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Effects of Pent-4-enoate on Cellular Redox State, Glycolysis and Fatty Acid Oxidation in Isolated Perfused Rat Heart

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The metabolic effects of pent-4-enoate were studied in beating and potassium-arrested perfused rat hearts. The addition of 0.8mM-pent-4-enoate to the fluid used to perfuse a potassium-arrested heart resulted in a 70% increase in the O_2 consumption and a 66% decrease in the glycolytic flux as measured in terms of the de-tritiation of [3-^{3}H]glucose, although the proportion of the O_2 consumption attributable to glucose oxidation decreased from an initial 30% to 10%. The pent-4-enoate-induced increase in O_2 consumption was only 15% in the beating heart. In the potassium-arrested heart, pent-4-enoate stimulated palmitate oxidation by more than 100% when measured in terms of the production of $^{14}CO_2$ from [1-^{14}C]palmitate, but in the beating heart palmitate oxidation was inhibited. Perfusion of the heart with pent-4-enoate had no effect on the proportion of pyruvate dehydrogenase found in the active form, in spite of large changes in the CoASH and acetyl-CoA concentrations and changes in their concentration ratios. The effects of pent-4-enoate on the cellular redox state were dependent on the ATP consumption of the heart. In the beating heart, pent-4-enoate caused a rapid mitochondrial NAD^+ reduction that subsequently faded out, so that the final state was more oxidized than the initial state. The arrested heart, however, remained in a more reduced state than initially, even after the partial re-oxidation that followed the initial rapid NAD^+ reduction. The ability of pent-4-enoate to increase or decrease fatty acid oxidation can be explained on the basis of the differential effects of pent-4-enoate on the concentration of citric acid-cycle intermediates under conditions of high or low ATP consumption of the myocardial cell. The proportion of the fatty acids in the fuel consumed by the heart is probably primarily determined by the regulatory mechanisms of glycolysis. When pent-4-enoate causes an increase in the citric acid-cycle intermediates, feedback inhibition of glycolysis results in an increase in the oxidation of fatty acids.

Pent-4-enoate is usually regarded as an inhibitor of the β-oxidation of fatty acids (Corredor *et al.*, 1967; Senior & Sherratt, 1978*a*,*b*; Senior *et al.*, 1968; Brendel *et al.*, 1969; Fukami & Williamson, 1971). Its effects seem to be tissue-specific, however. Gorin & Zendowski (1975) showed that pent-4-enoate is a poor inhibitor of fatty acid oxidation in the adipose tissue. It does inhibit hepatic gluconeogenesis (Williamson *et al.*, 1970), but its effects on glycolysis are poorly characterized. It does not influence the rate of glycolysis in the isolated cytosol fraction from skeletal muscle (Senior & Sherratt, 1968*b*).

It was demonstrated that pent-4-enoate is rapidly metabolized in the heart and that its metabolism leads to a large accumulation of citric acid cycle intermediates [see the preceding paper (Hiltunen, 1978)]. In the present paper we are able to demonstrate that pent-4-enoate is an effective inhibitor of glycolysis in cardiac tissue and under certain conditions this may lead to a compensatory stimulation of the oxidation of long-chain fatty acids.

Experimental

Reagents

The standard reagents were obtained from E. Merck A.G., Darmstadt, Germany; NAD^+ and CoA were from Boehringer G.m.b.H., Mannheim, Germany. Dithiothreitol, bovine serum albumin and enzymes were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. [3-^{3}H]Glucose and [1-^{14}C]palmitate were purchased from NEN Chemicals G.m.b.H., Dreieichenhain, Germany, and NCS solution for CO_2 collection was from The Radiochemical Centre, Amersham, Bucks., U.K. Pent-4-enoic acid was obtained from Fluka A.G., Buchs, Switzerland.

Animals and perfusion methods

Handling of the animals, preparation of the heart for perfusion, the experimental arrangement and perfusion media were as described in the preceding paper (Hiltunen, 1978). The fatty acids were removed from bovine serum albumin by the method of Chen (1967), and palmitic acid was bound to the albumin as described by Krebs *et al.* (1969). This freshly prepared stock solution was added to the perfusion medium to give final palmitate concentrations of 12.5 μM or 0.2 mM.

