SPONTaneous AGE-Related PERIPHERAL NEUROPATHY IN B6C3Fl MICE

Hajime TABATA1, Hisashi IKEGAMI1 and Kimio KARIYA2

1Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd.,
1-1-8 Azusawa Itabashi-ku, Tokyo 174-8511, Japan
2Faculty of Pharmaceutical Sciences, KobeGakuin University,
518 Arise, Ikawadani-cho, nishi-ku, Kobe, 651-2180, Japan

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ABSTRACT — Peripheral neuropathy, which accompanies aging, occurs during the long-term rearing of laboratory animals. The present study set out to delineate the clinical and functional features of this neuropathy. A total of 200 B6C3Fl female mice, in groups of 5 to 20 mice, were sacrificed and autopsied each week beginning at 5 weeks and continuing to 130 weeks of age. Examination for histopathologic changes was conducted on the dorsal nerve roots, sciatic nerves, peroneal nerves, tibial nerves, plantar nerves and brachial nerve plexuses. At 90 weeks of age or later, peripheral neuropathy, characterized by axonal degeneration and Schwann cell proliferation, were observed mainly in the sciatic nerves, brachial nerve plexus and peroneal nerves. These spontaneous age-related nerve lesions appeared in all animals by 100 weeks of age in all nerves, and increased with increasing age. The nerve lesions were most prominent in the distal sciatic nerve. The rectal and hind-limb surface temperatures, motor nerve conduction velocity, blood glucose and HbAic decreased with increasing age. Elevation of sorbitol contents in sciatic nerves and reduction of myo-inositol levels were also detected in 120-week-old mice. However, except for blood glycemic parameters, no correlation with peripheral nerve lesions could be demonstrated. Spontaneous hypoglycemia (<40 mg/dL) persisted throughout the day in a small percentage (<5%) of animals aged 80 weeks or more; these animals had extensive lesions in the peripheral nerves and showed decreased plasma levels of HbAic and fructosamines and increased plasma levels of ketones. These results suggest that spontaneous peripheral nerve disorders which accompany aging might worsen if spontaneous age-related hypoglycemia is also present. Such age-related changes must be taken into consideration in experimental studies performed on mice of this age.

KEY WORDS: Peripheral neuropathy, B6C3Fl mice, Aging

INTRODUCTION

Neuropathy with accompanying nerve fiber loss in the spinal root and peripheral nerves is recognized as a common spontaneous complication of the aging process (Thomas et al., 1994). During this process, the normal relationship between internodal length and fiber diameter is disrupted due to segmental demyelination and remyelination as well as axonal degeneration and regeneration, leading to increased variability in successive internodes both along and between individual fibers.

The cause of these changes is uncertain. It is not known whether they are the result of neuronal aging, giving rise to distal axonal degeneration and secondary demyelination, or whether they could be the result of local factors such as ischemia or the consequences of repeated minor trauma. Although spontaneous, age-related, peripheral neuropathy has been studied extensively in rats (Thomas et al., 1980; Mitsumori et al., 1981; Krinke, 1983; Kazui and Fujisawa, 1988; Knox et al., 1989; Bronson, 1990; Majeed, 1992b), there are few reports concerning this phenomenon in mice, even though B6C3Fl mice are widely used in carcinogenic-
MATERIALS AND METHODS

Animals and animal care

Two hundred female B6C3F1 mice (F1 offspring of a cross between C57BL/6NCrj and C3H/HeNCrj mice, bred by Charles River) were used in this study. Five-to-six-week-old B6C3F1 mice weighing 20 - 26 g were purchased from Charles River Japan, Inc in 1993 - 1996. All the mice were cared for using similar housing and diet conditions. Animals were housed in a barriered rodent facility maintained at a temperature of 23±3°C and relative humidity of 40% - 70%; the room was lit 13 hr per day (0800 - 2100) with fluorescent lighting. Groups of not more than five animals occupied stainless-steel cages (25×34×17 cm). The mice were provided with pelleted food (CRF-1, Oriental Industries, Japan) and tap water ad libitum.

