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Sodium-potassium-ATPase activity in the dorsal root ganglia of rats with streptozotocin-induced diabetes

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Summary. Sodium-potassium-ATPase activity was measured in excised dorsal root ganglia of streptozotocin-diabetic rats, 2 months after induction of diabetes. In comparison with age-matched controls, there was a decrease in both the total and ouabain-insensitive activity, indicating an overall reduction in ouabain-sensitive activity of 46%. This decrease may explain the reduced amino-acid uptake exhibited by diabetic sensory ganglia and could be relevant to the development of diabetic neuropathy.

Key words: Sodium-potassium-ATPase, dorsal root ganglia, streptozotocin diabetes, diabetic neuropathy.

Most studies on peripheral nerve function in streptozotocin-induced diabetes have concentrated on the changes in the peripheral nerve trunks. Nerve conduction velocity has been shown to be reduced [1, 2] and nerve fibre diameter is slightly but significantly less in diabetic compared with control animals. This reduction may be partly the result of a maturational deficit [3] and partially related to axonal 'dwindling' [4] or shrinkage [5]. Segmental demyelination does not occur in the earlier stages [6, 7] but degenerative changes including axonal loss and segmental demyelination have been described after prolonged alloxan diabetes in rats [8]. The transport of structural [9] and other proteins, including the enzyme choline acetyltransferase [10], by the slow transport system, is known to be reduced. There are conflicting reports relating to the fast transport system [11].

The synthesis of structural proteins and enzymes takes place virtually entirely in the neuronal perikarya. The events that occur in dorsal root and autonomic ganglia and in anterior horn cells in diabetes are therefore of interest, but few studies on this topic have been undertaken so far. Sidenius and Jakobsen [12] showed that perikaryal volume is less for anterior horn and dorsal root ganglion cells in early streptozotocin-induced diabetes in comparison with controls. Thomas et al. [13] recently reported that amino-acid uptake by dorsal root ganglia was impaired in streptozotocin diabetes, based upon the uptake of 4-amino [^{3}H]-butyric acid. The incorporation of [^{3}H]-leucine into protein was also found to be reduced: this could have been secondary to impaired uptake.

The present study has examined the activity of sodium-potassium activated adenosinetriphosphatase (Na^+, K^+-ATPase, EC 3.6.1.3) in dorsal root ganglia from streptozotocin-diabetic rats. The activity of this enzyme is known to be impaired in diabetic nerve [14, 15] and if it is also reduced in ganglia, this could provide a mechanism for the diminution in amino-acid uptake.

Materials and methods

Animal preparation

Diabetes was induced in mature male Wistar rats (aged 4 months, weight 230–280 g) by the intraperitoneal injection of a buffered solution of streptozotocin (55 mg/kg body weight). Control rats were administered an equivalent quantity of physiological saline (150 mmol/l).

Diabetic and control animals were housed separately in plastic metabolic cages and fed on 41 B Oxoid diet (Oxoid, Basingstoke, Hants, UK) with water ad libitum. Both groups were weighed regularly and the diabetic rats checked for glycosuria. Diabetic animals developed glycosuria in excess of 2% within a few days of the administration of streptozotocin and progressively lost weight. Both groups were maintained for a period of 2 months. When anaesthetized for removal of the dorsal root ganglia, a blood sample was taken from the tail vein and plasma glucose determined on a Beckman Glucose Analyser II (Beckman, Fullerton, California, USA).

Tissue collection

Under general anaesthesia with intramuscular Hypnorm (Janssen, Crown Chemicals, Lamberhurst, Kent, UK) and ether inhalation, laminectomy was performed and the five caudal pairs of lumbar dorsal root ganglia were excised under an operating microscope (Zeiss, Jena, DDR). At each session the ganglia from one diabetic and one

control animal were processed. The excised ganglia were immediately frozen on dry ice after removal of any attached pieces of spinal root and washing free of blood with homogenizing solution. The homogenates were prepared by six passes of a borosilicate manual homogenizer (Baird & Tatlock, Romford, Essex, UK) in 1 ml of a homogenizing solution comprising sucrose (250 mmol/l), EDTA (disodium salt; 1 mmol/l) and Tris base (20 mmol/l, pH 7.4) at 25 °C.