Pyruvate dehydrogenase activity

The activity of pyruvate dehydrogenase was measured with [1-^{14}C]pyruvate as described by Hiltunen & Hassinen (1976). The final reaction medium consisted of 69.6 mM-potassium phosphate, pH 8.0, 0.17 mM-EDTA, 1 mM-dithiothreitol, 1.5 mM-NAD^+, 1.4 mM-$MgCl_2$, 4.3 mM-[1-^{14}C]pyruvate (specific radioactivity 15000–17000 d.p.m./μmol), 0.42 mM-CoASH, 50 μg of lactate dehydrogenase, 10 μg of phosphotransacetylase, and tissue homogenate in an amount equivalent to 3.5–5.5 mg of protein. The total volume was 1.15 ml, and the incubation temperature 30°C. Tissue homogenate was incubated with 10 mM-Mg^{2+} for 40 min at 25°C to activate the pyruvate dehydrogenase fully for the determination of total activity. One unit of activity was defined as the decarboxylation of 1 μmol of pyruvate/min.

Protein concentration was determined by the biuret method and corrected for turbidity with KCN (Szarkowska & Klingenberg, 1963).

Glycolytic rate and liberation of $^{14}CO_2$ from [1-^{14}C]-palmitate in the heart perfusion

The rate of glycolysis was determined by measuring the amount of ^3H_2O formed from [3-^{3}H]glucose, basically by the procedure of Safer & Williamson (1973). It was assumed that the 3-^{3}H of the glucose equilibrates with water at the aldolase and triose phosphate isomerase steps (Katz & Rognstad, 1966) with a complete loss of ^{3}H into the water. The apparatus described by Moss (1964) was used for the collection of ^3H_2O.

To measure the amount of $^{14}CO_2$ liberated from [1-^{14}C]palmitate, the effluent was collected under heptane at 1 min intervals. A sample of the effluent was then transferred to special vessels, acidified and shaken for 20 min for the collection of the $^{14}CO_2$. Equipment and conditions for the collection of $^{14}CO_2$ in NCS solution and measurement of radioactivity were described previously (Hiltunen & Hassinen, 1976).

Surface fluorimetry and oxygen consumption

Whole-organ fluorimetry was performed with a laboratory-built fluorimeter (Hassinen *et al.*, 1974) modified for heart perfusion as described previously (Hassinen & Hiltunen, 1975). A tungsten iodide lamp was used as the light source, the primary filter being a Corning no. 5840 and the secondary filter a combination of Corning nos. 4303 and 3387 for the nicotinamide nucleotide fluorescence. The i.r. radiation of the light source was cut off by 1 cm of saturated $CuSO_4$ in water. A Farrand 465 nm interference filter and a Corning no. 3484 filter were used as the primary and secondary filters respectively for the measurement of flavin fluorescence. The O_2 concentration in the venous perfusate was monitored by using a Radiometer E5046 oxygen electrode.

Results and Discussion

Glycolytic flux and oxygen consumption

Addition of 0.8 mM-pent-4-enoate to the medium perfusing a potassium-arrested heart resulted in a 70% increase in the O_2 consumption and a simultaneous 66% decrease in the glycolytic flux (Fig. 1). The efflux of lactate plus pyruvate similarly declined from an initial 1.77 μmol/min per g dry wt. to 0.722 μmol/min per g dry wt. From these values it can be calculated that 30% of the O_2 consumption is due to the oxidation of external glucose to CO_2 and water in the potassium-arrested heart. After the addition of pent-4-enoate, the proportion of the O_2 consumption attributable to glucose oxidation was decreased to 10%. The pent-4-enoate-induced

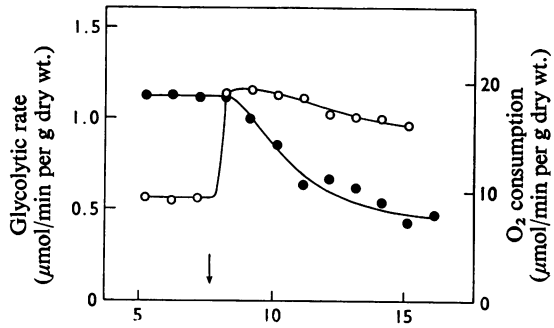

Fig. 1. *Effects of pent-4-enoate on O_2 consumption and glycolytic rate in a perfused potassium-arrested rat heart*

