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Ischemic and reperfusion injury of rat peripheral nerve

(ischemia/blood–nerve barrier/nerve conduction/blood flow)

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ABSTRACT **A rat model of severe nerve ischemia was used to study the effects of ischemia and reperfusion on nerve conduction, blood flow, and the integrity of the blood–nerve barrier. Conduction failure was consistently found in the sciatic–tibial nerve during 1- and 3-hr ischemic periods. Recovery of the compound muscle action potential was prompt and complete upon reperfusion following 1 hr of ischemia. However, after 3 hr of ischemia, recovery in the proximal portion of the sciatic nerve was <10%, and conduction block occurred in the distal portion of the nerve. Nerve blood flow was restored to only 55% and 45% of resting values following 1 and 3 hr, respectively, of ischemia and did not recover even after 2 hr of reperfusion. The blood–nerve barrier was not statistically impaired to the passage of [^{14}C]sucrose following 1 hr of ischemia but was significantly impaired after 3 hr of ischemia. The permeability–surface area product was consistently greater following 1 hr of reperfusion than during the immediate reperfusion period. These data indicate that severe ischemia of peripheral nerve results in reperfusion injury, conduction block, and blood–nerve barrier disruption. Microvascular events, which may occur during reperfusion, may be important in amplifying the nerve fiber damage that began during ischemia.**

Although severe nerve ischemia is known to result in conduction failure and fiber degeneration, the mechanism(s) of ischemic injury are largely unknown. The effects of ischemia are amplified in several tissues during reperfusion, a phenomenon referred to as reperfusion injury or reduced reflow (1–4). In other systems, especially intestine and heart, the ischemic insult is mediated by oxygen free-radical effects and, perhaps, by intracellular calcium influx during the period of ischemia and, to a greater extent, during the period of reperfusion. In this study, we investigated whether ischemia resulted in persistently low nerve blood flow (NBF), whether the injury was sufficient to cause conduction failure, whether the blood–nerve barrier (BNB) was disrupted, and whether reperfusion resulted in accentuation of the ischemic injury. Ischemia resulted in electrophysiological abnormalities, NBF impairment, and BNB disruption. Reperfusion resulted in progressive impairment of the BNB, suggesting that reperfusion injury to nerve capillaries had occurred.

METHODS

Model of Severe Nerve Ischemia. We used adult male Sprague–Dawley rats weighing 250–300 g. Our model of severe nerve ischemia was produced by ligation of the iliolumbar and inferior mesenteric arteries followed by the temporary occlusion of the abdominal aorta and both iliac arteries. The abdominal aorta was occluded immediately above the origin of the common iliac arteries. The iliac arteries were occluded separately, and we tied off all identifiable anastomotic vessels, including the iliolumbar and inferior mesenteric arteries. The aorta and iliac arteries were tied with a silk suture (6-0), using a slip-knot technique for rapid release, when needed. Measurements of the femoral blood pressure (BP) were used to monitor the completeness of the occlusion, and direct inspection of the sciatic nerve epineurial vessels showed that blood flow had been arrested. Sluggish flow was sometimes seen in these vessels several minutes after aortic occlusion, presumably due to partial reestablishment of anastomotic flow.

NBF Measurements. NBF was measured by microelectrode (tip size 2–5 μm) hydrogen polarography (5). The signal was input into a computer (IBM PC-XT) via an analog-to-digital converter for simultaneous display (Labtech Notebook) and storage (Lotus 123). A curve was fitted to the data by a nonlinear regression program based on the Marquardt algorithm.

NBF was measured before ischemia, immediately following the release of the aortic ligature (time 0 hr), and after 2 hr of reperfusion. The duration of ischemia was 1 hr in one group and 3 hr in a second group of rats. During the period of aortic occlusion, systemic BP was monitored via the carotid line, whereas the effectiveness of the occlusive noose was confirmed by the femoral arterial BP.

Nerve Conduction Studies. Electrophysiologic studies were performed on the sciatic–tibial nerve by using previously described techniques (6). In brief, the compound muscle action potential (CMAP) was recorded from the dorsum of the hind paw while the nerve was stimulated at the level of the sciatic notch and ankle. We measured CMAP amplitude and latency-to-onset after proximal and distal stimulation. The motor conduction velocity and the proximal/distal amplitude ratio were then calculated, the latter being used as an index of the presence of conduction block.

Nerve conduction studies were performed on two groups of rats. In the first group, the rats were subjected to 1 hr of ischemic stress followed by 3 hr of reperfusion. Measurements were made immediately before aortic occlusion and then every 5 min during ischemia and every 15 min during reperfusion. In the second group, the rats were subjected to 3 hr of ischemic stress followed by 3 hr of reperfusion. Measurements were made immediately before aortic occlusion and then every 5 min for the first hour and every 15 min thereafter during ischemia and then every 15 min during reperfusion.

