THE EFFECT OF ETHANOL ON HISTONE GLYCATION IN DIABETIC RATS

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Abstract — The glycation of liver histones was studied in rats with streptozotocin-induced diabetes, in ethanol (EtOH)-treated rats, and in EtOH-treated diabetic rats. In diabetes, the conditions of glucose-protein addition are more favourable extracellularly in serum and in erythrocytes than in the nucleus. This is indicated by the increased level of serum fructosamine and by the high level of glycated haemoglobin, while the glycation of intracellular histone is decreased. In the serum of diabetic rats, we found a relatively high acetaldehyde level, which resulted in elevated histone fluorescence. Fluorescence is an accepted marker of advanced glycation end-product (AGE), the intensity of which, according to our experiments is related not to the level of serum glucose, but to the level of acetaldehyde. The data obtained with histone proteins in diabetic rats treated with EtOH are in good agreement with the results of our earlier in vitro experimental results obtained with H1 histone: the reaction of the two aldehydes (glucose and acetaldehyde) in combination gives a lower glycohistone value than they do separately.

INTRODUCTION

The glycation of proteins of different tissues (Kohn and Schnider, 1982; van Boekel and Hoender, 1992; Pekiner et al., 1993), of haemoglobin of erythrocytes (Trivelli et al., 1971; Bunn, 1981) and of serum albumin (Day et al., 1979), and the importance of non-enzymatic glycation in the diagnosis of diabetes (Bunn, 1981) have been reported before. However, we failed to observe this phenomenon in the basic histone proteins of cell nuclei. This was surprising, especially as in this addition reaction, most of the dibasic lysine amino acid residues take part and, in the human body, it is the histone proteins of the nucleus that are the richest in lysine. This is why we began to study the bio-histochemistry and clinical relations of the so-called glycohistones (Lakatos and Jobst, 1989; Lakatos et al., 1994a) produced during the histone–glucose reaction with special regard to diabetes and ethanol (EtOH).

In the course of our preliminary work, we demonstrated glycohistone in histones of hepatic nuclei of patients dying of decompensated diabetes (Jobst et al., 1991; Jobst and Lakatos, 1996). At the same time, the spread of alcoholism in Hungary prompted us to examine the effect of EtOH and its metabolite, acetaldehyde (ACAD), on the glycation of histone proteins of diabetic rats.

Based on earlier experiments (Donohue et al., 1983; Tuma et al., 1991), we have reported a difference in the kinetics of separate and combined addition of glucose (Glu) and ACAD to histone proteins in vitro (Lakatos et al., 1994b). In the present experiments, we were able to measure fluorescence in histone proteins when incubated with ACAD but not with Glu.

ANIMALS AND METHODS

Animals and treatments

We used four groups of eight rats each of both sexes and mixed breed, weighing 300–400 g. Thirty per cent of the treated animals kept on a normal standard diet died during the 9-month experiment. The four groups were as follows.

Group 1. These were diabetic animals: during the first month, they received 10 mg of Streptozotocin (Stz) (Sigma) in 0.5 ml of citrate buffer subcutaneously twice. Five survived until the end of the experiment.

Group 2. These were EtOH-treated animals: EtOH 15% (v/v) was added to the drinking water supplied to the rats ad libitum, 4 days a week (Nicholls et al., 1994). Five rats survived until the end of the experiment.

Group 3. In addition to inducing diabetes with Stz as in group 1, the rats also received 15% EtOH in the drinking water ad libitum, as in group 2. Six rats survived until the end of the experiment.

The experiments were performed with the permission of the Scientific Council (ETT) of the Hungarian Ministry of Health and followed their ethical guidelines.

