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# *Adrenergic Vasoconstriction in Peripheral Nerves of the Rabbit*

*Dag Selander, M.D.,* Lars G. Månsson, M.Sc.,† Lilian Karlsson,‡ Joar Svanvik, M.D.§*

**The blood flow in the sciatic nerve of the rabbit was estimated from the wash out of intraneurally injected $^{133}Xe$. To avoid diffusion of the tracer into the surrounding muscular tissue, the nerve was covered by a gas-tight plastic film. Using this technique, the basal blood flow in the sciatic nerve was estimated to 35 ml · min$^{-1}$ · 100 g$^{-1}$. It was found that intraarterial norepinephrine and electrical stimulation of the lumbar sympathetic chain strongly reduced the wash out of $^{133}Xe$, which only can be explained by a pronounced reduction of the blood flow in the nerve itself. The blood flow again increased within 4 min of stopping the infusion of norepinephrine or the sympathetic stimulation. The prolonged effect and higher neurotoxicity of local anesthetics containing adrenaline may be explained by an alpha receptor–mediated vasoconstriction of the microvessels of peripheral nerves. (Key words: Measurement technique: Xenon wash out. Nerve: blood flow; sciatic. Sympathetic nervous system: catecholamines; norepinephrine.)**

CLINICAL EXPERIENCE indicates that adrenaline enhances the neural toxicity of local anesthetics.[1] In an experimental series of intraneural injections, the neurotoxicity of the local anesthetic bupivacaine was increased significantly by the addition of adrenaline (5 μg/ml).[2,3] This may be explained by a delayed clearance of the anesthetic agent due to both extraneural and intraneural vasoconstriction induced by the adrenaline. Using a vital microscopic technique, Lundborg[4] found a pronounced intraneural vasoconstriction in the rabbit tibial nerve after stimulation of the lumbar sympathetic chain. With histochemical techniques he also demonstrated the presence of adrenergic nerve endings in the vasa nervorum.

Blood circulation in the peripheral nerves has been investigated with intravital microscopy[4,5] and microangiographic techniques,[4,6] but these techniques do not allow quantitative measurements. A few attempts have been made to quantitate the blood flow through peripheral nerves. With indicator-fractioning techniques using iodide–antipyrine and $^{86}Rb$, Mandel *et al.*[7] studied the blood flow of the sciatic nerve in the rat and Tschetter *et al.*,[8] using a microsphere technique, estimated the blood flow in the sciatic nerve of the dog. These techniques do not provide information about changes in intraneural blood flow and do not allow studies of the time course of vasoconstriction. Utilizing a hydrogen polarographic method, Smith *et al.*[9] measured the blood flow in the sciatic nerve of the cat with good reproducibility.

In the present study, a $^{133}Xe$-wash-out technique was used to measure the blood flow in the sciatic nerve in the rabbit at basal conditions and during lumbar sympathetic stimulation or intraarterial infusion of norepinephrine.

## Materials and Methods

Twenty-one adult rabbits (2–3 kg) were anesthetized with an injection of sodium pentobarbital (30 mg/kg), with supplementary doses of 12–15 mg when required. After tracheotomy, a tube was inserted into the trachea. The left carotid artery was cannulated for continuous blood pressure monitoring (Statham® P23D transducer, Grass® Model 7 B Polygraph).

In seven animals the abdominal aorta was cannulated with a polyethylene catheter (PE 50) through the left femoral artery with its tip below the renal arteries for infusion of norepinephrine. In another seven animals the right lumbar sympathetic chain was exposed transabdominally; divided and bipolar silver electrodes were attached to the distal part.

Each sciatic nerve was exposed carefully through a lateral incision and mobilized without damaging the vascular supply to the nerve. The nerve was enclosed between two sheets of gas-tight plastic film (Mylar®, 12 μm, Du Pont) to prevent diffusion of the tracer gas. During preparation the nerve was soaked repeatedly with 37° C Tyrode's solution. After preparation, the nerve was covered by the muscles and skin flaps for 10 min to restore normal temperature of the nerve and surrounding tissue. After reexposing the nerve, 10–20 μl of a solution of $^{133}Xe$ in normal saline (approx. 440 Mbq/ml) was injected during 5 s into either a fascicle (diameter 1–1.2 mm) or the epineurium through a 0.3-mm needle. The nerve again was covered with Mylar®, carefully avoiding air bubbles between the sheets, and the muscles repositioned. During the experiments an intravenous infusion of 3–5 ml · kg$^{-1}$ · h$^{-1}$ of 5% glucose in normal saline was given.

