



CHRONIC EXPERIMENTAL DIABETES MELLITUS; QUANTITATIVE CHANGES IN DORSAL ROOT GANGLION CELLS

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ABSTRACT... Background: Multiple factors operate in the development of diabetic neuropathy. Sensory neurons are not protected by blood-brain or blood-nerve barrier; also the dorsal root ganglion cells (DRG) have a higher metabolic requirement than the nerve trunks. Oxygen level at the dorsal root ganglions also appears to be lower. All these physiological characteristics suggest that DRG may be particularly susceptible to damage in prolonged diabetic conditions. **Objectives:** To observe the quantitative cellular changes in dorsal root ganglion cells in rats with prolonged experimental diabetes. **Study Design:** An experimental study. **Setting:** Department of Human Anatomy, Faculty of Medicine, Umm al Qura University, Makkah, Saudi Arabia. **Period:** Fifteen months to complete. **Material and methods:** Observations were made on six control and six streptozotocin-treated male Sprague-Dawley rats after 12 months of diabetes. Cell count was done on silver-stained paraffin sections. DRG cells were arbitrarily grouped as large A-type and small B-type. Statistical examination of the cell count was done using a two-tailed t-test. Values were considered significant at $P \leq 0.05$. **Results:** In the control group of animals the mean total number was 15856.33 ± 552.538 while in the diabetic animals it was 11836.666 ± 583.177 ; the reduction in the number of cells was significant. The number of A-type and B-type cells and their percentages in the control group and the diabetic group of animals were 2753.833 ± 257.683 (17.36%), 13102.5 ± 443.092 (82.63%) and 1202.833 ± 87.082 (10.16%), 10633.833 ± 517.900 (89.83%) respectively. The differences in the number of A-type and B-type of cells when compared between control and diabetic groups of animals were statistically highly significant. **Conclusion:** Selective cells damage to DRG cells may be the harbinger of diabetic neuropathy in experimentally induced diabetic rats.

Key words: Dorsal root ganglion cells, experimental diabetes mellitus, quantitative cellular change, diabetic neuropathy

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INTRODUCTION

The precise mechanism of diabetic neuropathy is not known; several pathophysiological mechanisms are present which ultimately result in increased oxidative stress, with deficits in nerve blood flow and sensory conduction¹. Oxidative stress seems to be more intense at the dorsal root ganglion (DRG) as compared to at the nerve². Recent findings that experimental diabetes beyond 6 months may develop florid radicular pathology in rats³ coupled with observations of vacuolar type of degenerative changes of DRG neurons⁴ have led to the hypothesis that the prime target in diabetic neuropathies is the sensory neuron.⁵

Sensory neurons are not protected by blood-brain or blood-nerve barrier.⁶ Also the DRG have a higher metabolic requirement than the nerve trunks.^{7,8} Oxygen level at the dorsal root ganglions also appears to be lower⁹. All these physiological characteristics suggest that DRG may be particularly susceptible to diabetic microangiopathy.

It could be postulated, therefore, that direct targeting of the dorsal root ganglia could account for the early sensory neuropathy clinically observed in patients with diabetes mellitus.

The aim of the current study was to observe quantitative cellular changes in DRG cells in rats

with prolonged experimental diabetes.

MATERIALS AND METHODS

Animals

Twelve male Sprague-Dawley rats, weighing 250 ± 10 g were used. The animals were split in two groups of six animals each as control and experimental. Experimental diabetes was produced by an intraperitoneal injection of streptozotocin (STZ) 0.05 mol/l with citrate buffer and with a pH 4.5. Citrate buffer alone was administered intra-peritoneally to the control group. Ad libitum access to rodent laboratory chow and water was available to both the control and the diabetic animals. The rats were considered as diabetic if their fasting blood glucose level exceeded 16.7 mmol/l three days after injection of STZ and remained at this level till the time of surgery. The duration of experimental diabetes was twelve months for the diabetic rats.

