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ACTION OF INSULIN ON LIVER CARBAMOYL-PHOSPHATE SYNTHETASE II (GLUTAMINE-HYDROLYZING) ACTIVITY

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Carbamoyl-phosphate synthetase II (glutamine-hydrolyzing) (EC 6.3.5.5) (synthetase II), is the first and rate-limiting enzyme in the de novo UMP biosynthetic pathway. The present investigation showed that insulin has a regulatory action on hepatic synthetase II activity. When diabetes was induced with injection of different doses of alloxan the plasma insulin concentrations decreased in a dose-dependent fashion to 72, 38, 31 and 28% and concurrently the liver synthetase II activity decreased to 75, 43, 29 and 22% of the normal values. In diabetic rats dose response studies showed that with insulin injections of 4, 6, 8 or 10 U/day for 48 h the hepatic synthetase II activity increased to 81, 95, 99 and 103% of the control liver values. In the diabetic rats the insulin-induced rise in liver synthetase II activity was prevented by treatment of the rats with actinomycin.

Mammalian carbamoyl-phosphate synthetase II[1], the first and rate-limiting enzyme of de novo uridylate biosynthesis, exists in the cytosol as a multi-enzyme complex (M_r 200,000) with aspartate carbamoyltransferase (EC 2.1.3.2) and dihydroorotase (EC 3.5.2.3) the second and third enzymes, respectively, in this pathway (1-4). Investigations by Aoki and Weber (5,6) showed that synthetase II activity increased in all hepatomas studied and the rise correlated positively with tumor proliferative rates. Therefore, synthetase II is considered a neoplastic transformation- and progression-linked enzyme. Nutritional studies showed that synthetase II activity decreased during starvation and returned to normal values upon refeeding[2], indicating a possible regulatory role of insulin. This study presents evidence that liver synthetase II activity, at least in part, is controlled by the action of insulin.

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[1]The abbreviations used are: synthetase II, carbamoyl-phosphate synthetase II; PRPP, 5-phosphoribosyl 1-pyrophosphate.

[2]Aoki, T. and Weber, G. (unpublished observations).

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MATERIALS AND METHODS

Materials: Young male Wistar rats (Harlan) (90-120g) were maintained in individual cages with water and food ad libitum unless otherwise specified. After a 30 h starvation, diabetes was induced by i.p. injection of alloxan monohydrate (Sigma). Protamine zinc insulin (Lilly) was administered s.c. in two divided doses for 48 h. Actinomycin-D (Merck Sharp & Dohme) (4 µg/100 g) was injected i.p. in two divided doses for 48 h always 30 min prior to insulin administration.

Partial Purification: Liver synthetase II was purified from the 105,000 x g supernatant fraction of tissue homogenate by ammonium sulfate and hydroxylapatite fractionation and gel filtration on a Sephadex G-25 column as reported (5).

Synthetase II assay: Synthetase II activity was assayed with potassium bi [^{14}C]-carbonate as substrate by following formation of [^{14}C]citrulline in the presence of excess L-ornithine and ornithine carbamoyltransferase (5). The reaction was initiated by the addition of the enzyme, incubated for 15 min at 37°C, and terminated with 3 N formic acid. Kinetic assays, with PRPP, were also carried out to test whether changes in enzyme activity could be due to differences in the extent of activation by this effector. The radioactivity was measured in a Packard Tri-Carb scintillation spectrometer and synthetase II activity was calculated and expressed in terms of specific activity (nmol/h/mg protein).

Blood sugar and plasma insulin assays: Blood sugar was determined according to Nelson's adaption of the Somogyi method (7). Plasma insulin levels were measured by (^{125}I) insulin immuno-assay (8).

Results were statistically evaluated by the t test for small samples. Differences between means giving a probability of less than 5% were considered as significant.

RESULTS

Effect of Plasma Insulin Concentrations on Hepatic Synthetase II Activity: An alloxan dose response experiment determined the effect of diabetes and circulating insulin on synthetase II activity. After 30 h starvation, groups of rats were injected i.p. with alloxan (120, 150, 175, 200 mg/kg), controls with physiological saline and all groups were killed 48 h later. In normal control rats the liver synthetase II activity was 9.1 ± 0.5 nmol/h/mg protein, the concentration of blood sugar was 112 ± 3 mg%, and the plasma insulin was 53 ± 2.9 µU/ml. Increasing alloxan doses resulted in a progressive decrease in circulating plasma insulin to 72, 38, 31, and 28% and an increase in blood sugar to 188, 542, 692, and 754% of controls (Fig. 1). The hepatic synthetase II activity significantly decreased to 75, 43, 29, and 22% of normal activity, closely correlating with the fall in plasma insulin concentrations.

