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LETTERS TO THE EDITOR

Inhibition of Glutathione Reductase in Diabetics and Non-Diabetics

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Patients with diabetes mellitus, including those in the latent phase, have increased insulin antagonism associated with their plasma albumin compared with that from normal subjects [1, 10, 14, 15]. It is well recognized that diabetes mellitus is a familial disease, and this increased antagonism to insulin is apparently inherited as a Mendelian dominant character [16].

ENSINCK et al. [2] have shown that the reduced phenylalanine chain of insulin (B-chain) will bind to plasma albumin and render non-antagonistic albumin antagonistic. Therefore it seems a reasonable hypothesis that the genetic defect in diabetes mellitus in some way leads to increased amounts of circulating B-chain bound to albumin.

One of the initial steps in the degradation of insulin in the liver may be the reductive cleavage of its disulphide bonds with reduced glutathione resulting in the formation of the isolated A- and B-chains of the insulin molecule.

$$\text{Insulin} + 4(6)\text{GSH} \rightarrow \text{A(SH)}_{2(4)}\ \text{(reduced A-chain)} + \\ + \text{B(SH)}_2\ \text{(reduced B-chain)} + 2(3)\text{GSSG}$$

A liver enzyme, "glutathione-insulin transhydrogenase" GIT, which catalyses this reaction has been isolated and characterized [4, 5, 11, 12, 13]. The K_{mGSH} for this enzyme renders the reaction very sensitive to variations in the liver GSH concentration [6].

The reaction regenerating reduced GSH is catalysed by the enzyme glutathione reductase (GSSG-R) according to the following reaction

$$\text{GSSG} + \text{NADPH} \rightarrow 2\ \text{GSH} + \text{NADP}$$

Rat liver GSSG-R is inhibited by small amounts of reduced B-chain [7]. This inhibition has now been studied further [3]. The inhibition is of the irreversible type and best described by a velocity constant. The reaction between enzyme and inhibitor (B-chain) only takes place in the presence of small amounts of NADPH ($\lesssim 3 \times 10^{-7}$ M).

A possible pattern for the regulation of insulin reduction may be discerned here. The product of the GIT reaction (B-chain) inhibits the reaction leading to the regeneration of reduced GSH. Fall in reduced GSH again lowers the production of B-chain, and a simple feed-back mechanism seems possible.

In an attempt to evaluate whether a defect in this simple feed-back mechanism could explain the increased circulating B-chain-albumin supposedly found in diabetes, the liver GSSG-R activity and inhibition were examined in human liver autopsy material.

Pieces of liver about 10 g were obtained at autopsy from 12 subjects, 6 known diabetics and 6 not known to have diabetes. The livers were homogenized in 10 volumes 0.25 M sucrose and centrifuged for 15 mins. at 600 × G and 15000 × G for 10 minutes. The supernatant was dialysed against distilled water for 48 hours. A precipitate formed which was collected by centrifugation and dissolved in 2 ml TRIS — EDTA buffer (0.1 M — 5 mM pH 7.5) per gram liver. All procedures were carried out at 0 — 4°C [3, 9]. Protein was determined according to LOWRY et al. [8]. The extracts were tested for GSSG-R activity and inhibition by B-chain [3, 9], and the activity calculated as units per mg protein.

The activity varied, perhaps due to various times having elapsed between death and autopsy. Two of the extracts (1 diabetic, 1 non-diabetic) contained too little activity to be tested. For the non-diabetic group the mean activity was 0.0892 ± 0.01 (SEM) units per mg protein, for the diabetic group 0.0802 ± 0.02 units per mg protein.

When tested after 90 minutes incubation at room temperature in the presence of reduced B-chain (0.1 mg per ml) the inhibition of activity was the same in the two groups. In the non-diabetic group $45.1\% \pm 4\%$ activity was retained, in the diabetic $40.2 \pm 6.7\%$, the difference is not significant.

The K_m for GSSG was also determined in the two groups. No difference was found, K_{mGSSG} being approximately 3×10^{-5} M in both groups.

No differences were found between the diabetic and the non-diabetic group. These results, therefore, indicate that the primary defect in diabetes mellitus leading to increased amounts of circulating B-chain is not connected with glutathione reductase activity in the liver.

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Corrigendum Notice

Diabetologia **2**, 277–280 (1966) H. Baumung, K. Reissert und B. Rudas: „Veränderungen des Gehaltes der freien Fettsäuren und der Ketonkörper im Blut alloxandiabetischer Ratten nach i.v. Fettbelastung und Heparinapplikation". Figure 1.

The legend

——— normale Ratten
═══ normale Ratten mit Heparin behandelt
····· diabetische Ratten
:::::: diabetische Ratten mit Heparin behandelt

should read

——— normale Ratten
····· normale Ratten mit Heparin behandelt
═══ diabetische Ratten
:::::: diabetische Ratten mit Heparin behandelt.

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