GLUCOSE EFFECT ON GLYCOGEN SYNTHETASE AND PHOSPHORYLASE IN FETAL RAT LIVER

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1. Introduction

Glucose administration results in an increase of adult rat liver glycogen synthetase *a*, both in vivo [1,2] or in vitro [3]. Stalmans et al. [4] suggested that glucose results a conversion of glycogen phosphorylase *a* to phosphorylase *b*. As phosphorylase *a* inhibits glycogen synthetase phosphatase, this conversion releases this inhibition and results in an activation of glycogen synthetase. On the other hand, other investigators reported that glucose administration to fasted rats causes a direct conversion of synthetase *b* to synthetase *a* unrelated to a preceding decrease of phosphorylase *a* activity [5,6].

In the foetal rat liver a progressive increase in glycogen concentration occurs from the 18th day of gestation until birth which subsequently decreases within 24 h to about 25% of its former maximal level [7]. These changes in glycogen concentration are mainly due to the activation and inactivation of glycogen synthetase, whereas phosphorylase plays apparently a minor role in this process [7–9].

The present study presents evidence that in the foetal rat liver during enzyme development a changing pattern of glucose effect on glycogen synthetase and phosphorylase is demonstrable. Whereas on the 17th day of gestation glucose exerts a direct effect on glycogen synthetase *a* activity which is not accompanied by any change on phosphorylase *a* activity, on the 21st day, activation of glycogen synthetase is preceded by a decrease in phosphorylase *a* activity.

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2. Materials and methods

2.1. *Handling of animals and of livers*

Three month-old Wistar rats on the 15th day of gestation weighing about 280g were obtained from the Weizmann Institute of Science. The fetuses were delivered by caesarean section after anaesthesizing the mothers. Anaesthesia was induced by intraperitoneal administration of 30 mg chloral hydrate per 100g body weight. The foetal livers were rapidly removed into liquid nitrogen. Homogenization was performed in a motor homogenizer with 4 vols of ice-cold 0.05 M Tris buffer pH 7.4.

2.2. *Enzyme assay*

Glycogen synthetase *a* activity was measured by the incorporation of the [^{3}H]glucose moiety of labelled UDPG into glycogen [7]. Glycogen phosphorylase *a* activity was measured by the incorporation of glucose from glucose-1-P into glycogen as previously described [7]. Synthetase *a* and phosphorylase *a* activities were measured in liver homogenates incubated at room temperature and samples were taken at 15 min interval during one hour. Activation of synthetase (conversion of synthetase *b* to *a*) and the inactivation of phosphorylase (conversion of phosphorylase *a* to *b*) reflect their respective phosphatase activities.

2.3. *Materials*

Radioactive chemicals D[U-^{14}C]glucose-1-P and uridine diphospho[^{3}H]glucose were obtained from the Radiochemical Centre Amersham, England. All other chemicals were purchased from Sigma Co. St. Louis, USA.

3. Results and discussion

It is shown in fig.1 that glucose administration to the peritoneal cavity of the pregnant rat beginning from the 17th day post conception until birth causes two-fold increase in glycogen synthetase *a* activity in the foetal liver homogenate. When the homogenates are incubated at room temperature a gradual increase in the activity of synthelase *a* takes place, which reflects the activity of glycogen synthetase phosphatase. The fact that in the glucose treated animals, synthetase *a* activity continues to rise during the incubation period suggests that the glucose effect prior to the incubation was incomplete.

In contrast, glycogen phosphorylase *a* activity is not altered by the administration of glucose until the 21st day of foetal life (fig.2). In order to follow the

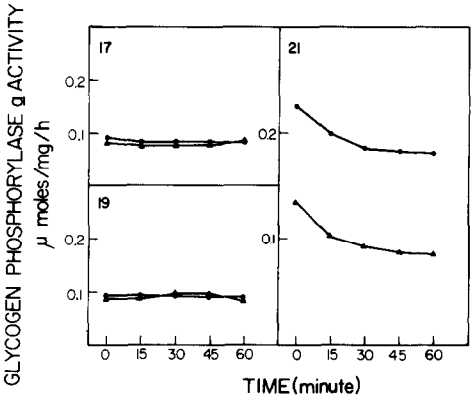

Fig.2. Glucose effect on glycogen phosphorylase *a* activity and its in vitro inactivation on different days of foetal age. Experimental conditions similar to fig.1. (●) Control animals. (▲) Glucose-treated animals.

