution oxygenated with 95% O_2 and 5% CO_2 at 37 ± 0.5°. The composition of the KH solution in millimoles was KCl, 4.75; KH_2PO_4, 1.19; $MgSO_4$, 1.19; $CaCl_2$, 2.54; NaCl, 118; $NaHCO_3$, 12.50; and glucose 10. The muscles were stimulated via bipolar platinum electrodes at 2.5 times threshold, 1 msec duration, and at a frequency of 1/sec. Stimulation was sufficient to produce maximum developed tension. The resting tension was adjusted until further increases failed to produce greater developed tension. Muscle tension was displayed on one beam of a Tektronix 502A dual-beam oscilloscope utilizing the bridge circuit of a Beckman RB Type Dynograph and a Grass FT-03 force transducer.

Transmembrane potentials were measured using glass microelectrodes. They were filled by Tasaki's method (7) with 3 *M* KCl. The tip diameters of the electrodes were 1μ or less and the electrode resistances were 15–40 MΩ. The electrical activity was displayed on the second beam of the oscilloscope and was photographed with the accompanying contraction.

After a 30- to 40-min equilibration period, mechanical and electrical activities were measured. This recording period was designated as the control period. The KH media in the bath was then replaced with KH solution which contained myocardial depressant factor (MDF) eluted on a Bio-gel P-2 column from 10 ml of cat plasma. The MDF was purified by ultrafiltration and gel filtration chromatography as previously described (4, 5, 8). Developed tension and transmembrane potentials were again recorded from 30 sec to 11 min after addition of MDF. At the end of this recording period, the muscle was rinsed several times with fresh KH solution over a period of 30 min before further observations were made.

Another series of papillary muscles were subjected to additions of calcium in the presence of MDF. After a maximum negative inotropic effect had been achieved with MDF, small increments of a concentrated $CaCl_2$ solution were added to the bath to increase the total calcium concentration stepwise from 2.5 to 15 m*M*.

Results. The effects of extracts of plasma

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TABLE I. Effects of MDF on Mechanical and Electrical Properties of Isolated Cat Papillary Muscles.

	Pre-MDF control	Maximum MDF effect	p value
Resting membrane potential (mV)	74.7 ± 1.3	74.4 ± 0.7	NS
Amplitude of action potential (mV)	83.7 ± 2.2	81.6 ± 2.6	NS
Duration of action potential[a] (msec)	156.5 ± 6.2	192.3 ± 6.9	$p < .01$
Developed tension (g/mm²)	3.24 ± 0.32	1.41 ± 0.18	$p < .001$

All values are means ± SEM for six muscles.

Muscles were maintained at 37° and stimulated at a frequency of 1/sec.

Maximum MDF effect obtained 6–11 min after addition of MDF to the bath.

[a] Duration measured from start of upstroke of action potential to a point equal to 80% repolarization back to the resting membrane potential.

from cats in late postoligemic shock, containing purified MDF, on the electrical and mechanical properties of isolated cat papillary muscles are summarized in Table I. MDF had no significant effect on the amplitude of either the resting membrane potential or the action potential. These values were obtained at the peak of the negative inotropic effect, approximately 6–11 min after addition of MDF to the bath. The developed tension had decreased an average of 58% at this time. Nevertheless, the duration of the action potential was significantly prolonged (*i.e.*, an average of 36 msec) at the peak of the MDF effect ($p < .01$).

Figure 1 illustrates a typical electrical and mechanical response of a cat papillary muscle to the addition of MDF. It can be seen that no obvious changes occurred in the amplitude of the resting or action potentials when MDF exerted a negative inotropic effect. Furthermore, MDF produced a relatively rapid effect on the duration of the action potential, so that a significant prolongation occurred by 180 sec. However, the maximum decrease in developed tension occurred at 6 min. The tracings shown were obtained from a continuous recording from a single cell near the surface of the papillary muscle. Recordings taken from cells throughout the muscle mass yielded essentially the same results.

