Diabetologia (1987) 30: 971

Diabetologia

Letters to the Editor

Sodium, potassium adenosine triphosphatase activity in peripheral nerve tissue of galactosaemic rats. Effect of aldose reductase inhibition

Dear Sir,

It is becoming increasingly clear that the galactosaemic animal model of neuropathy differs in a number of respects from experimental diabetic neuropathy. This was further shown in the paper by Lambourne et al. [1] where sodium, potassium adenosine triphosphatase activity in whole sciatic nerve was significantly increased in the galactosaemic animals. Activity was reduced in diabetic nerve, confirming previous observations [2, 3]. Both animal models show an impairment of nerve conduction velocity with evidence of polyol accumulation and reduced inositol concentrations.

We have recently shown that sodium, potassium adenosine triphosphatase activity is significantly reduced in the perineurium of streptozotocin diabetic rats [4]. We have measured the ouabain sensitive component of adenosine triphosphatase activity in endoneurial and perineurial homogenates of mature rats maintained on a 20% galactose diet for 8 weeks. It is possible that the perineurial component could contribute to whole sciatic nerve enzyme activity as measured by Lambourne et al. [1]. Our results are outlined in Table 1, and show that endoneurial enzyme activity is increased 2-fold ($p < 0.015$) compared with age-matched control animals, in keeping with the results on whole sciatic nerve [1]. Activity was increased in the perineurium, but the increase just failed to reach significance. We detected no significant change in enzyme activity in dorsal root or superior cervical sympathetic ganglia. Animals treated orally for 8 weeks with the aldose reductase inhibitor ponalrestat [the proposed INN for 'Statil' (ICI 128436/MK538)] at a dose of 400 mg/kg feed, showed no change in enzyme activity, although there was an appreciable but nonsignificant fall in activity in perineurium.

Contrary to the authors' comment [1], we previously found that myo-inositol levels in dorsal root ganglia, whilst being significantly reduced after 2 weeks of streptozotocin diabetes, had in fact returned to normal at the 8-week stage, even though there was evidence of increased polyol pathway activity in terms of fructose accumulation [5], and previous evidence of reduced sodium, potassium adenosine triphosphatase activity at this stage [6]. The relationship between inositol levels and adenosine triphosphatase activity was therefore questioned, although we could only speculate as to whether there was persisting change in smaller phosphatidyl inositol pools that may be more closely linked to enzyme activity [7].

Aldose reductase inhibition corrects the nerve conduction defect, and polyol and inositol changes in galactosaemic nerve [8], although we could not demonstrate an effect on sodium, potassium adenosine triphosphatase in the endoneurium. The relationship between polyol and inositol metabolism and functional nerve changes in this experimental model of neuropathy clearly differs from the diabetic model. The relationship is again different in the genetically diabetic (db/db) mouse which shows the nerve conduction defect [9], together with lowered inositol levels, but unchanged sorbitol and fructose levels in sciatic nerve [10].

We have also found that vasoactive intestinal polypeptide levels in sciatic nerves from experimental diabetic rats is increased 2-fold but levels in galactosaemic nerve remain unaltered (unpublished observation). This, together with the changes documented by Lambourne et al. [1] and our observations outlined above suggest that the galactosaemic model is not a suitable one for interpreting the changes that occur in diabetic neuropathy.

Yours sincerely,
J. G. Llewelyn, N. J. Patel, P. K. Thomas and D. Stribling

Table 1. Ouabain-sensitive component of sodium, potassium adenosine triphosphatase activity (expressed as nmol Pi·mg protein^{-1}·h^{-1})

	Controls	Galactosaemia	Galactosaemia with ponalrestat
Endoneurium	546.5±117 (7)	1188.3± 195 (7)[a]	1098.9±202 (7) NS
Perineurium	3224.2±643 (7)	5464.9±1322 (6)	3476.4±797 (6) NS
Dorsal root ganglia	2272.3±548 (7)	2773.1± 533 (7)	2366.7±295 (7) NS
Sympathetic ganglia	3462.9±452 (7)	3885.0± 785 (7)	4193.1±708 (7)

Results are expressed as mean±SEM and the number of animals in each group given in parenthesis.
[a] $p < 0.015$, NS = not significant (galactosaemia vs controls)

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Variability of the fructosamine assay in pregnancy

Dear Sir,

In a recent article Flückiger et al. [1] demonstrated a high diurnal variance of the fructosamine assay with a coeffient of variation of up to 10%. Swings of the plasma protein concentration largely but not exclusively accounted for this variance.

