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Effect of Growth Hormone on Glycogenesis in Rat Cerebral Cortical Slices

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Incubation of cerebral cortical slices of growth hormone treated diabetic and normal rats with U–C^{14} glucose showed a two-fold increase in glycogenesis in dialetic rats. Glucose-6-phosphatase activity was lowered while the activities of phosphoglucomutase and phosphorylase were elevated in the cerebral cortex of diabetic rats treated with growth hormone. However, glycogen synthetase activity was slightly depressed.

INTRODUCTION

Prasannan and Subrahmanyam[1] have observed an increase in the re-synthesis of glycogen in cerebral cortical slices. The incorporation of C^{14} from labelled glucose into glycogen and carbon dioxide and its stimulation by insulin *in vitro* has been reported by Visweswaran, *et al.*[2] Prior administration of growth hormone to normal rats resulted in an increase in the incorporation of C^{14} from U–C^{14} glucose into glycogen. However, this effect was not observed when growth hormone was added to the slices incubated in the medium. Vilee and Hastings[3] have proposed that growth hormone acts by releasing insulin bound to tissues. An insulin like activity of growth hormone on carbohydrate metabolism in adipose tissue was reported by Ketterer *et al.*[4] Since we have observed an increase in glycogenesis in normal rat cerebral cortex on treatment with growth hormone, it would be of interest to investigate the mode of action of growth hormone by studying the glycogenesis in cerebral cortical slices of diabetic rats treated with growth hormone.

EXPERIMENTAL

Diabetic rats were prepared by injecting 15 mg alloxan monohydrate/100 gm body wt. intraperitonially into rats which were fasted for 24 h Rats which exihibited a blood sugar (glucose oxidase method) of 360 to 600 mg % were used for these experiments.

Diabetic rats received bovine growth hormone 500 μg/100 gm body wt.

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intravenously 6 h before killing. The incorporation of C^{14} into glycogen and carbon dioxide as a result of incubation of these slices in the presence of U–C^{14} glucose was determined as follows. After incubation the cerebral cortical slices were removed from the Warburg flasks, rinsed in saline medium in two beakers and digested in 1 ml. of 30% alcoholic KOH[5], unlabelled glycogen (1.5 mgm in 0.2 ml) was added to the digest. It was centrifuged for 30 min. and the glycogen was washed with hot chloroform-methanol mixture (1 : 4 v/v) until the washings were free from radioactivity. The glycogen pellet was placed in boiling water for 10 min. to free it from adhering solvent, and dried over P_2O_5 in a desiccator overnight. The dry glycogen was dissolved in a few drops of water and oxidised to carbon dioxide by Van Slyke-Folch combustion fluid.[6] It was washed first with 50% acetone, then with 100% acetone and dried over silica gel in a desiccator overnight. The counts in the planchets were determined in a 2π Windowless gas-flow counter. The number of counts obtained at any time was at least eight times that of the background. Correction was made for background and self-absorption as described by Kamen.[7] The incorporation slice was thus determined and expressed as counts/min. per g wet wt. of cerebral cortex. The fluid in the centre well was carefully removed immediately after incubation and the well was rinsed several times with CO_2 free water. Sodium carbonate (6 mg) was added to the fluid and then converted into $BaCO_3$ in the plating apparatus as described above and counted. The incorporation of C^{14} into carbon dioxide was calculated and expressed as counts/min. per gm wet wt. of cerebral cortex.

Studies of Enzyme Activity

Hexokinase (E C 2.7.1.1) activity was estimated by the method of Bennett, *et al.*[8] using 0.15 M tris HCl buffer (pH 8.0), 20 mm $MgCl_2$, 1.0 mm NADP, G. 6 P. dehydrogenase 1 unit, 25 mm glucose and 9.0 mm ATP. Glucose-6-phosphate obtained by the hexokinase reaction was measured in the presence of excess glucose-6-phosphate dehydrogenase and NADP. The NADPH formed was estimated using a Beckman, DU Spectrophotometer. Glucose-6-phosphatase activity (E C 3.1.3.a) was determined by the method of Yeing *et al.*[9] by measuring the phosphate liberated at pH 6.8 (tris-maleate buffer 0.1 M) from 0.1 M glucose-6-phosphate. Inorganic phosphate liberated was determined by the method of Tausky and Shorr[10]. Cerebral cortical slices were prepared, weighed quickly on a wire grid and homogenized in water in Potter-Elvejhem homogenizer surrounded by ice. The homogenate was filtered through muslin and the filtrate was used for the estimation of glycogen phosphorylase (E C 2.4.1.1) activity by the method of Cori *et al.*[11] and phosphoglucomutase (E Co. 7.5.1) activity by the method of Najjar[12].

Glycogen synthetase (uridine-di-phosphoglucose-glycogen transglucosylase) (E C 2.4.1.11) activity was measured by the method of Breckenridge and Crawford[13].

