EFFECT OF DL-α-LIPOIC ACID ON THE CITRATE CONCENTRATION AND PHOSPHOFRUCTOKINASE ACTIVITY OF PERFUSED HEARTS FROM NORMAL AND DIABETIC RATS

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SUMMARY: Perfusion of the rat heart with lipoic acid inhibited the rise in acetyl-CoA and citrate normally induced by octanoate. With glucose as substrate, lipoic acid reduced cardiac muscle citrate and stimulated phosphofructokinase activity; glucose utilization and glycogenolysis were likewise increased. These effects were observed in normal hearts and in hearts from alloxan diabetic rats. It is suggested that lipoic acid stimulates glycolysis through the lowering of citrate, a known inhibitor of phosphofructokinase.

It is now well established that hexose phosphorylation is inhibited in heart muscle from diabetic rats and in normal hearts perfused with fatty acids (1-4). The defect results primarily from decreased phosphofructokinase (PFK) activity, which is believed to be due, in part, to elevated levels of intra-cellular citrate. Citrate has been shown *in vitro* to be an effective inhibitor of PFK (5-7), and several studies have shown that tissue PFK activity is inhibited when citrate is increased (6-11). The level of intra-cellular citrate is apparently dependent upon the concentration of acetyl-CoA (12,13), and it is assumed that one means by which fatty acids inhibit glycolysis is via elevation of acetyl-CoA and citrate (6,7,12,13).

Octanoic acid has frequently been used to demonstrate the effect of fatty acids on citrate and PFK. Octanoate is closely related in structure to lipoic (6:8 dithio-octanoic) acid, a co-factor of the pyruvate dehydrogenase complex. Because of this structural similarity it appeared of interest to investigate the

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effect of lipoic acid in the perfused heart. The results show that lipoic acid had effects entirely opposite to those of octanoate; there was a pronounced fall in the level of citrate, and a concomitant stimulation of PFK activity and glucose utilization. Others have reported that lipoic acid increases the oxidation of glucose in liver slices (19).

METHODS: Hearts from 300 to 350 g male rats were used for perfusion (14). Diabetes was induced by tail vein injection of alloxan (55 mg/kg), and hearts were perfused 44 to 48 hours later. Perfusion was terminated by rapid freezing (15). The analytical procedures have been described (13). DL-α-lipoic acid (Sigma) was added as the sodium salt to the perfusate to give an estimated final concentration of approximately 1.0 mM. The behavior of the hearts was not visibly affected by lipoate.

RESULTS: To examine the effect of lipoic acid on the levels of citrate and acetyl-CoA, hearts were perfused with or without lipoate for 10 minutes and then switched to another perfusion system containing 2 mM octanoate. Perfusion was continued for an additional 20 minutes. Table 1 shows that in hearts exposed to lipoic acid there was considerably less acetyl-CoA and less citrate than in hearts perfused with octanoate alone. The effect of lipoic acid on glucose utilization is shown in Table 2. It was found that both in the absence and presence of insulin there was an increase in glucose uptake. With insulin, lipoic acid caused some accumulation of lactate, but the actual percentage of extra glucose equivalents so diverted was quite small, since lipoate caused a significant reduction in cardiac glycogen, as well (Table 2).

Table 1. Effect of DL-Lipoic acid on octanoate-induced changes in acetyl-CoA and citrate in the perfused rat heart.

Hearts were perfused with or without 1 mM lipoate for 20 minutes and then switched to buffer containing 2 mM octanoate for 10 minutes. Values are mμmoles/g, dry wt, mean ± S.E.M. of 6 hearts.

	Control	Lipoic acid
Acetyl-CoA	218 ± 10	74 ± 4
Citrate	6210 ± 41	4120 ± 68

Table 2. Effect of DL-lipoic acid on glucose utilization and tissue glycogen in the perfused normal rat heart.

Hearts were perfused with 5.5 mM glucose with or without 1 mM lipoate and with or without insulin (10 mU/ml). Each value represents the mean ± S.E.M. of 5 hearts.