The disappearance of the $^{133}Xe$ from the site of injection was measured by a NaI(Te) scintillation detector placed above the nerve close to the leg.

* Associate Professor of Anesthesiology.
† Hospital Physicist.
‡ Research Assistant.
§ Associate Professor of Surgery.

Received from the Department of Anaesthesiology, Department of Radiation Physics, Laboratory of Rheology, and Department of Surgery I, Sahlgrenska Sjukhuset, University of Göteborg, Göteborg, Sweden. Accepted for publication June 7, 1984.

Address reprint requests to Dr. Selander: Department of Anesthesiology, Vastra Frolunda Sjukhus, P. O. Box 138, S-421 22 Vastra Frolunda, Sweden.

The scintillation detector was connected to a spectrometer (Beckman) and a linear ratemeter (Nukab, Göteborg, Sweden), and the gamma activity was registered graphically. The disappearance rate of the $^{133}Xe$ ($k_{Xe}$) was calculated using the least-squares method as the best fitting monoexponential function for each curve.

### Theoretic Considerations

The theory upon which this work is based was described by Kety.[10,11] The elimination of an inert gas from a constantly and uniformly perfused tissue can be described by the following equation, provided that the arterial concentration of the gas is neglectibly low during the wash-out period.

$$C_t = C_O \cdot e^{-kt} \quad (1)$$

$C_t$ and $C_o$ denote the tissue concentrations of the gas at times t and O and k denotes the clearance constant k that is closely related to the blood flow.

If the gas leaves a tissue compartment exclusively via the blood and if it can be assumed that a gas equilibrium between blood and tissue is reached in a fraction of a second, *i.e.*, with negligible concentration gradients in the tissue, then the blood flow (f) through the tissue can be calculated in ml · $min^{-1}$ · $100^{-1}$ g from the formula

$$f = k \cdot s/w \cdot 100 \quad (2)$$

where *s* denotes the tissue blood partition coefficient of the gas and *w* the density of the tissue.

### Estimation of the Partition Coefficient

The partition coefficient for Xe in nerve/blood was estimated in model experiments from the partition coefficient in blood/gas and in nerve/gas.

*The Blood–Gas Partition Coefficient:* Five milliliters of heparinized venous blood from each of six rabbits were collected in glass test tubes, the open end of which had been melted to a hour-glass-like shape. The opening was plugged with plasticine (Hartbutt's Plasticine,® Ltd.) and 2–10 MBq of $^{133}$Xenon gas was injected into the sample, and the narrow neck of the tube was closed by heating. The contents of the tubes then were equilibrated at 37°C for 2 h.

The emitted radiation from gas and blood was measured separately with a 1.5″ NaI(Te)-scintillation detector (aperture 3 × 12 mm), which was connected to a multichannel analyzer (MCA) (Canberra, series 30). $^{133}$Xenon emits radiation at two main energy levels: 81 keV $\gamma$-rays and about 31 keV x-rays; the MCA allowed each energy level to be measured separately. The blood–gas partition coefficient was calculated from the mean values of the 12 determinations (two in each sample).

*The Nerve Gas Partition Coefficient:* Sciatic nerves from rabbits were cut into 5–10-mm pieces and put in equal amounts into each of seven glass test tubes. The tubes were filled with saline to half their volume, and a brass metal net that fit the inside of the tube was put on top of the nerves to compress the nerve pieces during centrifugation. $^{133}$Xenon gas, 10 MBq, was injected into each tube, which was then closed. The samples were equilibrated for 2 h at 37° C. The gravity needed to compress the nerve pieces to a physiologic wet volume was not known. Therefore, various centrifugation speeds (200–2,500 rpm) were tested, each for 5 min before the emitted radiation in the gas and nerve phases were measured.

### Estimation of the Density of the Sciatic Nerve

The density of four sciatic nerves was estimated from the wet weight of the tissue and from the volume measured by immersion.

