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ESTIMATION OF PYRUVATE DECARBOXYLATION IN PERFUSED RAT SKELETAL MUSCLE

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Summary. By the determination of pyruvate dehydrogenase activity in tissue homogenates only limited information is gained on the actual metabolic flux. We therefore determined pyruvate decarboxylation in isolated rat hindlimbs non recirculating perfused with physiological (1-^{14}C)pyruvate levels. On the basis of perfusate pyruvate specific activity a $^{14}CO_2$ production of 15.8 ± o.5 nmol/min per g muscle was measured. However, by this method the actual pyruvate flux through the enzyme complex is underestimated by a factor of 7 due to the intracellular dilution of label.

Pyruvate, a key substrate of intermediary metabolism, is oxidatively decarboxylated by the mitochondrial pyruvate dehydrogenase complex (EC 1.2.4.1,EC 2.3.1.12,EC 1.6.4.3). Enzyme activity is regulated by a phosphorylation-dephosphorylation system and modified by changes in substrate, coenzyme, and ion concentrations (1-4). The activity state of the complex is usually determined in tissue homogenates under optimized conditions in vitro (5,6). By these assays only limited information is gained on the actual pyruvate flux through the enzyme in the intact tissue. Another approach is the determination of $^{14}CO_2$ release during perfusion of isolated organs with (1-^{14}C)pyruvate. The method has been widely used for the study of pyruvate metabolism in the perfused rat liver and heart (7-14) but has been scarcely applied to perfused skeletal muscle. In these systems the quantitative determination is complicated by $^{14}CO_2$ recycling due to $^{14}CO_2$ fixation and/or by the dilution of label due to the intracellular production of pyruvate. The present study was undertaken

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to determine the actual pyruvate flux in isolated rat hindlimbs non recirculating perfused with (1-^{14}C)pyruvate.

MATERIAL AND METHODS

Perfused hindlimb preparation. Hindlimbs of fed rats (Wistar - strain, 180-200 g body wt.) were prepared and non recirculating perfused with a standard medium comprising Krebs-Ringer-bicar - bonate buffer, pH 7.4, sodium pyruvate (0.15 mM and bovine serum albumin (60 g/l) as described (15). Additions to the influent were made with infusion pumps. Under standard perfusion condi - tions tracer doses of (1-^{14}C)pyruvate were infused to give final arterial specific activities of 80-90 dpm/nmol pyruvate. Oxygen concentration was measured with a Clark-type electrode. The effluent was fractionated and aliquots assayed for lactate (16) and pyruvate (17) by common spectrophotometric methods. The sources for the materials used were as in (15). Results are expressed as means ± standard deviation.

$^{14}CO_2$ release and pyruvate decarboxylation rates. For the determination of $^{14}CO_2$ one ml perfusate was incubated with an equal volume of 2 M citric acid in a CO_2 trap described previously (15). $^{14}CO_2$ release was calculated from the arterio-venous differences on the basis of the specific activity of (1-^{14}C)pyruvate in the perfusate, the flow rate and the perfused muscle mass. Likewise, the pyruvate decarboxylation rate was calculated on the basis of the specific activity of lactate in the effluent. Arterial $^{14}CO_2$ was below 1% of total radioactivity.

Separation of labelled metabolites. Effluent perchloric acid extracts were gassed with N_2 and neutralized with KOH. The amino acids were separated on an Amberlite IR 120 column, 200-400 mesh, H^+-form (18). The anionic fraction was neutralized and chromatographed on a Dowex 1X8 column, 200-400 mesh, formate form. Lactate was eluted with 0.6 M formic acid in a sharp peak and pyruvate with 6 M formic acid. Under standard conditions, the distribution of label in CO_2, pyruvate, lactate, and amino acids in the effluent was about 11%, 52%, 33% and 2%, respectively. For the determination of specific activity the lactate peak fractions were pooled and assayed for metabolite concentration and radioactivity. Alanine was specifically converted to lactate with alanine aminotransferase (EC 2.6.1.2) (19) and lactate dehydrogenase (EC1.1.1.27) and separated from the other amino acids as described above. No significant differences between the specific activities of lactate and alanine in the effluent could be observed.

RESULTS

Under standard conditions the pyruvate uptake (61 ± 14 nmol/min per g muscle, n=27) and lactate release (377 ± 82 nmol/min per g muscle, n=32) of rat hindlimbs remained constant up to 90 min of non recirculating perfusion. A stable plateau of labelled CO_2 and lactate release was reached 10 min after start of (1-^{14}C)pyruvate infusion (15). Creatine phosphate and adenine nucleotide levels as well as lactate and pyruvate concentrations of perfused muscle

