Experimental Hypoglycemic Neuropathy in Rat:
Nerve Blood Flow and Nerve Conduction Study

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Abstract
Severe hypoglycemia causes peripheral neuropathy. The role of ischemia in the development of hypoglycemic neuropathy has been postulated, although its underlying mechanism is uncertain. We, therefore, measured the blood flow of sciatic nerve in hypoglycemic rat. Severe hypoglycemia was induced for 3 h by insulin injection. Nerve blood flow (NBF) was measured using a laser Doppler flowmeter at mid- and lower thigh levels of sciatic nerve, and nerve vascular resistance (NVR) was calculated. NBF was significantly decreased and NVR was significantly increased in sciatic nerve during 3 h of hypoglycemia. Nerve conduction velocity in sciatic nerve was significantly slowed after 3 h of hypoglycemia. These results suggest that ischemia is implicated in the underlying mechanism of hypoglycemic neuropathy.

Key Words
hypoglycemia, peripheral neuropathy, ischemia, blood flow, rat

Introduction
Severe hypoglycemia causes both central and peripheral nervous system abnormalities⁵. Experimental hypoglycemic neuropathy has been induced by a single episode of severe hypoglycemia⁶. However, the underlying mechanism in the development of hypoglycemic neuropathy is still unknown. In experimental hypoglycemic neuropathy, Sidenius and Jakobsen demonstrated impaired fast axonal transport⁷. Yasaki and Dyck showed that the topographical distribution of nerve fiber abnormalities along the length of sciatic nerve in hypoglycemic rat was similar to that of acute ischemic neuropathy⁸. Insulin administration resulted in a reduction in nerve nutritive blood flow in both diabetic and non-diabetic rats⁹. We have demonstrated that intermittent hypoglycemic episodes induced swollen endothelial cells and reduced luminal area of endoneurial microvessels at the thigh level of rat sciatic nerve, and these vascular changes are most likely due to ischemic/reperfusion injury⁹. These studies suggested that nerve ischemia could play an important role in the development of hypoglycemic neuropathy. In the current study, we examined nerve blood flow and nerve conduction velocity (NCV) in hypoglycemic rats.

Materials and Methods

Animals
We used 12 male Sprague-Dawley rats (10–13 weeks) (Department of Laboratory Animal Sciences, University of Otago), and randomly assigned to the hypoglycemic group (n=6) and controls (n=6). The method to induce hypoglycemic episode has been described previously⁹. In brief, rats were fasted...
overnight and we injected regular insulin (Humulin R®, Eli Lily, Indianapolis, IN, USA) (7.5 U/kg BW) into the caudal vein. Blood glucose was kept < 3.0 mmol/L for 3 h with additional insulin injections. Subsequent doses of insulin depend on the level of blood glucose measured at each time point. Blood glucose levels were analyzed via tail prick samples prior to the insulin injection and every 30 min intervals thereafter utilizing a Diascan glucometer (Home Diagnostics, Eatontown NJ). At the end of 3-h hypoglycemia, 10% glucose was injected intraperitoneally. Saline was injected in control rats. We measured NBF in sciatic nerve, NCV in sciatic-tibial and caudal nerves, arterial blood pressure, and arterial blood gas before, during (every 1 h) and after 3 h of hypoglycemia. Animals were placed on a heating pad to maintain rectal temperature at 37.0 ± 0.5°C, and the skin temperature was kept at 36.5 ± 0.5°C using a lamp. This study was approved by the Committee on Ethics in the Care and Use of Laboratory Animals, University of Otago.

**Nerve blood flow (NBF) measurement**

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg BW). Tracheotomy tube was inserted, and right common carotid artery was cannulated with heparin-filled catheter for the recording of intra-arterial blood pressure and the sampling of arterial blood gases. Blood pressure was monitored continuously via a pressure transducer (Statham P23AC, Hato Rey, Puerto Rico), connected to a polygraph (Model 79 D, Grass Instruments, Quincy, Mass., USA). NBF was assessed with a laser Doppler flowmeter (Model LF 21, Advance, Tokyo, Japan) using a 1-mm diameter fibre-optic probe.12, 13 Right sciatic nerve at the thigh level was exposed and the probe was placed over the perineurium. NBF was then measured in 1-mm intervals at 6 points each of mid- and lower thigh levels. Blood pressure and NBF were monitored simultaneously with the MacLab Data Acquisition System (Analog Digital Instruments, Dunedin, New Zealand) using Chart program on an Apple Macintosh computer (Apple Computer Inc., Cupertino, Calif., USA). Blood pressure was divided by NBF to calculate the nerve vascular resistance (NVR) (measured in units of mmHg/ perfusion units). NVR is adjustable for the effect of blood pressure variability, and allows more valid comparisons between the two groups.