Effect of Different Doses of Insulin: To examine this observation in more detail, an insulin dose-response study was conducted. Alloxan diabetic rats were treated with twice daily s.c. injections of protamine zinc insulin for 48 h and

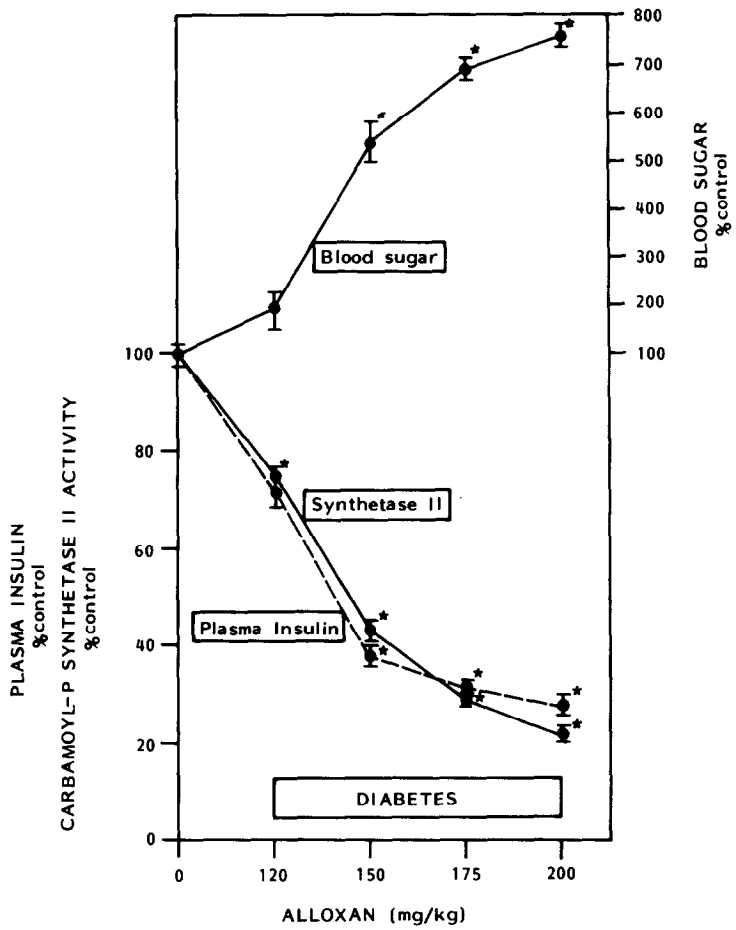

Fig. 1. Effect of diabetes on synthetase II activity, plasma insulin, and blood sugar levels. After 30 h starvation, as described in the text, diabetes was introduced by i.p. injection of different doses of alloxan (120, 150, 175, 200 mg/kg). In the control normal and untreated diabetic groups the concentrations of the blood sugar were 112 ± 3, 211 ± 77, 607 ± 117, 775 ± 39, 844 ± 35 mg %; insulin levels were 53 ± 2.9, 38.3 ± 8.1, 20 ± 4.1, 16.4 ± 4.5, 15.3 ± 2.7 μU/ml, respectively. The hepatic synthetase II activities were 9.1 ± 0.5, 6.8 ± 0.7, 3.9 ± 0.6, 2.6 ± 0.1, 2.0 ± 0.2 nmol/h/mg protein, respectively. Synthetase II activity, plasma insulin, and blood sugar were determined as described in "Materials and Methods". Bars indicate the means ± standard errors of 4 rats in each group. Asterisks denote values significantly different from those of the control group ($p < 0.50$).

then killed. In the control rat liver the synthetase II activity was 9.1 ± 0.1 nmol/h/mg protein and the concentration of blood sugar was 120 ± 4 mg%. In the un treated diabetic group the blood sugars were in the 400-500 mg% range and synthetase II activity decreased to 59% control activity. As the result of insulin treatment (4, 6, 8, or 10 U/day) the liver enzyme activity increased to 81, 95, 99, and 103% of normal activity in a dose-dependent manner (Fig. 2).

