Nerve and muscle blood flow during hindlimb ischemia and reperfusion in rats

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Animal models of peripheral nerve ischemia have yielded variable results. The question of whether post-ischemia re-establishment of blood flow to the nerves augments injury has not been examined. To study this question, the ipsilateral common iliac and femoral arteries were occluded with arterial snare for 3 hours in rats; 14C-butanol tissue distribution was then used to measure blood flow in both sciatic and posterior tibial nerve trunks and in both biceps femoris muscles during occlusion and reperfusion. Clinical limb function was graded serially, with the undisturbed contralateral limb serving as the study control. Nerve blood flow was reduced throughout the ischemic period and was only 20% of the control value in the posterior tibial nerve. Muscle blood flow was unchanged. All rats had functional impairment, with an average limb function score 

KEY WORDS: peripheral nerve - tibial nerve - sciatic nerve - regional blood flow - ischemia - reperfusion - rat

ISCHEMIA has been identified as a significant factor in the pathogenesis of a variety of clinical and experimental peripheral neuropathies. It is generally agreed that nerve tissue is moderately resistant to ischemia, however, as it has a relatively rich blood supply and substantial stores of high-energy phosphate compounds. Nerve tissue can also adapt rapidly to anaerobic metabolism and has a low energy requirement even when maximally functioning. These protective factors are at least in part responsible for the difficulties that have been encountered by previous investigators in attempting to develop consistent experimental animal models of peripheral ischemia. Technical factors associated with the measurement of regional nerve blood flow have also posed an obstacle. Nerve blood flow was not quantitated until 1977; since then, several methods of measuring blood flow in nerve tissue have been described, each of which possesses some disadvantages. Additionally, most animal models of nerve ischemia either fail to permit long-term follow-up monitoring or lack serial measurements of nerve blood flow. In the present study, based on data from pilot experiments, we quantitated in rats the effects on regional blood flow in the sciatic and tibial nerves of temporary occlusion of the ipsilateral common iliac and femoral arteries. The tissue distribution of 14C-butanol was used to measure nerve blood flow; this is a method with which we have had considerable experience. We correlated these blood flow measurements with follow-up observations of the functional status of the affected hindlimb, as we were particularly interested in determining whether there was clinical evidence of a so-called "reperfusion" injury in this model, as is known to occur in several other tissues, notably cardiac muscle, brain, and intestine.

Materials and Methods

Male Sprague-Dawley rats, each weighing between 300 and 420 gm, were used for this study. As described below, the common iliac artery was temporarily occluded at the level of the superior vesical artery and the femoral artery was occluded between its circumflex iliac and superficial epigastric arterial branches. The undisturbed contralateral hindlimb served as the study control.
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<table>
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<td><strong>Hindlimb function scoring system</strong></td>
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* Latex tubing, 8 mm in outer diameter, tautly positioned horizontally.

**Hindlimb Motor Function Assessment**

A modification of the numerical scoring system previously reported by LeMay, et al., was used to quantitate function in the test and control hindlimbs. Normal rats score less than 2, and the worst possible score is 12 (Table 1). We included an evaluation of the rats' ability to maintain a horizontal stance on an inclined plane for at least 5 seconds without falling, as described by Rivlin and Tator. A conservative estimate of normal. The rat was positioned so that the limb to be tested was lowest on the incline. All evaluations were carried out by an experienced observer who was blinded to the rat's history.