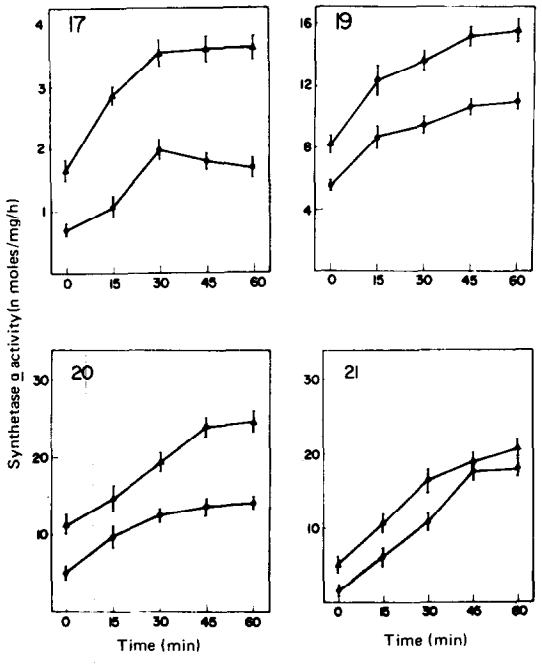

Fig.1. Glucose effect on glucogen synthetase *a* activity and its in vitro activation on different days of foetal age. 3 mg/g B. W. of glucose were injected into the peritoneal cavity of pregnant rats. After 10 min foetal livers were removed. Glycogen synthetase *a* activity in liver homogenates was examined instantly, and its in vitro activation was measured during incubation at room temperature for one hour. The figure on the left upper corner indicates foetal age. (●) Control animals. (▲) Glucose-treated animals.

time sequence of the stimulation induced by glucose on these enzymes, glucose was injected to the uterine vein, followed by periodic removal of fetuses at short intervals (fig.3).

It is striking on the 17th day, that whereas glucose has no effect on the activity of phosphorylase, synthetase *a* activity starts to rise already 1 min after glucose injection. In contrast on day 21 a different

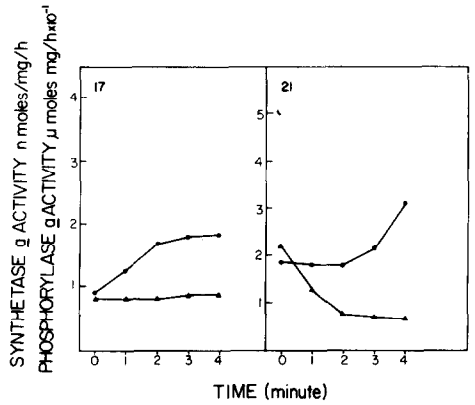

Fig.3. Glycogen synthetase *a* and phosphorylase *a* activities in foetal rat liver after glucose administration. 3 mg/g B. W. of glucose was injected into the uterine vein of the pregnant rat anaesthetised with chloral hydrate. After glucose injection, the foetuses were removed and the corresponding blood vessels were ligated. Foetal rat livers were removed to liquid nitrogen. Glycogen synthetase *a* (●) and phosphorylase *a* (▲) activities were examined as described in Materials and methods.

pattern is observed: glycogen synthetase *a* activity starts to rise only after a latent period of 2 min during which phosphorylase *a* activity decrease to 0.08 mmole/mg/H (fig.3).

Stalmans et al. [4] suggested that the primary mechanism by which the level of glucose controls glycogen metabolism in the adult rat is by inactivation of liver phosphorylase by its specific phosphatase which is enhanced by glucose. This inactivation is a prerequisite for the activation of glycogen synthetase. If such a mechanism operates, one has to assume, either that two different phosphatases are present in rat liver or that a single phosphatase exists, which displays a different affinity for glycogen synthetase than for glycogen phosphorylase. On day 21 of foetal rat life the glucose effect is similar to the one observed in the adult rat liver. Glycogen phosphorylase undergoes inactivation before glycogen synthetase is being activated. This sequence of events suggests that, as in the adult, phosphorylase *a* inhibits synthetase phosphatase, so that synthetase phosphatase activity does not start to operate before phosphorylase *a* activity has decreased to 0.08 mmole/mg/h. On the other hand, on day 17 no phosphorylase phosphatase activity can be found. Therefore, as expected glucose injection on day 17 resulted in an immediate activation of glycogen synthetase which is not preceded by an inactivation of glycogen phosphorylase. These findings do not contradict the statement of Stalmans et al. [4] in which he maintains that the inactivation of phosphorylase is a prerequisite to the activation of synthetase, since on day 17 phosphorylase activity is below the threshold of synthetase phosphatase inhibition. However these results suggest that two different phosphatases exist in foetal rat liver which display a different pattern of maturation. Synthetase phosphatase exists already on day 17 and can react directly to glucose activation, whereas phosphorylase phosphatase appears only at a later stage of development.

References

[1] De Wulf, H. and Hers, H. G. (1968) Eur. J. Biochem. 6, 558–564.
[2] De Wulf, H. and Hers, H. G. (1967) Eur. J. Biochem. 2, 50–56.
[3] Miller, B. T., Hazen, R. and Larner, J. (1973) Biochem. Biophys. Res. Commun. 53, 466–474.
[4] Stalmans, W., De Wulf, H., Hue, L. and Hers, H. G. (1974) Eur. J. Biochem. 41, 127–134.
[5] Nuttall, F. Q., Gannon, M. C. and Larner, J. (1972) Physiol. Chem. Phys. 4, 497–515.
[6] Nuttall, F. Q. and Gannon, M. C. (1972). Clin. Res. 20, 771–774.
[7] Pines, M., Bashan, N. and Moses, W. S. Biochem. Biophys. Acta, in the press.
[8] Devos, P. and Hers, H. G. (1974) Biochem. J. 140, 331–340.
[9] Schwartz, A. L. and Rall, T. W. (1973) Biochem. J. 134, 985–993.