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Glycogenolysis and Gluconeogenesis by Eel Liver Slices

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Abstract

Effect of epinephrine, insulin and 3′, 5′-c-AMP on glycogenolysis was investigated by eel liver slices and compared with the studies by perfused eel liver. Epinephrine and 3′, 5′-c-AMP stimulated glycogenolysis about 1.5 to 2 times and insulin suppressed glucose production in liver slices.

Gluconeogenesis from lactate was also studied by eel liver slices. Under the experimental condition the rate of gluconeogenesis by liver slices was not comparable to that measured in perfused liver.

Liver slices, perfused liver and isolated liver cells are useful for examination of the effect of hormone (glucagon, epinephrine or insulin) on glycogenolysis or gluconeogenesis and for studies of metabolisms in vitro. However it is due to experimental animal that which of these methods is most useful.

In this report we investigated whether eel, *Anguilla japonica,* liver slices were useful for studies of glycogenolysis and gluconeogenesis in vitro, and compared with the studies by perfused eel liver.

Experimental

Preparation of liver slices Eels (90 to 120 g) were obtained from fish market. They were kept in a brackish water water tank and fasted. Eels were anaesthesized in 0.8% urethane (ethyl carbamate) solution. The abdomen was opened through a midline incision from the anus. The hepatic portal vein was cut with scissors and the polyethylene tubing was inserted. The liver was perfused with KREBS-RINGER bicarbonate buffer oxygenated with O_2 and CO_2 (95: 5). Then the liver was removed and sliced with a razor on filter paper humidified with KREBS-RINGER bicarbonate buffer. Liver slices were immediately added to the test tubing containing 10 ml of the buffer oxygenated with O_2 and CO_2. The test tubing were incubated at 25°C.

Determination of glucose Glucose was determined by the calorimetric method using o-toluidine-borate reagent described by Sasaki[1].

Determination of lactate Lactate was determined with lactate dehydrogenase according to the method of Hohorst[2].

Technique of liver perfusion The perfusion apparatus and procedure have

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been described previously[3].

Chemicals Crystaline insulin (from bovine pancreas) was obtained from Sigma Chemical Co. Crystaline epinephrine was obtained from E. Merck AG. Crystaline 3′, 5′-c-AMP was generously provided by Yamasa Soybean Souce Co., Research Institute.

Results

Effect of epinephrine The increase of glucose production was observed by the addition of epinephrine (0.5 mM) and was about 2 times of that of control at 60 min after the addition of epinephrine (Fig. 1). In perfused liver the increase of glucose production by epinephrine was observed more remarkably than in liver slices. 10^{-2} mM of epinephrine caused an increase in glucose output in perfused liver[3].

Effect of insulin Insulin at a concentration of 4×10^{-3} mM (545 mμ units/ml) suppressed glucose production in liver slices (Fig. 2). However the suppression by insulin in liver slices was not strongly as in perfused liver. In perfused liver insulin at a concentration of 4.4×10^{-6} mM (0.6 mμ unit/ml) suppressed glucose production almost completely[3].

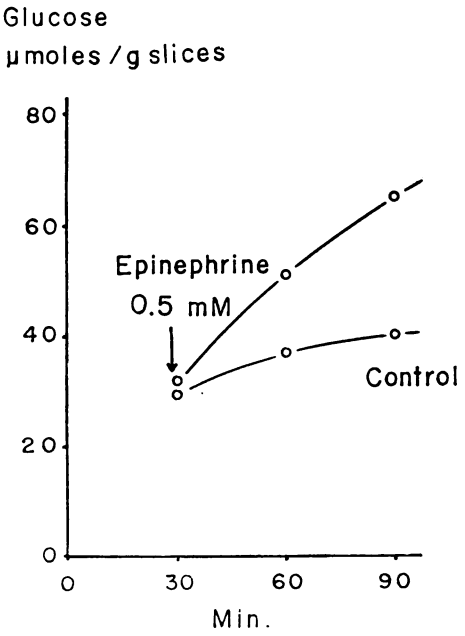

Fig. 1. Effect of epinephrine on glucose production. Epinephrine was added at 29 min to give final concentration indicated.

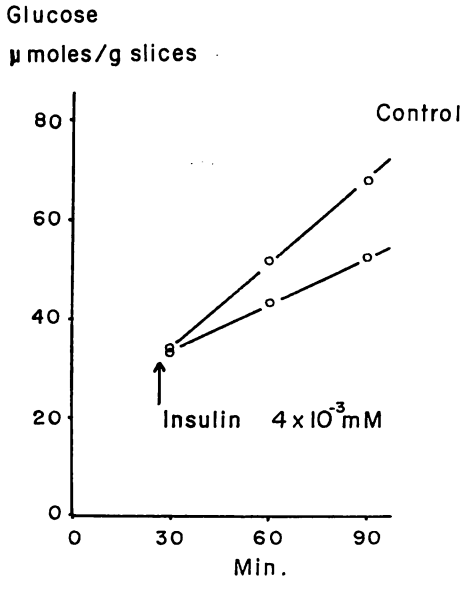

Fig. 2. Effect of insulin (4×10^{-3} mM, 545 mμ units/ml) on glucose production. Insulin was added at 29 min.