Laboratory procedures

Determinations were made once a week in the first month and then once a month for 9 months. After month 9, the rats were killed. Histones were isolated from the liver using the acid extraction method (Johns, 1971). In addition to body weight, Glu was also measured using the GOD–POD method (Trinder, 1969). Glycated haemoglobin (GHgb) and HgbA1C were determined by the Abbot IMX method (Wilson et al., 1993). Fructosamine was assayed by the NBT Roche method (Baker et al., 1991). Total protein was determined using the biuret method (Tietz, 1986). Serum ACAD was measured by gas chromatography (Duritz and Truitt, 1964). Glycohistone was determined by the fructosamine NBT test (fram) and by the fluorimetric method (flsc) described earlier (Lakatos and Jobst, 1989). Fluorimetry is the usual method for determining the advanced glycation end-product (AGE) of proteins (Gugliucci and Bendayan, 1995).

Tissue samples from livers and kidneys were fixed in formalin and alcohol. Alterations were evaluated in frozen
RESULTS

Table 1 shows the results of the final measurements. Average results ± standard deviation and percentage differences from controls are presented. In all three experimental groups, body weight decreased by about 30% ($P < 0.01$ in all cases).

In group 1 (diabetic rats), all characteristic values point to the development of diabetes. Besides high ACAD ($P = 0.01$), decreased H-fram ($P < 0.01$) and elevated H-flsc ($P = 0.07$) values were also observed.

In addition to a slight elevation in blood Glu ($P = 0.09$) in group 2 (alcoholic rats), the analytes indicative of glycation decreased slightly. The decrease may be ascribed to the high value of ACAD in alcohol-treated rats. The lower level of fructosamine also shows this. At the same time, a 50% higher value was obtained for H-flsc ($P = 0.06$).

In group 3 (diabetic rats given EtOH), all parameters indicative of the development of diabetes were also observed, as in group 1, but to a lesser extent. Contrary to our expectations and in spite of moderately higher ACAD, no values indicative of increased histone glycation were observed. We cannot explain why the level of the characteristic values of diabetes are lower than in group 1.

Microscopically, decreased glycogen, fatty degeneration and fibrosis were observed in the liver of rats treated with Stz with or without EtOH. The kidneys showed signs of heavy proteinuria. The thickened glomeruli were slightly lobulated and numerous loops were thickened. In EtOH-treated animals, diffuse fatty degeneration of the liver was dominant.

DISCUSSION

On the basis of our experimental results, we tried to clarify, in rats with Stz-induced diabetes: (1) to what degree histone proteins are glycated; (2) whether ACAD, a metabolite of EtOH, has an effect on glycation; (3) what effect EtOH has in diabetic rats on the uptake of Glu and glycation of histones?

Our answer to the first question is this: the conditions of glucose-protein addition are more favourable in serum and in erythrocytes than within the nucleus. This is proved by the elevation in the values of serum fructosamine and glycated haemoglobin. It is not known whether, during their synthesis, histones are glycated and enter the nucleus as glycohistones, or if they take up glucose non-enzymatically, i.e. glycation takes place in the nucleus. However, after comparison with control animals, we failed to observe increased glycation. This finding is not in agreement with what was observed earlier in human material, where we had noted a moderate increase in liver histone in patients dying of diabetes. (This would not be the first discrepancy between findings in human and animal experiments.)

In the second group, treated with EtOH only, the drug did not greatly influence the glycation of histones. This finding agrees with the observation made by Al-Abed et al. (1999) on haemoglobin in circulating blood. At the same time, the level of serum ACAD was elevated, which resulted in considerable fluorescence. It is of interest that we also found elevated ACAD values and elevated histone fluorescence, in the diabetic group. Since it is known from our earlier investigations (Lakatos et al., 1994b) that, in contrast to the histone–ACAD reaction product, the histone–Glu adduct does not fluoresce; a secondary reaction in response to the effect of ACAD, the formation of an AGE, may be an explanation.

An answer to question 3 is given by the result obtained for group 3. This agrees with our earlier in vitro data: the simultaneous effect of diabetes and EtOH, i.e. the reaction of Glu and ACAD together gives lower values for glycohistone than do the two aldehydes separately. We do not know why this is so; however, our finding is in good agreement with the latest results of Al-Abed et al. (1999).

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REFERENCES


