α-Glycerophosphate Dehydrogenase Inhibition in Rat Heart and Adipose Tissue (34541)

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Glucose and free fatty acids are known to be utilized as major sources of energy in heart and adipose tissue. It is also known that a reciprocal relationship exists between the consumption of these two oxidizable substrates since glycolysis is inhibited while fatty acids are being utilized. Free fatty acids which are produced as a result of lipolysis are either converted to acyl-CoA for the generation of energy or recycled to form triglycerides through reesterification with α-glycerophosphate. In heart and adipose tissue, which contain little or no glycerokinase, α-glycerophosphate is formed from glucose through dihydroxyacetone phosphate as an intermediate (1). Sodium octanoate was shown to inhibit hepatic and cardiac pyruvate kinase as well as several other key glycolytic enzymes in liver; and it has been suggested that free fatty acids act as an effective "metabolic directional switch" in the control of glycolysis and gluconeogenesis (2–4). NAD-linked α-glycerophosphate dehydrogenase, which catalyzes the interconversion of α-glycerophosphate and dihydroxyacetone phosphate has been studied in several rat tissues with respect to the regulation of its synthesis by thyroid hormones and the control of its activity by different metabolites (5–8). Fructose 1, 6-diphosphate, adenylic acid, and ADP all have been shown to be competitive inhibitors of muscle α-glycerophosphate dehydrogenase (9, 10). In the present report, we have studied the influence of sodium octanoate on α-glycerophosphate dehydrogenase activity in rat heart and adipose tissue.

Materials and Methods. Male Wistar rats, weighing approximately 180 g maintained on Master Laboratory Chow and water *ad libitum*, were used. The animals were stunned, decapitated, and exsanguinated. Heart and adipose tissue were rapidly excised, weighed, and placed in beakers chilled in crushed ice. The tissues were minced finely with scissors and 5% homogenates were prepared in 0.9% KCl solution, pH 7.4. Homogenization was effected with a Teflon plastic pestle turning at about 700 rpm for exactly 60 sec. The supernatant fluid was obtained by centrifuging the tissue homogenates at 100,000*g* at 0° for 30 min using a refrigerated IEC model B-60 centrifuge as described previously (11, 12). This preparation was always used fresh for the enzyme assay. The activity of α-glycerophosphate dehydrogenase was determined in the supernatant fluid under strictly linear kinetic conditions by measuring the formation of NAD from NADH in an assay system coupled with aldolase. The reaction mixture (final volume 3.0 ml) contained the following components in the given order of addition: Tris buffer, pH 7.4, 100 μmoles; NADH, 0.66 μmoles; aldolase, 50 μg of protein; 0.2 ml of the supernatant fluid corresponding to 10.0 mg fresh weight of tissue; and fructose 1,6-diphosphate, 108 μmoles. The blank reaction (the increase in NAD formation prior to the addition of fructose 1,6-diphosphate) was allowed to run for 3 min before the α-glycerophosphate dehydrogenase reaction was started by the addition of fructose 1,6-diphosphate. The changes in optical density were recorded for a period of 5 min and the enzyme activity was calculated as micromoles of substrate metabolized per gram of tissue per hour at 37°.

Results and Discussion. Sodium octanoate dissolved in distilled water (pH 7.4) was added in varying concentrations to the reac-

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TABLE I. Effect of Sodium Octanoate on α-Glycerophosphate Dehydrogenase Activity in Rat Heart and Adipose Tissue.[a]

Sodium octanoate (m*M*)	α-Glycerophosphate dehydrogenase (μmoles/g/hr)	
	Heart	Adipose tissue
None (control)	309 ± 7 (100)	414 ± 14 (100)
2.5	274 ± 6 (87)[b]	390 ± 10 (95)
5.0	236 ± 11 (77)[b]	340 ± 11 (82)[b]
10.0	202 ± 14 (64)[b]	300 ± 13 (73)[b]
20.0	158 ± 10 (51)[b]	279 ± 10 (68)[b]
40.0	101 ± 10 (32)[b]	159 ± 8 (39)[b]

[a] Varying concentrations of sodium octanoate were added directly into the reaction mixture just before the addition of fructose 1,6-diphosphate. Each value is the mean ± SE of three determinations of enzyme activity.

[b] Statistically significant difference as compared with the values of control rats ($p = <0.05$).

tion mixture and changes in α-glycerophosphate dehydrogenase activity of cardiac and adipose tissue are recorded in Table. I. A definite inhibition of the enzyme activity was observed at 5.0 m*M* concentration of octanoate. The activity of α-glycerophosphate dehydrogenase in both tissues declined progressively on augmenting the concentration of sodium octanoate, and in the presence of 40 m*M* octanoate, enzyme activity was decreased to 30–40% of the control values.