BNB Studies. We determined the permeability–surface area product (PA) of endoneurial capillaries by using [^{14}C]sucrose (30 μCi/ml of Ringer's solution; 1 μCi = 37 kBq) as the extracellular marker. The technique of Ohno *et al.* (7) with the modification of Phillips (8) was used to maintain a constant plasma isotope concentration. The nerve vascular bed was cleared by perfusion with mammalian Ringer's solution (145 mM NaCl/3.5 mM KCl/2 mM $CaCl_2$/6 mM

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Abbreviations: BNB, blood–nerve barrier; BP, blood pressure; CMAP, compound muscle action potential; NBF, nerve blood flow; PA, permeability–surface area product.

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Table 1. Arterial blood gases before ischemia and after reperfusion following 1 or 3 hr of severe nerve ischemia

Group	Duration of reperfusion	P_{O_2}, mmHg	P_{CO_2}, mmHg	pH
1 hr	Pre-ischemia	126.2 ± 5.3	36.6 ± 1.5	7.45 ± 0.01
	0 hr	139.4 ± 7.4	37.3 ± 2.4	7.31 ± 0.01*
	2 hr	131.1 ± 8.7	38.7 ± 2.0	7.35 ± 0.01†
3 hr	Pre-ischemia	122.8 ± 7.1	42.6 ± 2.1	7.45 ± 0.01
	0 hr	172.4 ± 11.1‡	34.0 ± 2.1§	7.32 ± 0.02¶
	2 hr	152.5 ± 10.2	36.9 ± 1.4	7.34 ± 0.03

For each group, $n = 8$ rats.
*pH for 1-hr group, 0 hr vs. pre-ischemia, $P < 0.001$.
†pH for 1-hr group, 0 hr vs. 2 hr, $P < 0.05$.
‡P_{O_2} for 3-hr group, 0 hr vs. pre-ischemia, $P < 0.05$.
§P_{CO_2} for 3-hr group, 0 hr vs. pre-ischemia, $P < 0.05$.
¶pH for 3-hr group, 0 hr vs. pre-ischemia, $P < 0.005$.

glucose/2 mM Hepes/7% albumin, adjusted to pH 7.4) at a pressure of 90–120 mmHg (1 mmHg = 133 Pa). A perfusion time of 20 min, which gives an adequate endoneurial radioactivity without backflux (9), was employed.

Rats were made ischemic for either 1 or 3 hr by occluding the abdominal aorta and both iliac arteries as well as collateral arteries. Upon release of the ligatures, for both the 1-hr and 3-hr groups, PA was determined immediately in one subgroup and after 1 hr of reperfusion in the other.

Statistical Analysis. For NBF data, each treatment group was compared with its control group by using a grouped *t* test. Data are expressed as mean ± SEM. For electrophysiologic data, each experimental group was compared with its control group by using a grouped *t* test. The null hypothesis was rejected for $P < 0.05$.

RESULTS

Arterial Blood Gases and Mean Arterial BP. The body weights were 289 ± 8 g and 293 ± 5 g for the 1-hr and 3-hr groups and were not significantly different. The arterial blood gas alterations were minor and all values remained within the physiologic range (Table 1). Mean arterial BP for the 1-hr group fell on average by 11 mmHg at 0 hr and 14 mmHg at 2 hr of reperfusion, while the 3-hr group fell by an identical amount at 0 hr and 25 mmHg at 2 hr, but none of the animals was hypotensive (Table 2).

NBF. The NBF data are summarized in Table 3. After 1 hr of ischemia, reperfusion resulted in a NBF restoration that was only 55% of the resting (pre-ischemia) value. Reperfusion for 2 hr did not restore NBF; instead, NBF fell further, to 45% of resting values. Following 3 hr of ischemia, reperfusion resulted in only 45% restoration of NBF, and NBF fell further to 38% of resting values with another 2 hr of reperfusion. The reperfusion values were all significantly lower than resting values ($P < 0.005$), but the 2-hr values were not significantly lower than the 0 hr values. In addition to the above experiments, NBF during ischemia was determined in two animals; the values were 0.9 and 3.0 ml per 100 g per min, values that are indistinguishable from complete cessation of NBF. Diffusion from the oil pool to air is responsible for the clearance curve under these circumstances (10).