The heart was first perfused with Krebs–Ringer bicarbonate buffer, pH 7.4, containing 10 mM-glucose (in this experiment [3-^{3}H]glucose, sp. radioactivity 8000 d.p.m./μmol) for 15 min, after which the perfusion was continued with the same fluid but containing 15 mM-KCl. From the time indicated by the arrow the perfusion was continued with 0.8 mM-pent-4-enoate. ●, Glycolytic flux; ○, O_2 consumption. Results are from a typical experiment.

increase in O_2 consumption in the beating heart was only 15% (Fig. 2). The latter value could well be explained by the lower energy equivalence of the oxygen consumption during the oxidation of pent-4-enoate (0.205 mol of O_2/mol of ATP when pent-4-enoate is oxidized to malate+CO_2) than during the oxidation of glucose (0.158 mol of O_2/mol of ATP).

Oxidation of external [1-^{14}C]palmitate

Estimation of the rate of oxidation of endogenous fatty acids in the perfused heart is a difficult task, principally because the rate of pent-4-enoate oxidation cannot be measured. To keep the metabolic situation near that found without external fatty acids, very low concentrations of labelled palmitate were used (12.5 μM). In the potassium-arrested heart, pent-4-enoate stimulated palmitate oxidation by more than 100% (Fig. 3), and stimulation was of the same magnitude when 0.2 mM-palmitate was used (Fig. 4). In the beating heart, however, pent-4-enoate inhibited palmitate oxidation (Fig. 5).

This observation, that pent-4-enoate can considerably inhibit or enhance fatty acid oxidation depending on the metabolic situation, may be explained on the basis of the data obtained concern-

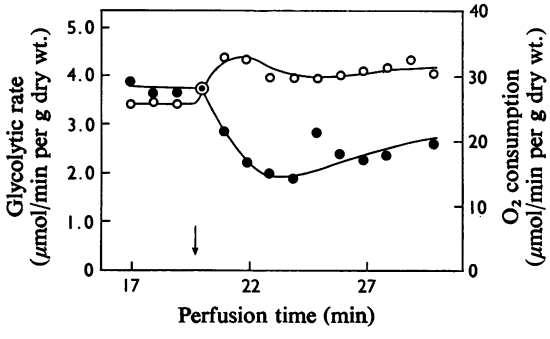

Fig. 2. *Effects of pent-4-enoate on O_2 consumption and glycolytic rate in a perfused beating rat heart*
The heart was perfused with Krebs–Ringer bicarbonate buffer, pH 7.4. The arrow indicates the beginning of perfusion with the same medium containing 0.8 mM-pent-4-enoate. ●, Glycolytic flux; ○, oxygen consumption. Results are from a typical experiment.

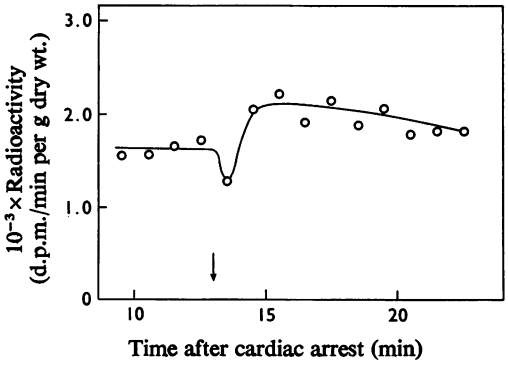

Fig. 3. *Effect of pent-4-enoate on $^{14}CO_2$ production from [1-^{14}C]palmitate in a perfused potassium-arrested rat heart*
A potassium-arrested heart was perfused with 10 mM-glucose and 12.5 μM-[1-^{14}C]palmitate (sp. radioactivity 184000 d.p.m./μmol). From the time indicated by the arrow the perfusion was continued with the same perfusion medium containing 0.8 mM-pent-4-enoate. Palmitate was bound to albumin as described in the Experimental section. Results are from a typical experiment.

