Rapid communications

Impaired induction of ornithine decarboxylase activity following nerve crush in the streptozotocin-diabetic rat

W.G. McLean, J.E. Chapman and N.A. Cullum

Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, UK

Summary. Ornithine decarboxylase activity was measured in the dorsal root ganglia from crushed and uncrushed contralateral sciatic nerve of control and streptozotocin-diabetic rats. A further group of diabetic rats was treated with insulin throughout the experiment. Ornithine decarboxylase activity in ganglia from uncrushed nerves was the same in diabetic and non-diabetic rats. A significant (greater than 4-fold) increase in mean levels of ornithine decarboxylase activity 72 h after crush injury was found in ganglia from crushed nerves in non-diabetic but not in diabetic rats. The enzyme activity

in ganglia from diabetic rats treated with insulin resembled that in non-diabetic rats. Twenty-four hours after crush injury, ornithine decarboxylase activity in ganglia from crushed nerves was higher in non-diabetic than in diabetic animals. This may be responsible for the delayed and defective nerve regeneration known to occur in peripheral nerve of the streptozotocin-diabetic rat.

Key words: Streptozotocin, diabetes mellitus, nerve regeneration, ornithine decarboxylase, neuropathy.

The defects in peripheral nerve in the streptozotocin-diabetic rat include a delay in regeneration following a crush injury [1, 2], and an impaired fibre maturation [2]. An early consequence of interruption of the continuity of peripheral nerve axons is an increase in the activity within the nerve cell bodies of ornithine decarboxylase, an enzyme associated with a number of growth-associated phenomena [3–5]. Inhibition of ornithine decarboxylase prevents outgrowth of new axons [5]. We undertook this study to determine if impaired induction of ornithine decarboxylase could explain the delayed nerve regeneration in the diabetic rat.

Materials and methods

Diabetes was induced in female Wistar albino rats (weight 200–210 g) by intravenous injection of streptozotocin (50 mg/kg) in citrate buffer (pH 4.0). Control rats received buffer alone. From the third day after injection of streptozotocin, one group of diabetic animals received twice daily intramuscular injections of (initially) 2.5 U/100 g Semitard (Novo, Copenhagen, Denmark) insulin in the morning and 2.5 U/100 g Mixtard (Novo) insulin in the evening. The insulin doses were subsequently adjusted to maintain non-fasting whole blood glucose levels at around 5 mmol/l. Fourteen days later, the extent of diabetes in all rats was assessed as weight loss and an increase in fasting blood glucose, measured with an Ames Glucocheck and glucose-sensitive reagent sticks.

Diabetic or control rats were anaesthetised with ether and the right or left sciatic nerve randomly selected and exposed under aseptic conditions. The nerve was crushed with 4/0 silk thread against a glass rod. The wound was closed and the rats allowed to recover for a period of 24 h or 72 h. The rats were then killed and the L4, 5 and 6 dorsal root ganglia supplying both the crushed and the contralateral uncrushed nerve were dissected out and placed on ice as rapidly as possible.

The three ganglia from each nerve were homogenised in 100 µl cold 25 mmol/l Tris buffer, pH 7.4, containing 1 mmol/l EDTA, 5 mmol/l dithiothreitol and 0.1 mmol/l pyridoxal-5'-phosphate. The homogenates were centrifuged at 32 600 g for 30 min at 4 °C. The supernatants were incubated in 5 ml autoclave bottles fitted in the neck with rubber tubing containing a strip of filter paper moistened with 40% KOH. The incubation mixture contained 200 µl 50 mmol/l Tris buffer, pH 7.1, 4 mmol/l EDTA, 4 mmol/l dithiothreitol, and 0.4 mmol/l pyridoxal-5'-phosphate, 50 µl of the ganglia supernatant and 0.2 µCi D,L-¹⁴C-ornithine (50 mCi/mmol; Amersham, Amersham, UK). The mixture was incubated at 37 °C with gentle shaking for 2 h. The reaction was stopped by injection of 0.1 ml H₂SO₄ directly into the reaction mixture, which was then allowed to stand overnight at room temperature. The filter papers, which contained the absorbed ¹⁴CO₂, were removed and the radioactivity measured by liquid scintillation counting. The protein content of the homogenate supernatant was determined by the method of Bradford [6].

Statistical analysis

Results were tested for statistical significance by the Wilcoxon Signed Rank Test for paired data and the Wilcoxon Rank Sum Test for unpaired data. Differences were considered to be statistically significant at the $p < 0.05$ level.

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co (Poole, UK).

Results

The mean plasma glucose of the diabetic animals at the time of operation was 26.03 ± 13.2 (SD) mmol/l while that of the non-diabetic rats was 5.92 ± 1.77 mmol/l. The value for insulin-treated diabetic rats was 5.51 ± 4.62 mmol/l. The mean weight loss in the diabetic rats was 15 g; the non-diabetic rats gained a mean of 10 g during the same period. Insulin-treated diabetic rats also gained weight.

