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Research Report

Ultrastructural and morphometric alterations in the aortic depressor nerve of rats due to long term experimental diabetes: Effects of insulin treatment

Fabrício S. Oliveira[a], *Randy A. Nessler*[b], *Jaci A. Castania*[c], *Hélio C. Salgado*[c], *Valéria P.S. Fazan*[b,d,*]

[a]Veterinary Medicine, Maringá State University—Umuarama Campus, Umuarama, Paraná, Brazil
[b]Central Microscopy Research Facility, The University of Iowa, Iowa City, IA, USA
[c]Physiology, School of Medicine of Ribeirão Preto—University of São Paulo, Brazil
[d]Neurosciences and Behavioral Neurosciences, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

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ABSTRACT

Most of the reports about an altered baroreflex attribute this condition to the diabetic efferent neuropathy of the aortic depressor nerve (ADN) (afferent arm of the baroreflex less explored). We evaluated the ADN ultrastructural alterations caused by long term experimental diabetes and the effects of insulin treatment. Wistar rats (N=14) received a single intravenous injection of streptozotocin (40 mg/kg) 12 weeks before the experiment. Control animals (N=9) received vehicle (citrate buffer). Insulin treated animals (N=8) received a single subcutaneous injection of insulin daily. Under pentobarbital anesthesia the ADNs were isolated and had their spontaneous activity recorded. Afterwards, proximal and distal segments of the nerves were prepared for transmission electron microscopy study. Morphometry of the unmyelinated fibers was carried out with the aid of computer software. ADN of the diabetic animals showed axonal atrophy for myelinated fibers, with more pronounced alterations of the myelin sheath, such as myelin infolding and out folding, presence of myelin balls and very thin myelin sheath in relation to the axonal size, particularly for the small myelinated fibers becoming evident. No differences were observed in myelinated fiber number and their density, as well as on the fascicular area. Unmyelinated fiber number was significantly larger in the diabetic group while fiber diameter was significantly smaller compared to control. This result suggests axonal atrophy or, if associated to the larger number of fibers present in this group, could indicate fiber sprouting. These alterations were more evident in the distal segments of the nerves and were moderated by insulin treatment.

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*Correspondence to: Department of Surgery and Anatomy, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Av. Bandeirantes 3900, 14049-900 SP, Brazil. Fax: +55 16 3633 0017.
E-mail address: vpsfazan@yahoo.com.br (V.P.S. Fazan).

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1. Introduction

Since 1960s, considerable research has been done on diabetic neuropathy, but a reliable description of the nerve alterations has not yet been presented. It has been clearly established, however, that an axonal loss occurs in the peripheral nerves and their trunks (Chopra et al., 1969; Greenbaum et al., 1964; Thomas, 1967), affecting both myelinated and unmyelinated axons (Behse et al., 1977), while being more evident in the distal segments of the nerve (Knopp and Rajabally, 2012).

Dysfunction of the baroreflex control of arterial pressure and heart rate have been thoroughly described, not only in diabetic patients (Low et al., 1975; Wang et al., 2012), but also in experimental models of diabetes (Fazan et al., 1997a, 1999a; McDowell et al., 1994a,b). Most reports on decreased baroreflex sensitivity in diabetic patients attribute this outcome to the autonomic neuropathy involving the efferent arm (vagus nerve and sympathetic nerves). The afferent arm of the baroreflex, i.e. the arterial baroreceptors (carotid sinus and aortic depressor nerves) has received less attention. The first detailed description of the morphological aspects and morphometric characteristics of the aortic depressor nerve (ADN) in rats was published in 1997 (Fazan et al., 1997b) but until now, the morphological ultrastructural involvement of the ADN in diabetes was not yet investigated.

It is well documented that the metabolic and morphological alterations due to diabetes can be prevented or even reverted by means of the insulin treatment (Jakobsen, 1979; Rodrigues Filho and Fazan, 2006) but this notion is still controversial. Recently, Fazan et al. (Fazan et al., 2009) demonstrated that endoneural blood vessels are susceptible to insulin treatment, with important ultrastructural alterations of the unmyelinated fibers. Also, a remarkable number of fibers undergoing Wallerian degeneration was described in nerves from diabetic animals treated with insulin (Sharma et al., 1985, Westfall et al., 1983).

The goals of this study were to assess the effect of long term experimental diabetes on the morphological aspects and morphometric parameters of the ADN in rats, at the ultrastructural level, as well as to investigate whether insulin treatment had a protective effect on ADN alterations caused by experimentally induced diabetes.

2. Results

Body weight and blood glucose levels obtained on the 3rd day after injections and on the experimental day, as well as the mean arterial pressure (MAP) and heart rate (HR) recorded on the experimental day are shown in Table 1. No differences were detected on body weight among groups at the beginning of the experiments and all three groups gained weight significantly during the 12-week period. Nevertheless, diabetic rats gained significantly less weight as compared to control and insulin treated animals. As expected, blood glucose level was significantly higher in both diabetic groups three days after STZ injection but it was corrected with insulin treatment. MAP was significantly reduced in both diabetic groups while the HR was reduced in the diabetic group as compared with the insulin treated group.

2.1. Morphology

The ADN of control animals showed normal morphological characteristics as previously described (Fazan et al., 1997b, 2001) in normotensive animals (Fig. 1).

The ADN of diabetic animals displayed axonal atrophy for the myelinated fibers, but the alterations were more evident on the myelin sheath, including infolding and out folding of the myelin sheath, presence of balls of myelin, myelin loops and splitting, and very thin myelin sheaths in relation to the axon size, particularly for small myelinated fibers. Several nerves presented myelinated axons much larger than would be expected, with some having almost no myelin sheath (Fig. 1). The unmyelinated fibers were more conserved in this experimental group in terms of morphology but clusters of very small unmyelinated fibers were present in all nerves of this group (Fig. 1). Some unmyelinated fibers also showed clear signs of axonal atrophy. Interestingly, the endoneural vessels, when present, had their morphology preserved (Fig. 1).

The ADN of the insulin treated group showed the same alterations described for the diabetic group, to a less extent. It was very common the presence of small myelinated fibers with loose or very thin myelin sheaths, suggesting remyelination (Fig. 1). Clusters of small unmyelinated fibers were

Table 1 – Body weight and blood glucose level on the 3rd day after streptozotocin (STZ) or vehicle injection, and on the experimental day (12 weeks after injection), together with average mean arterial pressure (MAP) and heart rate (HR) on the experimental day. Data are expressed as mean ± standard error of mean (SEM).

	Body weight	Blood glucose	MAP	HR
Control—3 days	199 ± 10	85 ± 6		
STZ—3 days	199 ± 9	519 ± 35[a]		
STZ+Ins—3 days	195 ± 8	525 ± 37[a]		
Control—12 weeks	535 ± 50[c]	73 ± 10[c]	140 ± 18	338 ± 21
STZ—12 weeks	233 ± 55[ac]	469 ± 90[a]	111 ± 10[a]	299 ± 22[a]
STZ+Ins—12 weeks	616 ± 46[abc]	132 ± 13[c]	115 ± 7[a]	327 ± 12[b]

STZ = diabetic group and STZ+Ins = Diabetic group treated with insulin.
[a] Significant difference compared to control group at the same experimental time.
[b] Significant difference compared to STZ group at the same experimental time.
[c] Significant difference compared to the same group at 3 days.

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