

Sensory nerve conduction in the caudal nerves of rats with diabetes¹

Condução nervosa sensorial no nervo caudal de ratos com diabetes experimental

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ABSTRACT

Purpose: To investigate sensory nerve conduction of the caudal nerve in normal and diabetic rats. **Methods:** Diabetes was induced in twenty 8-weeks old Wistar male rats. Twenty normal rats served as controls. Caudal nerve conduction studies were made before diabetes induction and the end of each week for six consecutive weeks. The caudal nerve was stimulated distally and nerve potentials were recorded proximally on the animal's tail using common "alligator" clips as surface electrodes. **Results:** After induction, nerve conduction velocities (NCV) increased slower in the diabetic than in the control group. Sensory nerve action potentials (SNAP) conduction velocities increased slower in the diabetic than in the control group (slope of regression line: 0.5 vs 1.3m/s per week; NCV in the 15th week = 39±3m/s vs 44±4m/s). Tukey's tests showed differences between groups at the 11th, 13th and 15th weeks old. From the 10th week on, SNAP amplitudes increased faster in the diabetic than in the control group (slopes of the regression line: 10 vs 8µV per week; SNAP amplitudes in the 15th week: 107±23µV vs 85±13µV). Differences at the 12th, 13th and 15th weeks were significant. **Conclusion:** In diabetic rats nerve conduction velocities were slower whereas amplitudes were larger than in normal rats.

Key words: Diabetes Mellitus, Experimental. Neural Conduction. Tail. Rats.

RESUMO

Objetivo: Investigar a condução nervosa sensorial do nervo caudal em ratos normais e diabéticos. **Métodos:** O diabetes foi induzido em vinte ratos Wistar com idade de oito semanas. Vinte ratos serviram como controle. Os estudos da condução do nervo caudal foram feitos antes da indução do diabetes e ao final de cada semana, durante seis semanas consecutivas. O nervo caudal foi estimulado distalmente e potenciais foram registrados proximalmente na cauda do animal, usando cliques comuns "jacaré" como eletrodos de superfície. **Resultados:** Após a indução, a velocidade de condução nervosa (VCN) aumentou mais lentamente no grupo diabético que no controle. Potenciais de ação dos nervos sensitivos (PANS) aumentaram a velocidade de condução mais lentamente no grupo diabético que no controle (linha de regressão: 0,5 vs 1,3m/s por semana; VCN na 15^a semana = 39±3m/s vs 44±4m/s). O teste de Tukey mostrou diferenças entre os grupos nas 11^a, 13^a e 15^a semanas de vida. A partir da semana 10, os PANS aumentaram mais rapidamente no grupo diabético que no controle (linha de regressão: 10 vs 8µV por semana; PANS na 15^a semana: 107±23µV vs 85±13µV). Diferenças na semana 12, 13 e 15 foram significativas. **Conclusão:** Nos ratos diabéticos a velocidade de condução nervosa foi mais lenta enquanto que as amplitudes foram maiores que em ratos normais.

Descritores: Diabetes Mellitus Experimental. Condução Nervosa. Cauda. Ratos.

Introduction

Peripheral polyneuropathy is a common complication of diabetes *mellitus*¹. It consists of demyelization and axonal degeneration of peripheral nerves¹, leading to slowing of nerve conduction velocity and reduction of the amplitude of the compound muscle and sensory nerve action potentials¹⁻⁸. These features of diabetic polyneuropathy are observed in humans diabetes^{1,9} and experimentally induced diabetes in animals²⁻⁸.

Along with the sciatic, fibular, tibial, sural, saphenous, and facial nerves^{3,6,10,11}, the caudal nerve has often been used for electrophysiological studies in rats^{2,4,5,12}, because the long tail allows accurate measurement of distances and the small diameter of the tail facilitates the placement of the stimulating and recording electrodes close to the nerve^{12,13}. Most animal nerve conduction studies use invasive techniques, with needle electrodes^{2-6,12,14,15} or surgical access to the nerve^{10,11}. A smaller number of studies use non-invasive techniques, with surface electrodes¹⁶⁻¹⁸.

The objective of this work was to investigate sensory nerve conduction of the caudal nerve in normal and diabetic rats.

Methods

Forty Wistar rats were used (*Rattus norvegicus*), male albino rats with 8-weeks old, from the Nutrition Department Animal Lab Center of the Federal University of Pernambuco. Animals were kept in collective plastic cages (maximum of 5 animals/cage), under a temperature of 23±1°C, an inverted 12h day/night cycle, with free access to water and food (Labina). Animals were randomly divided in two groups of 20 animals: The Control Group, diabetes free and the Diabetes Group, with non-treated experimentally induced diabetes. Diabetes was induced by a single intraperitoneal injection of 60mg/kg streptozotocin (Sigma Chemical Co., USA) diluted in buffer solution (sodium citrate, 10mM, pH 4.5) after 12 hours of fasting²³. Animals in the control group received the same buffer solution without streptozotocin. After 30 minutes both groups were fed normally. Blood glucose was measured 3 and 7 days after induction, with blood samples collected from the tip of the tail after 12 hours of fasting. Only animals with blood glucose above 200mg/dL (Glucosymmetry AccuCheck – Roche) were admitted in the Diabetes Group. Both groups received a weekly blood glucose check up.

