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Effect of Insulin Administration and Immobilization Stress on Liver Glycogen Metabolism in Diabetic Rats

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Abstract

Hepatic glycogen content as quantified both biochemically and histologically, was estimated in control rats, in alloxan - induced diabetic rats, in diabetic rats treated with 10 I.U. insulin for one week, and in diabetic rats exposed to stress in the form of immobilization for 15 minutes prior to their sacrifice. The data obtained in diabetic rats demonstrated a statistically significant reduction in liver glycogen content from control values, and histologically there was marked depletion in the majority of the hepatic cells. Administration of insulin to diabetic rats revealed a statistically significant increase in liver glycogen content above the levels obtained in both diabetic and control animals and histologically there was almost restoration of the glycogen content in the hepatic cells. This adds further confirmation as to that administration of insulin in the intact animal switched the liver from glycogenolysis to synthesis. The propable mechanisms underlying insulin mode of action were discussed. Exposure of diabetic rats to acute stress in the form of immobilization, resulted in a statistically significant reduction in liver glycogen content from both control and diabetic values. Histologically, there was marked depletion of glycogen in all liver cells which was far more greater than that observed in the hepatic cells from non-stressed diabetic rats. The probable causes of the augmented glycogenolysis in stressed diabetic rats were discussed.

Introduction

GLYCOGEN in liver is present as granules located mainly in the extramitochondrial phase of the cell [1]. It appears that most hepatic glycogen is located in parynchymal cells, and since parynchymal cells constitute about 90% of the liver mass [2], thus measurements of glycogenolysis in whole liver usually reflect events in parynchymal cells.

Liver glycogen phosphorylase is the rate limiting enzyme of glycogenolysis and exists in two forms, an active form "a" and an inactive form "b". Control of glycogenolysis in the liver is mainly achieved by altering the available amount of the "a" form of phosphorylase [3]. A number of altered physiological conditions cause changes in total hepatic phosphorylase activity. Streptozotocin-induced diabetes was found to reduce total activity [4] and hyperthyroidism was also observed to result in slight lowering. Hormones like epinephirine, angiotensin II and vasopressin produce an increase in cellular calcium which stimulates phosphorylase kinase and leads to activation of phosphorylase. The two best measures of liver glycogenolysis are the net decrease in glycogen content as well as the activity of phosphorylase "a" in rapidly frozen samples of liver [3,5].

Therefore, the present study, in the diabetic rat, aimed at evaluating the effects of insulin administration as well as immobilization stress on liver glycogen metabolism in terms of glycogen content as quantified both biochemically and histologically.

Material and Methods

Twenty four albino rats of the Wistar strain of both sexes, weighing from 150-200 g. were used.

four groups of experiments were performed:

1. Control group:

Included 6 animals which were non-diabetic and which received no insulin and were not stressed by handling.

2. Diabetic group:

Included 6 animals which were rendered diabetic by the i-p injection of freshly prepared alloxan (dissolved in normal saline) at a dose of 150 mg/Kg body weight. The diabetic state was confirmed by the presence of polyurea and glucosuria 72 h. after injection, using Glucotest strips (Boehringer Mannheim), and subsequent analysis of serum obtained at the time of death showed glucose levels of at least 400 mg/100 ml (further details were mentioned in Tadros et al., in Press [6]). The diabetic animals were left for one week during which they received no insulin treatment.

3. Diabetic group treated with insulin:

Included 6 animals which were rendered diabetic as mentioned in the above group,

and which were then maintained on 10 I. U. Lente insulin/day administered S. C. for one week (guided by Sundaresan at al., [7]).

4. Diabetic group exposed to acute stress in the form of immobilization:

Included 6 animals which were rendered diabetic as mentioned before and which were left for one week with no insulin therapy. On the 8th day before their sacrifice, the animals were exposed to acute stress in the form of immobilization for 15 minutes. The animals were immobilized by fixing their four limbs with adhesive tape while in the prone position [8].

All the animals were kept under the same conditions during the period of the study. On the 8th day following the development of the diabetic state, the animals were killed by a hit on the head, the throat was cut and blood was collected into dry clean centrifuge tubes. Blood was allowed to clot and then centrifuged, and serum was obtained for the estimation of serum glucose by the glucose oxidase method. Immediately after the sacrifice of the animals, the liver was removed and a piece of liver tissue was obtained for further histological examination. This was fixed in 85% alcohol and then processed by paraffin and stained by periodic Acid Shiff (PAS) reaction (After Pearse, [9]). The rest of the liver tissue was used for the estimation of liver glycogen content using the method adopted by Kemp and Kits Van Heijningen [10].

