Myocardial Lipases and Catecholamines in Burn Shock[1] (35574)

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Acute burn injuries induce changes in lipid metabolism that are described as an elevation in plasma free fatty acids (FFA) and a decrease in plasma cholesterol and phospholipids (1). The simultaneous increase observed in both plasma FFA and urinary catecholamines in burn injuries has been suggested to stem from stimulation of the sympathetic nervous system (2).

In addition to burn trauma, other states of stress such as hyperthyroidism (3), fasting (4, 5), and exercise (6) were found to alter myocardial muscle lipolysis. The present study was undertaken to explore whether the stress from acute burn shock would change cardiac lipase activity. In addition, myocardial concentrations of norepinephrine (NE) and epinephrine (E) were analyzed to determine if a relationship might be found between cardiac lipolytic activity and catecholamines in burn shock.

Methods. Mongrel dogs weighing from 12 to 15 kg were anesthetized by the intravenous administration of 30 mg/kg of sodium pentobarbital. The trachea was intubated from a midline neck incision and the right common carotid artery was cannulated for recording mean arterial blood pressure with a Hg manometer. At predetermined intervals, blood was drawn from the cannulated carotid artery to measure the hematocrit and to analyze plasma free fatty acids (FFA). In 9 of 33 dogs, burn shock was induced by exposing the lower half of the dog ventral surface for 3 min in a chamber containing infrared heat lamps. Heat from these lamps caused, within 30 sec, a rapid rise in surface skin temperature to 70°, while temperature in subcutaneous tissues remained close to the averaged recorded blood temperature of 40°. Blood temperature was measured from a thermistor in the inferior vena cava. Four hr after burn trauma, a left thoracotomy was performed with the animal under positive pressure respiration from a Harvard respiratory pump. Cardiac muscle samples were excised from the ventral lateral area of the left ventricle and frozen in liquid N_2 for analysis of E and NE by the method of Price and Price (7), using an Aminco-Bowman spectrophotofluorometer. In cardiac muscle excised from the same region of the left ventricle, cardiac lipolytic activity (CLA) was determined according to the method of Ho *et al.* (8). The tissue was rinsed in cold Krebs–Ringer bicarbonate solution, pH 7.4, blotted with filter paper and accurately weighed in 100- to 200-mg samples. The muscle was homogenized at 4° in a glass homogenizing tube containing 5 ml of Krebs–Ringer bicarbonate. Four ml of the homogenate was incubated in a medium containing 10.4 ml of 0.06 *M* phosphate buffer (pH 7.4), 3.2 ml of 1% Ediol (a coconut oil emulsion), 1.6 ml of 25% fatty acid-poor bovine serum albumin (Nutritional Biochem, Cleveland, Ohio) and 0.8 ml of fresh whole blood serum from the same dog. The incubated medium was gently shaken in a Dubnoff metabolic shaker for periods ranging from 10 to 60 min at 37°. After a predetermined period of incubation, 2.5 ml of the mixture was analyzed for FFA by the method of Dole and Meinertz (9) using phenolphthalein as the indicator (10). CLA was expressed as FFA liberated into the medium

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μEq/g/unit time. A solution of 800 μEq/liter of palmitic acid containing all of the components of the incubation mixture was prepared as a standard, with an equal volume of Krebs–Ringer bicarbonate substituted for the homogenate. In 6 of 9 dogs in burn shock, CLA was determined every 10 min of incubation. In 3 of the 6 dogs, additional determinations of CLA were made on duplicate homogenates after addition of 10 μg/ml of E. In the absence of burn injury, NE, E, and CLA were determined in 7 control dogs subjected to the same experimental procedures as the burned dogs.

To distinguish between a lipoprotein lipase (LPL) and an epinephrine-sensitive lipase (ESL), CLA in 2 of 5 additional control dogs was determined at a pH of 6.8, 7.4, and 8.5 with an incubation time of 10 and 60 min. To quantitate the contribution of myocardial LPL and ESL to total CLA, 0.2 *M* NaF (inhibitor of ESL) and 1 *M* NaCl and 1 mg/ml of protamine sulfate (inhibitors of LPL) were added to the lipolytic media prepared from the remaining 3 control dogs (3, 4, 11, 12). Epinephrine (10 μg/ml) and heparin (30 μg/ml) were used in these same dogs as activators of ESL and LPL, respectively (11, 12).

