Reduction of Ischemia-Induced Acyl Carnitine Accumulation by TDGA and Its Influence on Lactate Dehydrogenase Release in Diabetic Rat Hearts

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SUMMARY

The contribution of long-chain acyl carnitine to increase enzyme release during ischemia was investigated both in normal and diabetic rat hearts. 2-Tetradecylglycidic acid (TDGA) was used to inhibit acyl carnitine formation. Isolated working-heart preparations were perfused with glucose (11 mM) and palmitate (0.1 mM) in control and mild ischemic conditions. Ischemia induced lactate dehydrogenase (LDH) release from both normal and diabetic hearts, but the release was higher from the diabetics over a 15-min ischemic period. The ischemia-induced tissue accumulation of long-chain acyl carnitine also was greater in diabetic hearts compared with normal hearts. When TDGA was provided in the perfusate 10 min before the addition of palmitate, levels of acyl carnitine were significantly reduced (by ~80%) in the ischemic tissue of both groups of hearts. Similarly, LDH release from ischemic hearts was markedly decreased in the presence of TDGA. A positive correlation was shown between LDH release over the ischemic period and the tissue levels of acyl carnitine at the end of ischemia. Significant improvement in mechanical function with TDGA was only observed in ischemic diabetic hearts. There was absolutely no difference in high-energy compounds under a given perfusion condition, either with or without TDGA, between normal and diabetic hearts. It is concluded that lessening the accumulation of fatty acid intermediates, such as acyl carnitine, may be important to prevent or to limit the loss of sarcolemmal integrity under ischemic conditions, especially in diabetic hearts.
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Experimental evidence has indicated that an accumulation of intermediates of fatty acid metabolism may have a deleterious effect on myocardial function.[1–4] Studies on both in vivo dog and swine hearts[5,6] and the isolated perfused rat heart[7,1] have shown that both long-chain acyl-CoA and acyl carnitine derivatives accumulate in response to a decreased oxygen supply. Acyl carnitine accumulates in proportion to the amount of exogenous fatty acid present.[5,7] High tissue levels of these amphiphile compounds have been shown to interfere with various cellular functions by specifically inhibiting enzymes or nonspecifically altering the structure of membranes.[8–13]

Release of large protein molecules such as enzymes from the cells has been used as an index for the loss of sarcolemmal integrity.[14] Release of these proteins may occur before any evidence of ultrastructural damage could be detected.[15] Under ischemic conditions, release of enzymes was increased in hearts perfused with free fatty acid in addition to glucose, suggesting that more marked damage had developed.[14,15] The question then arises as to whether the loss of sarcolemmal integrity, reflected by enzyme release, results from the direct effect of fatty acid per se on the cell membrane[16] or from an indirect effect of accumulated metabolites such as acyl-CoA and acyl carnitine.[3]

In our study, the contribution of palmityl carnitine to increase enzyme release during ischemia was investigated. Palmityl carnitine levels were decreased by inhibition of its formation with 2-tetradecylglycidic acid (TDGA; McNeil 3802, Spring House, PA). This agent has been reported to specifically inhibit the transfer of acyl units from acyl-CoA to carnitine on the outer side of the inner mitochondrial membrane.[17,18] Moreover, the effect of ischemia and TDGA was investigated in hearts from both normal and diabetic rats. The diabetic situation was of particular interest because excessive oxidation of fatty acids occurs in association with elevated serum and tissue fatty acids.[19] In addition, diabetic hearts contain elevated levels of acyl carnitine.[20]

METHODS

Experimental protocol. Male Wistar rats weighing between 250 and 300 g were used. Diabetes was induced by femoral vein injection of 45 mg alloxan (Eastman Kodak, Rochester,

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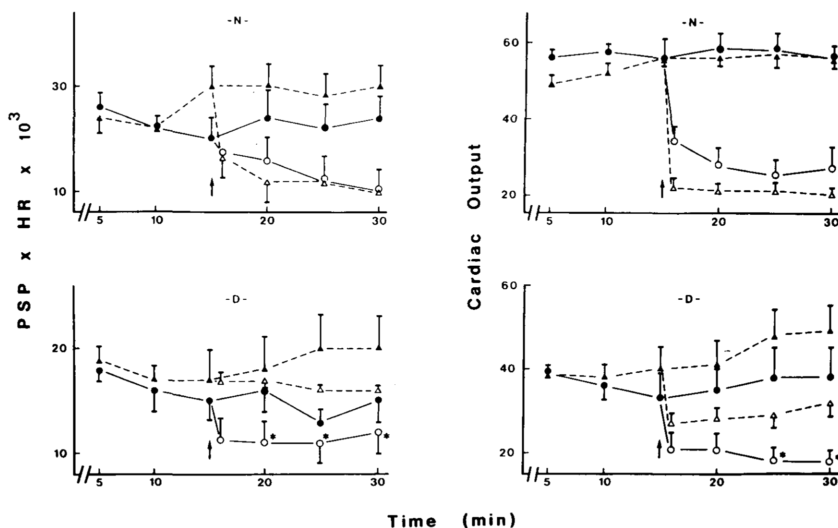