Observations and laboratory investigations

Animals were observed daily for clinical signs. Body weight and food consumption were measured weekly. Blood was collected every 3 months for blood chemistry analysis including glyceric parameters. Within 2 min of being taken from the cage, blood samples were obtained by a longitudinal (1 mm) incision at the tip of the tail. Bleeding from this wound was stopped easily by direct pressure. Blood glucose and HbA1c levels were measured using Antsense II and DCA2000 analyzers (both from Bayer-Sankyo Co., Ltd., Tokyo, Japan), with 6 μl of whole blood. Blood pH and hematocrit was measured using a CHIRON 348 (Chiron K.K., Tokyo, Japan) with 40 μl of whole blood. Another 100 μl sample of blood was drawn into a cooled, heparin-coated capillary tube and centrifuged at 4°C. The plasma was immediately analyzed for 1,5-anhydroglucitol, fructoseamine, triglycerides, cholesterol, phospholipids, nonesterified fatty acids, ketone bodies, 3-hydroxybutyrate and total protein levels in a HITACHI 7170 automated analyzer (Hitachi Ltd.; Tokyo, Japan) using special-purpose reagents (Wako Pure Chemical Industries, Ltd., Osaka, Japan). To assess the animals’ general health, sole and tail skin temperatures were measured monthly with a Braun-Thermo Scan PRO-1® infrared thermometer (Braun-Japan K.K., Tokyo, Japan). At 10, 63, 90 and 110 weeks, grip strength was measured with a grip strength meter for mice Model MK-380®; blood pressure and heart rate were measured with a BP monitor for mice and rats Model MK-1030® (both from Muromachi Kikai Co., Ltd., Tokyo, Japan); and the tail-flick test was performed with a MK-330B® tail-flick algnesia meter (Muromachi Kikai Co., Ltd., Tokyo, Japan).

On the day of sacrifice, rectal temperature was determined with a Termo-Finer CTM-303® electronic thermometer (Termo Corporation; Tokyo, Japan), and motor nerve conduction velocity (MNCV) was measured using the method described by Sharma and Thomas (1974). In brief, surviving animals were anesthetized with sodium pentobarbital (20 mg/kg, i.p.). To minimize the effects of anesthesia-induced low body temperature on conduction velocity, the body temperature of animals was maintained at 37-38°C with a thermoregulator and heating mat. A neural circuit consisting of the right sciatic and tibial nerves was stimulated at the sciatic notch (proximal end) and the posterior tibial nerve at the ankle (distant end), by means of monopolar needle electrodes from an MS92 electromyograph (Medelec; London, UK). Muscle action potentials were recorded from the interdigital plantar muscle of the right hindlimb using a bipolar needle electrode. The MNCV was calculated from the latency of the M-response and the distance between the two stimulation points.

At 10, 63 and 120 weeks, nerve sorbitol and myo-inositol levels were measured using high-performance liquid chromatography (HPLC) in 6 animals at each time point by the method of Miwa et al. (1988) with minor modifications. In brief, after sacrifice by ether, a part of the right sciatic nerve was isolated. The samples were homogenized in 0.1 mol/L HClO4 (0.5-1.0 mL) and centrifuged at 3000 rpm for 10 min at 4°C. The supernatant of 0.2 mL aliquot of the isolating supernatant was deionized by passage through AG501IIX-8D column (Bio-Rad: Hercules; California, U.S.A.) and the effluent was lyophilized. The residue was dis-
solved in a mixture of 80 μL of pyridine and 20 μL of phenylisocyanate, and then placed in hot water bath at 55°C for 1 hr. After cooling, 20 μL methanol was added and the mixture was again incubated at 55°C for 5 min. Finally, 100 μL acetonitrile was added and an aliquot of each sample was analyzed by HPLC.

Pathology

Ten to 20 animals were necropsied following sacrifice at 5, 10, 20, 63, 90, 95, 100, 105, 110, 115, 120, 125 and 130 weeks, and necropsy and histopathology examinations were also performed on 21 moribund animals that were sacrificed before scheduled necropsy. In total, 171 mice were examined in the course of this study. Animals were sacrificed by intracardiac perfusion under ether anesthesia, performed with 0.1 M phosphate-buffered 4% paraformaldehyde after an infusion of 0.01 M phosphate-buffered saline. The tibial nerve, peroneal nerve, proximal and distal (femoral part) sciatic nerve, and L4 to L6 lumbar dorsal roots were then resected, taking care not to stretch the tissue, from the same site in each animal and postfixed for twelve hr with 0.1 M phosphate-buffered 4% paraformaldehyde. After fixation, these nerves were cut into 5 mm lengths, embedded in paraffin or plastic (Historesin plus; Jung, Germany), and sectioned longitudinally. The sections were then stained with either hematoxylin and eosin or hematoxylin, eosin and Luxol fast blue for light microscopic examination. Some sections of the sciatic nerve were stained with anti-MAC3 (Pharmpingen, California, U.S.A.) to detect macrophages. In addition, other portions of the tibial nerve, peroneal nerve, proximal and distal sciatic nerve, and medial plantar nerve were resected and fixed for six hr in 0.1 M phosphate buffered 2.5% glutaraldehyde, post-fixed for two hr in 0.1 M phosphate buffered 1% osmium tetroxide, and dehydrated in serial alcohol solutions and propylene oxide prior to embedding in epoxy resin. Transverse sections (1 μm thick) were stained with toluidine-blue. Sciatic nerve and brachial plexus injuries were scored based on the following scale: none = normal; minimal = focal lesions in some nerve fibers; slight = diffuse lesions in some nerve fibers, present in less than 1/3 of the tissue area; moderate = diffuse lesions in some nerve fibers, present in 1/3 - 2/3 of the area; marked = diffuse lesions in some nerve fibers, present in more than 2/3 of the tissue area.