	No insulin		Insulin	
	Control	Lipoate	Control	Lipoate
Glucose uptake [a]	7.6 ± 3.0	23.6 ± 2.4	34.7 ± 1.2	48.5 ± 2.7
Lactate production [a]	0.6 ± 0.4	1.6 ± 0.5	4.3 ± 0.3	12.9 ± 0.8
Glycogen [b]	100 ± 12	69 ± 11	152 ± 10	115 ± 7

a. μmoles/30 minutes/heart.
b. μmoles/g, dry wt.

To determine if the increase in glucose utilization was brought about through greater PFK activity, several glycolytic intermediates were measured in normal hearts perfused with or without lipoic acid. Table 3 shows that glucose 6-phosphate and fructose 6-phosphate (F6P) were decreased, while fructose 1,6-diphosphate (FDP), dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate were elevated by lipoic acid. In the face of a faster rate of glycolysis (Table 2) these results indicate an increase in activity of PFK. The changes were associated with a fall in

Table 3. Metabolic intermediates in normal hearts perfused with or without lipoic acid.

Hearts were perfused as in table 2, with insulin. Values are mμmoles/g, dry wt, mean ± S. E. M. of 5 hearts.

	Control	Lipoate
Glucose 6-P	1319 ± 97	604 ± 70
Fructose 6-P	284 ± 29	133 ± 21
Fructose 1,6-diP	132 ± 5	233 ± 17
Dihydroxyacetone-P	134 ± 11	415 ± 37
Glyceraldehyde 3-P	38 ± 4	130 ± 22
Pyruvate	250 ± 15	171 ± 18
Lactate	4130 ± 269	7910 ± 222
Citrate	1586 ± 119	795 ± 57
α-Ketoglutarate	437 ± 33	231 ± 12

Table 4. Effect of DL-lipoic acid on glucose utilization and tissue glycogen in perfused hearts from alloxan-diabetic rats.

Hearts were perfused with 5.5 mM glucose with or without 1 mM lipoate. Insulin (10 mU/ml) was present in all perfusates. Each value represents the mean ± S.E.M. of 10 hearts.

	Control	Lipoate
Glucose uptake [a.]	17.8 ± 1.3	38.4 ± 1.2
Lactate production [a.]	20.4 ± 3.4	35.7 ± 5.1
Glycogen [b.]	196 ± 10	147 ± 17

a. μmoles/30 minutes/heart.
b. μmoles/g, dry wt.

citrate and α-ketoglutarate (Table 3). Effects similar to those seen in normal hearts were obtained in hearts from diabetic rats. Table 4 and Figure 1 show that glycolysis was increased and the step between F6P and FDP (i.e, PFK) was facilitated in diabetic hearts perfused with lipoic acid; citrate, α-ketoglutarate, and malate concentrations were reduced.

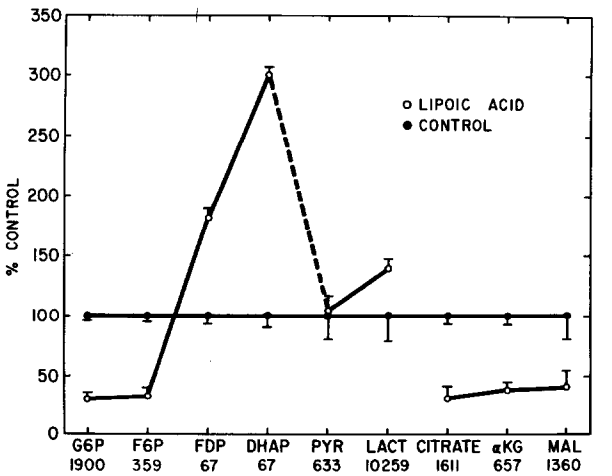

Figure 1. Levels of intermediates in hearts of alloxan diabetic rats after 30 minutes perfusion with 5.5 mM glucose and 1.0 mM DL-lipoic acid, plotted as percentages of levels in diabetic hearts perfused with glucose and no lipoate. Insulin was present in all perfusates. Control values (mμmoles/g dry wt) are given beneath each point; a range of 1 S.E.M. is shown. G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FDP, fructose 1,6-diphosphate; DHAP, dihydroxyacetone phosphate; PYR, pyruvate; LACT, lactate; α-KG, α-ketoglutarate; MAL, malate. The comparative rates of glycolytic flux are given in Table 4.