Histological preparation

Experimental and control animals were given pentobarbital sodium 50 mg/kg by intraperitoneal injection for anesthesia. Laminectomy was then done and the lumbar-5 dorsal root ganglia were exposed and fixed by formaldehyde in place for 20 min. Lumbar-5 DRG were then harvested, post fixed in 10% formaldehyde overnight, and processed for paraffin embedding. Four to six micron thick transverse sections were stained with silver nitrate.

Cell count

Serial sections were initially observed and compared at 10th, 20th, 30th, 40th and 50th section for the cell counts and diameter. In all these samples there was no significant difference in cell counts or their diameters. Therefore subsequent analysis was done using every 30th section which was found to be at the largest diameter of DRG.

Cells were counted at 20x magnification for all selected specimens. For the cell size, measurement of the largest diameter was made only on those cells which showed the nucleolus or part of the nucleolus. The cell count was

corrected by multiplying with a correction factor to make up for the split nucleoli.^{9,10}

Early observations revealed differences in the size of the DRG cells which were arbitrarily divided in two groups: those larger than 1500 micron² were named 'A-type' and those smaller than 1500 micron² were termed as 'B-type'.

Statistics

Total cell counts, large (A-type) and small (B-type) populations, were expressed as means \pm SE. Two-tailed t-test was used for comparisons. Significance was accepted at $P \leq 0.05$.

RESULTS

The weight of animals was reduced significantly (287 ± 23 g) as compared with the controls (532 ± 33 g) at the time of surgery after 12 months ($P \leq 0.01$). Fasting glucose level for the diabetic rats was 16.4 ± 4.2 mmol/l at the time of surgery.

The number and percentages of A-type (large) and B-type (small) DRG cells in the control and experimental groups are shown in Table I.

In the control group of animals the mean total number was 15856.33 ± 552.538 while in the diabetic animals it was 11836.666 ± 583.177 ; the decrease in the total number of cells was found to be significant (Table II). Similarly the number of A-type and B-type cells and their percentages in the control and diabetic animals were 2753.833 ± 257.683 (17.36%), 13102.5 ± 443.092 (82.63%) and 1202.833 ± 87.082 (10.16%), 10633.833 ± 517.900 (89.83%) respectively. The differences in the number of A-type and B-type of cells when compared between control and diabetic groups of animals were statistically highly significant (Table II).

DISCUSSION

Weight loss observed in the experimental group of animals was expected and is in agreement with previous observations on STZ-induced diabetic rats.^{14,17} It appears that our method of sampling, profile counting and application of correction factors was satisfactory for cell counting. Our

| Animals | Number of cells | | | Percentage | |
|----------------------|-------------------------|------------------------|-------------------------|------------|--------|
| | Total | A-type | B-type | A-type | B-type |
| Control: | | | | | |
| C-1 | 16542 | 2991 | 13551 | 18.08% | 81.91% |
| C-2 | 14356 | 1681 | 12675 | 11.70% | 88.29% |
| C-3 | 17765 | 2778 | 14987 | 15.63% | 84.36% |
| C-4 | 15490 | 2837 | 12653 | 18.31% | 81.68% |
| C-5 | 14398 | 2612 | 11786 | 18.14% | 81.85% |
| C-6 | 16587 | 3624 | 12963 | 21.84% | 78.15% |
| Mean \pm SE | 15856.333 \pm 552.538 | 2753.833 \pm 257.683 | 13102.5 \pm 443.092 | 17.36% | 82.63% |
| Experimental: | | | | | |
| D-1 | 12654 | 1178 | 11476 | 9.30% | 90.69% |
| D-2 | 10654 | 1122 | 9532 | 10.53% | 89.46% |
| D-3 | 9896 | 920 | 8976 | 9.29% | 90.70% |
| D-4 | 11475 | 1111 | 10364 | 9.68% | 90.31% |
| D-5 | 13754 | 1352 | 12402 | 9.82% | 90.17% |
| D-6 | 12587 | 1534 | 11053 | 12.18% | 87.81% |
| Mean \pm SE | 11836.666 \pm 583.177 | 1202.833 \pm 87.082 | 10633.833 \pm 517.900 | 10.16% | 89.83% |