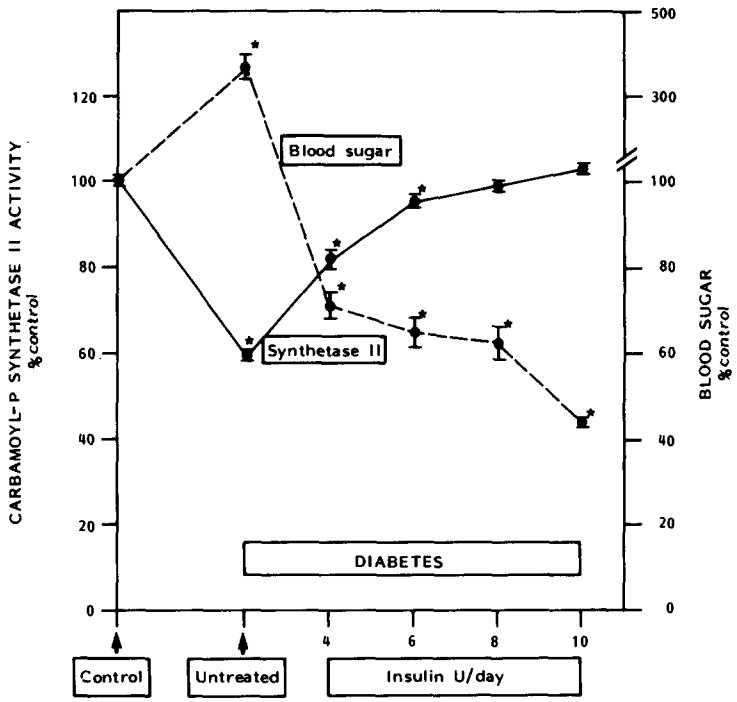

Fig. 2. Effect of diabetes and insulin on synthetase II activity and blood sugar. Groups of alloxan diabetic rats were treated twice a day with different doses of insulin (4, 6, 8, 10 U/day) for 48 h. The blood sugars in the normal controls and untreated diabetics were 120 ± 4 and 443 ± 42 mg % and the hepatic synthetase II activities were 9.1 ± 0.1 and 5.4 ± 0.2 nmol/h/mg protein, respectively. Blood sugar and synthetase II activity wer determined under standard conditions as described in "Materials and Methods". Bars indicate the means ± standard errors of 4 rats in each group. Asterisks denote values significantly different from those of the control group ($p < 0.50$).

Effect of Actinomycin on the Insulin Induced Rise in Liver Synthetase II Activity: In the diabetic group the hepatic synthetase II activity was decreased to 26% and blood sugar was 719% of the normal values. Insulin administration returned the synthetase II activity to above normal range and the blood sugar decreased to 38% of the normal concentration. When diabetic rats were given injections of actinomycin 30 min prior to insulin treatments the insulin-induced rise in liver synthetase II activity was blocked and the enzymic activity remained in the diabetic range (Fig. 3). The insulin induced decrease in blood sugar concentration was not affected by actinomycin treatment.

DISCUSSION

Synthetase II is known to increase in activity in presence of the allosteric effector, PRPP (5). In extracts of liver of normal, diabetic, and insulin-treated

