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Opposite effects of diabetes and galactosaemia on adenosine triphosphatase activity in rat nervous tissue

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Summary. This study measured the ouabain-sensitive adenosine triphosphatase activity in sciatic nerve, lumbar dorsal root ganglia and superior cervical ganglia from control rats, rats with 8 weeks streptozotocin-induced diabetes and rats fed a diet containing 20% galactose for 8 weeks. Whilst the sciatic nerves of the diabetic rats showed a 42% reduction in ouabain-sensitive adenosine triphosphatase activity, the galactose-fed rats showed an increase of 124% ($p<0.01$ and $p<0.005$, respectively, compared to controls). There was also a reduction (by 30% compared to controls; $p<0.05$) in the ouabain-sensitive adenosine triphosphatase activity of the dorsal root ganglia from the diabetic rats, but their superior cervical ganglia did not show a significant fall. The ganglia of the galactosaemic rats showed no change in ouabain-sensitive adenosine triphosphatase activity compared to controls. These changes coexisted with increases in appropriate polyol pathway metabolites in all tissues of both diabetic and galactosaemic rats. There were also depletions of myo-inositol in the sciatic nerves and dorsal root ganglia of diabetic and galactosaemic rats, but their superior cervical ganglia contained levels of myo-inositol which were similar to those of controls. The nerves of the galactosaemic rats showed increased water content; the nerves of the diabetic rats did not. The data argue against a simple relationship between myo-inositol depletion and impaired Na/K adenosine triphosphatase activity in association with exaggerated polyol pathway flux in peripheral nervous tissue.

Key words: adenosine triphosphatase, diabetic neuropathies, galactosaemia, myo-inositol, polyol pathway, streptozotocin-diabetes.

Impaired Na/K adenosine triphosphatase (ATPase) activity has been reported in sciatic nerve [1, 2], dorsal root ganglia [3] and superior cervical ganglia [4] from rats with streptozotocin-induced diabetes. Evidence links this impairment with a depletion of sciatic nerve myo-inositol [2], which in turn appears to be generated by increased flux through the polyol pathway [5]. Exaggerated polyol pathway flux is also generated by feeding galactose to non-diabetic rats; this also causes depletion of myo-inositol [6]. Consequently, it has been suggested that those complications of diabetes mellitus thought to arise from increased flux through the polyol pathway should be mimicked in galactosaemia in those species whose target tissues contain the first enzyme of the pathway, aldose reductase [7]. Since the impairment of Na/K ATPase is reckoned to be central to hypotheses explaining various complications of diabetic nerve [8], the present study was undertaken to determine whether galactose feeding interferes with Na/K ATPase activity in a range of peripheral nervous tissues from rats.

Materials and methods

The study comprised three groups of male Wistar rats which were age- (12 weeks) and weight- (260 to 275 g) matched at the start. Diabetes was induced in one group by intraperitoneal injection of streptozotocin (50 mg/kg body weight in saline) after an overnight fast. Three days later a drop of blood obtained by tail prick from the treated rats was screened for glucose by a strip-operated reflectance meter ('Reflolux': Boehringer Corporation Ltd. London, UK). Rats with blood glucose values of less than 15 mmol/l were rejected at that stage. A second group were given a diet comprising the normal laboratory food (Heygates 41B ('modified'): L. A. Pilsbury, Northampton, UK) mixed with D(+)galactose in the proportions 4 to 1 by dry weight. The third group (control) rats, like the diabetic rats, were given normal 41B diet. All rats had unrestricted access to food and water. The groups were maintained under these conditions for 8 weeks. This time was selected to achieve parity with other studies on dorsal root and superior cervical ganglia [3, 4]. At the end of this time the rats were killed by a blow to the head and bled from the thoracic cavity. The sciatic nerves, superior cervical ganglia and the L_4 and L_5 dorsal root ganglia were removed rapidly from both sides and placed on ice-cold stainless steel plates. A blood sample was centrifuged (9000 *g* for 1 min) and the plasma glucose determined by a hydrogen peroxidase/glucose oxidase (static phase)-operated glucose analyser (Model 23AM; Yellow Springs Instrument Company, Yellow Springs, Oh, USA).