In another series of experiments, purified extracts of plasma containing MDF were added to papillary muscles. After the resultant negative inotropic effect reached a stable maximum level, $CaCl_2$ was added to increase the external calcium concentration in a stepwise manner from 2.5 to 15.0 m*M*. Table II summarizes the effects of the addition of $CaCl_2$ on the contractile response to MDF. It is evident that even a small increment in the calcium concentration (*i.e.*, from 2.5 to 3.0 m*M*) exerted a noticeable increase in tension development. This was a rapid phenomenon, having a latency of 5–10 sec and reaching a plateau in 30–45 sec. Further increases in calcium exerted additional increases in tension development, so that at a calcium concentration of 7.5 m*M*, the effect of MDF had been completely reversed. Figure 2 shows a typical recording of this phenomenon.

A concentration of 15.0 m*M* (or six times the calcium concentration of the KH solu-

TABLE II. Effect of Calcium on Reversal of the Negative Inotropic Effect of MDF.

Calcium concentration (m*M*)	Presence of MDF	Developed tension (% of control)
2.5	0	100.0
2.5	+	36.0 ± 4.0
3.0	+	44.5 ± 3.7
3.5	+	63.9 ± 7.9
7.5	+	95.8 ± 6.6
15.0	+	126.6 ± 8.1

All values are mean equilibrium values ± SEM for eight muscles.

Mean control developed tension for the eight muscles was 1.88 ± 0.24 g/mm².

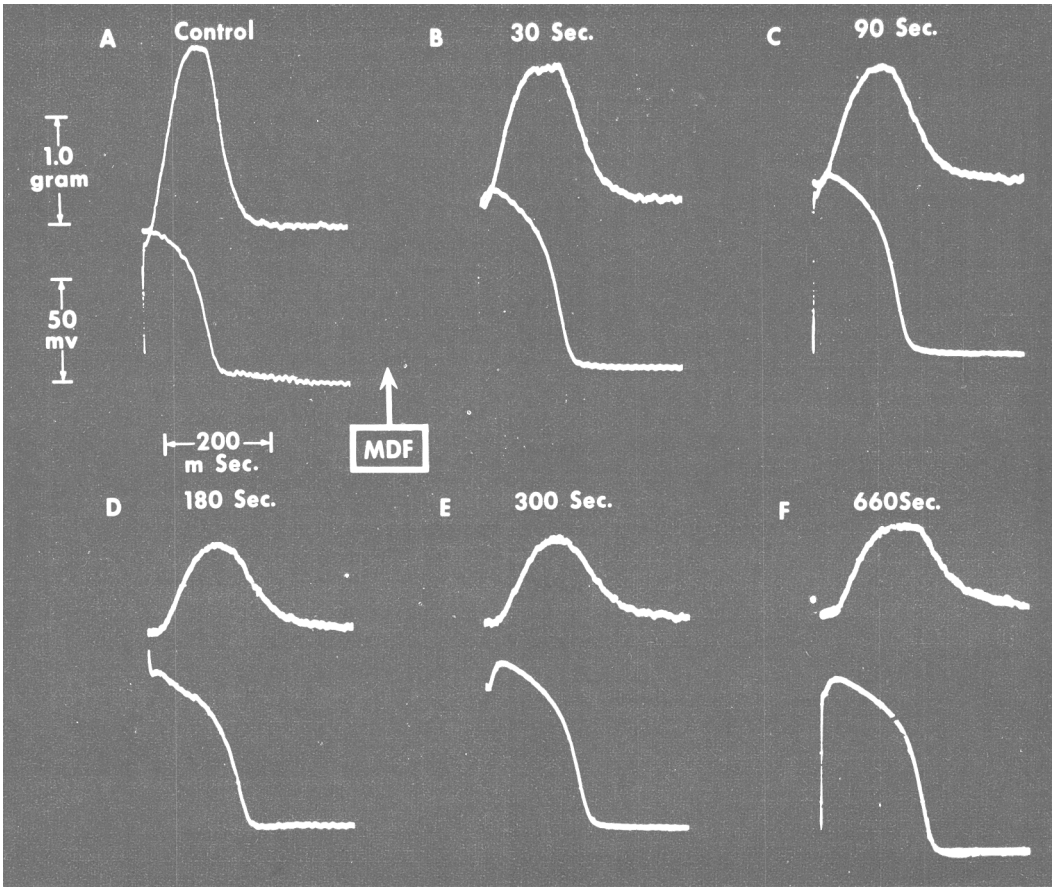

FIG. 1. Typical unretouched oscilloscope recording of contractile force (upper tracing) and the concomitant action potential of a single papillary muscle cell at different time periods from just prior to addition of MDF to 11 min after addition of MDF to the muscle bath. The muscle was electrically driven at a frequency of 1/sec at 37°.