Statistical analyses

All results are expressed as mean values with standard deviation. The values were tested for significant differences by student t-test (Gad et al., 1982). A probability value of p<0.05 was considered statistically significant.

RESULTS

Clinical observations and laboratory investigations

Signs observed during the study period, such as thin build, hair loss, hunched posture and tremor, are signs typically seen in aged mice and were not related to the development of peripheral nerve injury. Body temperature gradually decreased with age, HbAc level gradually decreased beginning at 60 weeks of age, and blood glucose and MNCV also decreased in 100-week or older mice. Additionally, a significant decrease in hematocrit was also observed in 130-week-old mice (Table 1, Fig. 1). Elevation of sorbitol contents in sciatic nerves and reduction of myo-inositol levels were also detected in 120-week-old mice (Table 2).

Histopathology

Light microscopic examination of hematoxylin and eosin stained sections revealed nerve fiber degeneration. Nerve fibers were in various stages of myelin breakdown, some in the early breakdown of the myelin sheath, others in the late stage of fragmentation into myelin ovoids. Degenerating nerve fibers appeared swollen with segmental demyelination, associated with the presence of ovoid bodies. Additionally, there were areas of axonal degeneration with myelin digestion chambers. Degeneration of myelin with breakdown of myelin into ellipsoids, myelin ovoids, spheroid bodies or myelin balls was confirmed by Luxol fast blue staining. Affected nerve fibers were degenerated in axon and/or in myelin as shown below.

Sciatic and peroneal nerves, and brachial plexuses: Nerve fiber degeneration with ovoid formation and remyelination characterized by Schwann cell proliferation were observed in all animals necropsied after 100 weeks of age (Photo 1 - 3). Dilatation of the myelin sheath with axonal atrophy was observed in a small number of nerve fibers in the sciatic nerve. Macrophages with foamy and abundant cytoplasm were often observed in these lesions (Photo 6). The nerve lesions were segmental at 100 weeks of age, but diffuse lesions, mainly consisting of remyelination, increased with increasing age. The nerve lesions were most prominent in the distal sciatic nerve.

Tibial nerve: No lesions were observed until 100 weeks of age, but segmental lesions, consisting of nerve fiber degeneration with ovoid formation and remyelina-
tion, were present in a small number of nerve fibers after 105 weeks of age (Photo 4). Affected nerve fibers were only sporadical even at 130 weeks of age, so the effect of aging on the severity of nerve lesions was unclear.

Medial plantar nerve: Nerve lesions, similar to those observed in the tibial nerve, were present after 90 weeks of age, although quite infrequently (Photo 5).

Dorsal ganglia root: Dilatation of myelin sheath with axonal atrophy was observed in nerve fibers after 100 weeks of age, becoming prominent after 120 weeks (Photo 8).

Incidence of spontaneous peripheral neuropathy

The incidence and severity of peripheral neuropathy in sciatic nerves and brachial plexuses is presented in Fig. 2, ranked by age. The data show that the incidence and severity of spontaneous peripheral neuropathy increased as mice aged. At 90 weeks of age or later, histopathologic lesions characterized by ovoid formation, remyelination characterized by Schwann cell proliferation, dilated myelin sheaths and atrophied axons were detected in sciatic nerves. At 96 weeks of age or later, the same findings were observed in the brachial plexus. These spontaneous age-related nerve lesions appeared in all animals by 100 weeks of age, and increased with increasing age. At 110 weeks or later, the number of Schwann cells also proliferated.

