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Gluconeogenesis in Perfused Eel Liver—Effect of starvation, amino-oxyacetate, D-malate and hormones

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Abstract

Any of them, ^{14}C-pyruvate, ^{14}C-lactate and ^{14}C-alanine, was incorporated into glucose linearly for 60 min by the perfused eel liver. Gluconeogenesis from ^{14}C-alanine after the starvation from 1 to 2 months was about 2 times as active as that after the starvation for one month. ^{14}C-Glucose synthesis from ^{14}C-lactate or ^{14}C-pyruvate was almost constant during the starvation for 2 months.

Gluconeogenesis from ^{14}C-pyruvate as well as from ^{14}C-lactate was inhibited by amino-oxyacetate by 40 to 50%. D-Malate did not effect on gluconeogenesis from either of these substrates.

Adrenalin at 5×10^{-5} M accelerated gluconeogenesis from ^{14}C-alanine by 50% and thyroxine also accelerated gluconeogenesis in the perfused eel liver.

Many teleost species are known to tolerate for a long period of starvation, and gluconeogenesis in their livers is stimulated during starvation[1.2.3]. In a previous investigation using the isolated perfused liver of the eel (*Anguilla japonica*), we have shown that the incorporation of lactate into glucose is almost the same as that in mammals[4.5]. Pyruvate or alanine is also an important precursor for gluconeogenesis in liver. So we investigated the rate of glucose synthesis from these precursors during the starvation of the eel.

In rat liver gluconeogenesis from lactate is inhibited almost completely by amino-oxyacetate, which is an inhibitor of transaminase, but no inhibition is obserbed in gluconeogenesis from pyruvate[6.7.8]. On the other hand D-malate inhibited gluconeogenesis from pyruvate[6]. Namely the metabolic pathway for the synthesis of glucose from lactate contains aspartate transaminase reaction, and that from pyruvate contains malate dehydrogenase reaction[9.10.11]. We found that in the eel liver amino-oxyacetate inhibited gluconeogenesis not only from lactate, but also from pyruvate. D-Malate did not inhibit gluconeogenesis from either of them.

Furthermore we investigated the effect of hormones, such as adrenalin and thyroxine, on gluconeogenesis. We examined previously that adrenalin stimulated the conversion of glycogen to glucose in the eel liver remarkably[4]. The stimulation by adrenalin was obserbed in the liver of the eel under the starvation, since the glycogen in the liver was maintained during the period[1.2]. Therefore, when we examined the effect of adrenalin on gluconeogenesis, the stimulation of the conversion of glyco-

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gen to glucose occured at the same time. To distinguish the glucose derived from glycogen, we used the rabelled substrate.

Experimental Procedure

Perfusion of the isolated liver The perfusion apparatus and procedure have been described previously[4]. The perfusion medium, KREBS-RINGER bicarbonate buffer (pH 7.4 at 25°C), was freshly made each day and oxygenated contineously with O_2 and CO_2 (95: 5). In all experiments the liver was initially perfused for 30 min with a recirculating medium. At the end of this period, one of them, sodium DL-(2-^{14}C)-lactate (10 mM, 0.85 μCi), sodium (2-^{14}C)-pyruvate (5 mM, 2.5 μCi) or L-(U-^{14}C)-alanine (5 mM, 0.5 μCi), added to the medium in the reservoir (50 or 100 ml.). In some experiments, in order to maintain a constant substrate level the perfusion was changed to a nonrecirculating or "flow-through" system.

Separation of ^{14}C-glucose from ^{14}C-labelled substrates ^{14}C-Glucose synthesized by the perfused eel liver was separated from the ^{14}C-labelled substrate by the treatment of two kinds of resins (Dowex 1×8, formate type and Dowex 50×8, H^+ type) as described previously[5]. 0.5 ml. of ^{14}C-glucose-fraction was added to 5 ml. of toluene containing 4 g. of 2, 5-diphenyloxazole (DPO), 0.1 g. of 1, 4-bis (2-(5-phenyloxazoyl)) benzene (POPOP) and 500 ml. of Triton X-100 per *litre* and the radioactivity was determined in a liquid scintillation spectrometer (Beckman LS-230).

Chemicals Radioactive compounds were obtained from the Radiochemical Center Amersham. Amino-oxyacetate (carboxymethoxylamine hemihydrochloride), D-malate and thyroxine were obtained from Wako Pure Chemical Industries, LTD. Adrenalin was obtained from E. Merck.