In another group of 12 normal dogs, a synthetic glyceride, rather than Ediol, was used as a substrate for measuring CLA. Duplicate left ventricular cardiac muscle samples were homogenized in Krebs–Ringer bicarbonate, pH 7,4. Four ml of the homogenate were added to an incubation mixture containing 6.6 ml of 0.06 *M* phosphate buffer (pH 7.4) and 1.6 ml of 10% fatty acid-poor bovine serum albumin. To this incubation mixture, the glycerides (monopalmitin, 1.8 mg/ml; dipalmitin, 2.0 mg/ml; or tripalmitin, 2.5 mg/ml, 99% pure from Sigma Chemical Co., St. Louis, Mo.) were added as a stable emulsion prepared according to the method of Yamamoto and Drummond (13).

Prior to incubation for 10 min at 37°, the lipolytic mixture was activated with 10 μg/ml of E. The hydrolysis of synthetic glycerides in E-stimulated lipolysis of cardiac homogenates prepared as described above, was also determined in the presence of adenosine triphosphate (ATP, 2.7×10^{-6} *M*) or

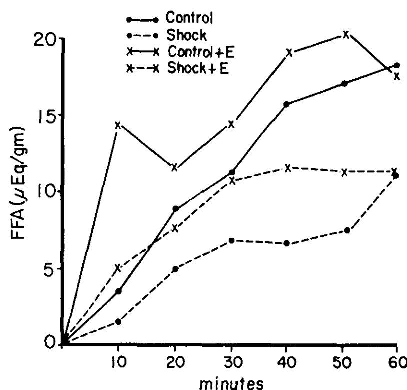

FIG. 1. A comparison in rate of cardiac lipolysis between 7 control dogs and 6 dogs subjected to burn shock. In 3 of 7 control dogs and in 3 of 6 burned dogs, the rate of lipolysis is compared after addition of epinephrine (10 μg/ml) to the media prior to its incubation.

cyclic 3′,5′-adenosine monophosphate (cyclic AMP, 2.5×10^{-3} *M*)

Results. In Fig. 1, the rate of change in CLA was compared between control dogs and dogs in burn shock. It can be noted that liberation of FFA from the onset of incubation in both groups proceeded at different rates, with the difference in CLA of the control group being greater from the 40- to 60-min periods. Differences in rate of lipolysis between both groups at 40 ($p<0.05$), 50 ($p<0.01$), and 60 ($p<0.01$) min were significant. Figure 1 shows that addition of E caused CLA in 3 of 7 control dogs to increase at the end of 10 min from an average of 3.6 in the nontreated samples to 14.5 μEq/g ($p<.05$). After 10 min of incubation, CLA rose slightly in dogs in burn shock (1.6 μEq/g), which more than doubled (5.0 μEq/g) when E was added to the media ($p<.05$). The addition of E to cardiac homogenates from control or burn shocked dogs caused insignificant differences from their respective controls in the rate of FFA liberated during subsequent incubation periods of 20, 30, 40, 50, and 60 min. The CLA at 60 min was found to be almost equal when E-treated and untreated homogenates were compared in either the control dogs or dogs in burn shock (Fig. 1).

The results from adding lipase inhibitors to normal cardiac muscle in Table I shows that

TABLE I. The Inhibition and Activation of Epinephrine-Sensitive Lipase (μEq/g) and Lipoprotein Lipase (μEq/g) in Normal Cardiac Muscle.

Dog	Control	NaF (0.2 *M*)	NaCl (1 *M*)	Protamine sulfate (1 mg/ml)	Epinephrine (10 μg/ml)	Heparin (30 μg/ml)
			10 min			
1	3.6	0	1.8	5.4	10.9	3.6
2	2.8	0	2.8	2.8	11.3	2.8
3	5.0	0	5.0	3.8	10.0	5.0
Mean	3.8	0	3.2	4	10.7	3.8
Change (%)		—100	—16	+5	+182	0
			60 min			
1	12.7	3.5	5.4	9.1	14.5	14.7
2	14.1	5.7	5.7	8.5	11.3	20.4
3	14.0	6.3	8.8	10.0	17.7	14.9
Mean	13.6	5.1	6.6	9.2	14.5	16.4
Change (%)		—63	—51	—32	+7	+21