Table 2. Mean arterial BP before ischemia and after reperfusion following 1 or 3 hr of severe nerve ischemia

Group	*n*	Duration of reperfusion	Mean arterial BP, mmHg
1 hr	8	Pre-ischemia	149 ± 4
		0 hr	138 ± 5*
		2 hr	135 ± 6
3 hr	8	Pre-ischemia	149 ± 4
		0 hr	138 ± 8
		2 hr	124 ± 6†

*For 1-hr group, 0 hr vs. pre-ischemia, $P < 0.05$.
†For 3-hr group, 2 hr vs. 0 hr, $P < 0.05$.

Nerve Conduction. Two groups of rats were used in this part of the study. One group was subjected to 1 hr of ischemia and the other to 3 hr of ischemia. Both groups then underwent reperfusion for 3 hr. Conduction failure regularly occurred throughout all segments of nerve within 30 min of occlusion in both 1- and 3-hr ischemic groups (Fig. 1). Following restoration of NBF, however, the two groups differed dramatically. Impulse transmission was rapidly restored with reperfusion following 1 hr of occlusion, and proximal and distal amplitudes were identical (Fig. 1 *Left*), indicating that focal conduction block did not occur with reperfusion.

In contrast, reperfusion following 3 hr of occlusion resulted in two interesting observations. Conduction failure continued, as indicated by persistently reduced CMAP amplitudes upon stimulation at the hip or at the ankle. Also, focal conduction block was present. This was indicated by the markedly lower CMAP obtained upon stimulation at the hip compared with at the ankle (Fig. 1 *Right*). The proximal/distal amplitude ratio, included as an index of conduction block, was only 0.19, 0.147, and 0.217, respectively, after 1, 2, and 3 hr of reperfusion. We have also extended the electrophysiologic studies to 24 hr ($n=5$) and 1 week ($n=3$). The mean ± SEM values for CMAP obtained upon stimulation at the hip and ankle were 10.6 ± 2.6% and 19.4 ± 3.4% of resting values for the 24-hr group and 13.7 ± 3.0% and 22.7 ± 6.2% for the 1-week group, demonstrating persistence of conduction failure. A single animal was studied 1 month after ischemia and the corresponding values were 26% and 34%.

BNB. One hour of ischemia did not result in a significant increase of the PA (Table 4). However, reperfusion resulted in a 71% increase, with the PA becoming significantly greater than the control value ($P < 0.05$). After 3 hr of ischemia, the PA was increased above control ($P < 0.05$) and 1 hr of reperfusion resulted in a further increase ($P < 0.001$).

Table 3. NBF before ischemia and after reperfusion following 1 or 3 hr of severe nerve ischemia

Group	*n*	Duration of reperfusion	NBF, ml per 100 g per min
1 hr	8	Pre-ischemia	14.6 ± 1.1
		0 hr	8.1 ± 0.7*
		2 hr	6.5 ± 1.2*
3 hr	8	Pre-ischemia	14.5 ± 0.7
		0 hr	6.5 ± 1.1*
		2 hr	5.5 ± 0.7*

*$P < 0.005$.

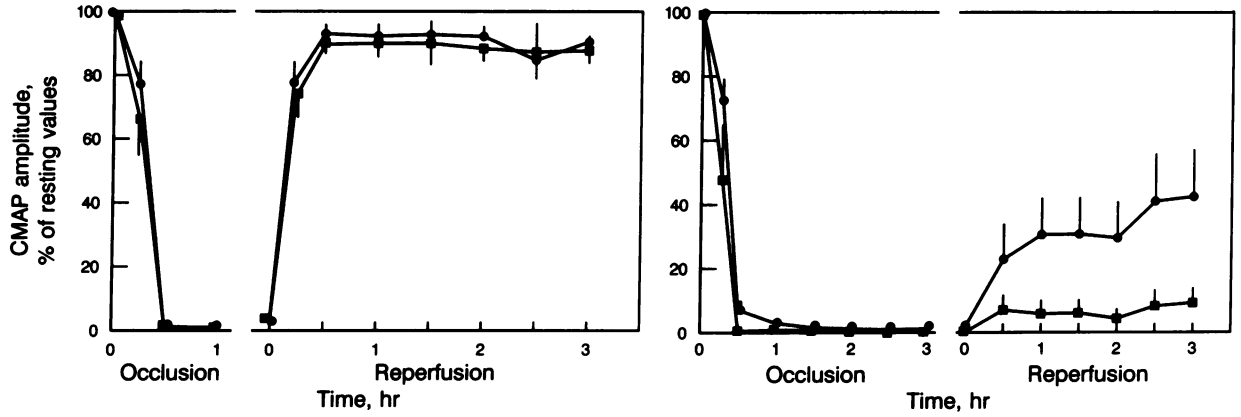

FIG. 1. CMAP amplitude on stimulation of the sciatic nerve at the proximal (hip, ■) or distal (ankle, ●) site during 1 hr (*Left*) or 3 hr (*Right*) of ischemia and 3 hr of reperfusion.