The effect of sodium octanoate on the affinity of α-glycerophosphate dehydrogenase for its substrate also was examined. Enzyme activity was assayed in the presence of varying amounts of fructose 1, 6-diphosphate with or without the addition of octanoate (10 m*M*). The reciprocal plots (Lineweaver-Burk) of velocity against substrate concentrations are given in Fig. 1. The increase in the apparent K_m value of both cardiac and adipose tissue enzyme observed in the presence of octanoate raises the possibility that free fatty acids might decrease the affinity of the enzyme towards its substrate. These plots which extrapolate to the same point on the ordinate also suggest that the observed inhibition of α-glycerophosphate dehydrogenase by octanoate is of the competitive type. The K_i values calculated from these data were 4.20×10^{-3} *M* for heart and 4.01×10^{-3} *M* for the adipose tissue enzyme.

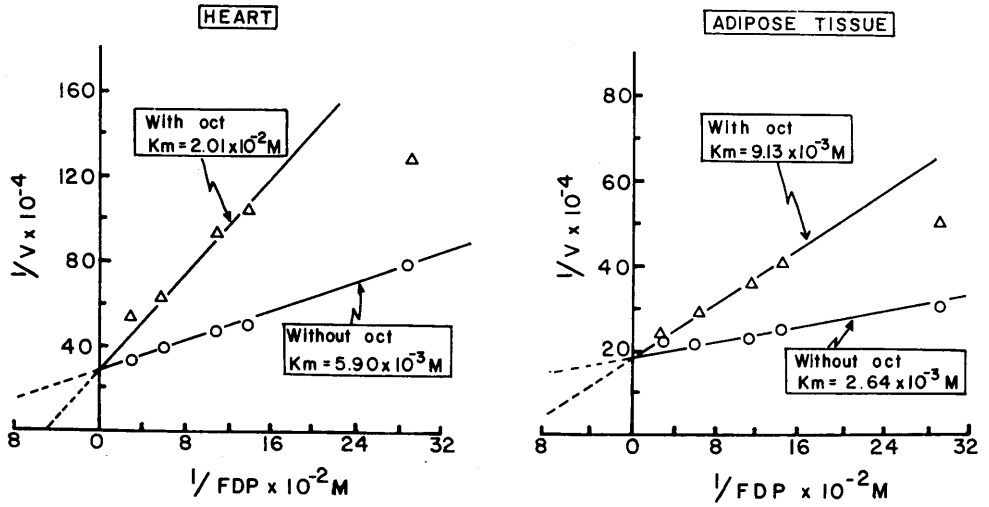

FIG. 1. Lineweaver-Burk plots showing competitive inhibition of α-glycerophosphate dehydrogenase by octanoate in heart and adipose tissue. The final concentration of sodium octanoate in the reaction mixture was 10 m*M*. Assay conditions were the same as described in the text excepting that varying concentrations of fructose 1,6-diphosphate were used. oct = octanoate.

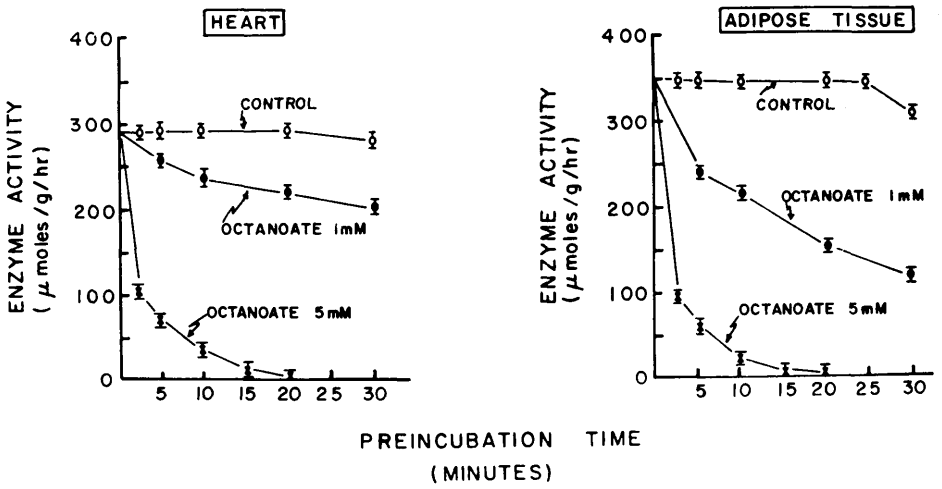

FIG. 2. Effect of preincubation with octanoate on α-glycerophosphate dehydrogenase activity in heart and adipose tissue. The supernatant fluids were preincubated for varying periods of time with either distilled water or sodium octanoate (1.0 or 5.0 m*M*). Each point represents the mean ± SE of four enzyme values.