Table-I. Showing the number and percentages of A-type (large) and B-type (small) DRG cells in the control and experimental groups

| Total cells | | A-type cells | | B-type cells | |
|-------------|-----------|--------------|-----------|--------------|-----------|
| t-value | p-value | t-value | p-value | t-value | p-value |
| 5.003539 | 0.000267* | 5.702196 | 9.900005* | 3.621975 | 0.002337* |

Table-II. Showing the statistical comparison of cell count between the control and experimental groups' t- and p-values and their significance after t-test

*significant at $p < 0.05$.

cell count in the control group of animals was in general agreement with previous studies using similar methods.^{11,12} Whereas our mean values for the total DRG cell count were 15856.33 ± 552.538 literature reports a range of thirteen thousand to eighteen thousand.¹¹⁻¹³ These reports by other investigators validate and support our methodology and observations.

The cut-off line between the large and small cells was 1500 micron^2 (with a maximal diameter of 20 microns); so that the DRG cells were arbitrarily classified into two groups: those larger than 1500 micron^2 were named 'A' and those smaller than 1500 micron^2 were termed as 'B'. The small B-type cells had a diameter much smaller than 20 microns.

We report a decrease in the number of A-type cells

and a relative increase in percentage of B-type cells in the diabetic animals which is in agreement with previous workers.^{4,10} Our observations are different from those reported by Sidenius and Jakobsen¹⁴ in short-term streptozotocin-induced diabetes in rats. Eighteen per cent reduction in cell volume affecting neurons of all sizes is reported by these authors. Our observations strongly indicate a loss that was confined to the large cell population rather than an even reduction as seen in case of atrophy. It seems plausible that the damage in our study appears to be selective rather than general as proposed by Sidenius and Jakobsen.¹⁴ Also the duration in our study was twelve months as compared to four months by these authors. The effects of diabetic conditions therefore are not comparable. The increase in percentage of the small B-type cells in the diabetic group is only relative to the

decrease of the large cells.

Correlations between electrophysiological characteristics and properties of the neurons and their axonal processes with the size of the DRG cells have been made in the rat and cat.^{15,16} The small (type B) neurons are mostly connected to unmyelinated nerve fibers, only a few being connected to the small myelinated fibers.¹⁶ The large (type A) neurons are connected to thick myelinated fibers and belong to the A-alpha and A-beta type of fibers. There is a linear relationship between the size of the neuron and the velocity of conduction by the axon.^{15,16} It is reported that changes following loss of neurons are associated with changes in both the dorsal and the ventral nerve roots indicated by demyelination of nerve fibers.^{3,17}

That these changes are functionally important has been shown by electrophysiological observations.^{18,19} In our study we have observed a loss of A-type cells in the DRG whereas the B-type cells remained unaffected indicating therefore that loss of cells appears to be selective rather than general.

Motor and autonomic fibers are also affected in experimental diabetes²⁰ and therefore DRG neurons should be considered as only one target. It needs to be considered if the sensory, motor and autonomic nerve fibers are also involved along with their parent cells in longstanding diabetes.

CONCLUSION

Decrease in A-type cells in our study indicates a selective damage to the dorsal root ganglion cells in prolonged experimentally induced diabetes mellitus in rats and may be the harbinger of diabetic neuropathy. However further studies to elucidate specific nerve fiber involvement and changes therein are warranted.

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“There is only one way to avoid criticism. do nothing say nothing and be nothing.”

Aristotle



AUTHORSHIP AND CONTRIBUTION DECLARATION

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