CLA after 10 min of incubation, pH of 7.4, was primarily due to ESL, since NaF, an inhibitor specific for ESL, completely blocked lipolytic activity. Sodium chloride (—16%) and protamine sulfate (+5%) were observed to have only a slight effect during this period of incubation. The addition of E prior to a 10-min period of incubation, almost tripled the average control CLA, while heparin had no effect (Table I). In incubating cardiac homogenates for 60 min at a pH of 7.4, CLA was found to be due to the presence of both ESL and LPL, since NaF reduced CLA activity by 63%, while NaCl and protamine sulfate had an inhibitory effect on lipolysis of —51 and —32%, respectively. Heparin increased LPL above control after 60 min of incubation in 2 of 3 dogs, suggesting that maximal activation of LPL requires a longer period of incubation than the 10 min required for maximal activation of ESL.

In Table II, incubation times of 10 and 60 min for cardiac muscle media were compared at a pH of 6.8, 7.4, and 8.5. Optimum pH for activation of CLA was about the same for a pH of 6.8 or 7.4 at 10 and 60 min of incubation. At a pH of 8.5, which is considered optimal for LPL (11), the contribution of LPL to total CLA was approximately 50% after 1 hr of incubation. These results taken together with the observations made from adding lipase inhibitors, suggest that the lipase primarily responsible for CLA after 10 min of incubation was almost entirely ESL, while for 60 min, both lipases appear to be present in significant concentrations (Table I).

TABLE II. The Effect of pH on Myocardial Lipase Activity (μEq/g) in Two Normal Dogs with Incubation Time of 10 and 60 min.

Dog	Incubation (min)	pH 6.8	pH 7.4	pH 8.5
1	10	5.9	5.9	2.6
	60	12.0	10.4	7.9
2	10	10.6	13.3	2.6
	60	12.0	10.4	5.2

As shown in Table III, the addition of monopalmitin as a substrate for measuring E-stimulated lipolytic activity of cardiac homogenates led to a marked increase in FFA after 10 min of incubation. The substitution of monopalmitin as a substrate for Ediol increased FFA from 6.4 to 17.1 μEq/g ($p<.01$), indicating that canine myocardial muscle contains a monoglyceride lipase. Lipolysis in cardiac homogenates containing the synthetic monoglyceride was not significantly increased from control by the addition of E (Table IV), nor by the addition of ATP or cyclic AMP (Table III), suggesting that the monoglyceride lipase is not activated by the adenyl cyclase system. Although of questionable significance, the results from adding

TABLE III. The Effects of ATP and Cyclic AMP on Epinephrine-Stimulated Lipolytic Activity (μEq/g) of Myocardial Homogenates Before and After Addition of Monopalmitin, Dipalmitin, or Tripalmitin.

	Tissue	Monopalmitin	Dipalmitin	Tripalmitin
Control	6.4 ± 1.4[a](11)[b]	17.1 ± 2.8 (9)	7.4 ± 1.4 (8)	5.1 ± 1.9 (6)
ATP ($2.7 \times 10^{-6}\,M$)	7.7 ± 1.6 (11)	19.3 ± 4.2 (10)	7.9 ± 1.8 (6)	8.6 ± 1.6 (8)
Cyclic AMP ($2.5 \times 10^{-3}\,M$)	8.7 ± 1.7 (12)	21.4 ± 4.3 (10)	10.8 ± 2.5 (7)	20.1 ± 3.6 (7)

[a] Mean ± SE.
[b] No. of dogs is given in parentheses.

TABLE IV. The Lipolytic Activity (μEq/g) of Epinephrine (E) on Normal Cardiac Homogenates Containing Monopalmitin.

Dog no.	Control	Monopalmitin	Monopalmitin + E
8	3.43	20.4	17.5
9	8.93	27.9	26.7
10	3.94	33.7	32.1

ATP to E-stimulated homogenates containing tripalmitin led to a slight rise in FFA. If the concentration of ATP added had been greater, a marked rise in hydrolysis of tripalmitin might have been observed. Rizack (14), using a higher concentration of ATP reported stimulation of E-sensitive lipolytic activity of cell-free extracts of adipose tissue. However, the addition of cyclic AMP to media containing tripalmitin led to a marked increase, from 5.1 to 20.1 μEq/g ($p<.01$) of FFA, while monopalmitin and dipalmitin were observed to increase only slightly ($p>.05$) from control.