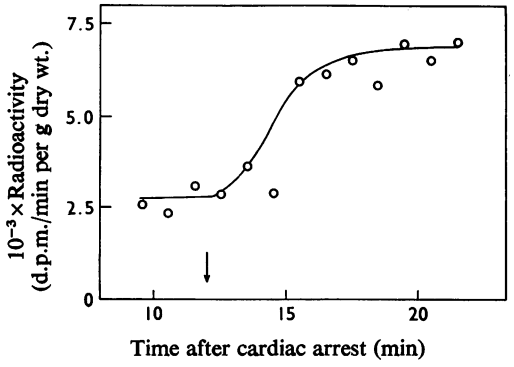

Fig. 4. *Effect of pent-4-enoate on $^{14}CO_2$ production from 0.2 mM-[1-^{14}C]palmitate in a perfused potassium-arrested rat heart*
The specific radioactivity of [1-^{14}C]palmitate was 36400 d.p.m./μmol. Other conditions are as in Fig. 3. Results are means from a typical experiment.

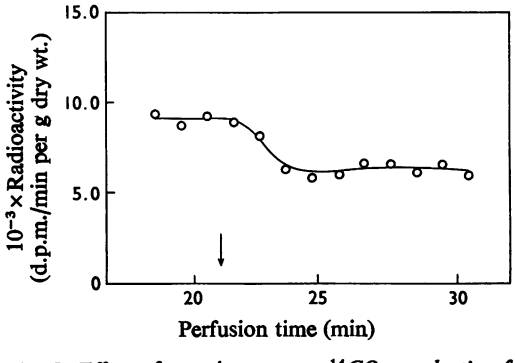

Fig. 5. *Effect of pent-4-enoate on $^{14}CO_2$ production from 12.5 μM-[1-^{14}C]palmitate in a perfused beating rat heart*
The specific radioactivity of [1-^{14}C]palmitate was 18400 d.p.m./μmol. Other conditions were as in Fig. 3. Results are means from a typical experiment.

ing the effects of pent-4-enoate on the concentrations of citric acid-cycle intermediates (Hiltunen, 1978). In the arrested heart, pent-4-enoate increases the pool of citric acid-cycle intermediates 16-fold, but in the beating heart only 5-fold. Thus glycolysis becomes inhibited by the high citrate concentration in the arrested heart, and the ATP demand is filled by a compensatory increase in fatty acid oxidation, whereas in the beating heart the inhibition of fatty acid oxidation by inhibitory products of pent-4-enoate oxidation over-rides the inhibition of glycolysis.

Pyruvate dehydrogenase interconversions

Senior & Sherratt (1968*a*) showed that pent-4-enoate inhibits the formation of $^{14}CO_2$ from [1-^{14}C]-pyruvate. It also has drastic effects on the tissue concentrations of acetyl-CoA and free CoASH, and influences the pyruvate concentration and the mitochondrial oxidation–reduction state (Hiltunen, 1978). Against this background it seems surprising that pent-4-enoate does not affect the proportion of the pyruvate dehydrogenase to be found in the active form (Table 1). The CoASH/acetyl-CoA ratio decreases from 8.0 to 1.1 after the addition of 0.8mM-pent-4-enoate to a beating heart, the phosphocreatine/creatine ratio increases, indicating a shift in the cellular energy state, the pyruvate concentration increases slightly, and the mitochondrial redox state shifts towards oxidation. The latter two effects probably compensate for the change in the CoASH/acetyl-CoA ratio, so that the net effect of the change in the effector concentrations is nil.

Cellular redox state

Since the activities of the strictly mitochondrial NAD^+-linked dehydrogenases are low in the heart, making the estimation of the redox potential of the mitochondrial NAD^+ couple difficult by methods involving metabolite determinations, surface fluorimetry of the heart was used to monitor the mitochondrial and cytosolic redox state.