Table 1. Monosaccharides and polyols in peripheral nervous tissues of control rats, rats with 8 weeks streptozotocin-diabetes and rats fed a diet containing 20% galactose for 8 weeks

	Tissue contents (nmol/mg[a]) of					
	Glucose	Galactose	Sorbitol	Dulcitol	Fructose	Myo-inositol
Sciatic nerve						
Controls (6)	6.45 ± 1.49	-	0.24 ± 0.10	-	1.92 ± 0.16	12.87 ± 0.26
Diabetic rats (9)	34.25 ± 2.53[d]	-	8.70 ± 0.57[d]	-	23.49 ± 0.77[d]	9.28 ± 0.27[d]
Galactosaemic rats (7)	6.80 ± 0.50	5.00 ± 0.82	-	34.39 ± 2.30	2.35 ± 0.19	8.10 ± 0.39[d]
Dorsal root ganglia						
Controls (14)	< 0.01	-	< 0.01	-	< 0.01	3.73 ± 1.32
Diabetic rats (10)	6.94 ± 2.35[d]	-	1.17 ± 0.57	-	1.70 ± 0.49[c]	2.12 ± 0.65[b]
Galactosaemic rats (8)	< 0.01	0.21 ± 0.26	< 0.01	4.53 ± 0.86	< 0.01	1.99 ± 0.53[c]
Superior cervical ganglia						
Controls (6)	0.34 ± 0.17	-	< 0.01	-	< 0.01	4.19 ± 0.40
Diabetic rats (9)	4.64 ± 1.14[c]	-	0.40 ± 0.20	-	0.22 ± 0.09[b]	4.89 ± 0.48
Galactosaemic rats (7)	< 0.01	0.36 ± 0.17	< 0.01	2.50 ± 0.45	< 0.01	5.01 ± 0.57

Data are mean ± SEM. Numbers of rats in brackets. [a] Contents are expressed per mg wet weight tissue for dorsal root ganglia and superior cervical ganglia and per mg dry weight for sciatic nerve. < 0.01 indicates chromatogram peaks below integration threshold; - indicates no trace of a peak.
Levels of significance: [b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.001$ versus controls

Table 2. Total and ouabain-sensitive ATPase in peripheral nervous tissues of control rats, rats with 8 weeks streptozotocin-diabetes and rats fed a diet containing 20% galactose for 8 weeks

	ATPase activity[a] (nmol ADP produced/h)	
	Composite	Ouabain-sensitive
Sciatic nerve		
Controls (6)	444 ± 47	114 ± 28
Diabetic rats (9)	281 ± 24[c]	66 ± 10[c]
Galactosaemic rats (7)	938 ± 132[c]	254 ± 30[d]
Dorsal root ganglia		
Controls (14)	410 ± 21	94 ± 8
Diabetic rats (10)	306 ± 23[c]	66 ± 9[b]
Galactosaemic rats (8)	348 ± 26	81 ± 11
Superior cervical ganglia		
Controls (6)	434 ± 54	139 ± 31
Diabetic rats (9)	377 ± 36	76 ± 10
Galactosaemic rats (7)	422 ± 23	118 ± 20

Data are mean ± SEM. Numbers of rats in brackets. [a] For dorsal root ganglia and superior cervical ganglia activity is per mg wet weight tissue, for sciatic nerve activity is per mg dry weight tissue. Levels of significance: [b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.005$ versus controls

The right side ganglia were homogenised in 0.5 ml buffer comprising (mmol/l) NaCl 130, KCl 30, Tris-Cl 20, $MgCl_2$ 3 and Tris-EGTA 1 at pH 7.6. The L_4 and L_5 dorsal root ganglia were pooled. A 1 cm portion of sciatic nerve was homogenised in 1 ml of the same buffer. Homogenates were centrifuged (180 *g* for 5 min at 4 °C) to sediment large cell debris. ATPase activity was determined in duplicate 30 µl samples of the supernatant in a total volume of 1.04 ml. The assay measured the rate of production of ADP by the standard method [9]. Reaction mixtures were stabilised for 20 min at 37 °C and the linear oxidation of NADH was then followed spectrophotometrically at 340 nm for a further 10 min. The reaction cuvette contained the buffer described above plus ATP 3 mmol/l, phosphoenolpyruvate 1 mmol/l, NADH 0.3 mmol/l, pyruvate kinase 45 µg/ml and lactate dehydrogenase (LDH) 24 µg/ml. Activity was measured against homogenate-free blanks. The fraction of total activity for each sample which was inhibited by 5 mmol/l ouabain was taken to represent the Na/K ATPase activity [2]. Ouabain and LDH were purchased from the Boehringer Corporation, London, UK; all other chemicals were obtained from the Sigma Chemical Company Ltd., London, UK.