**Measurement of NCV**

Nerve conduction studies in left sciatic-tibial and caudal nerves were undertaken before and during hypoglycemia. For the measurement of sciatic-tibial NCV, stimulating needle electrodes were placed at the ankle level of the tibial nerve. Electrical stimuli consisting of square-wave pulses of 0.05-ms duration were maintained at a supramaximal level which was adjusted in each rat. Nerve action potentials were recorded directly by platinum electrodes in the left sciatic nerve at the sciatic notch level. Caudal nerve action potential was measured according to the method described by Low et al.2 Stimulating electrodes were placed percutaneously 6 and 7 cm from the tip of the tail. Nerve action potential was recorded percutaneously 12 cm proximal to the point of the stimulation. NCV values were calculated by dividing the distance between stimulating and recording electrodes by the onset latency.

**Statistical analysis**

All statistical calculations were carried out on an Apple Macintosh computer using Statview. Two groups were compared using a two-way repeated measure analysis of variance (ANOVA). Differences occurring between the two groups were tested with an unpaired, two-tailed Student’s t-test. Data were expressed as mean ± S. D.

**Results**

**Blood glucose, blood gas analysis, and nerve conduction velocity (NCV)**

Blood glucose level fell below 3.0 mmol/L within 30 min after insulin injection, and was maintained for 3 h (Fig. 1). Blood gas data were not significantly different before and during hypoglycemic episodes: pH 7.42 ± 0.03 and 7.38 ± 0.03, PO2 96.9 ± 3.2 and 97.5 ± 3.0 mmHg, and PCO2 36.9 ± 2.2 and 38.7 ± 3.5 mmHg before and at 3 h of hypoglycemia, respectively. Sciatic NCV was slowed significantly at 3 h of hypoglycemia when compared with those in controls (36.3 ± 1.2 and 38.9 ± 1.1 m/s, p < 0.01, in hypoglycemic rats and controls, respectively) (Fig. 2). There were no significant differences of caudal NCV between hypoglycemic and control groups (at 3 h of hypoglycemia, 36.9 ± 3.3 and 39.0 ± 1.4 m/s, p > 0.05, in hypoglycemic rats and controls, respectively.

**Nerve blood flow (NBF)**

NBF at mid- and lower thigh levels of sciatic nerve was significantly reduced during 3 h of hypo-
glycemia (Figs. 3a and 3b). At both levels of sciatic nerve, NBF was decreased significantly after 1 h of hypoglycemia and reduced continuously at 2 and 3 h of hypoglycemia. Systematic blood pressure in hypoglycemic rats was not significantly different from those in control rats at 3 h of hypoglycemia, 28.2±9.2 and 8.9±2.6 mmHg/perfusion unit at mid-thigh level, \( p < 0.01 \), and 25.3±9.7 and; 7.7±1.5 mmHg/perfusion unit at lower thigh level, \( p < 0.01 \), in hypoglycemic rats and controls, respectively (Figs. 4a and 4b).

**Discussion**

We demonstrated that NBF was significantly decreased and NVR was significantly increased at the thigh level of sciatic nerve during severe hypoglycemic episodes. It is known that hypoglycemia may cause hypothermia which could induce nerve ischemia. In the current study, we monitored rectal and skin temperatures simultaneously while measuring NBF and NCV, and found no significant differences between experimental and control rats.