The changes observed in mean blood pressure and hematocrit in dogs subjected to burn shock confirm our previous findings as well as those reported from other laboratories (15–17). Mean arterial pressure during the first hour decreased in 9 dogs from an average of 154 ± 23 to 137 ± 25 mm Hg. Thereafter blood pressure gradually declined in the 2nd and 3rd hr to 132 ± 27 and 125 ± 21, respectively. The decline in blood pressure from control was not significant for the 3-hr period of burn shock, although after 4 hr, blood pressure decreased to an average of 121 ± 24 mm Hg ($p<.05$). The arterial hematocrit measured every hour for 4 hr of shock was noted to increase from an average control of 43.8 ± 5.8 to 53.5 ± 6.9 ($p<.05$); 57.9 ± 8.2 ($p<.01$); 57.1 ± 7.7 ($p<.01$); and 58.0 ± 7.7% ($p<.01$); respectively. In addition to confirming that the injury resulted in a state of acute burn shock characterized by a gradual decline in mean blood pressure and a rise in hematocrit, an elevated plasma level of FFA was found. After 4 hr of burn shock, plasma FFA rose from an average control level of 493 to 734 μEq/liter ($p<.01$).

In Table V, levels of NE and E are given for control dogs and dogs subjected to burn trauma. Control levels of myocardial left ventricular NE and E averaged 0.85 ± 0.10 and 0.12 ± 0.03 μg/g, respectively. In dogs subjected to burn injury, a slight but insignificant decline was noted in the average myocardial NE concentration (0.76 ± 0.07 μg/g), while no change was found in E levels (0.12 ± 0.02 μg/g).

Discussion. Other lipases have been reported for the rat myocardium since Korn (11) first described a heparin-activated lipoprotein lipase in the hearts of normal rats. In addition to LPL, Björntorp and Furman (12) reported a tissue lipase having a maximal activity in the presence of E between a pH of 6.5 and 7.0. In a more recent report, Yamamoto and Drummond (13) presented evidence which suggested that the ESL of Björntorp and Furman utilized a monoglyceride substrate. Although the lipase was significantly inhibited by NaF these authors did not determine the activity of the monoglyceride lipase to E. Other investigators (18) have shown that different lipases can account

TABLE V. Myocardial Left Ventricular Levels of Norepinephrine (NE) and Epinephrine (E) in Control Dogs and in Dogs Subjected to Burn Shock

Control (μg/g)			Shock (μg/g)		
No.	NE	E	No.	NE	E
1	0.88	0.07	8	1.04	0.13
2	0.97	0.11	9	0.65	0.07
3	1.32	0.16	10	0.76	0.28
4	0.44	0.07	11	0.85	0.14
5	0.65	0.05	12	0.70	0.05
6	0.76	0.16	13	0.34	0.09
7	0.93	0.25	14	0.73	0.12
			15	1.08	0.16
			16	0.73	0.05
Mean ± SE	0.85 ± 0.10	0.12 ± 0.03		0.76 ± 0.07	0.12 ± 0.02

for the CLA of the rat heart. This group demonstrated that specific lipases exist for triglycerides, diglycerides, and monoglycerides, with the rate of lipolysis for diglycerides being 2 to 3 times higher than triglycerides, while the rate of lipolysis of monoglycerides was 10 times higher than that observed for triglycerides.

On the basis of a limited number of experiments, an ESL can be postulated in heart muscle of normal dog. This lipase appears to be similar to the ESL originally described in adipose tissue by Rizack (19). Maximal ESL was found when the tissue homogenate was treated with E and incubated for 10 min at a pH of 7.4. In another series of experiments, the lipase during this period of incubation could be completely inhibited by NAF, while only slight inhibition was observed when adding NaCl or protamine sulfate (Table I). As suggested by Yamamoto and Drummond (13) the likelihood that ESL in the rat heart might be a monoglyceride lipase was not supported in the reported experiments when synthetic monopalmitin added to cardiac muscle homogenates was substituted for Ediol as a substrate. Epinephrine activation of the monoglyceride failed to increase the rate of lipolysis above that measured in control tissue samples (Table IV). Unlike the ESL described by Rizack (14, 19) in adipose tissue, monoglyceride lipase was not markedly stimulated by ATP or cyclic AMP (Table III). These observations confirm the work of Tsai *et al.* (20) where ATP or cyclic AMP were reported not to stimulate monoglyceride lipase activity in fractions where lipolytic activity, presumed to arise from a hormone-sensitive lipase, was enhanced by 30 to 40%. However in the reported experiments the addition of cyclic AMP as a cofactor to activation of a triglyceride lipase in E-treated homogenates was sufficient to induce a significant elevation in tripalmitin hydrolysis (Table III).