FIG. 1. Ventricular function of normal (N, *upper panel*) and diabetic (D, *lower panel*) hearts perfused in presence (*dashed line*) or absence (*solid line*) of 5.10^{-5} M TDGA. Hearts were perfused as control working preparations with left atrial pressure of 10 cmH_2O. Ischemia was induced at 15 min (*vertical arrow*, ○, △) by reducing coronary flow an average of 50%. In control hearts (●, ▲), perfusion was continued for 30 min with control rates of coronary flow. Product of peak systolic pressure (PSP) and heart rate (HR), used as an index of cardiac function is expressed as mmHg · min^{-1} · 10^{-3}; cardiac output is expressed as ml/min. Body weights were 280 ± 3 and 246 ± 5 g for control and diabetic rats, respectively. Blood glucose was 9 ± 0.3 and 35 ± 2 mM for controls and diabetics, respectively, in fed state. There were 6–8 hearts in each group. *$P < .05$.

NY) per kilogram body weight to overnight-fasted animals. Alloxan-treated and age-matched control animals were maintained on the same diet until they were killed 3 wk later. The diabetic state was assessed by measurement of nonfasting glucose concentration in blood samples collected at the time of heart excision. Only rats with plasma glucose levels >20 mM were considered diabetic.

Hearts removed from 3-wk diabetic and age-matched control rats were perfused in vitro by the working-heart technique of Neely et al.[21] The perfusate consisted of Krebs bicarbonate buffer maintained at 37°C and gassed with 95% O_2–5% CO_2 (pH 7.4). The buffer was supplemented with 1% bovine serum albumin (fraction V, Sigma, St. Louis, MD) and 11 mM glucose. After a 10-min initial working perfusion period, palmitate bound to albumin was added, at a final concentration of 0.1 mM. Palmitate was bound to albumin by adding sodium salt palmitate to a stirred, warm albumin solution. This solution was dialyzed three times against a large volume of buffer and filtered through 0.8-μM Millipore filters before use. The perfusate (150 ml) was recirculated. When used, TDGA 5.10^{-5} M (a gift from McNeil) was added to the perfusate when starting the initial 10-min working mode perfusion.

In the ischemic group, global ischemia was induced by the use of a one-way aortic valve that reduced coronary diastolic perfusion.[22] As a result, coronary flow initially decreased by 50%. Aortic pulse pressure and heart rate were monitored via a fluid-filled sidearm on the aortic cannula connected to a pressure transducer and recorded on a Gould 2200 apparatus (Gould, Cleveland, OH).

Enzyme assays. Arteriovenous differences of lactate dehydrogenase (LDH) activity were measured every 5 min on samples taken simultaneously of the aortic flow and of the coronary effluent, with measurements of coronary flow rate.[14] LDH activity was assayed spectrophotometrically by the method of Wroblewski and La Due.[23] Data are expressed in milliunits (mU) released during the 30-min working perfusion period.

Tissue metabolites. After perfusion, hearts were quick frozen with a Wollenberger clamp cooled to the temperature of liquid nitrogen. The frozen tissue was lyophilized and extracted with ice-cold perchloric acid (6% wt/vol). After centrifugation, 1 aliquot of supernatant was processed for creatine phosphate (CP) and ATP,[24] and the remainder was used, after alkaline hydrolysis, for the determination of acid-soluble CoA and carnitine, which includes both free and acetyl esters. Tissue levels of long-chain acyl-CoA and acyl carnitine were determined on the perchloric acid precipitate.[11] Acid-soluble CoA and long-chain acyl-CoA were assayed fluorimetrically with the α-ketoglutarate dehydrogenase reaction.[25] Acid-soluble carnitine and long-chain acyl carnitine were assayed as free carnitine, after hydrolysis, by a radioisotope procedure.[26]

Statistical analysis. All results are expressed as mean ± SEM. Data were analyzed with the Student's *t* test for comparison of the means of independent samples. Linear regression was performed with the least-squares method. The correlation coefficient (*r*) and the probability (*P*) that the slope differed from zero were calculated. Statistical significance was set at $P < .05$.