Monitoring of glycaemia in pregnancy usually employs an early morning measurement. Therefore, in an ongoing study of glycated proteins in pregnancy, we have measured fructosamine serially during an oral glucose tolerance test between 07.00 and 10.00 hours. Venous plasma fructosamine concentration was determined in duplicate in 60 outpatients at the 28th week of gestation after repeated venipuncture at 0, 1, and at 3 h. Methods were identical to the procedure used by Flückiger et al. with the exception that absorbance was measured at 550 nm in a Cobas Mira analyser and that sample and reagent volumes were smaller by a factor of 5. Means (±SD) of plasma fructosamine were 1.68 (0.13) mmol/l at 0 h, 1.70 (0.14) mmol/l at 1 h, and 1.68 (0.14) mmol/l at 3 h. The 120 individual values obtained at 1 h differed from the same individual's mean at 0 h by 2.7 (2.3)%; the 120 values obtained at 3 h differed by 2.7 (2.5)%. Figure 1 shows the distribution of the individual variations.

We conclude that in the morning hours a 3-h variance of the fructosamine assay does not exceed analytical variance. This conclusion must be confined to fasting women who do not ingest anything else than a glucose solution and remain in a sitting position. On the other hand, the nature of the fructosamine assay as an index of intermediate control of glycaemia and everyday laboratory practice make it unlikely that an afternoon value in the fed state will be performed very often. The dependence of the fructosamine assay under such conditions from the plasma protein concentration as shown by Flückiger et al. has recently been suggested also by others [2]. It is thus tempting to correct fructosamine values to the plasma protein concentration. Because the absolute values of fructosamine have already become familiar it appears reasonable to correct the fructosamine reading to 7 g/dl protein or 4 g/dl albumin so as to maintain the established range of normality. Such a normalisation is an inherent feature of the furosine assay [3]. This assay is time consuming in its original form. A recent modification, however, has made it faster and automated allowing its use in the clinical laboratory as an alternative to the fructosamine assay [4].

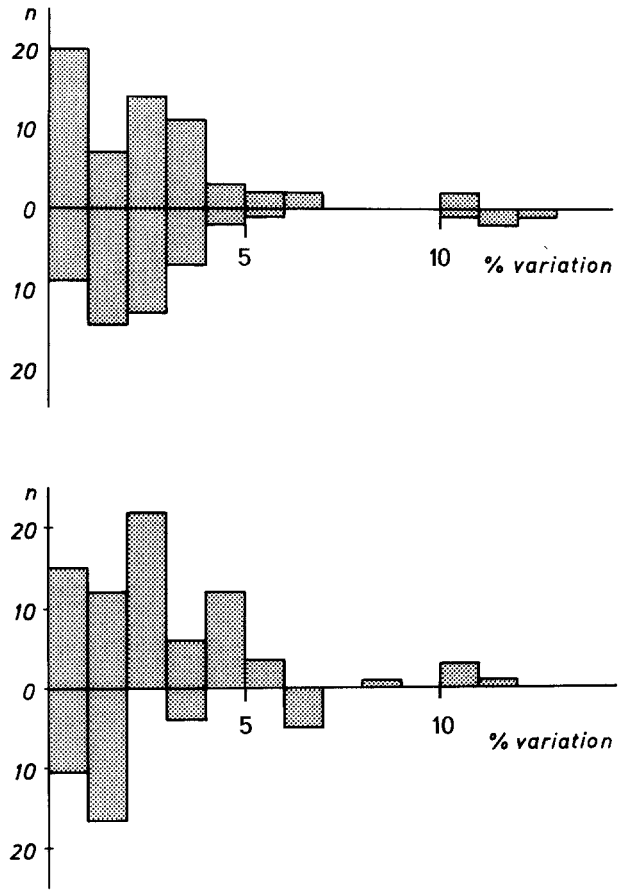

Fig. 1. Histogram of the percentage variation of individual values at 1 h (lower panel) and at 3 h (upper panel) from the mean of the fasting fructosamine result. Bars above abscissa signify positive variations, those beneath the abscissa signify negative variations

Yours sincerely,
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