The peripheral nerve degeneration in mice up to 63 weeks of age (n = 70) was 0% in both nerves, while in 91-95-week-old mice of age (n = 12) the percentage reached 33% in sciatic nerves and 0% in brachial nerves. In 96 to 100-week-old mice of age (n = 12) the percentage of nerves damaged reached 75% of sciatic nerves and 92% of brachial nerves, and in 100-week-old age or older the percentage of nerves damaged was almost 100% for both nerves.

| Table 1. Physiological and biochemical assessment of B6C3F1 female mice. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Age (weeks)** | **10-week old** | **63-week old** | **100-week old** | **130-week old** |
| N               | 10              | 10              | 10              | 10              |
| Body Weight (g) | 22.7            | 36.5**          | 38.5**          | 38.5**          |
| Food Consumption (g/animal/day) | 5.1            | 4.6            | 4.5            | 4.5            |
| Rectal Temperature (°C) | 38.5            | 38.4            | 38.0*          | 37.7*          |
| Sole Skin Temperature (°C) | 30.5            | 29.2            | 28.0*          | 26.5**          |
| Heart Rate (bpm) | 722             | 540*            | 550*           | 598*           |
| Blood Pressures (mmHg) | 113             | 83*             | 90*            | 96*            |
| Grip Strength (g) | 285             | 311             | 322            | 302            |
| Tail Flick Latency (sec) | 5.5             | 5.4             | 5.2            | 5.9            |
| MNCV (m/sec) | 55.5            | 51.5            | 44.0**         | 39.8**         |

**Blood Chemistry**

<table>
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<tr>
<th></th>
<th>10-week old</th>
<th>63-week old</th>
<th>100-week old</th>
<th>130-week old</th>
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<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>43.0</td>
<td>41.5</td>
<td>41.0</td>
<td>39.5*</td>
</tr>
<tr>
<td>pH</td>
<td>7.35</td>
<td>7.32</td>
<td>7.28</td>
<td>7.26</td>
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<td>Glucose (mg/dL)</td>
<td>138</td>
<td>126</td>
<td>110*</td>
<td>101**</td>
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<tr>
<td>HbA1c (%)</td>
<td>3.2</td>
<td>3.2</td>
<td>2.9*</td>
<td>2.7**</td>
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<tr>
<td>1,5-Anhydroglucitol (μg/mL)</td>
<td>10.1</td>
<td>10.7</td>
<td>11.2</td>
<td>9.6</td>
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<td>Fructosamine (μmol/L)</td>
<td>58.8</td>
<td>45.5</td>
<td>52.5</td>
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<td>Cholesterol (mg/dL)</td>
<td>125</td>
<td>144*</td>
<td>140*</td>
<td>128</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
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<td>150*</td>
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<td>107</td>
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<tr>
<td>Phospholipids (mg/dL)</td>
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<tr>
<td>NEFA (μEq/L)</td>
<td>611</td>
<td>772</td>
<td>871</td>
<td>575</td>
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<tr>
<td>T-KB (μmol/L)</td>
<td>200</td>
<td>127</td>
<td>179</td>
<td>255</td>
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<tr>
<td>3-OHBA (μmol/L)</td>
<td>175</td>
<td>104</td>
<td>151</td>
<td>195</td>
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<tr>
<td>Total Protein (g/dL)</td>
<td>5.4</td>
<td>5.3</td>
<td>5.4</td>
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Significantly different from values measured at 10 weeks. **p < 0.01, *p < 0.05.

HbA1c: Hemoglobin A1c, NEFA: Nonesterified fatty acids, T-KB: Total ketone bodies, 3-OHBA: 3-Hydroxybutyrate.
Spontaneous hypoglycemic animals

Spontaneous hypoglycemia occurred in a small percentage (<5\%) of animals from all lots of female B6C3F1 mice. A representative blood glucose profile from typical spontaneous hypoglycemic animals is presented in Fig. 3. The blood glucose level was low (<50 mg/dL) at 52 weeks of age. Additionally, these low glucose levels (<40 mg/dL) persisted throughout the day in 3.2\% percentage of animals aged 100 weeks or more. All hypoglycemic animals also had extensive lesions in the peripheral nerves and showed slightly decreased body temperatures; MNCV, blood HbA/c and fructosamines levels; and increased levels of ketones (>300 \( \mu \)mol/L). Light microscopic examination of hematoxylin and eosin stained sections and plastic embedded sections from these animals revealed considerable degeneration of nerve fibers. Examination of longitudinally arranged, teased peripheral nerve fibers (sciatic nerves) revealed segmental demyelination, swelling of nerve fibers which appeared intermittently, nerve fibers with paranodal demyelination, segmental demyelination, fragmentation of myelin, and ovoid, myelin ball or spheroid body formation (Photo 7). The swelling of nerve fibers varied in both severity and extent. In dorsal root ganglia, central loss of Nissl substances and peripheral location of nuclei in cytoplasm were observed after 120 weeks (Photo 9).