Results

Results are shown in Table (1) and in Figs. (1, 2, 3 and 4).

1. In the control group, the blood glucose level was 177.0 ± 6.66 mg.dL. The liver glycogen content was 7.846 ± 0.811 mg/g. wt. Fig. (1) shows the glycogen distribution in the hepatic cells of a control animal.

2. In the diabetic group, the blood glucose level was 624.8 ± 200.11 mg/dL. The liver glycogen content was 4.853 ± 0.982 mg/g. wt. which showed a statistically significant reduction from the control level ($p < 0.05$). Fig. (2) shows partial to marked depletion of glycogen in the hepatic cells from a diabetic animal.

3. In the group of diabetic rats which received insulin for one week at 10 I. U. S. C., the blood glucose level was 173.7 ± 21.27 mg/dL. The liver glycogen content was 16.435 ± 1.968 mg/g. wt. which revealed a statistically significant increase above control group ($p < 0.05$), and diabetic group ($p < 0.05$). Fig. (3) demonstrates very slight depletion and almost restoration of the glycogen content in the hepatic cells from diabetic animals treated with insulin.

4. In the group of diabetic rats which were exposed to acute stress in the form of immobilization for 1/4 h., blood glucose

Table (1): Liver Glycogen Content (mg/g. wt.) and Blood Glucose Level (mg/dL) in Control Rats, Alloxan Induced Diabetic Rats, Diabetic Rats Receiving 10 I.U Insulin S.C. Daily for one Week, and Diabetic Rats Exposed to Immobilization Stress for 15 min. Prior to Sacrifice.

	(1) Control rats	(2) Alloxan induced diabetic rats	(3) Diabetic rats receiving 10 I.U. insulin daily	(4) Diabetic rats exposed to immo. stress for 15 min.
	(+) (-)	*(+) (-)	*	*
Liver glycogen	7.846	4.853	16.435	3.265
Content (mg/g.w.t.)	± 0.811	± 0.982	± 1.968	± 1.320
Blood glucose	177.0	624.8	173.7	707.4
Level (mg/dL)	± 6.66	± 200.11	± 21.27	± 90.25

Values are for mean ± S.D.

* $p < 0.05$ (values in 2,3 and 4 compared to control value).

(+) $p < 0.05$ (Values in 1 and 2 compared to 3).

(-) $p < 0.05$ (Values in 1 and 2 compared to 4).

level was 707.4 ± 90.25 mg/dL. The liver glycogen content was 3.265 ± 1.320 mg/g. wt. which revealed a statistically significant reduction from control value ($p < 0.05$) and the value obtained in the diabetic group ($p < 0.05$). Fig. (4) demonstrates extremely marked depletion of glycogen in the hepatic cells from a diabetic animal exposed to acute stress.

Discussion

In the present study, liver glycogen content in diabetic rats revealed a significant reduction, as assessed both biochemically and histologically, compared to the control data. Histologically, the majority of the hepatic cells demonstrated marked depletion. These findings point to the impaired ability of the diabetic liver to synthesize glycogen and/or an enhancement of glycogenolysis.

Khandelwal et al. [4] reported that in the liver of diabetic rats, the content of inhibitors of enzymes (inhibitors of dephosphorylation of phosporylase a) is increased. Accordingly Hems and Whitton

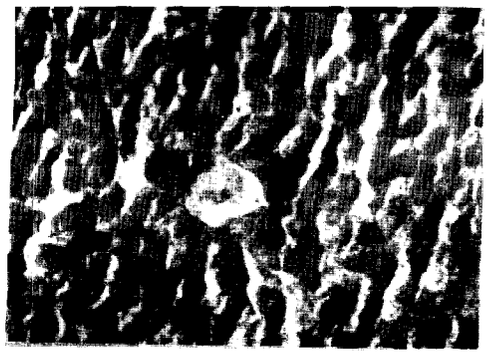

Fig. 1: Section in the liver of control rat showing the glycogen distribution in the hepatic cells (PAS reaction x 400).