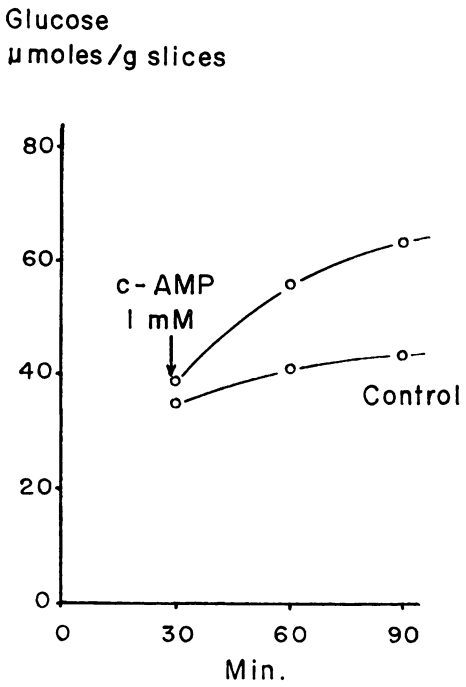

Fig. 3. Effect of 3′, 5′-c-AMP on glucose production. 3′, 5′-c-AMP was added at 29 min to give final concentration indicated.

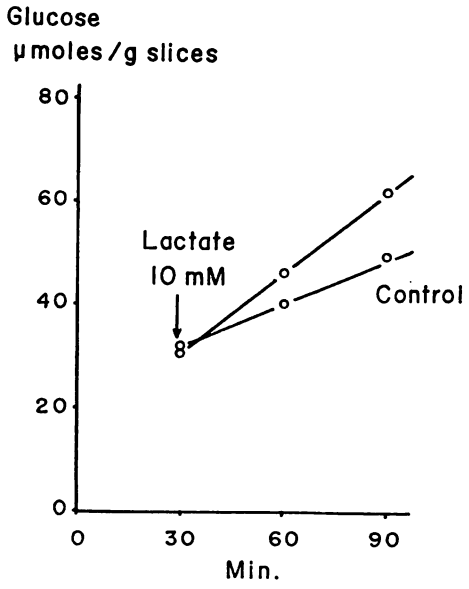

Fig. 4. Gluconeogenesis from lactate by eel liver slices. Lactate (10 mM) was added at 29 min.

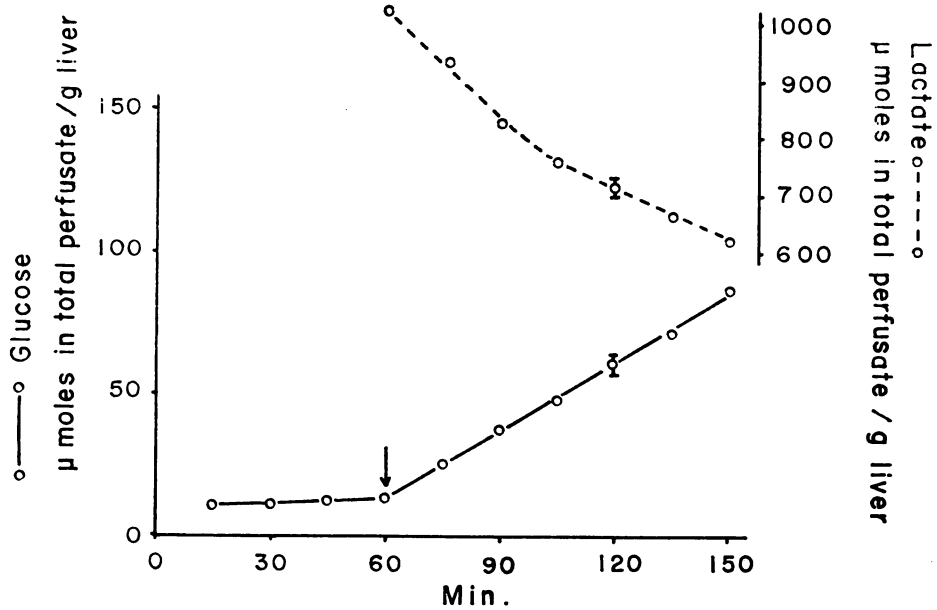

Fig. 5. Gluconeogenesis from lactate (10 mM) by the perfused eel liver. Lactate was added at 58 min. Values are means of four observations; the *vertical bars* indicate 2 S. E. M.

Effect of 3′, 5′-c-AMP The increase of glucose production was observed by the addition of 1 mM of 3′, 5′-c-AMP in liver slices (Fig. 3). In perfused liver 3′, 5′-c-AMP at a concentration of 10^{-2} mM caused an increase in glucose production more remarkably[3].

Gluconeogenesis from lactate Glucose production increased by the addition of 10 mM of lactate (Fig. 4). The increase of glucose production in liver slices was not so remarkably as in perfused liver. In perfused liver the rate of glucose formation was 0.90 μmol/min/g liver and the amount of lactate decerased at a rate of 2.07 μmoles/min/g liver (Fig. 5).

Discussion

The effects of epinephrine and insulin were observed in eel liver slices. Epinephrine at a concentration of 10^{-2} mM caused an increase in glucose output. Gluocse production was suppressed by 4×10^{-3} mM of insulin. Gluconeogenesis from 10 mM of lactate was also recognized. These findings provides possibilities to use liver slices for studies of glycogenolysis and gluconeogenesis in eel liver in vitro. However liver slices produced glucose from lactate at lower rate than perfused liver did and the effects of epinephrine, insulin or 3′, 5′-c-AMP were not so remarkably in liver slices as in perfused liver. These results may be improved by preparation of liver slices being thinner. It has been known that important caution to prepare liver slice is to slice the liver as thinly as possible.

Six to eight numbers of experiments are done at the same time by using liver slices prepared from one liver. In experiments using perfused livers it takes much time. However perfused liver is more organized than liver slices, so the physiological state of perfused liver is more like the state in vivo.

It must be considered that supposing results obtained by these two different methods are the same, physiological state between liver slices and perfused liver is not the same. For example it has been known that the effect of glucagon on glycogenolysis is different between liver slices and perfused liver of rat. And the rate of gluconeogenesis from lactate is different between perfused liver and isolated hepatocytes of rat[4].

References

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