Relation between Functional Deficit and Intraneural Local Anesthetic during Peripheral Nerve Block

A Study in the Rat Sciatic Nerve

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Background: During peripheral nerve block, local anesthetic (LA) penetrates within and along the nerve to produce the observed functional deficits. Although much is known about the kinetics and steady-state relation for LA inhibition of impulse activity in vitro in isolated nerve, little is known about the relation between functional loss and intraneural LA content in vitro. This study was undertaken to investigate the relation of functional change to intraneural LA.

Methods: A sciatic nerve block was performed in rats with 0.1 ml 1% lidocaine radiolabeled with 14 C. The total intraneural uptake of LA was determined at different times after injection, and the distribution of lidocaine along the nerve was assayed at different stages of functional block. Drug content was also compared with equilibrium lidocaine uptake in the isolated rat sciatic nerve.

Results: Total intraneural lidocaine in vitro increased to near steady-state in about 3 min, stabilizing at approximately 14.3 nmol/mg wet tissue for about 12 min before decreasing to zero at 70 min after injection. Although intraneural lidocaine was 1.6% of the injected dose during full block, only 0.3% was left when deep pain sensation returned and 0.065% was still detected when functions fully recovered. Despite these large differences in total lidocaine content, the longitudinal distribution remained constant. Intraneural lidocaine concentrations obtained at full block and partial recovery could be achieved in vitro by equilibration in 0.7–0.9 and 0.2–0.3 ms lidocaine, respectively.

Conclusions: During peripheral nerve block only a small amount of injected LA penetrates into the nerve. The intraneural content of LA correlates with the depth of functional block. (Key words: Anesthetics, local; lidocaine. Anesthetic techniques, regional; nerve block. Animals: rat. Nerve(s); neural function. Pharmacokinetics; lidocaine.)

WHEN peripheral nerve blocks are performed, a relatively large volume of a high concentration of local anesthetic (LA) is injected to ensure adequate anesthesia for surgery. For example, volumes of 20–30 ml 2% lidocaine (75 mg) are typically injected to block large nerves such as the sciatic nerve in humans, whereas less than 0.5 ml of only 0.4 mg lidocaine blocks impulse conduction in a desheathed rat sciatic nerve at 35°C in vitro. 1-3 The need for such large doses may be due primarily to pharmacokinetic factors; the human sciatic nerve is far more massive than the rat sciatic nerve and furthermore, only a small amount of the injected LA may reach the nerve. The nerve sheath (perineurium) is a very effective diffusion barrier to drugs and a substantial fraction of the delivered LA is removed by the systemic circulation and absorbed locally by the tissue surrounding the nerve. Indeed, the amount of injected LA that actually reaches the nerve and contributes to the block of neurologic function is unknown.

The neural LA content responsible for conduction block has been examined in previous studies in which it was correlated with changes in compound action potential (CAP) amplitude in isolated nerves in vitro, but the relation of CAP degradation to functional inhibition in vivo has never been established. It is in-

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correct to equate the kinetics of drug uptake assessed from these *in vitro* studies with LA kinetics *in vivo* because of the very different experimental conditions. For example, nerves *in vivo* are embedded within neighboring connective tissue and muscle, and are intimately associated with extra- and intraneural circulation. Furthermore, the nerves that are the source of CAPs that have been used to assess generalized electrophysiologic block *in vitro* contain a variety of axons that serve different neurologic functions in the respective animal species. Equating inhibition of A-fiber components of the CAP with "motor block" and C-fiber components with "sensory block" disregards our knowledge about fiber specialization and integrated actions of the nervous system. Finally, the conditions under which *in vitro* electrophysiologic studies were performed were rarely representative of the physiologic milieu (i.e., temperature, pH, CO₂, and glucose) in which those nerves function *in vivo* and these factors can profoundly affect potency. Another apparent difference separating functional deficits and CAP depression is found in the clinical evidence that afferent neural activity can still be recorded electrophysiologically when functional neuraxial block is achieved. Therefore, the degree to which impulse activity is reduced is not a proportional indicator of functional losses. For all these reasons, the drug uptake observed to block peripheral nerve conduction *in vitro* reflects poorly the amount necessary to block neurally mediated function.

The goal of these experiments was to correlate the intraneural content of LA with different functional stages of blockade during a sciatic nerve block in the rat, using a method where neurologic functions could be properly evaluated in the awake and unrestrained animal. We determined the net uptake and longitudinal distribution of radiolabeled lidocaine at times when full block, partial recovery and full recovery of different neural functions were observed. These amounts were compared with directly measured lidocaine uptake at equilibrium in the nerve *in vitro*, where drug concentration was controlled and constant.

Materials and Methods

*Lidocaine Uptake In Vivo*

**Animals.** Male Sprague-Dawley rats weighing 300–400 g were housed in the Brigham and Women's Hospital animal facilities with controlled room temperature (21 ± 0.5°C), controlled humidity, and a 12-h (6:00 AM–6:00 PM) light–dark cycle. Animal treatment for these experiments was approved by the Harvard Medical Area Committee on Animals. Experiments were performed between 9:00 AM and 3:00 PM at an ambient temperature of 21°C.