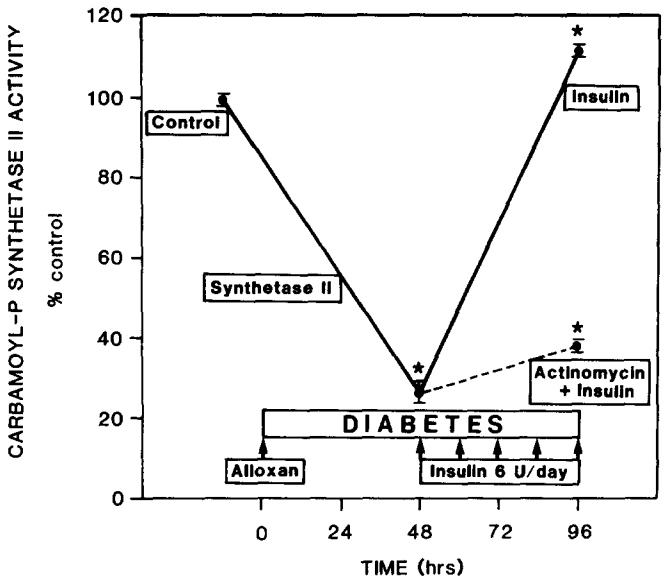

Fig. 3. Effect of actinomycin on the insulin-induced rise in synthetase II activity. The broken lines (---) represent diabetic animals injected with actinomycin prior to insulin administration. The solid lines (———) represent diabetic animals injected with insulin alone. In the normal control and untreated diabetic groups, the blood sugar concentrations were 107 ± 3.9 and 770 ± 60; the levels decreased in diabetic rats treated with insulin and with actinomycin plus insulin to 52 ± 4.9 and 53 ± 4.6 mg %, respectively. In normal control and untreated diabetic groups, liver synthetase II activities were 9.3 ± 0.1 and 2.5 ± 0.3 and they changed in diabetic rats treated with insulin and with actinomycin plus insulin to 10.4 ± 0.4 and 3.5 ± 0.2 nmol/h/mg protein, respectively. The dosages and procedures are described in "Materials and Methods". Bars indicate the means ± standard errors of 4 rats in each group. Asterisks denote values significantly different from those of the control group ($p < 0.05$).

diabetic animals, a similar extent of activation was observed after addition of PRPP. Thus, the changes in synthetase II activity in the diabetic and insulin-treated rat were not due to any possible differences in the extent of activation by PRPP.

Insulin regulates the activity and amount of certain key enzymes involved in carbohydrate and lipid metabolism (9-13). In this communication, evidence is provided that in diabetic rat liver synthetase II activity decreased proportionally with the fall in plasma insulin levels and returned to normal range upon the administration of insulin. The decrease in the activity of this rate-limiting enzyme could result in a reduced capacity of the de novo pyrimidine biosynthetic pathway, which should curtail UTP production in diabetic cells. This is in accord with the observation that in the liver of the diabetic rat the concentration of UTP was decreased to 54% and insulin treatment returned UTP pools to the range of the normal

liver (14). The responsiveness of synthetase II and the behavior of the UTP pool may relate to the insulin-induced liver glycogen deposition. The concentration of UTP should influence the availability of UDP-glucose required for the action of glycogen synthetase leading to glycogen deposition. Thus, our observation provides an enzymic basis for the regulation by insulin of hepatic pyrimidine biosynthesis and its linking with glycogen synthesis.

It is concluded that insulin regulates synthetase II activity, at least in part, and is required for maintaining normal activity of this enzyme in rat liver. Since previous investigation established that synthetase II activity was strictly proportional to the amount of enzyme added, it is assumed that the alterations in activity indicate changes in the enzyme protein amount (5). The specific activity of aspartate carbamoyltransferase changed in parallel with that of synthetase II under the conditions investigated in this study. This is further evidence that the multi-enzyme complex is altered in amount. Since actinomycin, an inhibitor of DNA-dependent RNA production, blocked the rise in synthetase II activity, it is assumed that the insulin-induced rise in enzyme activity was due to de novo biosynthesis of new enzyme protein. Further studies with immunotitration of purified enzyme should provide more stringent evidence whether this assumption is correct.

ACKNOWLEDGEMENTS

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REFERENCES

1. Tatibana, M., and Shigesada, K. (1972) Adv. Enzyme Regul. 10, 249-271.
2. Shoaf, W. T., and Jones, M. E. (1973) Biochemistry 14, 2622-2630.
3. Mori, M., and Tatibana, M. (1978) Eur. J. Biochem. 86, 381-388.
4. Coleman, P. F., Suttle, D. P., and Stark, G. R. (1978) Methods Enzymol. 51, 121-134.
5. Aoki, T., Morris, H. P., and Weber, G. (1982) J. Biol. Chem. 257, 432-438.
6. Aoki, T., and Weber, G. (1981) Science 212, 463-465.
7. Nelson, N. (1944) J. Biol. Chem. 153, 375-380.
8. Helding, L. G. (1972) Diabetologia 8, 260-266.
9. Dunaway, G. A., and Weber, G. (1974) Arch. Biochem. Biophys. 162, 699-737.
10. Steiner, D. F., and King, S., (1964) J. Biochem. 5, 1292-1298.
11. Weber, G. (1972) Israeli J. Med. Sciences 8, 325-340.
12. Weber, G., Singhal, R. L., and Srivastava, S. K. (1965) Adv. Enzyme Regul. 3, 43-75.
13. Ingebritsen, T. S., Geelen, M. J. H., Parker, R. A., Evenson, K. J., and Gibson, D. M. (1979) J. Biol. Chem. 254, 9986-9989.
14. Weber, G., Tzeng, D. Y., Lui, M. S., Kizaki, H., and Shiotani, T. (1980) Fed. Proc. 39, 636.