![Graphs showing Rectal Temperature, Sole Skin Temperature, Blood glucose, and MNCV](image)

**Fig. 1.** Relation of age to body temperature, blood glucose, and HbA/c levels and MNCV in 5 to 130-week-old female B6C3F1 mice. Mean±S.D. (n=6 - 200)

<table>
<thead>
<tr>
<th></th>
<th>10-weeks old</th>
<th>63-weeks old</th>
<th>120-weeks old</th>
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<tbody>
<tr>
<td>Sorbitol (mmol/mg)</td>
<td>0.111 ± 0.010</td>
<td>0.125 ± 0.010</td>
<td>0.141 ± 0.015**</td>
</tr>
<tr>
<td>myo-Inositol (mmol/mg)</td>
<td>3.46 ± 0.11</td>
<td>3.20 ± 0.18</td>
<td>3.01 ± 0.08**</td>
</tr>
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</table>

Significantly different from values measured at 10 weeks. **p < 0.01. Mean±S.D. (n=6)
DISCUSSION

The age-related histopathologic findings from this study are in close agreement with a previous report (Majeed, 1992a). The neuropathy in senescent mice is not species-specific and is less severe, even in susceptible B6C3F1 female mice, than neuropathy seen in rats or guinea pigs (Shimpo et al., 1987; Knox et al., 1989; Majeed, 1992b). Blood glucose level decreased with increasing age, and a relationship could be established between the appearance of spontaneous peripheral neuropathy and clinical glycemic parameters (Fig. 4). The rectal and hind-limb surface temperatures and MNCV also decreased with age, and the grasping power of hands and feet was lower in 100-week-old or older animals. Electrophysiologic changes in the nerves of elderly mice are consistent with those seen in CBA mice (Robertson et al., 1993), rats (Schmelzer et al., 1987).

Photos 1-5. Histopathology of hind-limbs nerves in an elderly B6C3F1 female mouse. 1-5 : Transverse section of proximal and distal sciatic nerves, peroneal nerves, tibial nerves and medial plantar nerves of a 110-week-old mouse. Myelin body formation (M), axonal atrophy with thin and dilated myelin sheath (A), schwann cells proliferation (S), loss of nerve fiber structure (L). Toluidine blue stain. Bar = 30 μm.
Mouse age-related peripheral neuropathy.

Photos 6–7. Histopathology of distal sciatic nerves in elderly B6C3F1 female mice.
6: MAC3 antigens (arrows) in vacuoles of nerve fibers (a) and dilated myelin sheath (b).
Anti-MAC3 with H&E. Bar = 50 μm.
7: Teased nerve fibers from 97-week-old hypoglycemic mice exhibiting segmental demyelination and axonal degeneration. Bar = 50 μm.

Photos 8–9. Histopathology of dorsal root ganglia from an elderly hypoglycemic B6C3F1 female mouse.
8: Transverse section of dorsal root and neuronal bodies in dorsal root of a 125-week-old hypoglycemic mouse. Axonal atrophy with dilation of myelin sheath (A). Toluidine blue stain. Bar = 50 μm
and in nerves of elderly patients (Dorfman and Bosley, 1979; Kimura, 1994). Patients over 60 years old exhibit a reduction of maximum conduction velocity of motor and sensory nerves, greater in the lower extremity and greater beyond the age of 60 years (Dorfman and Bosley, 1979). In rat caudal nerve there is a clearcut impairment in MNCV with increasing age similar to that seen in human nerves (Schmelzer and Low, 1987). The electrophysiologic changes in the nerves of elderly mice are, thus, consistent with those seen in rat caudal nerve and in nerves of elderly patients. Impaired conduction velocities in old animals may be attributed to the interaction of many factors including demyelination, remyelination, a disproportionate loss of large myelinated fibers, axonal atrophy, nerve regeneration and reduced peripheral nerve (Na', K')ATPase (Robertson et al., 1993). There was significant accumulation of sorbitol, and tissue myo-inositol levels were lower than those at 10 weeks. Polyol changes in mouse nerves were less marked than those in rat nerves. These findings may show that there is increased aldose reductase activity in peripheral nerve tissue of the aged mouse.