RESULTS

Cardiac function of perfused hearts from both normal and diabetic rats is shown in Fig. 1. For both groups of hearts, the presence of TDGA during normoxic perfusions did not induce a significant change either in the product of peak systolic pressure and heart rate or in the cardiac output. Diabetic hearts exhibited depressed function compared with normal hearts (34–44% decrease in the product of peak systolic pressure and heart rate and 20–32% decrease in cardiac output). Such depressed function has already been described in hearts from rats made diabetic with a similar dose of alloxan and could be totally reversed with insulin treatment.[27]

The reduction in function induced by ischemia in normal hearts was the same in the presence or absence of TDGA, whereas in the diabetics the presence of TDGA significantly attenuated the deterioration in function, as assessed by a better preservation of both the product of peak systolic pressure and heart rate and of cardiac output (Fig. 1).

There was a slow release of LDH from diabetic and normal hearts during control perfusions (Fig. 2). However, the total amount of the enzyme released over 30 min from both groups of hearts was the same, regardless of whether TDGA was

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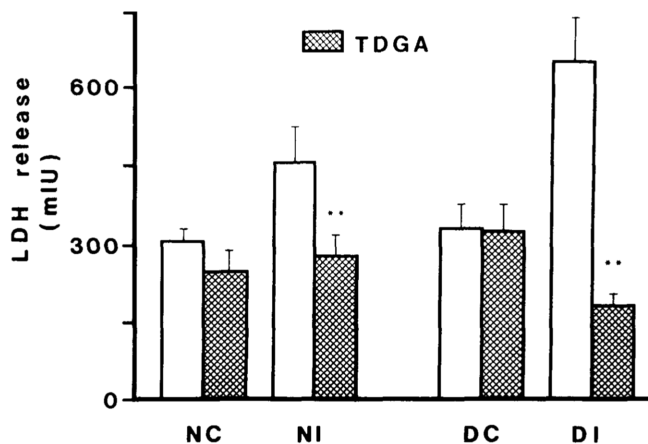

FIG. 2. Influence of TDGA on LDH release (expressed as mU) from normal (NC) and diabetic (DC) control hearts and from normal (NI) and diabetic (DI) ischemic hearts. There were 7–11 hearts in each group. **$P < .001$.

present. On the other hand, ischemia-induced enzyme release from diabetic hearts was significantly higher than the release in normal ischemic hearts. However, the LDH release could be considerably reduced for both the normal and diabetic ischemic hearts when TDGA was added to the perfusate. Release then returned to basal control levels, or even below for the diabetic ischemic hearts (Fig. 2).

Tissue levels of long-chain acyl carnitine esters in control and ischemic perfusion conditions are shown in Fig. 3*A*. In control diabetic hearts, levels were about twice those measured in control normal hearts. Fifteen minutes of ischemia resulted in increased levels of acyl carnitine in both normals and diabetics, but again levels were higher in the diabetic tissue. Acyl carnitine levels were markedly reduced when ischemic diabetic and ischemic normal hearts were exposed to TDGA. However, the higher the level, the more marked was the reduction. Tissue levels of long-chain acyl-CoA paralleled those of acyl carnitine in the different experimental conditions (Fig. 3*B*). Levels were higher in control diabetics than in control normal hearts and were also increased in the ischemic tissue of both groups, compared with their respective control. However, the reduction in tissue levels of acyl-CoA in hearts receiving TDGA and exposed to ischemia was less than for acyl carnitine. For example, the acyl-CoA decrease in ischemic hearts was 22 and 43% for normals and diabetics, respectively, whereas it was 87 and 77%, respectively, for acyl carnitine levels.

Tissue content analysis of energy metabolites showed that ATP and CP were reduced by the same order of magnitude in normal and diabetic hearts at the end of the ischemic period and that TDGA had no effect on that content (Table 1).

It has been shown that whole-heart values of ATP and creatine phosphate did not change in the opposite direction to LDH release.[28] These results were confirmed by our study, because we found no correlation between the total LDH released during ischemia and the residual ATP content. (Fig. 4*A*). On the other hand, a good positive correlation (r = .85) was observed between LDH release and acyl carnitine accumulated in the tissue at the end of ischemia (Fig. 4*B*).