Methods applied to handle and care for animals are in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animal standards and were approved by the Ethics Committee for Animal Experimentation of the Federal University of Pernambuco.

Electrophysiology

Caudal nerve conduction studies were made before diabetes induction and the end of each week for six consecutive weeks. After anesthesia with an intramuscular injection of a mixture of Xylazin Chlorhydrate (Rompum) and Ketamin (Ketalar), 0.2ml/100g¹⁹, animals were positioned ventrally for their tail to be completely loose and the tail was clean and fat free with alcohol at 70%.

An orthodromic technique was used¹⁹: The caudal nerve was stimulated distally (duration: 0.1ms; intensity: 10-20µA, supramaximal) and the sensory nerve action potentials (SNAP's) were recorded proximally (high-pass filter: 10Hz; low-pass filter: 10kHz) on the animal's tail. No movement of the tail was noted during stimulation. A Racia-Alvar Centor electromyograph was used. The SNAP parameters studied were peak-to-peak amplitude (µV) and nerve conduction velocity (m/s), derived from the onset latency (ms). Measurements were taken 4 times and the accepted result was their arithmetic average. Stimulation, recording and ground electrodes were identical standard "alligator" clips¹⁹. The distance between the stimulating cathode and the non-inverting recording electrode was 8cm, whereas the distance between the pair of stimulating and recording electrodes was 3cm. The ground was connected to the pair of alligator clips located between the stimulation and recording electrodes. The springs of the alligator clips were removed to prevent excessive compression of the tail.

Due to the almost linear relationship between the temperature of the tail and the nerve conduction velocity²⁰, before each recording the temperature of the tail was maintained at 31-32°C and confirmed before each recording with an infrared thermometer (Doc Thermo; Comdek Industrial Corp.). A dichroic lamp was used for heating.

Statistical analysis

Data were summarized by mean and standard deviation. The statistical significance of the differences was tested with mixed-model analysis of variance (ANOVA). Post-hoc comparisons were made by Tukey test. The critical *p* for both tests was 0.05.

Results

After two weeks of the induction period the animals in the experimental group showed increased levels of blood glucose, whereas those in the control group had normal glucose levels (Table 1).

TABLE 1 – Mean (standard deviation) of blood glucose levels (mg/100ml) by age (weeks) of diabetic and control rats

Age (Weeks)	8	10	11	12	13	14	15
Diabetics	83±10	251±111	249±129	251±121	349±151	314±109	324±101
Controls	79±9	78±21	76±14	80±8	81±16	79±12	68±10

SNAP conduction velocities of diabetic and control animals are shown in Figure 1. There was no significant difference between them at eight weeks of life (pre-induction): 36±2m/s *versus* 34±3m/s, respectively. After induction, SNAP conduction velocities increased slower in the diabetic than in the control group (slope of regression line: 0.5 *versus* 1.3 m/s per week; NCV in the 15th week = 39±3m/s *versus* 44±4m/s). *Post hoc* tests (Tukey) showed significant differences between groups at the 11th, 13th and 15th weeks.

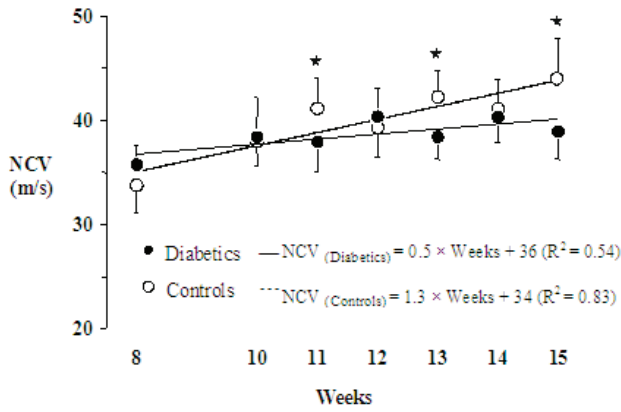


FIGURE 1 – Mean (standard deviation) of conduction velocities (m/s) by age (weeks) of diabetic (filled circles) and control (open circles) rats. Linear regression equations and square of Pearson correlation coefficient (R^2).

> Tukey, $p < 0.05$; diabetics rats *versus* controls rats.

Figure 2 shows SNAP amplitudes of diabetic and control animals. At the 8th week (pre-induction; baseline) the SNAP amplitudes were not different between the two groups ($33 \pm 7 \mu\text{V}$ *versus* $29 \pm 6 \mu\text{V}$). From the 10th week, SNAP amplitudes increased faster in the diabetic than in the control group (slopes of the regression line: 10 *versus* 8 μV per week; SNAP amplitudes in the 15th week: $107 \pm 23 \mu\text{V}$ *versus* $85 \pm 13 \mu\text{V}$). Differences at the 12th, 13th and 15th weeks were statistically significant (Tukey).