**Temporary Occlusion and Reperfusion of Common Iliac and Femoral Arteries**

Based on preliminary experiments, it was elected to temporarily occlude the ipsilateral common iliac and femoral arteries. An occlusion time of 3 hours was arbitrarily selected. In these experiments, 28 rats were anesthetized by inhalation of 1.5% fluothane. The occlusion was performed aseptically through a longitudinal groin incision using snares of soft silicone tubing after each rat had received 200 U/kg of bovine heparin intravenously to prevent clot formation from stagnant blood. Immediately following occlusion, the skin was sutured and the rat permitted to awaken; hindlimb function was assessed after 1 hour of occlusion. Four of the 28 rats were then reanesthetized with pentobarbital as described below and regional blood flows were measured. After 3 hours of occlusion, the remaining 24 rats were reanesthetized with fluothane, the snares were released, the skin was resutured, and anesthesia was discontinued. The re-establishment of arterial flow was visually confirmed by the observation of pulsations distal to the occlusion sites and by the rapid disappearance of cyanosis from the paw, which quickly became pink. If the femoral snare was the first of the two to be released, pulsations typically appeared at once in the femoral artery despite the proximal occlusion, indicating the presence of substantial collateral flow via the contralateral pelvic vasculature into the femoral artery.

Regional blood flow measurements were performed subsequently in 12 rats at the following intervals after snare release: 2 hours in five rats, 7 hours in two rats, and 21 hours in five rats. Eight other rats were observed for 6 days postoperatively. Their hindlimb function was noted at 24 and 48 hours and at 6 days. Four other rats were sacrificed at various postocclusion intervals just prior to obtaining flow measurements because of nonphysiological blood gas values or arterial hypotension.

**Regional Blood Flow Measurements**

Regional blood flow in the sciatic nerve trunk, the tibial nerve, and the biceps femoris muscle was measured using the 14C-butanol "indicator-fractionation" technique described in detail previously. Anesthesia was induced with intraperitoneal pentobarbital (50 mg/kg) after atropine (0.004 mg/kg) had been administered subcutaneously. Briefly, plastic catheters were inserted through right cervical and bilateral axillary incisions into the right jugular vein and into both axillary arteries, respectively. The mean arterial blood pressure was monitored by the left axillary catheter. A rectal probe was used to monitor body temperature, which was maintained within the normal range with an external heat source. Just before the measurements were made, 0.6 ml of arterial blood was sampled via the left axillary catheter and blood gas values were analyzed. One hour after induction of pentobarbital anesthesia, 25 μCi of 14C-butanol was rapidly injected through the right jugular catheter 5 seconds after the onset of free-flowing arterial hemorrhage from the previously unclamped left axillary catheter. Fifteen seconds after butanol injection, 1.0 ml of cyanoacrylate glue was injected into the right axillary catheter to arrest arterial flow. The resultant intraluminal coagulum extended beyond the aortoiliac bifurcation. Simultaneously, the left axillary catheter was clamped and the rat was sacrificed with an intravenous injection of concentrated KCl solution. Catheter positions and aortic occlusion were verified. The resumption of blood circulation distal to the temporary occlusion sites was confirmed by the presence of distal intraluminal coagulum.

The sciatic nerve trunks beginning at the sciatic notch and extending to their trifurcation were excised and divided into equal lengths. Samples were obtained from both tibial nerves at their midportion and from the biceps femoris muscle adjacent to the sciatic trunk. The order in which the samples were harvested was varied intentionally. All tissue was placed in preweighed glass vials. The arterial blood and tissue
samples were weighed, then solubilized for 48 to 72 hours and their radioactivity was determined by scintillation spectrophotometry.

Regional blood flow was calculated using the equation: 
\[ F_r = \frac{Q_r}{Q_x} \times 100 \]
where \( F_r \) is regional blood flow (ml min\(^{-1}\) · 100 gm\(^{-1}\)), \( Q_r \) is the rate of external hemorrhage (ml min\(^{-1}\)), \( Q_x \) is the indicator content in the tissue (cpm · gm\(^{-1}\)), and \( M_i \) is sample weight (gm).