### Statistical Analysis

The statistical significance of differences between means was determined with Student's *t* test for paired samples.

## Results

### The Nerve–Blood Partition Coefficient

The blood–gas partition coefficient for $^{133}$Xe from 12 determinations was 0.16 ± 0.003 SEM. The nerve–gas partition coefficient was estimated from 23 separate measurements after centrifugation for 5 min at 200, 400, 600, 800, 1,000, 1,500, 2,000, and 2,500 rpm. There was no evident correlation to centifugation rate, and the mean value was 0.55 ± 0.011. Based on the figures above, the nerve–blood partition coefficient was calculated to be 3.42. The hematocrit value in 18 animals was found to be 36 ± 3.2%. The density of sciatic nerves was estimated to 1.09 ± 0.05.

### Basal Blood Flow and Evaluation of the Method

The disappearance rate of the $^{133}$Xe-gamma activity was stabilized within 1–2 min of injection and appeared monoexponential within the first 10–15 min. After semilogarithmic plotting and analysis, the k value was found to be 0.097 ± 0.005 SEM (n = 7) after epineurial injection and 0.126 ± 0.017 (n = 7) after endoneurial (intrafascicular) injection (ns) (fig. 1). After the measurements, the rabbits were killed by an overdose of barbiturate. The wash-out of tracer then was