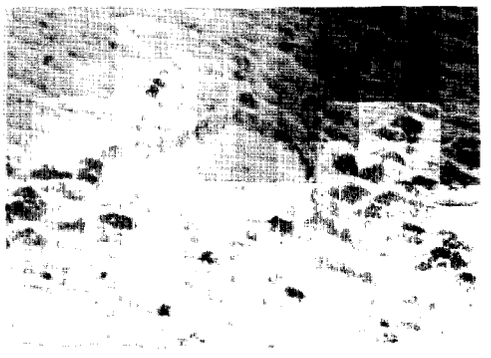

Fig. 2: Section in the liver of an alloxan-induced diabetic rat showing partial to marked depletion of glycogen in the hepatic cells (PAS reaction x 400).

Fig. 3: Section in the liver of an alloxan-induced diabetic rat receiving 10 I.U. insulin/day for one week, showing very slight depletion and almost restoration of the glycogen in the hepatic cells (PAS reaction x 400).

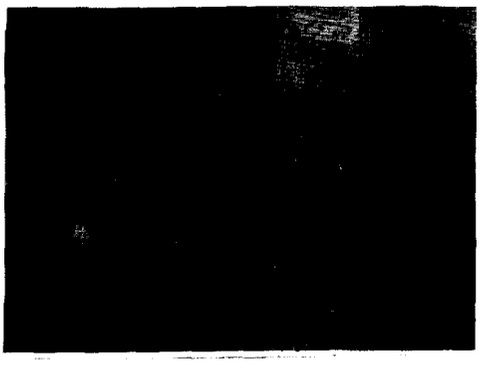

Fig. 4: Section in the liver of an alloxan-induced diabetic rat which was exposed to 15 minutes immobilization stress immediately prior to sacrifice, showing extremely marked depletion of glycogen in the hepatic cells (PAS reaction x 400).

[3] concluded that this could contribute to the inability of the liver in diabetes to synthesize and store glycogen.

Also, the plasma levels of catecholamines and renin may increase in diabetics [11,12]. Accordingly, catecholamines and angiotensin II could act on the liver to contribute to glycogenolysis and glucose release in diabetes.

Bahnak and Gold [13] observed that the rates of both synthesis and degradation of liver phosphorylase were increased in alloxan - diabetic rats, although the increments in the rate of degradation was greater, thus accounting for the reduced total phosphorylase activity in diabetes.

Roesler and Khandelwal [14] showed that in streptozotocin diabetic rats, there was a 67% reduction in total hepatic phosphorylase activity as well as 68% decrease in phosphorylase protein. This was also acompanied with a significantly elevated circulating glucagon to insulin molar ratios to 2.03 ± 0.26. Accordingly, the authors suggested that conditions which lead to an increase of the glucagon to insulin molar ratios to values above 1.0 cause a significant reduction in the liver phosphorylase protein level.

When diabetic rats were treated with 10 I.U. Lente insulin for one week, the liver glycogen content showed a significant increase compared to the levels in diabetic and control animals. Histologically, there was almost restoration of the glycogen content in the hepatic cells. The increased amounts of liver glycogen under the effect of insulin occurs through inhibiting the enzyme phosphorylase and enhancing the activity of both the enzymes glucokinase which phosphorylates glucose inside liver cells and the enzyme glycogen synthetase which polymerises the monosaccharide units to form the glycogen molecule [15].

Glucagon acts on the liver to stimulate glycogenolysis and gluconeogenesis via stimulation of adenylate cyclase and the CAMP produced stimulates CAMP-dependent protein kinase leading to activation of glycogen phosphorylase [16].

The role of insulin in the regulation of hepatic glycogenolysis has been the subject of various studies. Insulin was found to depress glucagon-induced increases in hepatic CAMP [17], inhibits glucagon activated adeylate cyclase [18] or enhancing the destruction of CAMP phosphodiesterase in isolated hepatocytes and hepatic plasma membrane [13].

However, insulin antagonism has been found independent of changes in CAMP. Hers et al. [20] reported that in the diabetic rat, where there is elevated serum glucagon levels, insulin requires only 10-20 minutes to elicit full inactivation of phosphorylase and activation of glycogen synthase, while 30 minutes are required before any decrease in elevated hepatic CAMP levels is noted. These results indicate that

insulin can antagonise the actions of glucagon without affecting adenylate cyclase or CAMP phosphodiesterase. Also, Werve et al. [21] observed that injection of insulin in the intact animal switches the liver within a few minutes from glycogenolysis to glycogen synthesis without any change in hepatic CAMP. Furthermore, Gabbay and Lardy [16] reported that insulin antagonised the glycogenolytic actions of CAMP in isolated hepatocytes even in the presence of the phosphodiesterase inhibitors which indicate that insulin acts on CAMP-dependent protein kinase independent of changes in CAMP levels.