Ganglia and nerves from the left side were prepared for estimation of monosaccharides and polyols as described elsewhere [10]. Again the L_4 and L_5 dorsal root ganglia were pooled. Chromatograms were run on a Shimadzu GC-8A gas chromatograph fitted with a 25-µm fused silica capillary column (DBI-30N: J. and W. Scientific Inc., Rancho Cordova, Calif, USA).

Statistical analysis

All data are expressed as mean ± SEM. Means were compared by unpaired Student's t-tests. A p value of < 0.05 was considered statistically significant.

Results

The diabetic rats lost 56 ± 6 g during the experiment and were hyperglycaemic at death (plasma glucose was 46.2 ± 4.9 mmol/l). The control and galactose-fed groups gained 53 ± 4 g and 46 ± 6 g, respectively, and were normoglycaemic at death (plasma glucose values were 6.5 ± 0.3 and 5.9 ± 0.7 mmol/l, respectively). The polyol and monosaccharide data are shown in Table 1. Diabetes and galactosaemia both induced formation of polyol (sorbitol and dulcitol respectively) in all three tissues. There was also an accumulation of fructose in the tissues from diabetic rats. In the sciatic nerve and dorsal root ganglia of both groups of experimental rats polyol accumulation was associated with a depletion of myo-inositol. This depletion was greater in association with larger amounts of polyol in the tissues from the galactose-fed rats. There was no myo-inositol depletion in the superior cervical ganglia.

Table 2 shows the data from the ATPase measurements. The sciatic nerves of the diabetic rats showed marked reductions in total and ouabain-sensitive

ATPase activity. This effect was also seen in the lumbar dorsal root ganglia, but the analogous numerical reductions in activity in the superior cervical ganglia were not significant. The sciatic nerves from the galactosaemic rats showed marked increases in both total and ouabain-sensitive ATPase activity. There were no changes in either total or ouabain-sensitive activity in either type of ganglion from the galactosaemic rats.

The sciatic nerves of the galactosaemic rats showed a significant increase in water content (2.51 ± 0.06 mg H_2O/mg dry nerve for galactosaemic rats against 1.87 ± 0.03 for controls; $p < 0.001$). This did not occur in the nerves of the diabetic rats (1.83 ± 0.04 mg H_2O/mg dry nerve).

Discussion

This study reveals a marked difference between sciatic nerve of diabetic and galactosaemic rats in their ATPase activities as measured in vitro. This is in spite of the presence of marked depletions of myo-inositol in the contralateral sciatic nerves of both groups of rats. This finding does not support the notion that a straightforward relationship exists connecting myo-inositol depletion to impaired Na/K ATPase [8]. There are clear indications that prevention of the myo-inositol depletion can forestall the development of impaired Na/K ATPase in diabetic rats [2, 5]. However, a similar relationship, between Na/K ATPase activity and myo-inositol levels, does not hold when the polyol-induced myo-inositol depletion is generated by galactose-feeding. We are inclined to wonder whether the increased water content of the nerves from galactosaemic rats might be related to the altered ATPase activity. An interesting possibility is that increased ATPase activity might in some way participate in the elevation of endoneurial sodium content [11] and that electrolytes could synergise with polyol to induce water uptake.

Our findings, along with those of others [12], show that exaggerated polyol pathway flux is linked to myo-inositol depletion in dorsal root ganglia. But, unlike another study [4], we did not find a similar link in the superior cervical ganglia. In spite of the similar association of dulcitol accumulation with myo-inositol depletion in sciatic nerve and dorsal root ganglia, the lack of effect of galactosaemia on the Na/K ATPase in the ganglia was in marked contrast to its effect on sciatic nerve. This provides further evidence against a straightforward relationship between myo-inositol levels and Na/K ATPase activity.

In conclusion, we suggest that much remains to be learned about the links between polyol pathway flux, myo-inositol depletion and the Na/K ATPase of peripheral nerve in the rat. We also argue that there are basic differences in the consequences of exaggerated polyol pathway flux in the peripheral nerves of galactosaemic and diabetic rats, and that galactosaemia does not provide an acceptable model for diabetes. Both cause a nerve conduction velocity deficit which is blocked by aldose reductase inhibition [10, 13]; impaired Na/K ATPase cannot be responsible in both cases.

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