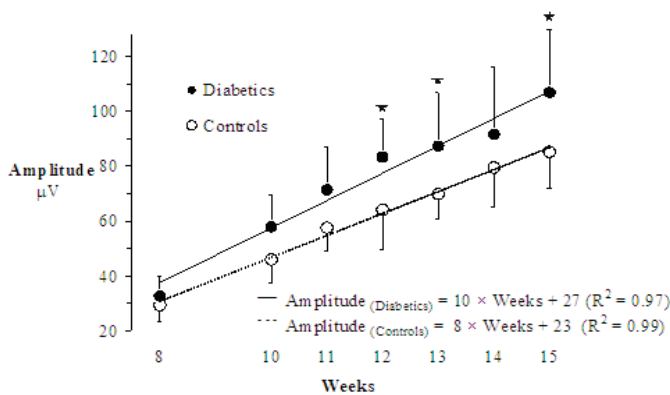


FIGURE 2 – Mean (standard deviation) of amplitudes (μV) by age (weeks) of diabetic (filled circles) and control (open circles) rats. Linear regression equations and square of Pearson correlation coefficient (R^2).

> Tukey, $p < 0.05$; diabetics rats *versus* controls rats.

Rats with diabetes lost weight during the experiment, whereas those in the control group gained weight (Figure 3). In the 15th week, the average weight of the diabetic rats was about 40% lower than the weights of the control rats.

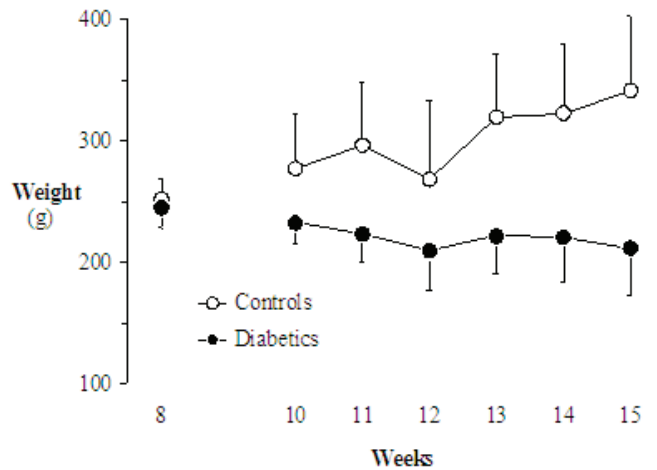


FIGURE 3 – Mean (standard deviation) of the weight (g) by age (weeks) of diabetic (filled circles) and control (open circles) rats.

Discussion

The rat caudal nerve has good characteristics for studying nerve conduction using non-invasive techniques. Its superficial trajectory allows easy stimulation and recording of potentials with surface electrodes²¹ and its long trajectory (10-15cm) allows accurate measurement of distances¹⁸.

In our data SNAP conduction velocities increased slower with age in the diabetic rats than in the normal rats. This was an expected result. Several authors obtained similar results studying the caudal nerve^{2,4,5,20} or other nerves^{3,6,7}. The slower conduction velocity is probably due to an abnormal development of the myelin sheath in the peripheral nerves of the diabetic rats.

Caudal nerve SNAP amplitudes increased faster in the diabetic rats than in the normal rats. This was an unexpected result. We expected a decrease in the rate of growth of the SNAP amplitude in the diabetic rats, due to the axonal loss and temporal dispersion of SNAP related to the diabetic polyneuropathy. Zochodne and Nguyen²² reported larger caudal nerve SNAP amplitudes in diabetic rats compared to non-diabetic rats. However, they did not discuss this result nor state if this difference was statistically significant.

The higher amplitude of the SNAP in the diabetic rats could be caused by a lower temperature of the animal's tail. Low temperature is known to cause SNAP amplitude to increase and nerve conduction velocity to fall²⁰. However, we monitored the temperature of the tail and kept it fairly constant.

Most probably the reason for this finding was the smaller development of the tail diameter in the diabetic rats, related to those in the control group. In a volume conductor, the amplitude of the SNAP (a quadrupole source) decreases in the inverse ratio of the cube of the distance between the nerve and the registry point²³. Therefore, the smaller diameter of the tail, and consequently the shorter distance between the caudal nerve and the recording electrode in the diabetic rats, would result in larger SNAP amplitudes.

As we did not measure the diameter of the animal tails,

these variations can be indirectly estimated by variations in the animal's weight. For any linear dimension of a body of any shape is proportional to the cubic root of its volume²⁴. Because the weight of a body is proportional to its volume, the diameter of the rat's tail is proportional to the cubic root of the rat's weight. Our rats with diabetes lost weight during the experiment, whereas those in the control group gained weight. Thus, it is reasonable to suppose that the diameter of the tails, and consequently the distances between the caudal nerve and the recording electrodes, were significantly smaller in the diabetic rats than in the control rats. Authors that use needle type electrodes for recording^{2-6,12,14,15} did not observe this phenomenon and the amplitudes behaved as expected, probably because the recording electrode was always placed close to the nerve being studied, independently of the weight of the animal.

Conclusion

The nerve conduction in diabetic rats was slower whereas amplitudes of action potentials were larger then compared with normal rats.

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