MITOCHONDRIAL REDOX STATE AND THE REGULATION OF GLUCONEOGENESIS IN THE ISOLATED, PERFUSED CAT LIVER

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1. Introduction

Previous studies from this laboratory [1–4] have advanced the hypothesis that the oxidation–reduction state is a major factor regulating the rate of PEP† formation by isolated guinea pig, rabbit and human liver mitochondria. The difference in the regulation of gluconeogenesis in the perfused guinea pig and rabbit liver as compared to rat liver [5] underscores the importance of mitochondrial PEPCK, which makes up 50–60% of the total hepatic activity of this enzyme in many mammalian species [6, 7]. In this paper we have tested the influence of redox alterations in the mitochondria on the overall rate of glucogenesis in the perfused liver of the cat, a non-ruminant carnivorous animal in which the intracellular distribution of hepatic PEPCK [7] is similar to that of the human. The results show that, as in guinea pig liver, shifting the mitochondrial oxidation–reduction potential towards increased reduction inhibits gluconeogenesis. The gluconeogenic flux via mitochondrial PEPCK accounts for at least half of the overall rate of glucose production from lactate.

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† *Abbreviations*: PEP: phosphoenolpyruvate; PEPCK: phosphoenolpyruvate carboxykinase; β-OHB: β-hydroxybutyrate; AcAc: acetoacetate.

2. Methods

Livers were perfused with a non-recirculating hemoglobin-free Krebs–Ringer bicarbonate buffer, pH 7.4, as described previously [5] using the Scholz perfusion apparatus [8–10]. The cats weighed 450–700 g and were fasted for 48 hr before the experiment. The perfusion protocol is summarized in the legend to fig. 1. The oxygen tension in the effluent medium was continuously monitored by a Clark-type oxygen electrode. Glucose, acetoacetate and β-hydroxybutyrate in the perfusate were measured enzymatically as described previously [5].

3. Results

Fig. 1 shows that low concentrations of octanoate inhibited glucose production from lactate in the perfused cat liver. This inhibition was reversed upon termination of the fatty acid infusion. Similar concentrations of octanoate have been shown to stimulate gluconeogenesis in the perfused rat liver [5]. The inhibition of gluconeogenesis in the perfused cat liver was accompanied by an increased production of ketone bodies and by a 2-fold increase in the β-OHB/AcAc ratio in the perfusate. Both the β-OHB/AcAc ratio and the ketone body level in the perfusate fell to control levels when the fatty acid infusion was terminated. Subsequently the rate of glucose production returned to near control levels.

A clear-cut association between the intramitochondrial generation of reducing equivalents and inhibition of gluconeogenesis is illustrated in fig. 2 in which β-hydroxybutyrate was used as a source of mitochondrial

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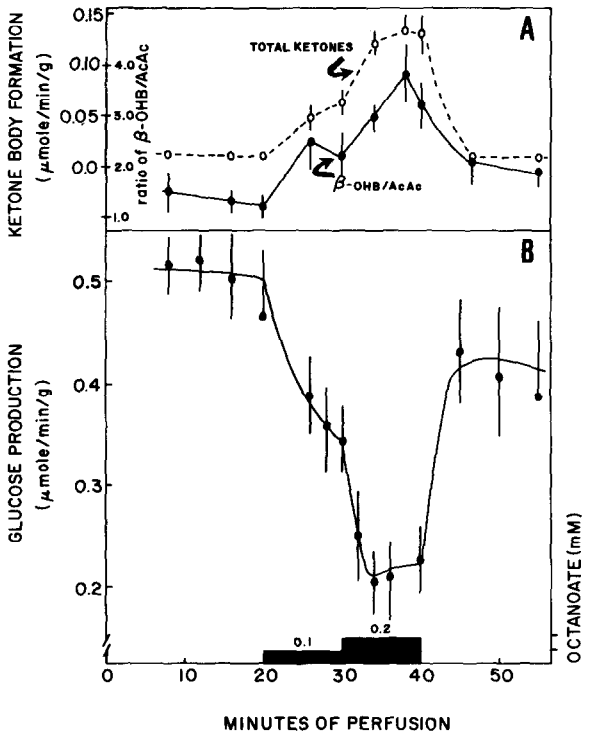

Fig. 1. Effect of octanoate on glucose production from lactate by the perfused cat liver. Livers from fasted (48 hr) cats were perfused with Krebs–Ringer bicarbonate buffer alone until endogenous glucose production was reduced to a minimum (0.061 ± 0.014 μmole/min/g). The zero time in the figure indicates the time of addition of lactate (2 mM). After a 20 min perfusion with substrate, octanoate was introduced from an infusion pump to deliver the indicated concentrations at the portal vein input. Points plotted are means ± S.E.M. (vertical bars) for 6 livers in (A) and 5 in (B).