tion) increased the tension significantly above that of the pre-MDF control value. The positive inotropic effects of higher concentrations of calcium were exerted within the same time as the smallest increment in calcium. Thus, calcium can reverse the depressant effect of MDF in isolated cat papillary muscles.

Discussion. The role of toxic factors is becoming increasingly important in the pathogenesis of hemorrhagic shock (4, 5, 9–11). A myocardial depressant factor (MDF) has been shown to depress the contractile response of cardiac muscle (2), the blood pressure of intact cats (3), and the phagocytic function of the reticuloendothelial system (6). The complete spectrum of activities of MDF is probably not fully known. However, since myocardial depression is commonly observed in the late stages of hemorrhagic shock (9, 12–16) the mechanism of this depression is of direct importance in understanding this phenomenon.

The data in this report indicate that the mechanism of the negative inotropic effect of MDF is not via electrical properties of the cardiac muscle cell membrane. This is based on the observations that the resting membrane and action potential undergo no significant alteration in magnitude at any time during the negative inotropic effect of MDF. The only significant effect of MDF on the electrophysiological properties studied was a prolongation of phase 2 (*i.e.*, the plateau) of the

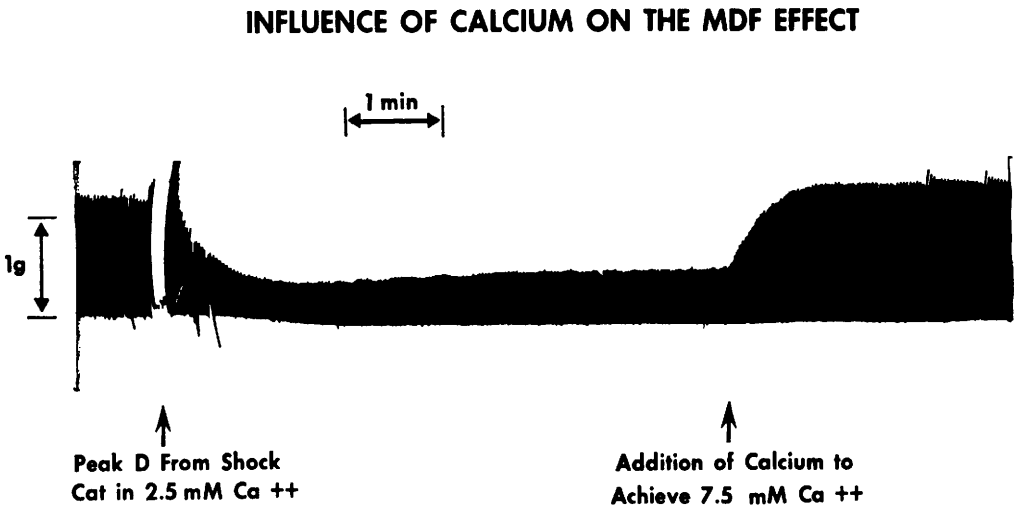

FIG. 2. Typical response of an isolated cat papillary muscle to a column eluate containing MDF (first arrow) showing the negative inotropic effect of MDF. The peak depressant activity of 68% occurred within 2 min and after a slight increase leveled off at 7 min to a 53% depression. At that point (second arrow), 0.5 ml of a concentrated $CaCl_2$ solution was added to the bath to elevate the final calcium concentration to 7.5 m*M*. The tension increased back to control levels within 1 min.

action potential. However, this would tend to increase developed tension, since a prolongation of the action potential would presumably increase the duration of the active state (17). Thus, none of the data presented here can be interpreted as evidence for a failure of the membrane excitability mechanisms in response to MDF.

