Rapid communications

Chronic low frequency electrical activation for one week corrects nerve conduction velocity deficits in rats with diabetes of three months duration

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Summary. This study examined the effect of chronic electrical activation on conduction velocity deficits after three months of streptozotocin-induced diabetes. There were 30% and 20% reductions in conduction velocity in diabetic animals for tibialis anterior and saphenous nerves, respectively ($p < 0.01$). Unilateral electrical stimulation of the common peroneal nerve, which contains axons supplying tibialis anterior but not saphenous nerve, was carried out in a group of diabetic and a group of normal control rats. Stimulation was given over seven days, at 10 Hz for 8 h/day. Final experiments were carried out at least 17 h after the last stimulation session. In normal rats stimulation had no effect on conduction velocity in

either nerve. In diabetic animals, however, tibialis anterior conduction was within the normal control range for the stimulated nerve. In contrast, the contralateral unstimulated nerve had reduced conduction velocity ($p < 0.001$), which was within the unoperated diabetic control range. There were no effects on saphenous nerve conduction, comparing stimulated and unstimulated legs. We conclude that chronic increases in nerve electrical activation promote mechanisms that reverse conduction deficits in diabetic rats.

Key words: Diabetic neuropathy, chronic electrical stimulation, nerve conduction, streptozotocin-diabetes.

Reduced nerve conduction velocity (NCV) is an early neuropathic change in diabetic patients and animals. Its aetiology is unclear although several hypotheses have been considered. One viewpoint is that neuropathy results from hyperglycaemia stimulating the polyol pathway, which leads to loss of myo-inositol from nerve fibres. This in turn reduces nerve Na-K ATPase pump activity: fibres become Na loaded, Na channels inactivate, and NCV decreases [1]. Another hypothesis suggests that reduced nerve blood flow caused by rheological changes coupled with vasa nervosum microangiopathy leads to endoneurial hypoxia sufficient to impair function [2].

In this communication we present data demonstrating that chronic nerve stimulation normalises NCV in streptozotocin-diabetic rats. We employed nerve stimulation to activate skeletal muscles as part of an investigation into changes in capillarisation and oxidative potential [3]. We also monitored NCV in stimulated and unstimulated nerve branches, and this previously unreported effect of electrical activation may have some bearing on the mechanisms underlying diabetic neuropathy.

Materials and methods

Male Sprague-Dawley rats aged 19 weeks, obtained from the Aberdeen University breeding colony, were divided into normal and diabetic groups. Normal animals acted as onset controls and were studied in final experiments one week later. A further group of normal rats was not subjected to stimulation or any operative procedures but was left for a further three months to act as unstimulated age-matched controls. The other animals were given a single i.p. injection of streptozotocin (45 mg/kg) in 20 mmol/l sodium citrate buffer (pH 4.5). Diabetes was verified 24 h later by the presence of hyperglycaemia (tail prick samples) and glycosuria (Visidex II and Diastix; Ames, Slough, UK) and animals were monitored regularly thereafter. Just before final experiments, samples were taken for plasma glucose estimation.

Diabetic rats were left untreated for 3 months. Fine stainless steel wires, teflon insulated except at the very tip, were implanted, under sterile conditions, either side of the common peroneal nerve at the popliteal fossa in the upper calf of one leg. These wires were routed subcutaneously to the back of the neck and externalised. Animals were stimulated for 8 h per day for seven days in their home cages. They were unrestrained and food and water were available ad libitum. Stimulus pulse-width was 0.3 ms and current was adjusted for just maximal contraction of tibialis anterior muscle by palpation and by observing the toe dorsiflexion produced. Stimulus frequency was 10 Hz. A group of normal onset control rats was also implanted with electrodes and stimulated using the same regime.

Fig. 1. Conduction velocity in normal control and diabetic nerves. Top graph, tibialis anterior nerve; bottom graph, saphenous nerve. Dashed horizontal lines represent unstimulated age-matched normal control mean $\pm$ SEM ($n=20$). Continuous horizontal lines represent unstimulated diabetic control mean $\pm$ SEM ($n=20$). Open columns represent means ($\pm$ SEM, vertical lines) for stimulated normal onset-control ($n=9$), left side, and stimulated-diabetic ($n=9$) groups. Hatched columns are for respective unstimulated contralateral legs. Unoperated diabetic group vs age-matched control group, $p < 0.01$ for both nerves. Stimulated leg vs unstimulated leg for tibialis anterior in stimulated-diabetic group, $p < 0.001$. Stimulated tibialis anterior vs unoperated diabetic control, $p < 0.01$. Saphenous nerve in stimulated-diabetic group vs age-matched normal control $p < 0.01$, both legs. Saphenous nerve in stimulated-diabetic group vs stimulated onset-control group, $p < 0.05$, both legs