Assay of ATPase activity

Total ATPase activity was determined in duplicate in 800 µl of buffer containing a final concentration of NaCl (100 mmol/l), KCl (15 mmol/l), ATP (vanadate-free; 5 mmol/l), $MgCl_2$ (5 mmol/l), EDTA (disodium salt; 3 mmol/l) and Tris base (50 mmol/l, pH 7.2) at 25 °C. Ouabain-insensitive activity was determined similarly in duplicate in 800 µl of the same buffer, but with equimolar choline chloride instead of KCl, in the presence of 1 mmol/l ouabain solution. To initiate the reaction, 200 µl of the crude homogenate was added to 800 µl of buffer, with or without 1 mmol/l ouabain or an equivalent volume of 150 mmol/l saline. The samples were incubated at 37 °C in a shaking water bath for 20 min. The reaction was terminated by the addition of 1 ml (15%, w/v) trichloracetic acid solution and immediately centrifuged at 500 *g* for 5 min. Inorganic phosphate (P_i) was determined spectrophotometrically by the manual stannous chloride-hydrazine method [16]. Protein estimations for each tube were measured according to the method of Lowry et al. [17]. Ouabain-sensitive Na^+, K^+, -ATPase activity is the difference between total ATPase and ouabain-insensitive activity and is expressed as µmol P_i released from ATP per g protein per h at 37 °C. All values obtained were the mean of the two estimations.

Statistical analysis

The comparisons between the results from the diabetic and control animals were made on mean values using Student's t-test for unpaired data. Results are expressed as mean ± SEM unless otherwise stated.

Results

Ten pairs of control and diabetic rats were studied when the latter had been diabetic for 2 months. The operations on the pairs were performed in random order. At the time of the terminal studies, the control animals weighed 413–652 g and the diabetic animals 232–317 g. Approximately half of the diabetic animals had developed cataracts. The plasma glucose levels for the two sets of animals are shown in Table 1.

ATPase activity

Mean ouabain-sensitive Na^+, K^+, -ATPase levels were consistently less in the ganglia from the diabetic compared with the control animals (499.4 ± 43.3 versus 917.6 ± 45.7 µmol $P_i \cdot g^{-1}$ protein $\cdot h^{-1}$; $p < 0.001$; Table 1, Fig. 1). Table 1 also shows that the total and ouabain-insensitive ATPase activity was reduced in the diabetic group. The reduction in ouabain-insensitive ATPase activity amounted to 17%. The results in Table 1 are tabulated in order of increasing weight of test animal. It is evident that in the control group there is a general trend towards higher ATPase activity in the heavier animals which is not apparent in the diabetic group.

Table 1. ATPase activity in rat dorsal root ganglia

Ganglia	ATPase activity (µmol $P_i \cdot g^{-1}$ protein $\cdot h^{-1}$)			Plasma glucose (mmol/l)
	Total ATPase	Ouabain-insensitive ATPase	Ouabain-sensitive ATPase	
Control ganglia ($n = 10$)	4483	3886	597	13.7
	6185	5420	765	11.9
	4634	3746	888	8.8
	5154	4169	985	11.9
	6536	5632	904	10.5
	6075	5086	989	8.4
	5851	4909	942	8.0
	6274	5274	1000	13.5
	5953	4961	992	11.1
	6123	5009	1114	9.1
Mean ± SEM				10.7 ± 0.66
Diabetic ganglia ($n = 10$)	4635	4095	270	44.0
	4330	3833	497	34.0
	3417	3021	396	32.0
	4811	4416	396	41.5
	4651	4246	405	32.0
	3870	3263	607	36.0
	4874	4365	509	34.0
	4985	4451	534	42.6
	4707	4014	690	28.8
	4988	4298	690	31.0
Mean ± SEM				35.6 ± 1.7