The flavoprotein fluorescence of intact tissues is elicited mainly by the mitochondrial lipoamide dehydrogenase that is in equilibrium with the mitochondrial NAD^+ couple (Hassinen & Chance, 1968). Flavoprotein fluorescence thus serves as an indicator of the redox state of the mitochondrial NAD^+ couple. The fluorescence excited at 360nm is

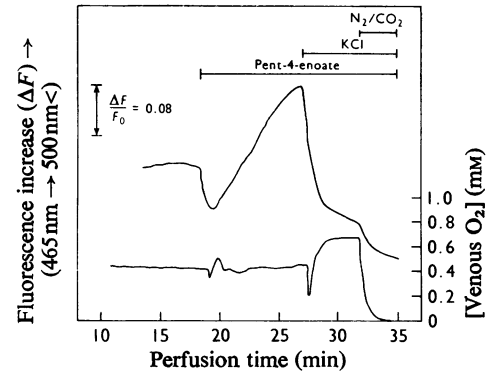

Fig. 6. *Effects of pent-4-enoate, cardiac arrest and anoxia on the redox state of fluorescent flavoproteins in a perfused rat heart*

The upper curve shows the surface fluorescence of the heart and the lower curve O_2 concentration in the venous perfusion fluid. The heart was initially perfused with Krebs–Ringer bicarbonate solution containing 10mM-glucose. The perfusion was continued with the same medium containing 0.8mM-pent-4-enoate, 15mM-KCl and with the same medium equilibrated with N_2/CO_2 (19:1) during the periods indicated by the horizontal bars. The traces are simultaneous recordings of a typical experiment. Fluorescence emission above 500nm was measured.

Table 1. *Pyruvate dehydrogenase activity in isolated rat heart perfused with* 0.8mM-*pent-4-enoate*

Hearts from rats fed *ad libitum* were perfused as described in the Experimental section. After pre-perfusion for 15min, the hearts were arrested or allowed to beat and the perfusion was continued for another 15min with pent-4-enoate where indicated. Values are means±S.E.M. for the number of independent biological experiments given in parentheses. One unit of activity was taken as 1 μmol of pyruvate decarboxylated/min. [P (versus control heart)* <0.05; P (versus beating heart under same conditions) ††† <0.0005.]

	Pyruvate dehydrogenase activity (munits/mg of protein)			
	Beating		Arrested	
	Actual activity	Total activity	Actual activity	Total activity
Control	7.9±1.2 (7)	21.0±1.3 (7)	1.3±0.3††† (5)	20.7±1.1 (7)
0.8mM-Pent-4-enoate	10.8±0.8* (5)	19.0±0.8 (5)	0.91±0.10††† (7)	20.2±0.2 (7)

due to the total cellular NADH+NADPH, allowing no means of making deductions about the compartmentalization of the redox changes.

The effects of pent-4-enoate on the redox state were dependent on the cellular energy state. In the beating heart, pent-4-enoate caused a rapid reduction of flavoproteins and nicotinamide nucleotides, which then faded out rapidly, resulting in oxidation compared with the basal redox state (Figs. 6 and 7).

In the arrested heart, pent-4-enoate also caused a rapid initial reduction of nicotinamide nucleotides, and this was only partially reversible, so that the result was a more reduced state than initially (Fig. 8). The redox state of the flavoproteins was restored after an initial reduction to roughly the same value as before the addition of pent-4-enoate (Fig. 9).

The effects on the mitochondrial redox state are in accordance with the effects on the fatty acid oxidation. Extensive oxidation of the flavoproteins (i.e. mitochondrial NADH) appears when pent-4-enoate inhibits fatty acid oxidation (in the beating heart) and only the initial flavoprotein reduction occurs when it enhances fatty acid oxidation (in the arrested heart).

The temporal pattern of the changes in the redox state and simultaneous changes in O_2 consumption merits some discussion. The intense mitochondrial NAD(P) reduction in the arrested heart lasts about 5 min, then it returns to near its initial value, whereas O_2 consumption also shows an initial burst coincident with the NAD(P) reduction, after which it remains stimulated. In the beating heart the reductive phase of the pent-4-enoate effect lasts about 3 min, then extensive flavoprotein (and NADH) oxidation occurs. The temporal pattern of the effects on the O_2 consumption is more complex; after an initial burst of respiration lasting a few seconds, an inhibition of about 1 min follows, after which O_2 consumption is stabilized almost to the initial value. However, the possibility that pent-4-enoate may affect the mechanical performance of the heart means that the data on O_2 consumption must be interpreted with caution.