Fig. 2. Incidence and severity of spontaneous peripheral neuropathy in B6C3F1 female mice (n = 171). Nerve injuries were scored based on the following scheme: minimal = focal lesions in some nerve fibers; slight = diffuse lesions in some nerve fibers, present in less than 1/3 of the tissue area; moderate = diffuse lesions in some nerve fibers, present in 1/3 - 2/3 of the area; marked = diffuse lesions in some nerve fibers, present in more than 2/3 of the tissue area.

Fig. 3. Representative blood glucose levels from a typical spontaneous hypoglycemic animal in female B6C3F1 mouse.

Fig. 4. Correlation between severity of peripheral neuropathy and blood glucose or HbA1c level in elderly female B6C3F1 mice (110 - 130 weeks-old, n = 59)
Mouse age-related peripheral neuropathy.

However, no correlation with peripheral nerve lesions and the above findings could be demonstrated individually, except for glycemic parameters.

The peripheral neuropathy described is spontaneous in origin and age-related. It was recently reported (Tabata et al., 2000) there were no differences in histopathologic findings associated with type of cage floor, but there has been no reported information on the influence of diet, drinking water or housing on the development of peripheral neuropathy in mice. Careful analysis of these factors may delineate the pathogenesis of this condition.

**Spontaneous hypoglycemic neuropathy**

The blood glucose level was sometimes lower (<50 mg/dL) in 2.2% animals at 52 weeks of age. Additionally, lower glucose levels (<40 mg/dL) persisted throughout the day in 3.2% of animals aged 100 weeks or more. These animals had extensive lesions in the peripheral nerves and exhibited decreased levels of HbA1c and fructosamines as well as increased levels of ketones. Severe hypoglycemia is most frequently encountered in relation to insulinomas or the accidental or deliberate injection of insulin, and is dominated by cerebral manifestations. There have been occasional descriptions of muscle weakness and wasting following severe prolonged hypoglycemia. A number of these patients have complained of distal paresthesias in the limbs, but few have demonstrable sensory loss.

Peripheral nerves are known to depend on glucose as the major substrate for energy production, and therefore hypoglycemia may reduce or inhibit of ATP synthesis. Failure of energy metabolism is known to affect axonal transport resulting in altered intra-nuclear concentrations of various nerve metabolites. Some authors have postulated that peripheral vasoconstriction leading to neural ischaemia may participate in the nerve fiber degeneration. The small amount of animal work, involving the production of acute or repeated episodes of hypoglycemia, indicates that neuroglucopenia has marked effects on peripheral nerves. Sidenius and Jakobsen (1983) demonstrated nervous system abnormalities in animals after administration of insulin; the effect of sustained hypoglycemia at a level between 18 to 49 mg/dL for a period of at least three days was examined in awake rats. In the present study, preparations of teased and isolated myelinated fibers of the sciatic nerve showed axonal degeneration. However, interpretation of studies must be made with care. Hypoglycemia reduces basal metabolic rate and causes hypothermia. In small animals, the greater the ratio of surface area to body mass, the greater the heat loss from the core and the more dependent the animal on thermogenesis.

Animal studies indicate that damage to peripheral nerves in hypoglycemia arises from a proximal focus, with degenerative changes spreading to individual cell bodies and axons (Mandelbaum et al., 1983; Sidenius and Jakobsen, 1983; Mohsei and Hidebrand, 1998). Several studies report changes in the characteristics of fast anterograde axonal transport. There are reductions in the apparent velocity of fast transport of radiolabeled unidentified proteins during hypoglycemia in nondiabetic rats (Sidenius and Jakobsen, 1987), streptozotocin-diabetic rats, and genetically diabetic BB rats (Mendell et al., 1981). Hypoglycemia in nondiabetic rats also causes reduced anterograde axonal transport of acetylcholinesterase activity (Tomlinson and James, 1984), although this effect is attenuated if the rats are warmed to limit hypothermia. Thus, experimental hypoglycemia can cause very marked hypothermia, and experiments should control for this. It thus appears that neuroglucopenia causes peripheral neuropathy, or at least defects that could be progenitive for neuropathy, but we know far less about the process in animals than we do about the selective cell-body degeneration that occurs in the nervous system. More work is needed.

**REFERENCES**


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