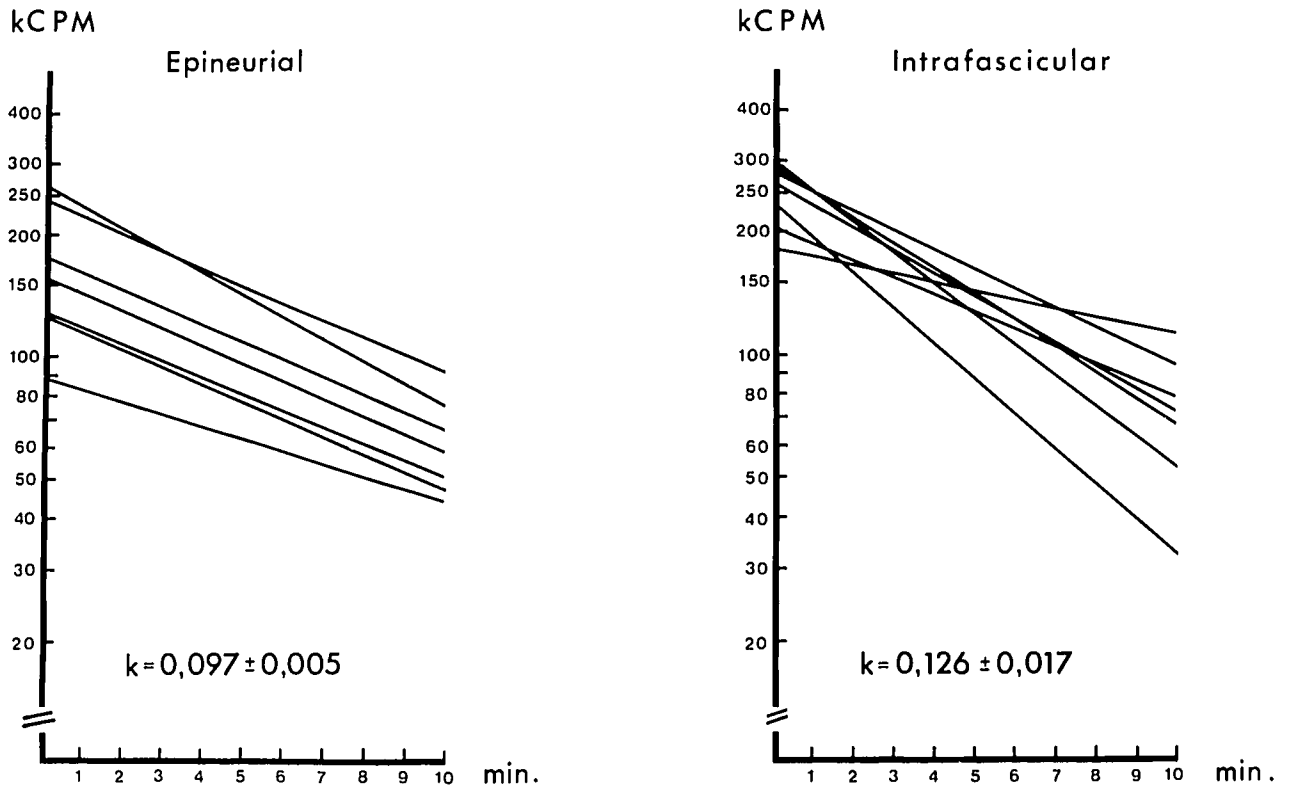


FIG. 1. Local radioactivity, semilogarithmically plotted, *versus* time after epineural or intrafascicular injection into the sciatic nerve. The appearance of the $^{133}Xe$ wash-out showed a stable monoexponential decay during the first 10–15 min.

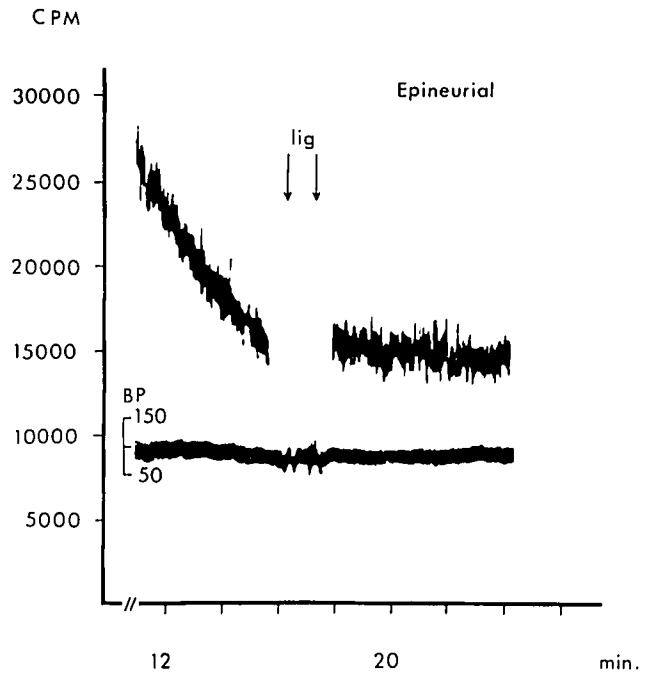


FIG. 2. A control experiment demonstrating that the wash-out of $^{133}Xe$ ceases when the blood circulation in the sciatic nerve but not in the surrounding muscle is stopped by ligation of the nerve. BP denotes arterial blood pressure.

found to level off, and no noticeable disappearance of $^{133}Xe$ was seen.

To test the possibility of tracer diffusion from the sciatic nerve into the surrounding muscles, despite the covering sheet of Mylar® in five experiments, the nerves were ligated on both sides of the place of injection without removing the Mylar®. The radioactivity curve then leveled off and no noticeable disappearance of tracer was detected. A representative recording can be seen in figure 2. From the k values obtained, the basal blood flow in the sciatic nerve was calculated to $30.4 \pm 1.6$ (SEM) ml · min$^{-1}$ · 100 g$^{-1}$ when the tracer was injected in the epineurium and $39.5 \pm 5.3$ ml · min$^{-1}$ · 100 g$^{-1}$ when the tracer was injected into a fascicle, giving a mean value of 35 ml · min$^{-1}$ · 100 g$^{-1}$.

### The Effects of Intraarterial Norepinephrine

During intraarterial infusion of norepinephrine (1–3 $\mu$g/min) in seven experiments, there was a distinct decrease in the disappearance rate of $^{133}Xe$. The k value decreased from $0.082 \pm 0.007$ (SEM) to $0.035 \pm 0.010$ ($P < 0.02$) corresponding to a blood flow reduction from $25.7 \pm 2.2$ to $11.0 \pm 3.1$ ml ·

$min^{-1} \cdot 100\ g^{-1}$. A representative experiment can be seen in figure 3. In three experiments the disappearance rate of $^{133}Xe$, again increased 2–4 min after stopping the norepinephrine infusion, but the average blood flow as calculated for all seven animals did not significantly increase within 10 min.