DISCUSSION

The concentration of TDGA used in this study (5.10^{-5} M) had no deleterious effect on the mechanical function of the working heart. On the contrary, a slight but significant improvement of function was observed in ischemic hearts of alloxan-diabetic rats receiving TDGA (Fig. 1). Our results are in agreement with those of Pearce et al.,[29] who showed no deleterious effect on function in the presence of TDGA at 5.10^{-5} M, a concentration that permitted the complete inhibition of long-chain fatty acid oxidation. However, this was true only with heart preparations at low work loads, which were similar to our experimental conditions.

The better preservation of function in diabetic hearts exposed to ischemia in the presence of TDGA (Fig. 1) may partly result from the inhibition of fatty acid oxidation and the concomitant stimulation of glucose utilization.[30] However, no improvement of function with TDGA occurred in normal ischemic hearts, which also rely essentially on glucose to meet the energy demand in this situation. There is no apparent reason for the rate of glucose utilization to be higher in diabetic hearts than in normal hearts under mild ischemic conditions, and in fact it was shown to be even less.[20]

The fact that whole-tissue contents of ATP and CP were

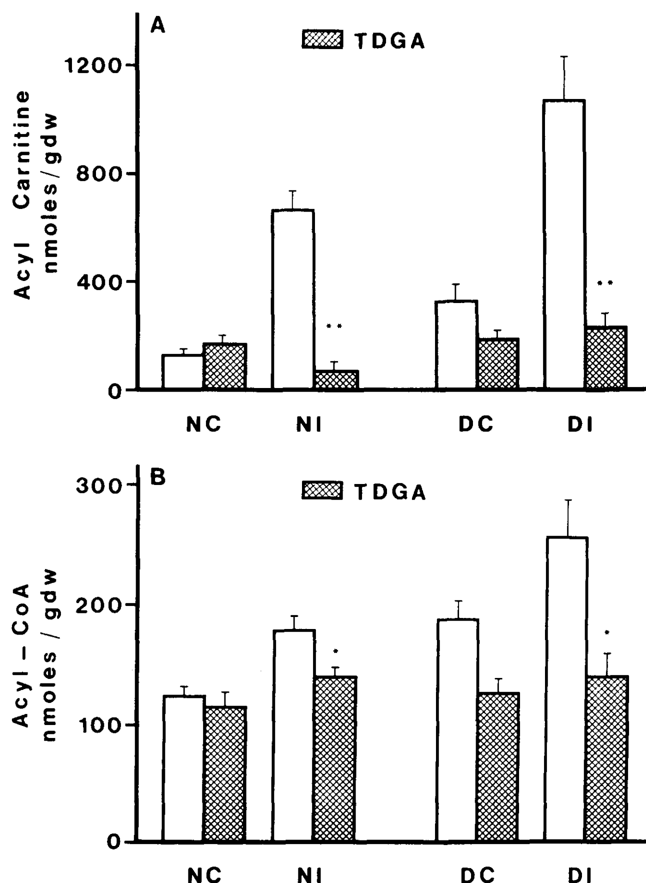

FIG. 3. Effect of TDGA on tissue contents of long-chain acyl carnitine (*A*) and long-chain acyl-CoA (*B*) (expressed as nmoles/g dry wt) in normal (NC) and diabetic (DC) control hearts and in normal (NI) and diabetic (DI) ischemic hearts. There were 6–8 hearts in each group. **$P < .01$.

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essentially the same for both normals and diabetics under a given perfusion condition cannot explain the differences in function. Likewise, the ATP and CP concentrations do not explain the differences in lactate dehydrogenase release measured over a 15-min ischemia between normal and diabetic hearts or the marked decrease in LDH release when TDGA was provided in the perfusate.