There was no significant difference between the diabetic and non-diabetic rats in the ornithine decarboxylase activity of ganglia from uncrushed nerves contralateral to the crushed nerves (Fig. 1). The operative procedure per se did not affect ornithine decarboxylase activity as the activity was not significantly different from that in ganglia from a group of non-diabetic rats subjected to no procedures prior to death: mean activity 3.1 ± 1.2 (SEM) units. Nor was the total protein content of the ganglia significantly altered by crush injury or by diabetes.

At 24 h after crush injury, there was an apparent increase in ornithine decarboxylase activity in non-diabetic animals which did not reach statistical significance on account of marked variations in the extent of induction between animals. At 72 h after injury, however, the increase in ornithine decarboxylase activity was statistically significant ($p < 0.05$) (Fig. 1), by paired analysis.

Neither 24 h nor 72 h after crush injury was there any significant change in ornithine decarboxylase activity in the diabetic rats; mean values in ganglia from crushed nerves were less than in ganglia from uncrushed nerves. The activity in ganglia from crushed nerves of diabetic rats was significantly less than that in non-diabetic rats 24 h after nerve crush, despite the lack of difference in ganglia from uncrushed nerves.

Mean values for ornithine decarboxylase activity in ganglia from diabetic rats treated with insulin were 5.23 ± 1.32 (SEM) units from uncrushed nerves and 9.41 ± 3.33 units from nerves crushed 72 h previously. This was not significantly different from the values in non-diabetic animals (Fig. 1).

When the activity in crushed nerves was expressed as a percentage of that in the uncrushed nerve in the same animal, the non-diabetic rats produced values in the range 67 to 702% (mean 243) and 122 to 3270% (mean 946) at 24 h and 72 h respectively. Diabetic rats produced values in the range 16 to 318% (mean 97) and 42 to 291% (mean 110), while diabetic rats treated with insulin gave values in the range 29 to 2112% (mean 330) at 72 h only ($n = 13$). The changes were significantly different between non-diabetic and diabetic rats at the $p < 0.05$ level at 24 h and the $p < 0.01$ level at 72 h after injury.

Fig. 1. Ornithine decarboxylase activity in pooled L4, 5 and 6 dorsal root ganglia from non-diabetic and diabetic rats in which one sciatic nerve had been crushed $\square$ 24 h or 72 h previously as indicated. The contralateral sciatic nerve served as uncrushed control $\square$. Results are expressed as units ornithine decarboxylase where 1 U = 1 pmol ornithine decarboxylated per milligramme total soluble protein per hour. Although data were not normally distributed, SEM provide an indication of variation between animals. Statistically significant differences at the $p < 0.05$ (*) and $p < 0.005$ (**) level are indicated. $n = 7$ for each group at 24 h and $n = 8$ for each group at 72 h

Discussion

Ornithine decarboxylase activity was low in dorsal root ganglia from uncrushed nerves and close to the level of sensitivity of the assay method (around 1 pmol·mg protein⁻¹·h⁻¹ = 1 U). This probably explains the high degree of variation between nerves and the fact that the data were not normally distributed; non-parametric statistical analysis was therefore used throughout. No absolute values exist in the literature for ornithine decarboxylase activity in rat dorsal root ganglia. The changes in activity resulting from nerve injury do appear, however, to be markedly greater than those recently published by Wells [7] using the same model, and are more in line with the changes found in dorsal root ganglia of the frog [8].

The results indicate a marked impairment of the induction of ornithine decarboxylase activity in sensory fibres of rat sciatic nerve in streptozotocin-induced diabetes up to three days after crush injury. It is unlikely that streptozotocin itself directly influenced the enzyme activity since it was identical in ganglia from uncrushed nerves in diabetic and non-diabetic animals. Also, the ornithine decarboxylase activity in ganglia from diabetic rats treated with insulin closely resembled that from non-diabetic rats, for both uncrushed nerves and nerves crushed 72 h previously. The mean increase in enzyme activity in that group was not as high as that in non-diabetic animals at the same time point. This might be expected in the absence of perfect control of blood glucose throughout the 14 days of diabetes. It should be noted that, possibly for that reason, none of the values in the insulin-treated diabetic ani-

mals was significantly different from that in the other groups.

The factors which stimulate ornithine decarboxylase activity in response to nerve injury are not yet understood, but are believed to rely on retrograde axonal transport of a signal to the nerve cell body from the site of injury [8]. Possible candidates for the signals are nerve growth factor and insulin, (or other insulin-like growth factors), both of which have growth-promoting effects including induction of ornithine decarboxylase [9, 10]. Insulin deficiency and inhibition of the retrograde axonal transport of nerve growth factor along with other proteins are features of streptozotocin-induced diabetes [11].

Further experiments will be necessary to determine if the impairment is permanent or if it takes the form of a delay in the induction following the crush. In either case, the defect may explain the delayed and deficient regenerative response in the sciatic nerve of the diabetic rat.

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Dr. W.G. McLean
Department of Pharmacology and Therapeutics
University of Liverpool
P.O. Box 147
Liverpool, L69 3BX
UK