Results

Effect of starvation on gluconeogenesis Precursor for gluconeogenesis, such as pyruvate, lactate or alanine, was incorporated into glucose linearly by the perfused eel liver for 60 min as shown in Fig. 1. Effect of starvation on gluconeogenesis from these precursors was investigated by using eels under the starvation for 2 months. At the period of starvation for one month the rate of incorporation of ^{14}C-lactate and ^{14}C-pyruvate into glucose was 4.9 and 13.1% respectively. After the starvation from 1 to 2 months, the rate of ^{14}C-glucose-synthesis from ^{14}C-lactate or ^{14}C-pyruvate was not changed. But the starvation from 1 to 2 months accelerated ^{14}C-glucose synthesis from ^{14}C-alanine by 100% (Table 1).

Effect of amino-oxyacetate or D-malate on gluconeogenesis from ^{14}C-pyruvate or ^{14}C-lactate In mammals such as rat and guinea pig amino-oxyacetate inhibits gluconeogenesis from lactate almost completely, but there is no its effect on gluconeogenesis from pyruvate[6,7]. On the other hand D-malate inhibits glucose-

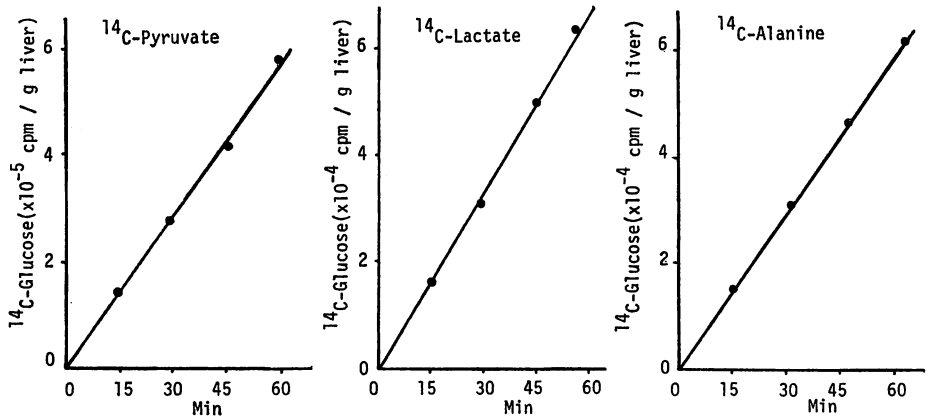

Fig. 1. Time course of the incorporation of (2-^{14}C)-pyruvate (5 mM, 2.5 μCi), DL-(2-^{14}C) -lactate (10 mM, 0.85 μCi) and L-(U-^{14}C)-alanine (5 mM, 0.5 μCi) into glucose by perfused eel liver.

Table 1. Effect of starvation on the rate of ^{14}C-glucose synthesis from labelled substrates. Means±s.e.m. are given with the numbers of observation in parentheses.

Periods of Starvation (month)	^{14}C-lactate (%)	^{14}C-pyruvate (%)	^{14}C-alanine (%)
0–1	4.89±2.13(5)	13.15±3.95(3)	5.11±0.68(3)
1–2	5.71±3.07(3)	17.15±7.32(3)	13.09±2.69(3)

$$\% = \frac{\text{^{14}C-glucose dpm/g liver/hr}}{\text{total dpm labelled substrate added initially}} \times 100$$

synthesis from pyruvate, but there is no inhibitory effect of D-malate on gluconeogenesis from lactate[6]. These differences are due to the difference between the metabloic pathways for phosphoenolpyruvate formation from lactate and that from pyruvate[8.9.10.11].

In the perfused eel liver amino-oxyacetate at 1 mM inhibited ^{14}C-glucose synthesis from either ^{14}C-lactate or ^{14}C-pyruvate as shown in Fig. 2. However the inhibition was 40 to 50%. On the other hand no inhibition by D-malate was observed in gluconeogenesis from ^{14}C-pyruvate or ^{14}C-lactate.

Effect of adrenalin or thyroxine on gluconeogenesis from ^{14}C-alanine

In spite of a long period of starvation glycogen in eel liver is stored. Adrenalin at 10^{-2} mM promoted the conversion of glycogen to glucose remarkably in the perfused liver of the eel under the starvation as described previously[4].