Final experiments were performed under i.p. urethane anaesthesia (1–1.5 g/kg) as described previously [4]. For stimulated rats 17–41 h elapsed between the last stimulation session and NCV measurements. Briefly, the sciatic nerve was exposed between the sciatic notch and the knee. Tibialis anterior muscle was exposed in the calf. Bipolar electrodes were placed around the nerve at notch and knee to deliver just supramaximal stimulation (0.1 ms pulses at 1 Hz). A concentric bipolar electrode was used to monitor tibialis anterior electromyogram activity. Conduction was also measured in the saphenous nerve. It was exposed at the top of the leg for stimulation, and at the ankle for recording compound action potentials using a unipolar platinum wire electrode.

Statistical analysis

All data are expressed as mean $\pm$ SEM. Between-leg, within-animal comparisons were made using Student's paired t -tests. Between group comparisons were performed using one-way analysis of variance (ANOVA). If a significant ($p < 0.05$) result was obtained, differences were assigned to individual groups using Duncan's multiple range test.

Results and discussion

Diabetic animals, irrespective of treatment, were hyperglycaemic having plasma glucose values of 45.9 ± 3.2 mmol/l. They had a 31% weight loss with a starting weight of 546 ± 15 g. There was no effect from the operative procedure on body weight.

Data for tibialis anterior and saphenous NCV are illustrated in Figure 1. Neither nerve showed changes in NCV with age in normal control rats, indicating that nerves were fully grown at the start of the study. Thus, the results are relatively uncomplicated by developmental factors [4]. Chronic stimulation had no significant effect on NCV in normal control rats.

In unoperated diabetic rats conduction was reduced by approximately 30% in tibialis anterior and 20% in saphenous nerve compared to normal control rats. However, with stimulation, conduction was within the normal range for tibialis anterior. In contrast, NCV in the unstimulated contralateral leg was reduced in all nine rats and fell within the unoperated diabetic range. Saphenous NCV was not affected by common peroneal nerve stimulation; NCV in both legs was similar, and was not significantly different from unoperated diabetic values.

Conduction deficits in streptozotocin-treated rats have been normalised by insulin treatment which results in near normoglycaemia [5]. They are also improved by aldose reductase inhibition and dietary myo-inositol supplementation [1]. Thus, it is unlikely that the deficits themselves resulted from a direct action of streptozotocin on axons or Schwann cells.

Electrical activation reversed the conduction deficit in the stimulated nerve branch but had no effect on the saphenous nerve of the same leg, or on either nerve in the contralateral leg. This rules out generalised effects of the procedure, such as stimulation-induced release of blood-borne factors, and strongly suggests that the mechanism depends on increased action potential production. In these experiments, the beneficial activation was conducted centrally, the stimulation site being distal to the section of nerve in which NCV was measured.

In terms of current hypotheses, we have no evidence that electrical activity affects nerve polyol or myo-inositol levels. However, it may be that stimulation leads to upregulation of membrane Na-K ATPase pump density or activity, perhaps by a mechanism involving enhanced phosphoinositide turnover and diacylglycerol production [6]. This needs further investigation.

A known effect of stimulation is to increase tissue blood flow. In acute experiments on anaesthetised diabetic rats, 10 Hz stimulation for 15 min led to a two fold decrease in vascular resistance and a concomitant increase in sciatic nerve blood flow [7]. If such an increase was maintained during the chronic stimulation regime used in the present study, more long-lasting vascular changes would be expected. The mechanical effect of chronically increased blood flow is a basic

stimulus which increases the percentage of capillaries perfused and also encourages the formation of new vessels [8]. In skeletal muscle, 10 Hz stimulation leads to increased capillary density after a few days [3]. Thus, if hypoxia is a major factor in the development of nerve conduction deficits [2], the beneficial effects of stimulation could have a vascular basis. This remains to be tested experimentally.

In conclusion, the results demonstrate that electrical activation normalises NCV in diabetic rats. This model could help determine the relative contributions of biochemical mechanisms and nerve hypoxia to the aetiology of diabetic neuropathy. To elucidate whether stimulation has a role in helping prevent neuropathy in patients requires further study.

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