Thus, one must look to other processes for the mechanism of the negative inotropic effect of MDF (*i.e.*, excitation-contraction coupling, direct effect on the contractile machinery, or other mechanisms. The reversal of the negative inotropic effect by free calcium ions tends to substantiate this concept. However, since Ca^{2+} would increase contractility under any conditions, it does not differentiate between these two possibilities.

It is of interest that the phenomena reported here were not frequency-dependent, since the same relationships occurred at stimulation frequencies of 0.5 and 1.0/sec. That is, a similar negative inotropic effect occurred in the absence of any electrophysiological change other than a prolongation of the action potential at both frequencies.

The negative inotropic effect of MDF commences within 30 sec but requires 6–11 min to reach a maximum. This negative inotropic effect can be completely reversed by one or two washes with KH solution. It is interesting that calcium restores developed tension more rapidly than the decline in developed tension in response to MDF.

The mechanism of MDF action may involve binding of free calcium in the sarcoplasm or sarcoplasmic reticulum; however such an explanation requires additional evidence. One must use caution in the interpretation of the calcium results, since these data do not conclusively show that MDF competitively blocks the intracellular actions of calcium. The additional calcium added to the bath may circumvent a depressant effect of MDF which may act via a non-calcium-dependent mechanism.

It is of interest that another myocardial toxic agent, streptolysin 0, was also without electrophysiological effect in isolated electrically driven ventricular myocardial preparations (18). However, the cardiotoxic effect of streptolysin 0 appears to be related to a disruption of the cardiac conduction system and not to a basic defect in the contractile machinery of the myocardium (18).

Summary. The electrophysiological effects of a myocardial depressant factor (MDF) were studied on isolated cat papillary muscles.

MDF decreased developed tension 58% within 6–11 min. There were no significant changes in the amplitude of either the resting membrane potential or the action potential of individual myocardial cells over this 11-min period. There was a significant prolongation of the action potential within 3 min of addition of MDF to the papillary muscles. However, this prolongation of the action potential cannot account for the negative inotropic effect of MDF. Addition of free calcium to the bathing medium from 2.5 m*M* to 7.5 m*M* completely and rapidly reversed the negative inotropic effect of MDF. We conclude that MDF acts at a site other than the excitation of the cell membrane, possibly by depressing excitation-contraction coupling or by impairing the contractile machinery directly.

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. Lefer, A. M., and Martin, J., Amer. J. Physiol. **216**, 314 (1969).

4. Lefer, A. M., and Martin, J., Circ. Res. **26**, 59 1970.

5. Lefer, A. M., and Martin, J., Amer. J. Physiol. In press, 1970.

6. Lefer, A. M., and Blattberg, B., J. Reticuloendothel. Soc. **5**, 54 (1968).

7. Tasaki, K., Tsukahara, Y., Ito, S., Wagner, M. J., and Yu, W. Y., Physiol. Behav. **3**, 1009 (1968).

8. Brand, E. D., Cowgill, R., and Lefer, A. M., J. Trauma. **9**, 216 (1969).

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

10. Fukuda, T., Nature **205**, 392 (1965).

11. Williams, L. F., Goldberg, A. H., Polansky, B. J., and Byrne, J. J., J. Surg. Res. **9**, 319 (1969).

12. Crowell, J. W., and Guyton, A. C., Amer. J. Physiol. **201**, 893 (1961).

13. Regan, T. J., LaForce, F. M., Teres, D., Block, J., and Hellems, H. K., Amer. J. Physiol. **208**, 938 (1965).

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

15. Wiggers, C. J., Amer. Heart J. **33**, 633 (1947).

16. Lefer, A. M., Craddock, G. B., Cowgill, R., and Brand, E. D., Amer. J. Physiol. **211**, 687 (1966).

17. Brady, A. J., Physiol. Rev. **48**, 570 (1968).

18. Reitz, B. A., Prager, D. J., and Feigen, G. A., J. Exp. Med. **128**, 1401 (1968).

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