The blood supply along the length of sciatic nerve shows the watershed zone at the thigh level: between territories supplied by superior gluteal and internal iliac arteries. Yasaki and Dyck showed morphological abnormalities of nerve fibers, e.g., axonal degeneration, at the thigh level of the sciatic nerve after 12 h of severe hypoglycemia. In experimental ischemic neuropathy, selective damage to the central region of endoneurial fascicle (ischemic core) has been demonstrated at the thigh level of rat sciatic nerve. In our previous study, we observed endothelial swelling of endoneurial microvessels at the thigh level of sciatic and tibial nerves after 4 daily episodes of 3-h hypoglycemia. These vascular changes have been shown in ischemic nerves. In the current study, we demonstrated reduced NBF and increased NVR in hypoglycemic sciatic nerves. Another study showed that sciatic NBF in acute hypoglycemic rats was significantly reduced as compared to those in controls. These results suggest that hypoglycemia causes ischemic injury in rat sciatic nerve. If blood pressure significantly increased or decreased, a rapid change in NBF in the same direction was seen within seconds, supporting previous studies which reported that peripheral NBF does not autoregulate in the blood pressure range of 50–120 mmHg. That is the reason we measured NVR values in this study. While NBF and NVR were altered significantly after 1 h of insulin injection, NCV in sciatic nerve was slowed significantly after 3 h of hypoglycemia. This discrepancy is related to physiological resistance to
ischemia in peripheral nerve because of its low energy needs and extensive anastomosis.

The mechanism of nerve ischemia caused by hypoglycemia has not been understood. Insulin-induced hypoglycemia is due to counter-regulatory events such as increase in catecholamine, and rise in glucagons, growth hormone, cortizol, and pancreatic polypeptide. Hypoglycemia leads to increment in catecholamine and may result in reduced NBF. Exogenous insulin administration reduced NBF in both non-diabetic and diabetic rat sciatic nerves. Yasaki et al. hypothesized that in-

Fig. 3. Mean nerve blood flow of rat sciatic nerves at the mid-thigh [a] and lower thigh [b] levels in hypoglycemic (open circles) and control (closed circles) groups. *p<0.001, **p<0.0001.

Fig. 4. Mean nerve vascular resistance in rat sciatic nerve at mid-thigh [a] and lower thigh [b] levels of hypoglycemic (open circles) and control (closed circles) groups. *p<0.01, **p<0.001.
sulin-induced severe hypoglycemia might produce either disruption of nerve energy metabolism or alternation of microvascular ischemia in peripheral nerves\(^6\). As to the mechanism of hypoglycemic neuropathy, one study suggested that nitric oxide (NO) generation plays a role on reduction of nerve blood flow caused by vasoconstriction in acute hypoglycemic rats\(^7\). In this study, both NBF and nerve blood velocity in the sciatic nerve were reduced in experimental acute hypoglycemic rats given NO synthases inhibitor. The results of our study were similar to his study. But Hata showed that NBF in the sciatic nerve was decreased by using a laser Doppler flowmeter, and did not calculate NVR to take blood pressure changes into account\(^8\). Peripheral nerve does not autoregulate its blood flow\(^9\), so we calculated NVR to take blood pressure changes into account and allow comparisons between experimental groups. We think the relation of sympathetic nerve function and NO on vasoconstriction of peripheral nerve in acute hypoglycemia still need to be clarified.

In conclusion, we showed reduced NBF in hypoglycemic rat, and nerve ischemia would be implicated in the pathogenic mechanism of acute hypoglycemic neuropathy.

Acknowledgements

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References


実験的低血糖性末梢神経障害における神経血流量と神経伝導速度

抄録

重度の低血糖は末梢神経障害を生じ、その低血糖性末梢神経障害の発症に虚血の関与が推測されている。今回、低血糖ラットの坐骨神経の神経血流量（nerve blood flow, 以下 NBF と略す）と神経伝導速度（nerve conduction velocity, 以下 NCV と略す）を測定した。レギュラーインスリン静注により 3 時間の低血糖発作を惹起し、レーザー血流計を用いて坐骨神経の大腿レベルでの NBF、および坐骨・脛骨神経の NCV を測定した。さらに血圧を測定し神経血管抵抗（nerve vascular resistance, 以下 NVR と略す）を計算した。低血糖により坐骨神経の NBF は直ちに対照群に比べ有意に低下し、NVR は有意に高値となった。これらの変化は 3 時間の低血糖の間持続した。坐骨・脛骨神経の NCV は 3 時間の低血糖後有意に低下した。これらの結果は、虚血が低血糖性末梢神経障害発症のメカニズムに関与する可能性を示唆する。

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