As shown in Fig. 2, the incorporation of ^{14}C-alanine into glucose was promoted by adrenalin in the perfused liver. As adrenalin has contracting action on the blood vessel, the flow rate of the perfused medium in the hepatic vein decreased (Fig. 4). So in the experiments of the effect of adrenalin the calculation of the incorporation

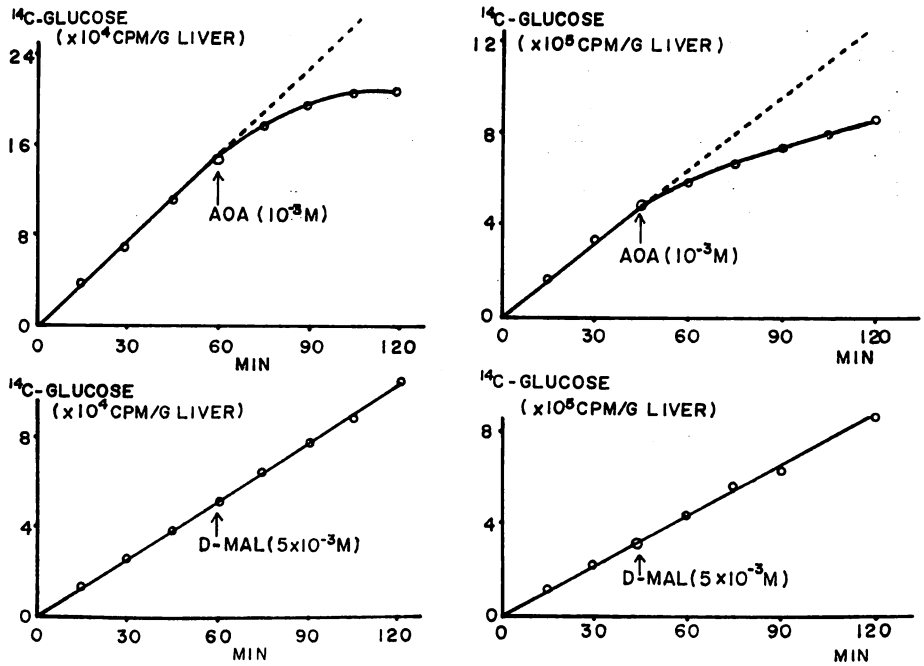

Fig. 2. Effect of amino-oxyacetate or D-malate on ^{14}C-glucose synthesis from DL-(2-^{14}C)-lactate (6 mM, 1.25 μCi) or (2-^{14}C)-pyruvate (3 mM, 1.25 μCi). (left: ^{14}C-glucose synthesis from ^{14}C-lactate. right: ^{14}C-glucose synthesis from ^{14}C-pyruvate.)

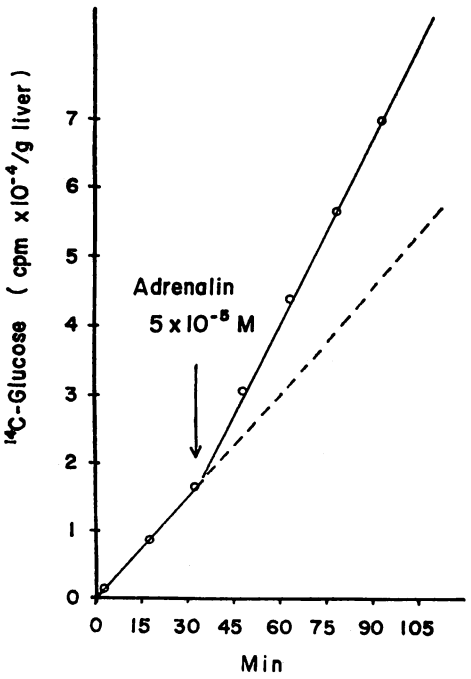

Fig. 3. Effect of adrenalin on ^{14}C-glucose synthesis from L-(U-^{14}C)-alanine (2.5 mM, 0.13 μCi).

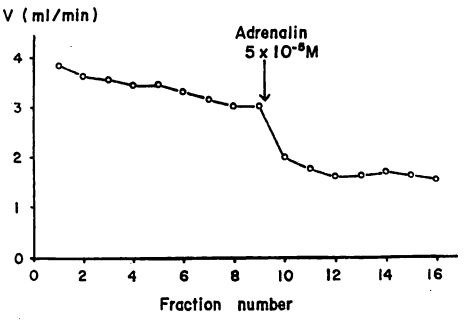

Fig. 4. Effect of adrenalin on the flow rate of the perfused medium in the hepatic vein.