In dogs subjected to acute burn shock, CLA was observed to be significantly below the activity measured in normal dogs (Fig. 1). The decline in CLA would indicate that an alteration in cardiac lipid metabolism occurs in burn shock. The decrease in ESL activity may result in a decline of available FFA for energy production, since hydrolysis of intracellular esterified fatty acids has been suggested to occur in other tissues by activation of ESL (19).

In the *in situ* dog heart, Ballard *et al.* (21) and Goto *et al.* (22) have observed a myocardial uptake of esterified fatty acids, although in a more recent report, Regan *et al.* (23) observed a minimal myocardial extraction of this substrate in the control state. However, the uptake and oxidation of esterified fatty acids by the isolated perfused heart have been repeatedly confirmed in which LPL has been implicated as playing a major role (24). Although conclusive evidence in lacking on the uptake of esterified fatty acids by the *in situ* heart, a decrease in cardiac LPL would suggest an inability of the heart to extract and transport esterified fatty acids in burn shock.

Several factors may be responsible for altering CLA in burn shock. Myocardial NE

and E concentrations in the reported experiments do not appear to be one of these factors, since levels of these catecholamines did not change from control. In other forms of shock, such as hemorrhagic shock, marked alterations in cardiac metabolism were observed (25) although myocardial NE and E levels remained normal (26). Therefore the analysis of NE and E in heart muscle may not be sufficient to point to a metabolic abnormality, since it is generally accepted that several pools of catecholamines exist in different functional compartments of the heart (27). Pools of catecholamines having different functions in cardiac muscle may indicate that an increase in the nonactive fraction (granulated vesicles) could mask a decrease in the "free" or chemically active fraction having a metabolic regulatory role. Humoral substances, such as histamine, serotonin, and bradykinin, found in high concentrations in burn shock (17, 28), may have a depressing action on CLA in the reported experiments. In addition to humoral substances, a toxic factor arising from tissue destruction by burns (29, 30) may also be involved in inhibition of CLA. On the other hand, the inhibition may also arise indirectly from the decrease in plasma albumin reported by other laboratories to occur in burn shock (31). The likely decline in plasma albumin before a significant rise in plasma FFA could result in a significant fraction of these acids not being bound to albumin. It follows that these nonprotein bound FFA may become highly toxic to the heart in burn shock (32). Whether the myocardial toxic properties of plasma unbound FFA or any of the factors mentioned above have a role in depressing CLA in burn shock remains a theoretical concept.

Summary. In anesthetized dogs subjected to 4 hr of shock from infrared burns, lipolytic activity of cardiac muscle was found significantly below the activity determined in normal heart muscle. Myocardial homogenates from control and burned dogs responded to the addition of epinephrine with an increase in rate of lipolysis after incubation for 10 min. Incubation of the media for 1 hr showed a further increase in lipolysis, although no difference was noted in activity between epinephrine-treated and untreated homogenates from either the control or burn group of dogs. The addition of inhibitors or activators to the lipolytic mixture prepared from normal hearts suggested that cardiac lipolytic activity results from both an epinephrine-sensitive lipase and a lipoprotein lipase. The epinephrine-sensitive lipase could not be classified as a monoglyceride lipase, since epinephrine as well as ATP and cyclic AMP failed to increase the rate of lipolysis in cardiac muscle homogenates containing monopalmitin substituted for Ediol as a substrate.

Myocardial norepinephrine and epinephrine levels in burned dogs were not different from those found in control dogs. The absence of a change in these cardiac catecholamines leaves the possibility that other humoral or toxic substances may be responsible for altering myocardial lipolysis in burn shock.

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