myo-Inositol metabolism and (Na$^+$ + K$^+$)-ATPase activity in relation to peripheral nerve function in rats with streptozotocin-induced diabetes

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Experimental diabetes in the rat is associated with a well-characterized decrease in motor nerve conduction velocity (Gillon et al., 1983). In rat sciatic nerve, induction of diabetes results in marked elevation of the concentrations of glucose, sorbitol and fructose, whereas the concentration of myo-inositol is reduced (Palmano et al., 1977). Diabetic nerve malfunction may occur as a result of a relationship between the increased polyol pathway activity, the decreased free myo-inositol concentration and an alteration in the activity of the enzyme (Na$^+$ + K$^+$)-ATPase. The present study was designed to examine the possibility of such a correlation and to investigate a report by Greene & Lattimer (1983) that (Na$^+$ + K$^+$)-ATPase activity is lower in sciatic nerve homogenates from diabetic rats compared with that in homogenates from control rats. The steady-state rate of energy utilization is known to be decreased in diabetic nerves (Greene & Winegrad, 1981). This study compares the rates of ouabain-sensitive 86Rb$^+$ uptake by endoneurial preparations from diabetic and control rats.

Diabetes was induced in male Wistar rats (253-314 g) by a single intraperitoneal injection of streptozotocin (60 mg/kg body wt.) in 0.2 ml of 50 mM-sodium citrate buffer. Age- and weight-matched controls received a similar volume of buffer alone. Animals whose blood glucose concentration exceeded 16 mm 2 days after injection were included as diabetics in the study. Diabetic and control animals were divided into two groups and used at either 4 or 6 weeks after injection. Sciatic nerves were removed from the rats under pentobarbitone anaesthesia (60 mg/kg body wt., by intraperitoneal injection). Two ligatures (No 5/0 nylon silk) were applied to each nerve before excision, one at the sciatic notch and the other approx. 4 cm distal to the notch. The epineurium and the perineurial membrane were removed from the ligated nerves by the method of Gillon & Hawthorne (1983) and two more ligatures were applied at the centre of the resulting endoneurial preparation. The nerve was bisected between these ligatures to yield two endoneurial preparations from each sciatic nerve. Endoneurial preparations were incubated at 37°C for 20 min in Krebs-Henseleit bicarbonate Ringer solution (Krebs & Henseleit, 1932) containing 4% (w/v) bovine serum albumin, 5 mM-glucose and 50 μM-myoinositol with a gas phase of O$_2$/CO$_2$ (19:1, v/v) and in the presence of 86Rb$^+$ final concentration: 5 μCi/ml using 6.0 mM-RbCl (1 μCi/ml) stock solution in the 4 week study and 2.5 μCi/ml using 3.6 mM-RbCl (1 μCi/ml) stock solution in the 6 week study. The proximal endoneurial preparation from each sciatic nerve was incubated in the presence of 1 mM-ouabain. 86Rb$^+$ uptake was terminated by washing the endoneurial preparations twice in ice-cold 0.1 M-MgCl$_2$, pH 7.0. The tissue was homogenized in 1.5 ml of 11 mM-ZnSO$_4$/10 mM-Ba(OH)$_2$, and the supernatant taken for measurement of Cerenkov radiation. Ouabain-sensitive 86Rb$^+$ uptake was calculated by subtracting 86Rb$^+$ uptake by the proximal endoneurial preparation in the presence of ouabain from 86Rb$^+$ uptake by the distal endoneurial preparation in the absence of the inhibitor.

During the course of the study, the control rats increased in weight whereas the diabetic rats lost, on average, 18% and 25% of their original weights over the 4 and 6 week periods respectively. The plasma glucose concentration was significantly elevated in the diabetic rats. Sciatic nerves from both groups of diabetic rats had increased concentrations of free glucose, sorbitol and fructose, but had decreased concentrations of free inositol compared with nerves from age-matched controls. Table 1 shows that 4 weeks after injection of streptozotocin there is no significant difference between the rates of 86Rb$^+$ uptake by endoneurial preparations of sciatic nerves from diabetic and control rats. However, 6 weeks after injection of streptozotocin, the rate of 86Rb$^+$ uptake was significantly reduced in diabetic nerves compared with controls. This difference reflects a decrease in the ouabain-sensitive component of 86Rb$^+$ uptake. The rate of 86Rb$^+$ uptake by endoneurial preparations in the 4 week study is higher than that in the 6 week study since the rate of influx of 86Rb$^+$ increases as a function of 86RbCl concentration (Rozenburg & Heppel, 1975) and a higher concentration of 86RbCl was used in the shorter trail. The rate of ouabain-sensitive 86Rb$^+$ uptake by sciatic nerve endoneurial preparations provides a measure of Na$^+$-K$^+$ pump activity in the tissue. The decrease in this activity observed after 6 weeks of diabetes corroborates the finding of Greene & Lattimer (1983) that (Na$^+$ + K$^+$)-ATPase activity measured enzymically, is reduced in crude sciatic nerve homogenates from diabetic rats. This decreased Na$^+$-K$^+$ pump activity may be related to the conduction defect in diabetic nerve.