### The Effects of Electrical Stimulation of the Lumbar Sympathetic Chain

In seven experiments, the wash-out of $^{133}Xe$ was recorded before, during, and after electrical stimulation of the ipsilateral lumbar sympathetic chain (8 Hz, 5 V, 5 ms). A representative experiment can be seen in figure 4. Within 1 min after starting the stimulation, a significant decrease in the disappearance rate of $^{133}Xe$ was seen and the curves leveled off in all cases. On stopping stimulation, the disappearance rate of $^{133}Xe$ again increased within 2–4 min. The k values changed from $0.060 \pm 0.01$ (SEM) to $0.004 \pm 0.001$ ($P < 0.01$) to $0.040 \pm 0.006$, corresponding to a blood flow of $18.8 \pm 3.1$ to $1.3 \pm 0.3$ to $12.6 \pm 1.9$ ml $\cdot$ $min^{-1} \cdot 100\ g^{-1}$. The k value after stimulation did not differ significantly from the prestimulation value.

## Discussion

When radioactive $^{133}Xe$ dissolved in saline was injected locally into the sciatic nerve, a monoexponential wash-out of the tracer was detected during the first 15 min. The traumatic effect of the injection itself was reduced by using thin needles and small injected volumes. Since it is known that even a small increment of volume in the intrafascicular space profoundly increases the pressure,[12] one series of experiments was performed where the tracer was injected into the soft connective tissue in the epineurial space. The k values in this series of experiments did not differ significantly from those obtained when the fascicle was perforated, probably due to the fast intercompartment diffusion of Xe.

The basal blood flow values obtained with this technique in the epineurial and intrafascicular studies averaged 35 ml $\cdot$ $min^{-1} \cdot 100\ g^{-1}$ nerve tissue. However higher than the initial blood flows in the experiments with infusion of norepinephrine or sympathetic stimulation, there was no significant difference between the resting values of the four groups. These values should be compared with those obtained in the sciatic nerve of the rat with an indicator fractioning technique[7] of 11 ml $\cdot$ $100\ g^{-1} \cdot min^{-1}$ and those obtained with the microsphere technique of Tschetter *et al.*[8] in the sciatic nerve of the dog of 4.7 ml $\cdot$ $100\ g^{-1} \cdot min^{-1}$. In a more recent study,[9] using hydrogen clearance, the

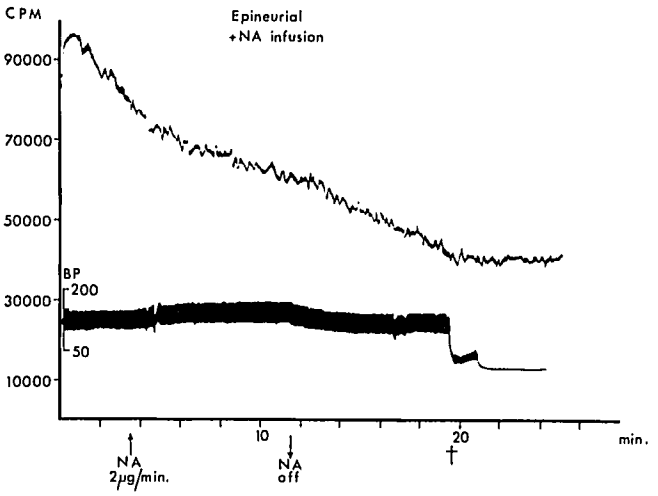


FIG. 3. The effect of intraarterial norepinephrine (NA) 1–3 μg/min on the wash-out of Xe from the sciatic nerve. BP denotes arterial blood pressure.

basal blood flow in the sciatic nerve of the cat was estimated to be 43 ml $\cdot$ $min^{-1} \cdot 100\ g^{-1}$. The basal intraneural blood flow level as estimated in the present study agrees with those obtained by other clearance techniques but is at variance with the single study where microspheres were used. These variations may be explained by differences in experimental design but will not invalidate the qualitative responses obtained. The human cerebral blood flow as measured by local injection of $^{133}Xe$ was found to be $83 \pm 5$ ml $\cdot$ $min^{-1} \cdot 100\ g^{-1}$ in gray matter and $25 \pm 3$ and $13 \pm 3$ in the subcortical and central white matter, respectively.[13]