The theoretical basis for this methodology is a derivation of this equation from the Fick principle, and its validity in the tissue studied are given in detail in previous publications.  \[ Y. Kinoshita and W. W. Monafo \]

If, as was done here, arterial blood at a known flow rate is collected after injection of marker into the heart for a brief period prior to arrest of the circulation, this "artificial organ" also has no venous indicator content. Its flow rate and indicator content can be inserted into the equation, which is then solved to determine the flow in the tissue (either nerve or muscle). The method measures the composite blood flow in nerve (the sum of epineural and endoneurial flow); it is comparatively simple to perform and does not require prior surgical exposure of the tissue. The resulting values for nerve blood flow are in the range that others have reported in experiments using hydrogen polarography or iodoantipyrene distribution.  \[ 24,35 \]

**Data Analysis**

The blood flow measurements and physiological data are given as the mean ± the standard error of the mean. A paired t-test was used to compare values in the test versus the control limb and an unpaired t-test to compare the changes in blood flow over time. The nonparametric data from the hindlimb scoring system were compared over time using the Wilcoxon rank-sum test. The null hypothesis was rejected at p less than 0.05.

**Results**

**Regional Blood Flow**

One hour after occlusion was commenced, blood flow was depressed in the entire sciatic-tibial nerve trunk but was hardly changed in muscle (Fig. 1). In the tibial nerve, mean flow was 3.9 ± 1.3 ml min\(^{-1}\) · 100 gm\(^{-1}\), which was only about 20% of the control value of 15.2 ± 3.2 ml min\(^{-1}\) · 100 gm\(^{-1}\) (p < 0.02).

Figure 2 shows blood flows during reperfusion. After either 2 or 21 hours of reperfusion, blood flow was little changed from that of the control side in the proximal sciatic nerve trunk. After 2 hours of reperfusion, blood flow was increased in the distal sciatic nerve but the apparent increase after 21 hours was not significant.

In the tibial nerve, there was a distinct hyperemic flow response to about twice the control value after both 2 and 21 hours of reperfusion; in both rats in which measurements were made following 7 hours of reperfusion, blood flow was similar to that observed at 2 hours (flows in ml min\(^{-1}\) · 100 gm\(^{-1}\): in the distal sciatic nerve, 19.8 and 20.6 occluded vs. 9.8 and 8.8 control; in the tibial nerve, 43.8 and 33.5 occluded vs. 15.4 and 12.1 control). In the biceps femoris muscle, flow was increased after 2 hours of reperfusion. After 7 hours, flow was 8.0 and 4.6 ml min\(^{-1}\) · 100 gm\(^{-1}\) occluded versus 4.1 and 3.0 ml min\(^{-1}\) · 100 gm\(^{-1}\) control; after 21 hours of reperfusion, flow was not significantly different from control values.

**Hindlimb Function**

Hindlimb function is shown in Fig. 3. Function on the control side was normal in all rats. During occlusion of the ipsilateral common iliac and femoral arteries, function scores ranged from 5 to 10, with an arithmetic mean of 7.5. All rats had an obvious gait disturbance and lacked toe spread, but pain sensation appeared to be preserved. The inclined-plane test results were also abnormal initially in 76% of rats. The grasp response was almost invariably abnormal.

One hour after declamping, the mean function score in the 24 rats had improved to 5.2 (p < 0.001 when compared to the score during occlusion). Progressive improvement occurred during the next 6 days. Three of the eight rats observed for 6 days ultimately scored in the normal range. At 6 days, the mean score of the test side in eight rats was 2.1, or still marginally abnormal.

**Physiological Variables**

There were no statistical or physiologically important differences in the status of the various experimen-
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Fig. 2. Bar graph showing regional blood flows during reperfusion. Shaded bars indicate the side occluded for 3 hours; unshaded bars indicate the contralateral control side. Statistical significance: * = p < 0.05 and ** = p < 0.01 when compared with the control side. Vertical bars = standard error of the mean. NS = no statistical significance.

Fig. 3. Scatterplot showing changes in the hindlimb function score during and after 3 hours of arterial occlusion. The horizontal bars indicate the mean at each interval.