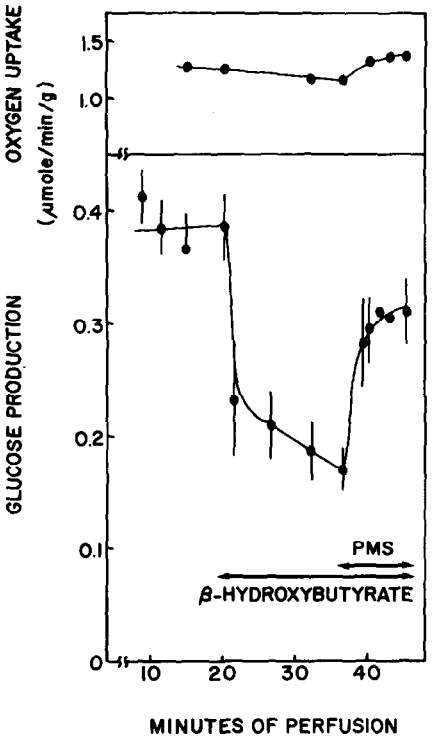

Fig. 2. Reversal of β-hydroxybutyrate-induced inhibition of glucogenesis in cat liver by phenazine methosulfate (PMS). Livers were perfused as described in the legend to fig. 1. β-Hydroxybutyrate and PMS were introduced as indicated. PMS (8 μM) had no effect on glucose production from lactate alone. Points plotted are means ± S.E.M. (vertical bars) for 4 livers.

NADH. A concentration of 8 mM β-hydroxybutyrate caused a 50% inhibition of glucose production. In similar experiments with rat liver, this metabolite was shown to enhance gluconeogenesis [5]. That the inhibition of gluconeogenesis in cat liver was due to the production of excess reducing equivalents is indicated by the reversal of the β-hydroxybutyrate-induced inhibition by the artificial electron acceptor, phenazine methosulfate (fig. 2).

The results in figs. 1 and 2 are better understood in the light of our earlier experiments [1–4] which implicate mitochondrial PEPCK in gluconeogenesis. To evaluate the extent to which this enzyme contributes to overall gluconeogenesis, the cytosolic formation of PEP via cytosolic oxalacetate can be blocked by the use of aminooxyacetate, an inhibitor of aspartate aminotransferase [11]. Fig. 3 shows that in the presence of 0.2 mM aminooxyacetate, glucose production from lactate was inhibited by about 60% in cat liver whereas in rat liver, which has negligible activity of mitochondrial PEPCK, gluconeogenesis was inhibited by more than 90%. In livers from fed cats in which the cytosolic activity of PEPCK is much smaller than in the fasting condition [7] the inhibition by aminooxyacetate was only 20–30%.

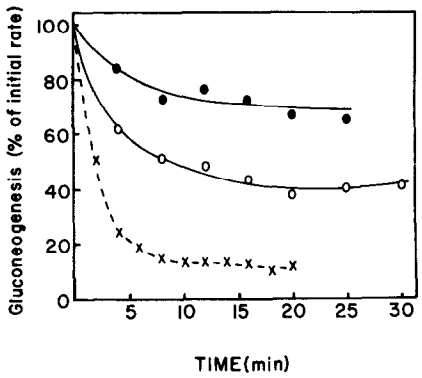

Fig. 3. A comparison of the effect of aminooxyacetic acid on gluconeogenesis from lactate in the perfused liver of the cat (o—o—o) and the rat (x—x—x). Aminooxyacetate was maintained at 0.2 mM by constant infusion. The closed circles (•—•—•) represent perfusions with livers from fed cats. Values plotted are means for 4–6 experiments.

4. Discusssion

In isolated mitochondria from the guinea pig or rabbit liver [2, 3] decreasing the proportion of NAD^+ relative to NADH lowers the steady state concentration of oxalacetate within the matrix space by the interaction of the NAD^+/NADH ratio with malate dehydrogenase [12]. Consequently, the rate of mitochondrial PEP synthesis falls. Jomain-Baum and Hanson [4] have recently reported an inhibition of PEP formation in isolated human liver mitochondria by octanoate at a concentration (0.2 mM) which inhibits gluconeogenesis in the perfused guinea pig liver [5]. The inhibition of gluconeogenesis in the perfused cat liver by mitochondrially-generated NADH (figs. 1 and 2) is similar to the effect of fatty acids on gluconeogenesis in the perfused guinea pig liver [5, 13]. These data support the contention that the oxidation–reduction state of the NAD^+-system in the mitochondria is of significant regulatory importance for overall hepatic glucose production in mammalian species which have substantial capacity for mitochondrial PEP formation. Similar results are obtained irrespective of the dietary habit of the animal. For example, fatty acids have also been reported to inhibit glucose production in portocaval shunted dogs [14]. The mitochondrial content of hepatic PEPCK in the dog [15] is similar to that of the guinea pig. In the rat, an animal that has been extensively used as a model for studying gluconeogenesis [16, 17], mitochondrial reducing equivalents have a stimulatory influence on glucose production [5, 18–20]. The almost identical response to fatty acids by mitochondria from both human and guinea pig liver [4] suggests that fatty acids may inhibit hepatic gluconeogenesis in the human.

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