To rule out the possibility that PFK activity was stimulated in these experiments through a rise in AMP (16), the adenine nucleotides were measured. The levels of ATP, ADP, and AMP were found not to be altered in either normal or diabetic hearts by perfusion with lipoic acid.

During the course of this study a wide range of citrate concentrations was encountered. It appeared worthwhile to correlate statistically these values with the hexose monophosphate/hexose diphosphate ratios in order to more accurately assess the relationship between intra-cellular citrate and PFK activity. In hearts from both normal and diabetic rats a close correlation was found to exist between these two parameters ($r = 0.984$ and 0.929 for normal and diabetic hearts, respectively). The data (not shown) suggest that at moderately high citrate levels PFK activity may be more inhibited in diabetic than in normal hearts.

DISCUSSION: As referred to earlier, various circumstances have been described wherein decreased PFK activity has been associated with an elevated level of intra-cellular citrate. The opposite condition of depressed citrate levels and accelerated PFK activity has not been as easily demonstrated. Salas et al (17) reported that in yeast citrate is lowered by oxygen lack, but this has not been found in heart muscle (13); anoxia apparently stimulates PFK in the latter tissue via AMP (4) rather than through removal of citrate. The present results show that lipoic acid is a citrate-lowering agent, and that there is greater PFK activity and greater glucose utilization in hearts perfused with lipoate. The fall in citrate induced by lipoic acid is assumed to have resulted from a reduced concentration of acetyl-CoA (see Table 1).

There are several mechanisms that can be postulated through which lipoic acid might decrease the cardiac muscle content of acetyl-CoA. Formation of lipoyl-CoA would remove CoA which is necessary for fatty acid and pyruvate oxidation; alternatively, acetylation to form S-acetyldihydrolipoate (18) would serve to trap acetyl groups. There is also the possibility that lipoic acid or one of its derivatives might directly inhibit fatty acid oxidation. At a low concentration (0.1 mM) lipoic acid was not effective at decreasing citrate (data not shown), so that sequestering of acetyl groups by the higher concentration (1.0 mM) of

lipoic acid may be a more likely explanation. The oxidation of pyruvate was not impaired by lipoate (data not shown; see also Ref. 19), giving partial evidence against lipoyl-CoA formation as a mechanism of action.

Physiologically, lipoic acid functions in the oxidative decarboxylation of pyruvate and α-ketoglutarate, suggesting that excess lipoic acid might facilitate these reactions. Additionally, since pyruvate oxidation is inhibited by fatty acids (20) the question arises as to whether such inhibition may be partially due to competition with lipoate. However, lipoic acid is not easily disocciated from its enzyme binding site (21), and the fact that in the present work pyruvate oxidation was not significantly increased by lipoate mediates against its having acted here in its role as an enzyme co-factor. Nevertheless, Bauman and Hill (22) have presented data showing that exogenous lipoate may function in this manner. They found that 5-methoxyindole-2-carboxylic acid specifically blocked liver mitochondrial oxidation of both pyruvate and α-ketoglutarate, and that this inhibition was reversed *in vitro* by lipoic acid. It may be that exogenous lipoate may act in several but opposite ways. If lipoic acid were to facilitate pyruvate oxidation, the level of citrate would be expected to rise (10,23,24) and PFK activity to be inhibited. However, this effect would be opposed by the action of lipoate to lower citrate and stimulate PFK, as shown in the present experiments. The result which is manifested may depend upon the experimental preparation and the concentration of lipoic acid employed.

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