Peripheral Neuropathy in Rats Induced by Insulin Treatment

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SUMMARY
The effect of sustained insulin-induced hypoglycemia on peripheral nerve function and structure was examined in rats. After a period of hypoglycemia (<2.5 mmol/L) of at least 72 h, axonal degeneration and reduction of the maximal amplitude of the evoked muscle action potential occurred, the two abnormalities being correlated negatively (r = -0.99, P = 0.00097). One of five rats developed paresis of both hindlegs as well as nerve damage and perikaryal alterations of lower motor neurons. DIABETES 32:383-386, April 1983.

Since peripheral nerve metabolism is dependent on glucose utilization, a potential cause of peripheral neuropathy is sustained periods of hypoglycemia. Nonetheless, very little is known about hypoglycemic nerve damage. A few case reports describe the occurrence of paresis in patients with insulinomas, but only scanty information on histologic and neurophysiologic abnormalities in such patients is available. Jaspan et al. described axonal degeneration in a sural biopsy from one patient, signs of denervation at electromyography in all of the patients, and reduced motor nerve conduction velocity in three of four patients. However, there are no reports in the literature on hypoglycemic damage of peripheral nerves induced by insulin treatment.

Whether sustained periods of hypoglycemia exert a deleterious effect on peripheral nerves has been an increasingly important question after the insulin pump has been introduced in the treatment of diabetes. We are currently studying this question in an animal model and have observed that a few days of profound hypoglycemia produce classic axonal degeneration (unpublished observation). We now for the first time report on the occurrence of muscle weakness and structural damage of peripheral nerve fibers and their cell bodies induced by insulin treatment.

MATERIALS AND METHODS
Five 60–75-wk-old male Wistar rats from our own inbred colony were treated once daily with a modified and very long-acting insulin (Ultralente, pH 5.5, NOVO, Denmark) during 6–7 days and had free access to food and water. Six to seven days after the last dose of insulin was given, muscle action potentials of the anterior tibial muscle on the right side were recorded and subsequently the left sciatic nerve was removed for quantification of nerve fiber degeneration. One animal developed a severe paresis of both hind limbs, and tissues from the lumbar enlargement of the spinal cord as well as from the tibial nerve were taken out after whole body perfusion through the heart with a 2% glutaraldehyde fixative (0.1 M cacodylate, pH 7.35).

Insulin treatment. To study the effect of one daily injection of the applied insulin preparation on the variation of blood glucose during a 24-h period in hypoglycemic animals, blood glucose was measured every 2–3 h in an additional group of seven rats. At the start of the experiment the mean blood glucose value was 2.3 mmol/L (range: 1.7–2.8 mmol/L); at the end it was 2.2 mmol/L (range: 2.0–2.5 mmol/L). Since blood glucose levels remained constantly depressed during the 24-h period, measurements in the experimental group were performed only once daily. Blood samples were taken from the tail veins at 4 p.m. and glucose levels were determined with a reflectance meter (Glucometer, Ames, Elkhart, Indiana). Insulin (200–300 μl, 1 IU/25 μl) was dosed according to blood glucose determinations measured immediately before the subcutaneous injection. In all animals a 72-h experimental period of severe hypoglycemia (<2.5 mmol/L) was intended.

Muscle action potentials. To quantify the extent of motor weakness, evoked maximal compound muscle action potentials were registered. The rats were anesthetized with pentobarbitate, and a bare needle electrode (cathode) was inserted into the vicinity of the sciatic nerve near the greater trochanter of the femur. A second needle (anode) was placed...
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For a 72-h period were obtained, whereas the lowest values for the other animals varied around 2 mmol/L.

In rat no. 1 (solid line) glucose values lower than 1.4 mmol/L were developed. In rat no. 5 (dashed line) glucose values above 1.4 mmol/L were obtained. Figure 1 illustrates the blood glucose values for the first 10 days of the experiment. It appears that blood glucose levels in rat no. 1 varied between 1.0 and 1.4 mmol/L for a 72-h hypoglycemic period, whereas nadirs close to 2.0 mmol/L were obtained in the other four animals.

The rat with the most profound hypoglycemia (no. 1) developed paresis of both hindlegs during the experimental period, starting at the nadir of hypoglycemia. The muscle weakness was most pronounced distally with absence of dorsiflexion of the feet during active movements. Knee flexors and extensors were also weak, whereas the movements of thighs and forelimbs maintained their normal strength. Furthermore, two rats (nos. 2 and 3) developed a characteristic grunting 7 days after the first dose of insulin, but displayed no conspicuous signs of peripheral neuropathy.

The maximal muscle action potential of the anterior tibial muscle is shown for each rat in Table 1. Two rats (nos. 1 and 2) had reduced muscle action potentials (>2 SD from the control value), whereas the logarithm of the amplitude of the muscle action potential (Figure 3).

The table gives the geometric mean blood glucose values for the hypoglycemic period for each rat. Figure 1 illustrates the blood glucose values for the first 10 days of the experiment. It appears that blood glucose levels in rat no. 1 varied between 1.0 and 1.4 mmol/L for a 72-h hypoglycemic period, whereas nadirs close to 2.0 mmol/L were obtained in the other four animals.

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**Figure 1.** Daily blood glucose values in five rats given insulin for 6-7 days. In rat no. 1 (solid line) glucose values lower than 1.4 mmol/L for a 72-h period were obtained, whereas the lowest values for the other animals varied around 2 mmol/L.