Abbreviations used: (Na$^+$ + K$^+$)-ATPase, sodium ion-plus-potassium ion-dependent adenosine triphosphatase.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>86Rb$^+$ uptake by endoneurial preparations of sciatic nerve from diabetic and age-matched control rats</th>
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<tbody>
<tr>
<td>Duration of diabetes</td>
<td>Total 86Rb$^+$ uptake</td>
</tr>
<tr>
<td>Control (8)</td>
<td></td>
</tr>
<tr>
<td>Diabetic (11)</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Control (10)</td>
<td></td>
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<tr>
<td>Diabetic (9)</td>
<td>6 weeks</td>
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</table>

Results are expressed as means ± s.d. in pmol/min per mg wet wt. of tissue for the numbers of animals given in parentheses. Significance limits between diabetic and age-matched control groups were determined by student's t test *P < 0.05, **P < 0.01.

Some effects of trishydroxymethylaminomethane (‘Tris’) on the activity of urease

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The choice of buffer is difficult when working with urease (EC 3.5.1.5). It has been shown to be affected by so many different ions (Reithel, 1971) that most of the usual buffer systems in the pH range 7–9 are unsuitable. Tris (trishydroxymethylaminomethane) has been reported to have no inhibitory effects on urease (Wall & Ladleier 1953). However, Tris has been shown to inhibit a number of enzymes including glutamate dehydrogenase (Sund, 1970), pyruvate kinase (Kapoor & Tronsgaard, 1972) and adenylate cyclase (Young & Stansfield, 1978). During studies using highly purified urease, Tris was found to inhibit urease activity and this inhibition has been investigated.

Urease was extracted from Jack bean meal (Sigma, Poole, Dorset) by the method of Blakeley et al. (1969) and purified by twice recrystallizing from acetone (20%), followed by filtration through a Millipore membrane (0.22μm). The purified enzyme was dissolved in a solution of Tris/H2SO4, pH 7.8 (5 mM), EDTA (1 mM) and dithiothreitol (1 mM). This method gave urease of specific activity approx. 110 kat/kg of enzyme protein. Rates of urea hydrolysis were determined by two methods. Reaction volumes (4ml) contained urea (62.5 mM), Tris/H2SO4, pH 7.8 (0–100 mM) or analogue, double-distilled water and 10 μl (20 nkat) of urease to initiate the reaction.

The reaction was monitored continuously with a Radiometer Autotitrator pH-stat and 50 mM H2SO4 to titrate the ammonia released. Alternatively a discontinuous assay was used when ammonia produced was estimated by the method of Chaney & Marbach (1962). Tris solutions were prepared using Trizma (Sigma) and formaldehyde could not be detected in the solutions. Tris solutions were adjusted to pH 7.8 with phosphoric acid, hydrochloric acid and sulphuric acid. The effects on the activity of urease of increasing concentrations of these solutions, triethanolamine sulphate and triethylamine sulphate were investigated and typical results are shown in Fig. 1.

All the solutions containing Tris, whichever anion was present, inhibited in the same manner. Both assay procedures gave the same results. Triethanolamine inhibited urease in a manner very similar to Tris but triethylamine did not inhibit urease, suggesting the hydroxyl groups are important for inhibition. 2-Amino-2-methylpropandiol buffered with sulphuric acid also inhibited urease activity but 2-amino-2-methylpropanol, 2-methylpropanol and tert-butylamine did not. It is concluded therefore that the presence of two hydroxyl groups are important for inhibition.

The inhibition was found to be dependent on pH, being most pronounced at pH 7.8, which is also the pH optimum for urease activity. At plus or minus one pH unit of this value urease was unaffected by Tris. It was also noted that the Tris concentration necessary to completely inhibit urease activity at pH 7.8 increased as the buffer solution aged. The decrease in inhibitory property of Tris solutions corresponded with an increase in detectable aliphatic reducing groups. This suggests that the hydroxyl groups of the Tris are being converted to aldehydes and impurities which prevents Tris binding.

The distance between the hydroxyl hydrogen of one methoxy group and the hydrogen of another is the same as the distance between the hydrogens of a water molecule. It is therefore suggested that Tris inhibits urease by binding at the water site on the enzyme. Only urease of high specific activity is inhibited by Tris, therefore impure urease is possibly associated with another protein or lipoprotein which prevents Tris binding.