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DOLICHOL SYNTHESIS IN LIVER SLICES OF STREPTOZOTOCIN DIABETIC RATS

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Abstract. The rate of dolichol synthesis in normal and diabetic liver slices in the presence or absence of insulin was investigated with radiolabeled acetate and mevalonate as substrates. Cholesterol and dolichol syntheses were found low in diabetic rat liver slices when incubated either with 1-^{14}C-acetate or 2-^{3}H-mevalonate. In the presence of insulin, cholesterol and dolichol synthesis in diabetic rat liver slices returned to normal in nine hours when incubated with 1-^{14}C-acetate; however, with 2-^{3}H-mevalonate, synthesis of cholesterol and dolichol normalized in about three hours. These studies show that dolichol synthesis in rat liver slices is dependent on insulin.

Introduction. It is recognized that the addition of oligosaccharide side chains to asparagine residues of proteins in the synthesis of glycoproteins is a co-translational event, and it proceeds by an en bloc mechanism in which oligosaccharides are preassembled on a lipid carrier, dolichol phosphate, prior to transfer (1). Presumably, this mechanism acts in a cyclic manner, wherein dolichol phosphate is thought to be regenerated after oligosaccharide transfer. For the most part, cholesterol shares a common biosynthetic pathway with ubiquinone and dolichol. Evidence in the literature supports the conclusion that β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) reductase is the major rate-limiting enzyme in the synthesis of cholesterol (2), and this enzyme is known to be under dietary and hormonal control (3). It has been shown in smooth muscle cells that HMG-CoA reductase regulates not only cholesterol but dolichol synthesis (4); whereas in the rat liver, this has been shown not to be the case (5).

Although it is known that hepatic HMG-CoA reductase activity and cholesterol biosynthesis are markedly reduced in diabetes (6,7), very little is known about whether diabetes causes an impairment in hepatic dolichol biosynthesis.

In the present study, we have focused on the rate of dolichol synthesis with radiolabeled acetate and mevalonate as substrates in normal and diabetic rat liver slices in the presence or absence of insulin.

Materials and Methods. Experiments were performed using 200-250 g Sprague-Dawley male rats. Diabetes was induced via intraperitoneal administration of streptozotocin (50 mg/kg body weight). Forty-eight hours after injection of streptozotocin the animals were fasted overnight and blood glucose levels were estimated by dextrostix strips. Animals having greater than 200 mg/dl fasting blood sugar levels were used in the experiment.

Three to four weeks after the induction of streptozotocin diabetes, animals were anesthetized with ether and sacrificed. The livers were perfused, and dissected. Liver slices (0.5 g) were incubated in vitro in 5 ml of Hanks-balanced solution containing 20 mM HEPES buffer (N-2-hydroxyethylpiperazine -N'-2-ethane-sulfonic acid). The incu-

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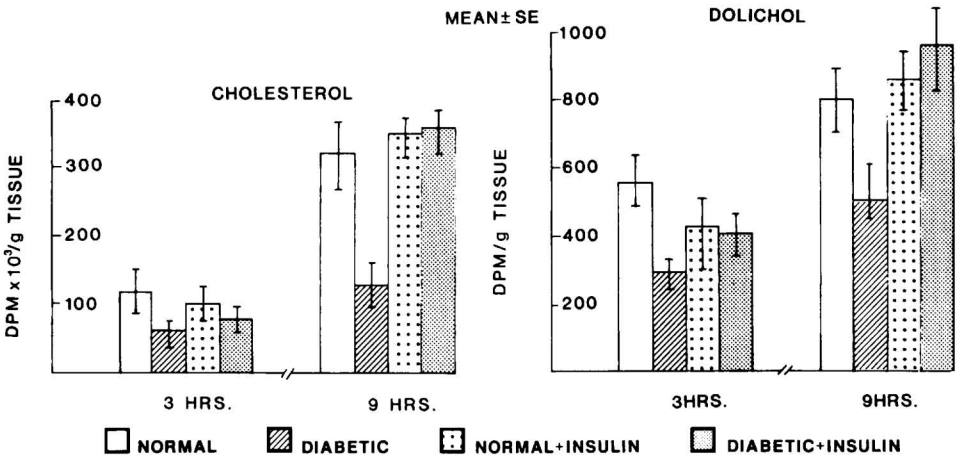