Finally, Hartmann et al. [22] investigated the inhibitory action of insulin on basal and glucagon activated glycogenolysis in clutured rat hepatocytes. The authors found a 50% inhibition in basal and glucagon activated phosphorylase activity as well as a complete block of glucagon-stimulated glucose release. Accordingly, the authors concluded that their results point to a direct regulatory role of insulin in the control of hepatic glycogen breakdown even when acting as the sole hormone.

In the present study, in the diabetic rats which were exposed to acute stress in the form of immobilization for 15 minutes, the liver glycogen content revealed a singificant reduction from both control and diabetic values. Histologically, all the liver cells were markedly depleted of glycogen. Depletion was far more greater than that observed in the hepatic cells from non-stressed diabetic rats.

Nakhooda et al. [23], reported that immobilization in rats was an extremely effective stimulus to typical stress responses, including 1.4 to 2.5 fold increases in individual catecholamines, 72 mg/dl increments in mean blood glucose level, 94 pg/ml increase in glucagon, suppression of the insulin response to the hyperglycemia and rise in lactate and pyruvate. The authors added, that the rise in blood glucose level in the diabetic rats was more than two fold that in normal rats whereas the rise in glucagon was similar. They concluded that hyperglucagonemia was more effective in the diabetic rat in increasing liver glucose production. Also the authors pointed to a role for α and β receptors in the stressed diabetic rats, whereas in the normal rat stress related increases in glucagen secretion have an alpha component, in the diabetic rat, glucagon secretion has a β-stimulating component and could also have an α-inhibitory component. The authors postulated that insulin deficiency itself could result in altered receptor behaviour. An alternative possibility is an altered prestressed adrenergic tone, for although circulating catecholamine levels were not different in normal and diabetic rats, local release of norepinephrine could have been increased in diabetic rats [24].

Younan et al. [25] observed that immobilization stress in the rat resulted in a

transient activation of the pituitary-thyroid axis, as reflected by significant increments in serum TSH and T_4 levels after 5 minutes of immobilization. Thyroid hormones aggrevate experimental diabetes and thyrotoxicosis makes clinical diabetes worse as the hormones cause some degree of hepatic glycogen depletion most probably by potentiating the effects of catecholamines [26]. If such mechanisms are operating in our diabetic stressed rats, then it would offer an additive clarification to the observed markedly depleted glycogen in the liver of these animals.

References

1. CARDELL, R. R., JR.: Smooth endoplasmic reticulum in rat hepatocytes during glycogen deposition and depletion. Int. Rev. Cytol., 48: 221-279, 1977.

2. BLOUIN, A., BOLENDER, R. P. and WEIBEL, E. R.: distribution of organelles and membranes between hepatocytes and non-hepatocytes in the rat liver parynchyma. A stereological study. J. Cell. Biol., 72:441-445, 1977.

3. HEMS, D.A. and WHITTON, P.D.: Control of hepatic glycogenolysis. Physiol. Rev., 60: 1, 1980.

4. KHANDELWAL, R. L., ZINMAN, S.M. and ZEBROWSKI, E. J..: The effect of streptozotocin-induced diabetes and of insulin supplementation on glycogen metabolism in rat liver. Bioch. J., 168: 541, 1977.

5. EXTON, J. H.: Mechanisms involved in adrenergic phenomena: role of calcium ions in actions of catecholamines in liver and other tissues. Am. J. Physiol., 238: E3, 1980.

6. TADROS, T. G. EL-BARADIE, T., ZIADA, G. and GODA, S. A.: Thyroid hormone levels and myocardial protein in diabetic rats treated with insulin and T_4. (In Press).

7. SUNDARESAN, P. R., SHARMA, V. K. GINGOLD, S. I., and BANERJEE, S.P.: Decreased B-adrenergic receptors in rat heart in streptozotocin induced diabetes: Role of thyroid hormones. Endocrinology, 114: 1358, 984.

8. KVETNANSKY. R. and MIKULAJ. L.: Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. Endocrinology, 85: 783-793, 1970.

9. PEARSE, A. G. E.: Histochemistry: Theoretical and applied. Vol. 1. 3rd edition (reprinted by Churchill. Livingstone, Lond.), 1975.