tissue were within the range of in vivo values (data not shown). In perfusions with physiological pyruvate levels (0.15 mM) the $^{14}CO_2$ release of fed rats was 15.8 ± 2.6 nmol/min per g muscle (n=32). On the basis of the specific activity of effluent lactate a pyruvate decarboxylation rate of 115 ± 26 nmol/min per g muscle (n=23) was calculated. The oxygen consumption of 380 ± 24 nmol per min per g muscle (n=5) was mainly attributable to pyruvate oxidation. In experiments with 24 h fasted rats the $^{14}CO_2$ release was 10.6 ± 0.7 nmol/min per g muscle and the pyruvate decarboxylation rate 66 ± 10 nmol/min per g muscle (n=5). The results are further supported by experiments with perfusate glucose concentrations up to 20 mM. The $^{14}CO_2$ release decreased with increasing perfusate glucose, but the pyruvate oxidation rate remained essentially unaltered and was not significantly different from the rate in perfusions without glucose (data not shown). The effect of increasing perfusate pyruvate concentrations on the $^{14}CO_2$ release and pyruvate decarboxylation rates of perfused rat hind-limbs is shown in Fig. 1. Up to 2 mM perfusate pyruvate the difference between $^{14}CO_2$ release and pyruvate decarboxylation was highly significant ($p < 0.001$ by Student's t-test). The ratio of pyruvate decarboxylation to $^{14}CO_2$ release decreased from 7 at 0.15 mM pyruvate to about 1 at 10 mM pyruvate.

DISCUSSION

It is evident from the pyruvate uptake and lactate release rates in glucose free perfusions that there was a considerable dilution of label within the muscle cell. The assumption that the intracellular pyruvate labelling was reflected by the specific activity of effluent lactate seems justified for the following reasons: (a) The determinations were performed in the isotopic and metabolic steady state of perfusion. (b) Under the conditions employed lactate was exclusively produced from the

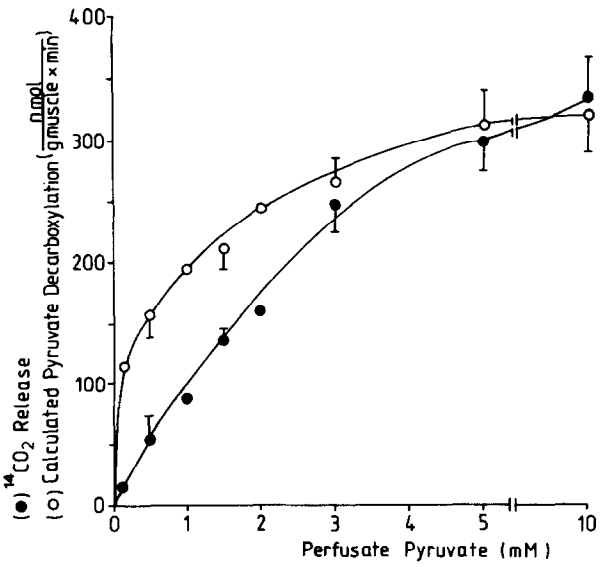

Fig. 1. Effect of increasing (1-^{14}C)pyruvate concentrations on the $^{14}CO_2$ release and pyruvate decarboxylation rates in isolated non recirculating perfused hindlimbs of fed rats. The $^{14}CO_2$ and the pyruvate decarboxylation rates were calculated from the $^{14}CO_2$ production on the basis of the specific activity of perfusate pyruvate and effluent lactate, respectively. Results are means ± standard deviation of at least three experiments.

intracellular pyruvate pool. (c) Tissue pyruvate and lactate levels remained in the normal range during perfusion. (d) The lactate dehydrogenase activity in skeletal muscle is sufficient to assure rapid distribution of label in the lactate and pyruvate pools (2o). (e) The dilution of label was reduced with increasing perfusate pyruvate concentrations. The $^{14}CO_2$ release and pyruvate decarboxylation rates approached at high perfusate pyruvate and were practically identical at 1o mM pyruvate. (f) Recently evidence was presented for the metabolic compartmentation of pyruvate in the perfused rat heart (21). The specific activity of alanine was shown to be closer correlated to the specific activity of intracellular pyruvate than lactate. In the present experiments significant differences between the specific activities of lactate and alanine could not be ascertained. (g) Perfusate oxygen supply of about 8oo nmol/min per g muscle accounts for the

oxidation of about 320 nmol pyruvate/min per g muscle. This value is in good agreement with the experimental results in perfusions with 10 mM pyruvate. (h) Disturbances from $^{14}CO_2$ recycling are negligible, because the CO_2 fixation is very low in skeletal muscle (22,23).

In comparison to fed rats, the pyruvate decarboxylation rate was reduced by about 50% in 24 h fasted rats. An appropriate decrease of the active form of the pyruvate dehydrogenase complex has been reported for the gastrocnemius muscle (24) and the perfused hindquarter of starved rats (25). The lacking effect of glucose on the pyruvate decarboxylation of perfused rat hindlimbs is similar to the observation of other authors. In experiments with insulin free incubated rat adipose tissue glucose concentrations up to 22.5 mM had no influence on the activity state of the adipose pyruvate dehydrogenase complex (26).

In conclusion, the present experiments with isolated perfused rat hindlimbs show that pyruvate flux through the pyruvate dehydrogenase complex was not reflected by the $^{14}CO_2$ production from (1-^{14}C)pyruvate. This was most distinct at physiological pyruvate concentrations. To consider the dilution of label the specific activity of intracellular pyruvate should be measured. This implies the withdrawal of freeze clamped tissue and is not possible with sufficient accuracy because of low tissue concentrations and disturbances from extracellular (1-^{14}C)pyruvate. In this study lactate has been shown to reflect intracellular pyruvate specific activity. This should be useful for further studies on the regulation of pyruvate fluxes in intact rat skeletal muscle.

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