Figure 1: Effect of insulin on cholesterol and dolichol synthesis in normal and diabetic rat liver slices. Liver slices were incubated with 1-^{14}C-acetate in the presence or absence of insulin (10^{-5} M); at 3 and 9 hours of incubation, tissues were analyzed for cholesterol and dolichol as described in the text. Cholesterol and dolichol syntheses were significantly lower in diabetic liver slices; the addition of insulin to medium restored to normal levels by nine hours. Each bar represents the mean ± SE of five animals.

bation was carried out in a Dubnoff shaker water bath under 95% O_2 and 5% CO_2 at 37°C for three hours.

These incubation studies were carried out separtely with 2 mM acetate containing 1-^{14}C-acetate (2.5 µc/ml) (sp. act. 52.5 mCi/mmol) from New England Nuclear and 1 mM mevalonolactone containing DL-2-^{3}H-mevalonic acid lactone (1 µci/ml) (sp. act. 4.28 ci/mmol) from Amersham Searle. Crystalline insulin dispersed in saline at a final concentration of 10^{-5} M was added to the medium. (Crystalline porcine insulin, Lot #615-07J-50, was a gift from Lilly Laboratories). In control experiments, insulin was omitted. After incubation the liver slices were washed twice in ice cold water and suspended in 2.5 ml water. Five ml of 10% alcoholic KOH and 1 ml of 15% pyrogallol in methanol were added to the suspension. The liver slices were saponified by boiling the entire mixture at 100°C for 45 minutes. After saponification, nonsaponifiable lipid fraction was extracted as per the method of Wong et al. (9). Separation of the dolichols from cholesterol was achieved by the method of Wong and Lennarz (10). Briefly, the lipid extract was applied to a Sep-Pak C_{18} reverse-phase cartridge type column; final products were eluted sequentially with methanol, acetone and dichloromethane to elute cholesterol, dolichol and dolichol phosphate, respectively, with a recovery of about 95%. Radioisotope counting was performed with a Beckmann LS-250 liquid scintillation counter using an InstaGel liquid scintillation cocktail.

Results. Relative Rate of Flux of Acetate and Mevalonate into Cholesterol and Dolichol in Normal and Diabetic Rat Liver Slices in the Presence or Absence of Insulin: Figure 1 shows total cholesterol and dolichol syntheses in normal and diabetic liver slices incubated with 1-^{14}C-acetate. Radiolabeled ace-

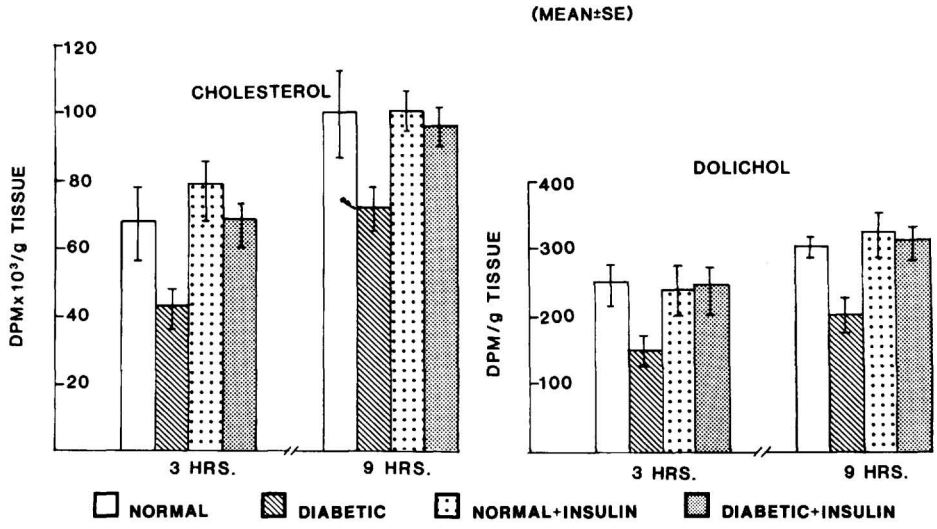

Figure 2: Effect of insulin on cholesterol and dolichol synthesis in normal and diabetic rat liver slices incubated with 2-^{3}H-mevalonate. Experimental procedures were similar to those described in the legend for Figure 1. As in Figure 1, cholesterol and dolichol syntheses were significantly lower in diabetic liver slices but were returned to normal levels in three hours with insulin in the medium.