DISCUSSION

Special problems are incurred in the study of peripheral nerve ischemia. Unlike brain and heart, where ischemic effects are readily produced, peripheral nerve is relatively resistant because of its low energy needs and extensive anastomoses (11). Thus, early attempts to produce experimental ischemia were largely unsuccessful. More recently, however, models of ischemia have been developed (12–15). For physiologic studies of nerve ischemia and the effects of reperfusion injury, it was necessary to develop a model of near-total yet reversible ischemia. Our model of severe nerve ischemia, produced by ligation of collateral arteries followed by the temporary occlusion of the abdominal aorta and both iliac arteries, consistently blocked NBF. This model also consistently produced ischemic conduction failure within 30 min with prompt recovery of impulse transmission following 1 hr of ischemia but persistent conduction failure and conduction block following 3 hr of ischemia. Reperfusion failure was evident in peripheral nerve after 1 hr and, especially, 3 hr of ischemia. NBF was not completely restored with reperfusion and there was a suggestion of progressive reduction in NBF with increasing duration of reperfusion.

The Po_2 alterations following ischemic stress were mild, and since the values for all animals remained within the physiologic range, these changes should not have been responsible for the observed NBF alterations. The reductions in pH and Pco_2 likely represented changes of metabolic acidosis due to gut ischemia, since the intestines were partially devascularized with ligation of the paraaortic vessels. However, the changes were mild, and arterial blood gases remained within the physiologic range and should not have affected NBF (5). Mild reductions of mean arterial blood pressure (Table 2) in the reperfusion phase may have affected NBF. However, based on previous calculations, the reduction was too small to account for the persistent reduction in NBF (5).

Table 4. PA following ischemia and reperfusion in rat sciatic nerve

Group	*n*	Duration of reperfusion	PA × 10^5, ml per g per sec
Control	6	—	2.5 ± 0.4
Ischemic			
1 hr	5	0 hr	2.8 ± 0.4
		1 hr	4.8 ± 1.2*
3 hr	6	0 hr	4.9 ± 1.2*
		1 hr	7.5 ± 1.1†

*$P < 0.05$ vs. control.
†$P < 0.001$ vs. control.

We used the BNB as a physiologic index of oxygen free-radical effects because oxygen free radicals are known to increase microvascular permeability (16–18). The PA may be increased as a result of an increase in permeability or of an increase in the surface area of endoneurial capillaries. In this study, NBF (Table 3) remained persistently reduced with reperfusion. Therefore, the progressive increase in PA (Table 4) must have been due to an increase in permeability.

The persistent failure of impulse transmission following 3 hr of ischemia with reperfusion had the characteristics of conduction block. Conduction block has been demonstrated in peripheral nerve following experimental occlusion of the vasa nervorum (19). This block occurred between identical stimulation sites as those used by us and was not associated with morphologic alterations. The site of the block appears to be at the distal sciatic level and the physiologic basis likely relates to this being the region of greatest ischemia following aortic occlusion (20, 21). Focal conduction failure can be due to focal metabolic failure, focal demyelination, or axonal degeneration. The results at 24 hr and especially those at 1 week shed some light on the likely mechanism. The persistent conduction failure on proximal stimulation and the progressive reduction in CMAP on distal stimulation indicate that the electrophysiologic abnormalities were likely due to axonal degeneration rather than transient conduction block, since a metabolic block would be transient and the CMAP with distal stimulation should remain unchanged wih focal demyelination.

The effects of ischemia are amplified in several tissues during reperfusion, a phenomenon referred to as reperfusion injury or reduced reflow (1–4). Our results suggest that such a phenomenon exists in nerve if the ischemia is nearly total and of long duration, since it occurs following 3 hr but not 1 hr of occlusion. An abstract has appeared reporting edema and focal demyelination following reperfusion, changes that were additional to the effects of ischemia alone (22). The molecular mechanisms underlying reperfusion injury in nerve remain to be elucidated. Based on the progressive increase in PA with reperfusion and the preliminary results of an increase in conjugated dienes and malondialdehyde with reperfusion (N. L. Parinandi, personal communication), we propose that nerve ischemia may be due to the generation of oxygen free radicals that damage the endothelial barrier and, subsequently, endoneurial contents. The mechanisms are likely to be similar to those involved in heart, gut, and brain but to be modified in terms of the threshold of each tissue to ischemic and reperfusion damage. The elucidation of the mechanisms of reperfusion injury and strategies for ameliorating this damage are particularly relevant in nerve, where the ischemic threshold is much higher and the rate of development of ischemic and reperfusion injury occurs over

many hours, thus permitting ample time to institute treatment, should it become available.

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