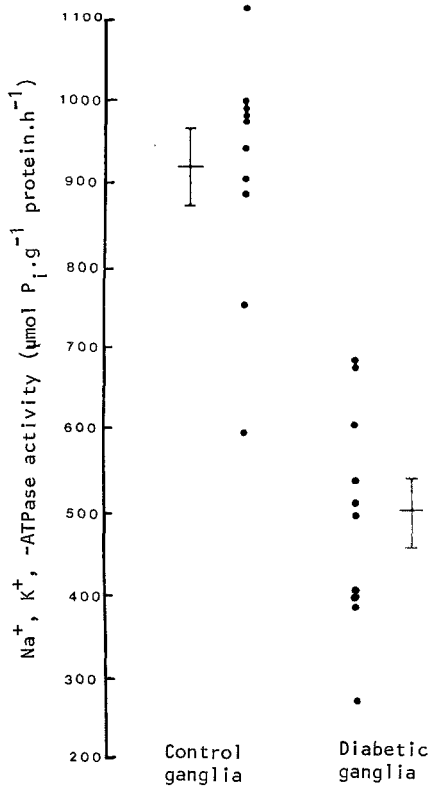

Fig. 1. Na^+, K^+,-ATPase activity in dorsal root ganglia of control and streptozotocin-diabetic rats. The bars indicate mean ± SEM

Discussion

The metabolic alterations in diabetic nerve have been clarified to a considerable extent in recent years. The use of endoneurial preparations has established that energy utilization in nerve, like that in brain, is independent of insulin control. It has been shown to be related to exogenous glucose supplies and mainly to be regulated by local energy requirements [18, 19]. Nevertheless, energy and substrate utilization in resting peripheral nerve is reduced by about 25–30% in streptozotocin-diabetic rats, despite increased tissue concentrations of glucose and fructose [20]. This reduction has been equated with an alteration in nerve Na^+, K^+,-ATPase activity: the decrease in steady-state resting energy utilization in diabetic nerve is strictly explicable in terms of a reduction in ouabain-sensitive Na^+, K^+,-ATPase activity [21]. The defect in ATPase activity is not affected by insulin in acute experiments in vitro [15]. It is evident that it represents membrane-associated ATPase activity as it is demonstrable in nerve homogenates [14, 15] when water-soluble modulators are controlled. The same is true for the present observations.

The defect in Na^+, K^+,-ATPase activity has been found to be reversed by correction of the reduced *myo*-inositol concentration known to exist in diabetic nerve [15], possibly through regulation by membrane phosphatidylinositol [22, 23]. The reduced tissue concentrations of *myo*-inositol in nerve are likely to be related to competitive inhibition of uptake by glucose on a sodium-dependent carrier mechanism [24]. This carrier mechanism secondarily could be impaired by a reduction in the Na^+, K^+,-ATPase activity upon which the mechanism relies. It is relevant in this context that the sodium dependent *myo*-inositol uptake remains impaired in acute in vitro experiments even if the glucose concentration is normalized [21].

The accumulation of sorbitol in nerve [25] may also impair *myo*-inositol uptake. Reduction in nerve sorbitol levels by the administration of an aldose reductase inhibitor will increase *myo*-inositol levels without changes in the degree of hyperglycaemia [26, 10].

In relation to the present experiments, it is not yet known whether *myo*-inositol levels are reduced and sorbitol levels increased in diabetic dorsal root ganglia. If this were so, as in peripheral nerve, it could provide an explanation for the reduced Na^+, K^+,-ATPase activity. Amino-acid uptake by cells is dependent on a sodium gradient maintained by Na^+, K^+,-ATPase activity [27]. The reduced amino-acid uptake by dorsal root ganglia in streptozotocin-diabetic rats found by Thomas et al. [13] could thus be explained. In its turn, this impaired uptake could have important metabolic consequences, for example through reduced synthesis of structural proteins and enzymes and their reduced delivery to the axons via the axonal transport system. This might constitute a contributory factor in the development of diabetic neuropathy.

The present results have shown that Na^+, K^+,-ATPase activity in the dorsal root ganglia tends to increase with weight in the control rats (but not in the diabetic animals). Age cannot be directly responsible as the animals were age-matched and initially were all of closely similar weight (230–280 g). The difference in the final weights of the control animals is presumably the consequence of individual variations in rates of maturation. The present results additionally have indicated that ouabain-insensitive ATPase activity is less in diabetic sensory ganglia, suggesting that the activity of other membrane ATPases is also reduced.

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