tate incorporation into dolichol and cholesterol is significantly reduced in diabetic liver slices (cholesterol synthesis: 117.4 ± 16.6 vs 61.8 ± 11.6 DPM x 10^3/g tissue, normal vs diabetic, $p < 0.01$; dolichol synthesis: 560 ± 120 vs 280 ± 120 DPM/g tissue, normal vs diabetic, $p < 0.01$). Cholesterol and dolichol syntheses were reduced by 47% and 49%, respectively. The addition of insulin to the media containing diabetic liver slices induced dolichol and cholesterol syntheses to normal levels in approximately nine hours; whereas with with the normal liver slices, insulin did not have any effect.

Figure 2 shows total cholesterol and dolichol syntheses in the presence or absence of insulin with 2-^{3}H-mevalonate as substrate. With radioactive mevalonate as substrate, cholesterol and dolichol syntheses were reduced significantly (cholesterol synthesis: 66.8 ± 13.2 vs 42.0 ± 5.2 DPM x 10^3/g tissue, normal vs diabetic, $p < 0.01$; dolichol synthesis: 240 ± 50 vs 140 ± 20 DPM/g tissue, control vs diabetic, $p < 0.01$) to about 33% and 40%, respectively. Reduced levels of cholesterol and dolichol syntheses in diabetic liver slices during the entire nine-hour incubation period were maintained. Addition of insulin to the medium containing diabetic liver slices induced cholesterol and dolichol syntheses within three hours.

Discussion. The biosynthesis of asparagine-linked oligosaccharide (1) involves several stages, the initial steps being synthesis of dolichol, then dolichol phosphate and dolichol pyrophosphate oligosaccharide, respectively. These results show that the initial step in glycoprotein synthesis, namely dolichol synthesis, is regulated by insulin.

Although, for the most part, cholesterol and dolichol syntheses share a common biosynthetic pathway, it is interesting to note that the flux of ace-

tate and mevalonate into cholesterol synthesis is many-fold greater than dolichol synthesis. This is in agreement with earlier findings that cholesterol is synthesized in large quantities by the liver (11); whereas dolichol is required only in catalytic amounts, since it is recycled by the dolichol cycle after it has been converted to dolichol phosphate (1).

HMG-CoA reductase is an inductible enzyme that catalyzes and regulates the formation of mevalonate, which is involved in the synthesis of both cholesterol and dolichol. Diabetic liver slices synthesized less cholesterol and dolichol either from acetate or mevalonate than the control. These results support the earlier findings that cholesterol synthesis is regulated mainly at the HMG-CoA reductase step, and secondarily at the squalene synthetase step (12). Since dolichol synthesis is reduced to the same degree with both substrates in the diabetic rat liver slices in comparison to normals, it is possible that dolichol synthesis is regulated at the dolichol synthetase step. Moreover, it is possible that in diabetic liver the enzyme or enzymes involved in dolichol synthetase complex might have reduced activity or may have a higher Km for farnesyl pyrophosphate than squalene synthetase, which could result in the conversion of more farnesyl pyrophosphate to cholesterol than to dolichol.

In the presence of insulin diabetic liver slices normalize cholesterol and dolichol syntheses within nine hours with acetate as substrate. The possible reason for this lag period could be that many steps, in addition to HMG-CoA reductase, are dependent on insulin when acetate is used as the substrate. With mevalonate as the substrate, in the presence of insulin, cholesterol and dolichol syntheses are normalized within three hours. It is possible that the regulatory steps after mevalonate, including dolichol synthetase, are induced within three hours.

These results show that dolichol, and possibly enzymic glycosylation of proteins, is dependent on insulin.

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