10. KEMP A. (AMSTERDAM) and KITZ VAN HEIJNINGEN, A. J. M.: Colorimetric-micromethod for determination of glycogen in tissues. Biochem. J., 56: 646, 1954.

11. CHRISTENSEN, N.JJ.: Plasma norepinephrine and epinephrine in untreated diabetics, fasting and after insulin administreation. Diabetes, 23: 1-8, 1974.

12. CHRISTLIEB, A. R., ASSAL, J. R., KATSILAMBROS, N., WILLIAMS, G. H., HOZAK, G. P. and SUZUKI, T: Plasma renin activity and blood volume in uncontrolled diabetes. Ketoacidosis, a state of secondary aldosteronism. Diabetes, 24: 190-193, 1975.

13. BAHNAK, B. R. and GOLD, A. H.: Effect of alloxan diabetes on the turnover of rat liver glycogen synthase. Comparison with liver phosphorylase. J. Biol. Chem., 257: 8775, 1982.

14. ROESLER, W. J. and KHANDELWAL, R. L.: Regulation of rat liver glycogen phosphorylase concentration by in vivo relative levels of glucagon and insulin. Endocrinology, 121: 227-232, 1987.

15. GUYTON, A. C.: Effect of insulin on carbohydrate metabolism. In Insulin, Glucagon and Diabetes Mellitus Chapter 78, P. 924. In Textbook of Medical Physiology, 7th edition W.B. Saunders Company Philadelphia, 1986.

16. GABBAY, R.A. and LARDY, H. A.: Site of insulin inhibition of CAMP-stimulated glycogenolysis. J. Biol. Chem., 259: 6052, 1984.

17. JEFFERSON, L. S., EXTON, J. H., BUTCHER, R. W. SUTHERLAND, E.W. and PARK, C.R.: Role of Adenosine 3'-5'- Monophosphate in the effects of insulin and anti-insulin serum on liver metabolism. J. Biol. Chem., 243, 1031-1038, 1968.

18. ILLIANO, G., and CUATRECASAS, P.: Modulation of adenylate cyclase activity in liver and fat cell membranes by insulin. Science, 175, 906-908, 1972.

19. LOTEN, E. G., ASSIMACOPOULOS-JEANNET, F. D., EXTON, J. H., and PARK, C. R.: Stimulation of a low Km Phosphodiesterase from liver by insulin and glucagon. J. Biol. Chem., 253, 746-757, 1978.

20. HERS, H. G., STALMANS, W., DE WULF H. LALOUX, M. and HUE, L.: In Metabolic Interconversion of Enzymes (Fisher, E. H., Krebs, E. G., Neurath, H., and Stadtman, E. R. eds) PP. 89-98, Springer-Verlag, Berlin, 1974.

21. VANDE WERVE, G., STALMANS, W., and HERS, H. G.: The effect of insulin on the glycogenolytic cascade and on the activity of glycogen synthetase in the liver of anaesthetized rabbits. Biochem. J., 162, 143-146, 1977.

22. HARTMANN, H., PROBST, I., JUNGERMANN, K. and CREUTZFELDT, W.: Inhibition of glycogenolysis and glycogen phosphorylase by insulin and proinsulin in rat hepatocyte cultures. Diabetes, 36: 551-55, 1987.

23. NOKHOODA, A. F., SOLE, M. J. and MARLISS, E. B.: Adrenergic regulation of glucagon and insulin secretion during immobilization stress in normal and spontaneously diabetic BB rats. Am. J. Physiol. 240: E373-E378, 1981.

24. SOLE, M. J., LO, C. M., LAIRD, C. W., SONNENBLICK, E. H. and WURTMAN, R. J.: Norepinephrine turnover in the heart and spleen of the cardiomyopathic Sytian hamster. Circ. Res., 37: 855-862, 1975.

25. YOUNAN, N., ABDEL RAHMAN, Y., MALEK, A., ISSA, M., TADROS, T. G., SHEHATA, R. and YOUSSEF, H.: Effect of immobilization stress and B-adrenergic blockade on pituitary thyroid axis. Med. J. Cairo Univ., 55: 245-252, 1978.

26. GANONG, W.F.: Endocrine regulation of carbohydrate metabolism. In endocrine function of the pancreas and the regulation of carbohydrate metabolism, Chapter 19, P. 292. Review of Medical Physiology 13th edition, Lange Medical Publications, Los Altos, California, 1984.