On the other hand, our results show a clear association between the amount of enzyme released and the tissue content of long-chain fatty acyl carnitine (Fig. 4*B*): LDH release increased in conditions of elevated acyl carnitine, and conversely, it decreased when acyl carnitine levels were lessened. Because acyl-CoA levels paralleled those of acyl carnitine in the different experimental conditions, an association could also be shown between enzyme release and the total tissue content of long-chain fatty acyl derivatives. However, most of the acyl-CoA increase during ischemia was shown to occur in the mitochondrial matrix,[31] and cytosolic levels of acyl-CoA should not be expected to be high enough to exert a detrimental effect on the cell membrane. The situation is reversed for the acyl carnitine, which is mainly located in the cytosol. Thus, a reduction of ischemia-induced enzyme release could occur without a change in circulating fatty acid concentration but by the prevention of acyl carnitine formation. Fatty acyl esters have been implicated as perturbants of various cellular functions by specifically inhibiting enzymes or by altering the structure of membranes.[8-13] Our results suggest that perturbation of myocardial sarcolemma may occur in the presence of high levels of acyl carnitine, which may alter membrane fluidity through detergent-like properties.[3,13,32] This action may then sensitize the fragilized sarcolemma of ischemic cells, inducing further alterations in membrane fluidity, which lead to a leakage of large protein molecules. Finally, improvement in function with TDGA in ischemic diabetic hearts could also be associated, to a certain extent, with a decline in acyl carnitine. Such an association was shown to occur in regionally ischemic swine hearts when tissue levels of acyl carnitine had been reduced with oxfenicine.[33]

In conclusion, the results of our study suggest that lessening the accumulation of fatty acid intermediates, such as long-chain acyl carnitine, may be of importance to preserve sarcolemmal integrity under ischemic conditions, especially in diabetic hearts.

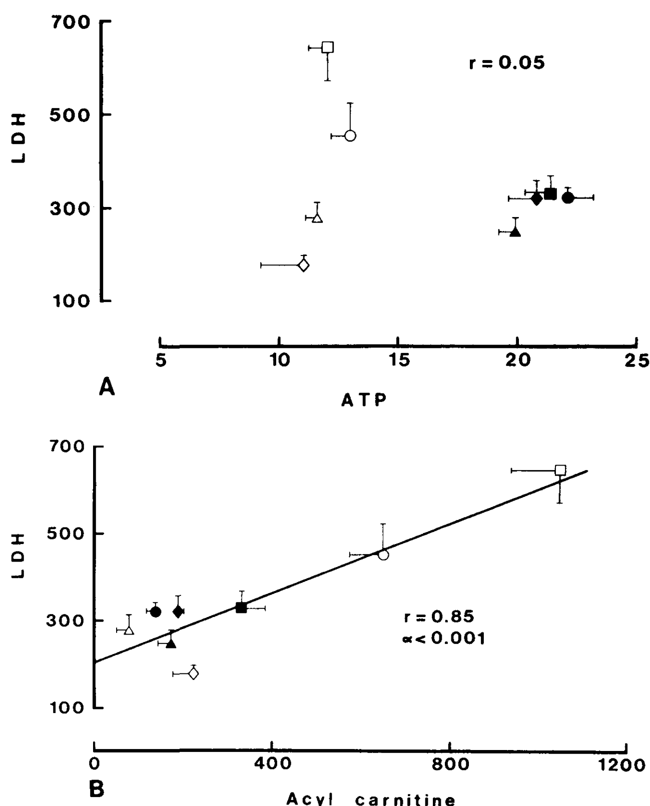

FIG. 4. LDH release is plotted as function of average tissue content of ATP (*A*) or acyl carnitine (*B*) in normal and diabetic control and ischemic hearts that have or have not received TDGA. Data in *panel A* are same as shown in Table 1 and Fig. 2; *in panel B*, data are from Figs. 2 and 3. ●, Normal control; ▲, Normal control, plus TDGA; ■, diabetic control; ◆, diabetic control, plus TDGA; ○, normal ischemic; △, normal ischemic, plus TDGA; □, diabetic ischemic; ◇, diabetic ischemic, plus TDGA.

TABLE 1
Tissue content of ATP and creatine phosphate (CP) in hearts from normal and diabetic rats under control and ischemic perfusion conditions

Preparation	TDGA	ATP	CP
		μmol/g dry wt	
Normal			
Control	−	22.1 ± 1.1	22.1 ± 1.8
	+	19.9 ± 0.7	19.9 ± 0.7
Ischemic	−	13.0 ± 0.8	12.0 ± 0.8
	+	11.6 ± 0.8	10.9 ± 0.7
Diabetic			
Control	−	21.3 ± 1.0	21.7 ± 0.7
	+	20.8 ± 1.2	21.8 ± 1.7
Ischemic	−	12.0 ± 0.8	12.0 ± 0.9
	+	11.0 ± 1.8	12.5 ± 0.6

Hearts were (+) or were not (−) exposed to TDGA. Values are means ± SEM for 9–15 hearts.

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