The existence of adrenergic nerve endings in the walls of intraneural microvessels was demonstrated by

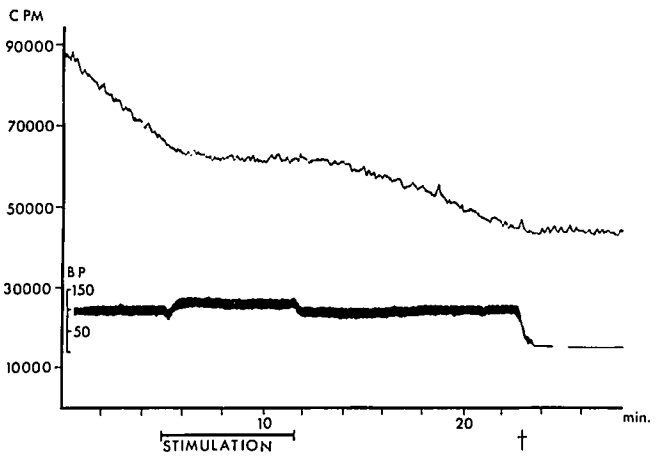


FIG. 4. The effect of electrical stimulation of the ipsilateral lumbar sympathetic chain on the wash-out of Xe from the sciatic nerve. BP denotes arterial blood pressure.

Lundborg.[4] He also demonstrated with intravital microscopy that stimulation of the lumbar sympathetic chain results in a marked constriction of the vasa nervorum of the tibial nerve of the rabbit. In the present study, it was found that norepinephrine at a dose of 1–3 μg/min into the aorta reduced the blood flow in the sciatic nerve from 25.7 to 11.0 ml · $min^{-1}$ · 100 $g^{-1}$ and that electrical stimulation of the lumbar sympathetic chain (8 Hz, 5 V, 5 ms) reduced the sciatic blood flow from 18.8 to 1.3 ml · $min^{-1}$ · 100 $g^{-1}$. When the stimulation was stopped, a wash-out of the tracer was seen again within a few minutes. The increased neurotoxicity of an intraneurally injected local anesthetic with epinephrine thus could depend on a prolonged exposure to the local anesthetic due to a decreased neural blood circulation. Fink *et al.*[14] showed in an *in vivo* study in the rat that addition of epinephrine 1 : 200,000 slowed the decline of intraneurally injected lidocaine about four times.

The wash-out technique with diffusible tracers has some inherent sources of error. It was demonstrated by Sejrsen and Tonnesen[15] that there is a shunting by diffusion of $^{133}Xe$ in the skeletal muscle from arteries to veins. The size of this shunting was estimated to be 11% of the tracer passing through the tissue. In this study, where the tracer is locally injected, a diffusion of gas from the veins into the arteries can be expected. This means that the arterial concentration of the tracer may not be negligible and therefore Kety's criteria are not fullfilled. The tracer in this way may leave the tissue slower than expected, and the blood flow therefore will be underestimated. This is particularly true when the transit of blood through the tissue is slow and the proportion of tracer shunted by diffusion is greater.

There seems to be no way in this experimental situation to quantify shunting by diffusion of gas from the veins to the arteries in the nerve. The vascular anatomy of the nerve has been studied by Lundborg,[4] who has shown that the vessels are arranged mostly parallel to the axis of the nerve but occasionally obliquely or perpendicular to the axis. Anastomoses frequently are found. At intravital microscopy, no special direction of blood flow seems to predominate in any segment of the nerve. There are no reasons on morphologic grounds to suspect that shunting by diffusion in the nerve is more pronounced than in skeletal muscle. In the present situation the blood flow values obtained during basal conditions are greater than expected and shunting by diffusion should be small. During adrenergic vasoconstriction, however, the blood flow could be underestimated. The fact that there is no wash-out of tracer when the lumbar sympathetic chain is stimulated implies that the blood flow is reduced markedly.

Experimental and clinical evidence has shown that the neurotoxicity of local anesthetics after intraneural injection is increased when combined with epinephrine. It seems probable that the admixture of a drug that can stimulate the alpha-receptors of the vasa nervorum reduces the blood flow through the nerve and thereby enhances the anesthetic drug toxicity.

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