Since preincubation of the supernatant fluid with free acids was shown to result in pronounced inhibition of hepatic and cardiac pyruvate kinase (2–4), the influence of incubation with octanoate on α-glycerophosphate dehydrogenase was investigated in heart and adipose tissue. Two ml of the supernatant fluid was preincubated at 37° for varying periods of time with either 1.0 ml of distilled water or sodium octanoate (1.0 or 5.0 m*M*). At the end of incubation, an aliquot of this mixture was used for assaying enzyme activity. The results presented in Fig. 2 demonstrate that when supernatant fluids were preincubated for 30 min without octanoate, there was little if any, loss of enzyme activity in either tissue. However, when sodium octanoate (1 m*M*) was included in the preincubation mixture, enzyme activity decreased progressively and after 30 min, was reduced by 30% in heart and 62% in adipose tissue. A more rapid and pronounced inhibition of the cardiac and adipose tissue enzyme was produced when the concentration of octanoate was augmented to 5.0 m*M*. In this case, enzyme activity decreased by almost 90% with 15 min of preincubation, and was completely inhibited when the supernatant fluids were incubated with octanoate for a 20-min period.

Since preincubation of the supernatant fluid with octanoate resulted in a much greater inhibition of α-glycerophosphate dehydrogenase, the possibility that a more active metabolite may have been formed from the free fatty acid during the incubation period, was considered. Formation of acetyl-CoA through acyl-CoA is an important metabolic step in the utilization of free fatty acids. It is conceivable that during the course of incubation, octanoate may have given rise to acetyl-CoA which in turn, might produce the observed pronounced inhibition of α-glycerophosphate dehydrogenase. This was examined by studying the direct effect of acetyl-CoA on enzyme activity in both heart and adipose tissue. When 0.2 m*M* acetyl-CoA was added directly to the assay mixture just before the addition of the substrate, enzyme activity was inhibited by 50% as compared to the control values. These results indicate that acetyl-CoA in a concentration range almost 100 times less than that of octanoate is capable of producing a similar inhibition of α-glycerophosphate dehydrogenase as that observed after the direct addition of the free fatty acid.

Weber *et al.* (2, 3) presented extensive evidence which indicates that the rapid action of free fatty acids in promoting glucone-

ogenesis may be explained, at least in part, by their inhibitory effects on the key enzymes of glycolysis which oppose the key enzymes involved in the process of gluconeogenesis. Under conditions of gluconeogenesis such as those during starvation and diabetes, the high rates of gluconeogenesis are associated also with large amounts of free fatty acids released by lipolytic hormones into the plasma. In consequence, fatty acids may function during acute adaptation as a "metabolic directional switch" by acting as allosteric inhibitors of hepatic key glycolytic enzymes (2, 3). Free fatty acids have also been shown to inhibit isocitrate dehydrogenase and fumarase which might contribute to a decrease in the activity of Krebs cycle (2). The present study indicates that sodium octanoate inhibits α-glycerophosphate dehydrogenase. The possibility can be raised that the generation of energy from carbohydrates and lipids is controlled by free fatty acids through their regulatory action on certain sites in the process of glycolysis and glycerophosphate and citric acid cycles. Additionally, NAD-linked α-glycerophosphate dehydrogenase in cytoplasmic fraction is known to regulate electron transport between extramitochondrial NAD and intramitochondrial cytochrome system (7, 13). The marked inhibition of α-glycerophosphate dehydrogenase by free fatty acids might change the NADH-NAD ratio in cell cytoplasm and thus affect the interconversion of carbohydrates and lipids.

Summary. Sodium octanoate, when added in increasing concentrations directly into the reaction mixture, resulted in progressive inhibition of α-glycerophosphate dehydrogenase activity in rat heart and adipose tissue. A complete inhibition of the cardiac and adipose tissue enzyme was produced when the supernatant fluids were preincubated with octanoate (5 m*M*) for a period of 20 min. It is suggested that the free fatty acids play an important regulatory role in controlling the interconversion of glucose and triglycerides through negative modulation of α-glycerophosphate dehydrogenase.

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