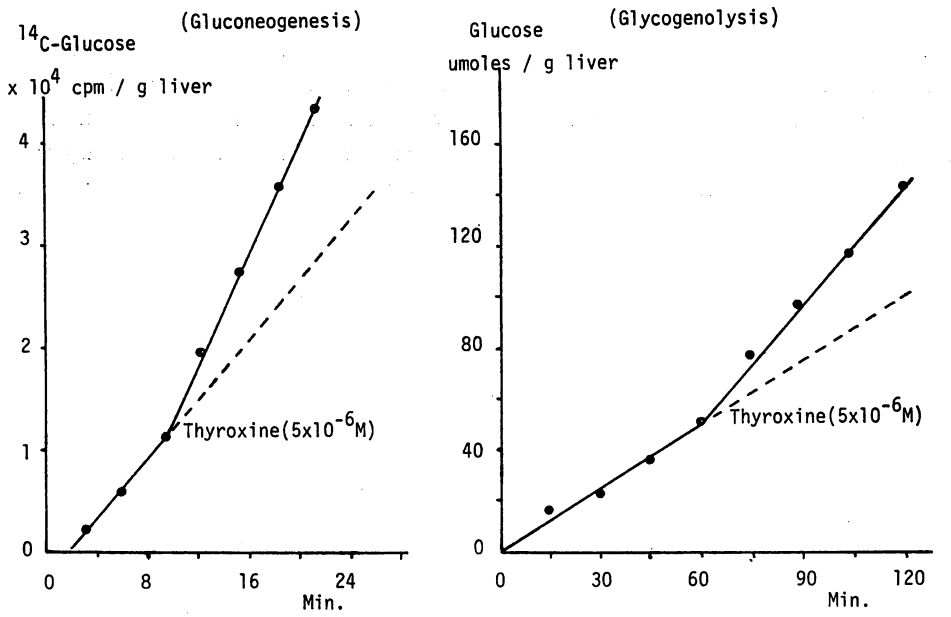

Fig. 5. Effect of thyroxine on ^{14}C-glucose synthesis from L-(U-^{14}C)-alanine (2.5 mM, 0.13 μCi) and on glycogenolysis in the perfused eel liver.

of ^{14}C-alanine into glucose took account of the flow rate of the perfused medium. Thyroxine as well as adrenalin promoted glycogenolysis and gluconeogenesis from ^{14}C-alanine in the liver perfused with nonrecirculating medium (Fig. 5). But thyroxine promoted the conversion of glycogen to glucose not so remarkably as adrenalin.

Discussion

When eels changed from yellow eels, which is the sexually immature form, to silver eels leaving the coasts, they apparently cease to eat. As LARSSON *et al.*[1,12] reported about metabolic effects of starvation of eel or metabolic and hematological studies on the yellow and silver phases of the European eel, we don't know how to supply the energy during the migration of eel.

From result of the effect of starvation on gluconeogenesis in the perfused eel liver, amino acids seem to be an important sourse of glucose during the migration. During the starvation from 1 to 2 months, ^{14}C-glucose synthesis from ^{14}C-alanine increased by 100% as shown in Table 1. But ^{14}C-glucose synthesis from ^{14}C-lactate or ^{14}C-pyruvate was almost constant during the starvation for 2 months.

Amino-oxyacetate inhibited ^{14}C-glucose synthesis from either ^{14}C-lactate or ^{14}C-pyruvate. In mammals there is no reports about the inhibition of gluconeogenesis from pyruvate by amino-oxyacetate. Though the metabolic pathway for the synthe-

sis of glucose from glucogenic precursors is not yet known in the eel liver, it is suggested from the results obtained in rat liver that gluconeogenesis from pyruvate as well as lactate contains aspartate transaminase reaction in the eel liver. It is known in mammals that the enzymes of this pathway are distributed between the mitochondria and the cytoplasm, and that pyruvate carboxylase is found exclusively in the mitochondria and phosphoenolpyruvate carboxykinase is in the cytoplasm in the rat, in the pigeon it is mainly mitochondria and in the guinea pig, phosphoenolpyruvate carboxykinase is almost evenly distributed between the mitochondria and cytoplasm[13]. There is no investigation about the distribution of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in the liver of fish. We are now under the investigation about the distribution of these two enzymes.

LARSSON[1] reported that the eels increased their level of plasma free fatty acids as a response to adrenalin treatment. Adrenalin at 5×10^{-5} M promoted ^{14}C-glucose synthesis from ^{14}C-alanine. But its effect on gluconeogenesis was not so strong as that on the conversion of glycogen to glucose.

Thyroxine promoted the migration of fishes of salmonids from river to sea[14], or promoted the synthesis of purines in yamame, *Onchorhynchus masou*[15]. As shown in Fig. 5, thyroxine as well as adrenalin promoted ^{14}C-glucose synthesis from ^{14}C-alanine in the perfused liver of the eel. However we don't know the effect of adrenalin or thyroxine on gluconeogenesis *in vivo* during the migration of the eel. As described by BUTLER[16], further studies on the effect of adrenocortical steroids in gluconeogenesis will be necessary.

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