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Reference value (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>wk</td>
<td>75</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Weight at start</td>
<td>g</td>
<td>495</td>
<td>515</td>
<td>505</td>
<td>505</td>
<td>545</td>
<td>470 ± 31</td>
</tr>
<tr>
<td>Weight at end</td>
<td>g</td>
<td>410</td>
<td>490</td>
<td>435</td>
<td>485</td>
<td>540</td>
<td></td>
</tr>
<tr>
<td>Total insulin dosage</td>
<td>IU</td>
<td>47</td>
<td>49</td>
<td>47</td>
<td>89</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Blood glucose at start</td>
<td>mmol/L</td>
<td>4.9</td>
<td>5.3</td>
<td>4.7</td>
<td>4.9</td>
<td>4.0</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Blood glucose at end</td>
<td>mmol/L</td>
<td>6.4</td>
<td>4.1</td>
<td>4.2</td>
<td>5.1</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Episodes of hypoglycemia (&lt;2.5 mmol/L)</td>
<td>mmol/L</td>
<td>1.4 (4)</td>
<td>2.2 (2)</td>
<td>2.0 (4)</td>
<td>2.2 (5)</td>
<td>2.2 (2)</td>
<td></td>
</tr>
<tr>
<td>1st episode, geometric mean (duration in days)</td>
<td>mmol/L</td>
<td>1.4 (4)</td>
<td>2.2 (2)</td>
<td>2.0 (4)</td>
<td>2.2 (5)</td>
<td>2.2 (2)</td>
<td></td>
</tr>
<tr>
<td>2nd episode, geometric mean (duration in days)</td>
<td>mmol/L</td>
<td>1.4 (4)</td>
<td>2.2 (2)</td>
<td>2.0 (4)</td>
<td>2.2 (5)</td>
<td>2.2 (2)</td>
<td></td>
</tr>
<tr>
<td>Evoked muscle action potential</td>
<td>mV</td>
<td>7</td>
<td>33</td>
<td>51</td>
<td>71</td>
<td>63</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>Axonal degeneration</td>
<td>%</td>
<td>73.3</td>
<td>16.8</td>
<td>11.5</td>
<td>2.9</td>
<td>1.7</td>
<td>0.9 ± 2.1</td>
</tr>
<tr>
<td>Demyelination</td>
<td>%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.7</td>
<td>0.4 ± 0.7</td>
</tr>
</tbody>
</table>

**RESULTS**

During a 24-h period of hypoglycemia blood glucose values remained remarkably constant, the values for the individual rats being 2.0 ± 0.2, 2.0 ± 0.3, 2.1 ± 0.2, 2.3 ± 0.3, 2.4 ± 0.4, 2.4 ± 0.3, and 2.5 ± 0.2 mmol/L; the ranges were 1.7-2.2, 1.7-2.5, 1.8-2.4, 1.9-2.6, 1.8-3.0, 1.9-2.8, and 2.1-2.8 mmol/L, respectively.

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**Figure 2** shows the type of abnormalities found in teased fiber preparations. It appears from Table 1 that axonal degeneration was present in all test animals, and that three rats (nos. 1, 2, and 3) had an increased number of degenerated fibers (>2 SD from the control value). There was a negative statistically significant correlation between the number of degenerated fibers and the logarithm of the amplitude of the muscle action potential (Figure 3).
FIGURE 2. The type of changes observed in teased fiber preparation of the sciatic nerve 6–7 days after a 3–5-day period of hypoglycemia (<2.5 mmol/L). A normal fiber with a Ranvier node in the middle is seen at top (1). Fiber 2 shows early signs of axonal degeneration with separation of small myelin segments, and fibers 3 and 4 are later stages in the same axonal process.

Figure 4 shows a cross-section of the tibial nerve from the disabled rat. Many abnormal profiles suggestive of axonal degeneration were present. In the same rat the motor neurons of the lumbar enlargement of the spinal cord displayed uniform alterations. Figure 5 shows the type of abnormalities observed. The chromophilic substance (rough endoplasmic reticulum) was retracted from the periphery of the perikaryon and concentrated centrally. No obvious changes of the nucleus or the nucleolus were recognized.

DISCUSSION
The present article on the effect of sustained hypoglycemia on peripheral nerve has shown that insulin treatment in rats can cause muscle weakness, axonal degeneration, and lower motor neuron changes. The fact that hindleg paresis was
observed in the rat with the most severe depression of blood glucose levels and developed during the hypoglycemic period makes it likely that this alteration is responsible for the nerve damage. Furthermore, we have never observed spontaneously occurring muscle weakness in our colony of rats inbred for more than 20 yr.

The distribution of muscle weakness in the disabled rat is typical of distal axonopathy. Recently, Sterman has observed a chromatolytic response of dorsal root ganglion cells of rats during advanced distal axonopathy induced by 2,5-hexanedione. However, that this disease entity can be associated with specific structural changes of the nerve cell bodies is a new observation of the present article.

Similar changes of nerve cell bodies have been observed by Agardh et al. in neurons of the cerebral cortices of rats exposed to profound hypoglycemia with isoelectric EEG for 1/2–1 h. The changes were considered characteristic for the condition since they are unlike any other reaction of the nerve cell to injury. The structural alteration was visible immediately after the hypoglycemic period and was fully reversible after 1 h of normoglycemia. In contrast, the present study demonstrated the somal alteration after 1-wk recovery.

Further studies in our laboratory have shown that axonal degeneration occurs to the same degree in the purely sensory sural nerve as in the mixed sensory motor tibial nerve (Jakobsen and Sidenius, to be published).

To elucidate whether insulin-induced neuropathy might contribute to the peripheral nerve damage present in long-term diabetics more studies on the relation between axonal degeneration and duration and severity of hypoglycemia are needed.

ACKNOWLEDGMENTS
We are grateful to Anette Larsen and Birthe Saugbjerg for skilful technical assistance and to Karin Wiedemann for careful preparation of the manuscript. This work was supported by NOVO.

REFERENCES