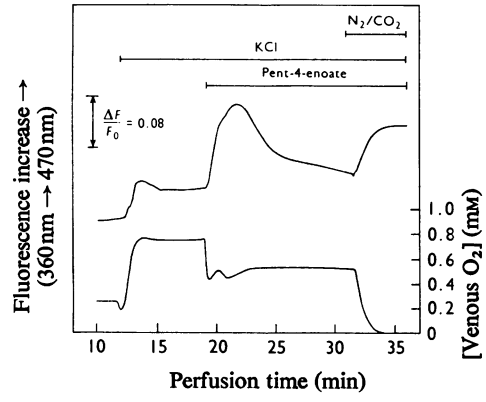

Fig. 8. *Effects of cardiac arrest and pent-4-enoate addition on the redox state of nicotinamide nucleotides in a perfused rat heart*

The upper curve shows surface fluorescence of the heart measured at wavelengths corresponding to the nicotinamide nucleotides. The lower curve shows O_2 concentration in the venous perfusion fluid.

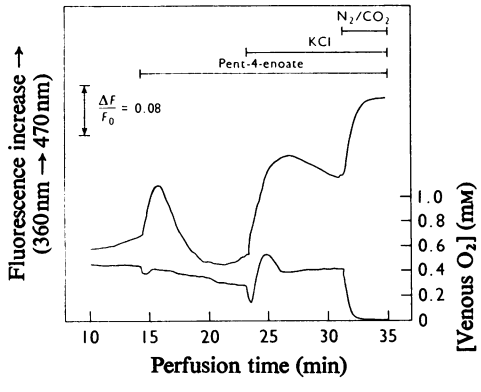

Fig. 7. *Effects of pent-4-enoate, cardiac arrest and anoxia on the redox state of nicotinamide nucleotides in a perfused rat heart*

The upper curve shows surface fluorescence of the heart measured at wavelengths corresponding to the nicotinamide nucleotides. The lower curve shows O_2 concentration in venous perfusion fluid. Conditions were as in Fig. 6.

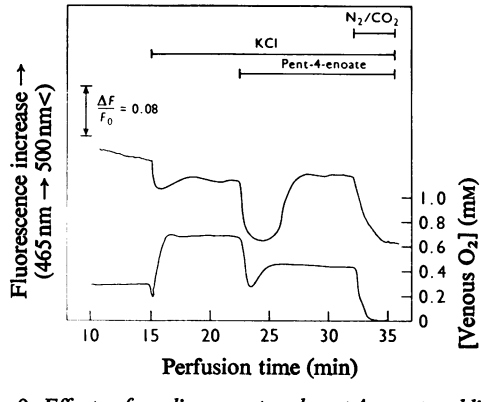

Fig. 9. *Effects of cardiac arrest and pent-4-enoate addition on the redox state of fluorescent flavoproteins in a perfused rat heart*

The upper curve shows surface fluorescence of the heart measured at wavelengths corresponding to flavoprotein fluorescence. The lower curve shows O_2 consumption in the venous perfusion fluid.

If the inhibitory action of pent-4-enoate on fatty acid oxidation is due to inhibitory intermediates arising from the β-oxidation of pent-4-enoate one could deduce that further metabolism of these intermediates is rapid in the heart. This is also in accordance with the observed accumulation of citric acid-cycle intermediates and an increased concentration ratio of phosphocreatine/creatine in hearts perfused with pent-4-enoate.

Conclusions

Pent-4-enoate is metabolized rapidly in cardiac muscle in comparison with other tissues. Its metabolic effects are dependent on the magnitude of the cellular O_2 consumption, the latter being a function of the energy demand of the cells. Its effects on glycolysis are probably mediated by the accumulation of citrate, the latter being due to the metabolism of the C_3 fragment of pent-4-enoate via the propionate pathway. During pent-4-enoate oxidation, the regulation of glycolysis is more efficient than either the regulation or inhibition of fatty acid oxidation. Under certain conditions this may lead paradoxically to a stimulation of fatty acid oxidation by pent-4-enoate. Owing to the efficiency of the propionate pathway, pent-4-enoate can be used by the heart as an energy-yielding oxidizable substrate.

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