Discussion

Most investigations of nerve ischemia have focused on the associated morphological and neurophysiological changes in the sciatic and tibial nerve segments of the rabbit, rat, or cat. No single animal model has been widely used. Nerve ischemia or infarction has been variously produced by ligating most or all of the limb arterial supply,1,5,12,16,18,19 by extensive mobilization and isolation of the nerve from its mesoneurium,1,25 by microsphere embolization via the femoral artery,29 by the intra-arterial administration of arachidonic acid,30,31 by the use of exsanguination to induce hypotension,41 and by the establishment of a proximal femoral arteriovenous fistula.36 The multitude of models suggests that there are important disadvantages in each. The literature on peripheral nerve ischemia and the measurement of nerve blood flow has been comprehensively reviewed by Low, et al.22

Several findings and concepts have emerged from
TABLE 2

Physiological variables in 12 rats following reperfusion*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time of Reperfusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2 Hrs</td>
</tr>
<tr>
<td>no. of rats</td>
<td>5</td>
</tr>
<tr>
<td>arterial blood pressure (mm Hg)</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>heart rate (beats/min)</td>
<td>410 ± 17</td>
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<tr>
<td>body temperature (°C)</td>
<td>37.5 ± 0.1</td>
</tr>
<tr>
<td>arterial blood (mm Hg)</td>
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<tr>
<td>pH</td>
<td>7.39 ± 0.02</td>
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<tr>
<td>PaCO₂</td>
<td>38.8 ± 0.7</td>
</tr>
<tr>
<td>PaO₂</td>
<td>81.6 ± 2.5</td>
</tr>
<tr>
<td>body weight (gm)</td>
<td>360 ± 21</td>
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<tr>
<td>duration of anesthesia (min)†</td>
<td>56 ± 6</td>
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* Values are mean ± standard error of the mean. Hemodynamic and arterial blood gas values were measured just prior to blood flow measurement.
† Indicates the interval between induction of pentobarbital anesthesia and blood flow measurements.

these investigations. They can be summarized as follows: 1) the dual, richly interconnecting longitudinal and segmental blood supply provides significant protection and is likely to be partially responsible for the variability of injury severity observed by most investigators; 2) nerve blood flow must be greatly reduced (by 60% to 70% or more) in order for ischemic changes to occur; 3) diffusion of oxygen and possibly other substrates in small animals may ameliorate or prevent ischemic changes that would otherwise occur; 4) infarction typically occurs in the central zones of the large fascicles; 5) pathological changes in ischemic nerve tissue are distinct from Wallerian degeneration; 6) a watershed area of the circulation is present in the region of the distal sciatic nerve trunk and its proximal branches, principally the posterior tibial nerve; and 7) little is known about the molecular mechanisms of ischemic nerve injury.

On a clinical level, paresis may not have a neural cause as ischemic muscle injury is frequently also present.34 Some evidence suggests that muscle is less resistant than nerve to ischemia;42 however, Chervu, et al.,4 concluded the opposite. The relative sensitivity to ischemia of nerve, muscle, and the various subtypes of both structures has received little attention and therefore remains to be determined.

The phenomenon of reperfusion injury has been well documented in brain, heart, and intestine tissue.13,15,38 Schmelzer, et al.,34 reported evidence suggesting that, as in those tissues, reperfusion of nerve tissue following ischemia may also amplify the original injury by disruption of the blood-nerve barrier, possibly via the generation of oxygen free radicals.34 As they point out, the relative resistance of nerve to ischemia (usually several hours elapse before conduction failure) makes therapeutic measures aimed at minimizing injury due to reperfusion a practical clinical maneuver in theory, provided that such therapy were available.

The ischemia-reperfusion model developed by Schmelzer, et al.,34 included prior ligation of the ileocolic and inferior mesenteric arteries, followed by temporary occlusion of the terminal aorta and both (possibly common) iliac arteries. Following 3 hours of ischemia, muscle compound action potentials showed only minimal recovery; nerve blood flow returned to only 45% of the control value after 2 hours of reperfusion. We note, however, that the rats in that study were not heparinized prior to aortoiliac occlusion so that thrombosis due to stagnation in occluded vascular segments might have occurred, a phenomenon we observed in our pilot experiments. As those authors pointed out, transient reactive hyperemia could have resolved prior to the single measurement of nerve blood flow made after 2 hours of reperfusion; however, clinical experience with tourniquet-induced ischemia suggests that reactive hyperemia would be expected, at least in non-neural soft tissues. The 21-hour duration of elevated nerve blood flow during reperfusion that we documented after a duration and severity of ischemia similar to the study by Schmelzer, et al. (and which may have persisted even longer), conflicts directly with their findings. On the other hand, we also recognize that although our heparinized rats did not develop thromboses at the snare sites, they were themselves in an altered physiological state.