RESULTS AND DISCUSSION

The incorporation of C^{14} from U–G^{14} glucose into glycogen (Table 1) in

cerebral cortical slices of diabetic rats is markedly diminished compared to the incorporation in normal tissue. Treatment of diabetic rats with growth hormone increases almost twofold the incorporation of C^{14} of glucose into glycogen (Table 1). It further results in a very marked decrease in the activity of glucose-6-phosphatase and a moderate increase in the activities of phosphoglucomutase and phosphorylase. However, glycogen synthetase is depressed by about 20% (Table 2).

TABLE 1
EFFECT OF GROWTH HORMONE ON THE INCORPORATION OF C^{14} FROM U-C^{14} GLUCOSE INTO GLYCOGEN AND CO_2 BY CEREBRAL CORTICAL SLICES OF ALBINO RATS

Cerebral cortical slices (about 50 mg) from rats were incubated in 3.5 ml of medium containing U-C^{14} glucose for 2 h Activity added 3,376,000 Cpm., Temp. 38°C, gas phase O_2.

Mode of treatment	Incorporation of C^{14} counts/min/g wet wt. of slice	
	Glycogen	Carbon dioxide
I. Normal (6) administered growth hormone	23,220 ± 2,372*	852,300 ± 74,460
500 μg 6 h before killing (6)	46,879 ± 3,378	944,700 ± 62,874
II. Diabetic (5) administered growth hormone	13,840 ± 2,254	244,900 ± 1644
500 μg 6 h before killing (5)	26,928 ± 1,067	250,820 ± 14,317

Numbers in parentheses indicate the number of animals.
*Mean ± S.E (a) in 0.2 ml of 0.9% saline adjusted to pH (7.4) with 0.1 N NaOH.

TABLE 2
ENZYME ACTIVITIES IN CEREBRAL CORTEX OF DIABETIC AND DIABETIC RATS TREATED WITH GROWTH HORMONE.

Enzyme studies	Activity/gm wet wt./ h		
	Diabetic	Treated with growth hormone 6 h before killing	'P'
1. Hexokinase (μ moles of NADPH produced)	810 ± 31*	837 ± 42	P > 0.1
2. Glucose-6-phosphatase (μ moles of inorganic phosphate liberated from G. 6 P.)	37 ± 4	14 ± 0.7	P < 0.001
3. Phosphoglucomutase (decrease in μ moles of acid labile phosphate)	135 ± 3.5	171 ± 9	P < 0.001
4. Phosphorylase (μ moles of inorganic phosphate liberated)	564 ± 12	811 ± 41	P < 0.001
5. Glycogen synthetase (μ moles of UDP liberated)	30.5 ± 1.4	24.7 ± 1.71	P < 0.02

*Mean ± S.E.

A markedly depressed utilization of glucose for glycogenesis in cerebral cortical slices of diabetic rats compared to those of normal rats (horizontal columns 1 and 3 of Table 1) is observed. It was observed that while growth hormone injected into the normal animal increases C^{14} incoporation into glycogen, growth hormone added to the slices does not produce any such effect. Similarly administration of growth hormone to diabetic rats in this series of experiments resulted in a dramatic increase in glycogenesis. It is possible that the action of growth hormone on glycogenesis is probably due to a direct action and not mediated through pancreas.

A closer examination of Table 2 shows that treatment with growth hormone brings about changes in enzyme activities in diabetic rats. It is possible that the larger net amount of glucose-6-phosphate available as a result of diminished glucose-6-phosphatase activity but unchanged hexokinase activity (Table 2) is rapidly converted into glucose-1-phosphate by the elevated phospho-glucomutase activity in growth hormone treated diabetic rats thereby giving rise to elevated glycogen synthesis observed in the above experiments.

REFERENCES

1. K.G. Prasannan and K. Subrahamanyam, *Indian J. Biochemistry*, **2**, 94 (1965).
2. P. Visweswaran, K.G. Prasannan and K. Subrahmanyam, *J. Neurochemistry*, **16**, 1389 (1969).
3. C.A. Villee and A.B. Hastings, *J. Biol. Chem.*, **179**, 673 (1949).
4. B. Ketterer, P.J. Randle and F.G. Young, *Ergebn. Physiol.*, **49**, 156 (1957).
5. H. Mcilwain and M.A. Tresize, *Biochem. J.*, **63**, 250 (1956).
6. W. Sakami, Handbook of Isotopic Tracer Methods, Western Reserve Univ. School of Med., p. 125 (1955).
7. M.D. Kamen, Istotopic Tracers in Biology, Academic Press, New York, p. 89 (1957).
8. E.L. Bennett, J.B. Drori, D. Kretch, M.R. Rosenzwelg and S. Abraham, *J. Biol. Chem.*, **237**, 1758 (1962).
9. D. Yeing, R.S. Stanley and I.T. Oliver, *Biochem. J.*, **105**, 1219 (1967).
10. Tausky and E. Shorr, *J. Biol. Chem.*, **202**, 675 (1953).
11. G.T. Cori, D. Illingworth and P.J. Keller, Methods of Enzymology, (Ed.) S.P. Colowick and N.O. Kaplan, Academic Press, New York, Vol. 1, p. 200 (1957).
12. V.A. Najjar, in *Ibid.*, p. 294.
13. B.M. Breckenridge and E.J. Crawford, *J. Biol. Chem.*, **235**, 3054 (1960).

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