Zollman, et al.,47 examined the effect of ischemia and reperfusion on energy metabolism in rat nerves. They found that reperfusion after 3 hours of ischemia resulted in rapid normalization of energy substrates. Within 1 hour after reperfusion, for example, creatine phosphate values had improved from 41% to 83% of resting values, and adenosine triphosphate from 44% to 112%. Lactate levels remained increased at 319% of the resting values after 1 hour of reperfusion, but after 3 hours were 165% of resting values. The authors concluded that ischemic nerve conduction failure has an energetic basis and that reduction of creatine phosphate by about 59% is required for conduction failure.

The present data confirm the vulnerability of the distal sciatic-posterior tibial nerve segment to ischemia, as has been previously pointed out by Sladky, et al.,35 and by Korthals and Wisniewski.19 The serial measurements reported here of nerve and muscle blood flow during reperfusion are the first to be published. They document clearly the presence of a brisk hyperemic flow response in the distal sciatic-posterior tibial nerve.
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segment to about double the baseline value after 2 hours of reperfusion. The increase in nerve blood flow persisted for at least 21 hours.

Blood flow in the biceps femoris muscle was apparently unaffected during occlusion, but this muscle participated in the posts ischemic flow response although in an apparently less pronounced and more transient fashion than in the nerve tissue. We note, however, that the single measurement of muscle blood flow during occlusion was made only 1 hour after the snare was tightened. A later decline in flow might therefore have been missed. The modest two-fold increase in muscle blood flow that was documented after 2 hours of reperfusion is consistent with the presence of a transient post-occlusive hyperemic phase, only the final phase of which was documented. In muscle, reactive hyperemia typically peaks at levels 10-fold or more above baseline within minutes after reperfusion begins. After 21 hours of reperfusion, muscle blood flow was unchanged from the control side. It may be pertinent to this difference in the response of nerve versus muscle that hemodynamic autoregulation of flow is normally present in skeletal muscle but not in nerve. We found previously that hemodynamic autoregulation of nerve blood flow was not demonstrable in rats subjected to hemorrhagic hypertension, but that muscle blood flow remained constant through a broad range of mean arterial blood pressure (38 to 132 mm Hg). Similarly, Low and Tuck found no evidence for the hemodynamic autoregulation of nerve blood flow and also noted that, unlike in brain, respiratory acidosis or hypoxia did not result in an increase in flow to nerve tissue.

Clinically, limb function in our rats tended to improve progressively, beginning immediately after reperfusion had commenced. This observation is not suggestive of an amplified insult, as in reperfusion injury, but the possibility remains that reperfusion injury did occur and that, in its absence, recovery would have been even more rapid than was observed.

Whether the limb function deficits we observed were due entirely or in part to ischemic injury to the muscle has not yet been determined. However, the coexistence of limb dysfunction and severe flow impairment in the posterior tibial nerve during the occlusive period in the absence of an apparent decrement in muscle flow (Fig. 1) suggests that nerve ischemia was responsible at least in part. The relatively rapid recovery of motor function in our rats is similar to that described by Denny-Brown and Brenner after tourniquet injury in cats. As in their subjects, sensation appeared to be preserved in our rats. Whether there is a differential fiber sensitivity to ischemia determined by fiber diameter or function remains uncertain.

With the model we describe, the ischemic interval can be varied as desired and presumably lengthened until frank nerve infarction regularly occurs. Being nonlethal, the model permits longitudinal assessment of molecular events associated with both ischemia and reperfusion, and correlation of those findings with clinical and/or electrophysiological functional parameters in both nerve and muscle.

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