**Preparation of Radiolabeled Lidocaine.** A 37 mm lidocaine solution (1%) was prepared by dissolving 10 mg lidocaine HCl powder (MW270.8, Sigma Chemical, St. Louis, MO) per milliliter 0.9% NaCl buffered with 5 mm piperazine-N,N'-bis (2-ethane-sulfonic acid) (Sigma Chemical). The pH of the solution was adjusted to 6.9 with NaOH. [¹⁴C]Lidocaine (0.1 mCi/ml ethanol; Du Pont New England Nuclear Research Products, Boston, MA) was added to the lidocaine solution at 1:100 dilution to a final radiochemical activity of 5 × 10⁻³ mCi/ml.

**Injection of Lidocaine.** A unilateral sciatic nerve block was performed under brief general anesthesia: the rat's muzzle was held in a beaker containing a cotton ball saturated with sevoflurane or isoflurane (Abbott Pharmaceuticals, Chicago, IL) until corneal reflex disappeared, at which point the general anesthesia was removed. In a standardized way, 0.1 ml 1.0% [¹⁴C]lidocaine HCl (pH = 6.9) was injected with a 25G needle, bevel turned to the femoral head, between the greater trochanter and the ischial tuberosity. When the needle encountered the body of the ischium, the LA was injected over a period of approximately 3 s (rate = 33 μl/s). Using this technique a complete motor and sensory block could be reliably induced with 0.1 ml 1% lidocaine in all animals included in the study.

**Evaluation of Peripheral Nerve Block.** Neurologic function of proprioeception, motor function, and nociception was done as soon as the animal awoke from general anesthesia in a modification of the examination described by Thalhammer et al. Each test was done at 2 min intervals until all functions were fully blocked and every 3–5 min throughout the recovery of those functions. The animals were moving freely in a large open box (1 m × 1 m) during testing.

An assessment of the successful hind-limb blockade could be done as soon as the animal emerged from general anesthesia by observing spontaneous locomotor activity of the animal trying to right itself. Paddling and dragging of the treated leg as the animal tried to ambulate could be observed within 1 min of the lidocaine injection.

When the animal was fully awake (1–2 min), neurologic evaluation of both treated and contralateral control limbs would proceed as follows.
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Posture and Gait. Onset of block was evaluated by observing abnormal resting posture of the injected foot (plantar eversion) and gait abnormality as the animal started to ambulate.

Proprioception. Proprioception was evaluated with two tests used in veterinary neurology:

1. Tactile Placing Response. The rat was kept in a normal resting posture, hind-paw toes plantar-flexed with their dorsi placed on the table. With intact proprioception, the animal would immediately reposition its toes. If proprioception was impaired, this postural correction was absent.

2. Hopping Response. The rat was held with the upper body upright and the body weight resting on the tested hind limb. As its whole body was moved laterally, the animal's normal response would be to hop with the weight-bearing limb in the direction of the movement to avoid falling over. With block of proprioception, the animal would lose this response and the leg would drag on the table.

Motor Function. When an animal's forelegs are lifted off a supporting surface its tibiotarsal joint is extended to maintain posture (extensor postural thrust). In our experiments, block of motor function was defined as the complete absence of extensor postural thrust.

Nocifensive Response. The presence (+) or absence (−) of response to nocuous stimulation was tested for cutaneous and deep pain in the area innervated by branches of the sciatic nerve without physical restraint while the animal was in a resting posture.

Withdrawal of the limb after briefly squeezing (<2 s) a digit or a skinfold on the lateral aspect of the foot with serrated forceps was noted as a positive response, respectively, to deep or superficial nocuous stimuli. To ascertain that the lack of withdrawal was the result of a sensory block and not a motor block, the nocuous stimulus was applied similarly to the foot's medial aspect, which is innervated by the saphenous nerve. By using the hip flexors (femoral innervation) the animal could withdraw its leg even when the sciatic nerve was blocked. The afferent aspect of response to nocuous stimuli was considered absent when the animal could withdraw its leg after application of the stimulu to the medial side (saphenous) of the foot but not to the lateral side (sciatic).

A complete absence of response to all tests described above was defined as a "full functional block," response only to deep pain stimulation as "partial recovery" from block, and resumption of all functions as "full recovery."

Nerve Dissection and Measurement of Lidocaine Uptake. The course of lidocaine uptake was assessed using two protocols that differed in the criteria for timing of nerve uptake.

Total Lidocaine Uptake versus Time. In these experiments the animals were killed, regardless of the stage of functional block, at specific times after injection: 3 min (n = 11), 4 min (n = 3), 5 min (n = 5), 8 min (n = 3), 10 min (n = 3), 12 min (n = 2), 15 min (n = 3), 30 min (n = 3), 37 min (n = 3), 45 min (n = 3), 60 min (n = 2), 70 min (n = 3).

Uptake of Lidocaine Related to Functional Block. The animals were killed at three stages of the functional block: (1) during full block at 10, 15, and 30 min after injection; (2) as soon as the withdrawal response to deep pain stimulation returned (partial recovery, assessed by pinching the digit every other minute, starting 25 min after injection); and (3) when all functions had fully recovered (determined by testing all functions every 5 min after the return of deep pain).

As soon as a given stage of functional block was reached, the animals were killed by deep inhalation anesthesia with the saturated cotton ball (see above). Cardiorespiratory arrest was obtained in less than 30 s.

The sciatic nerve was dissected in situ in less than 3 min, from 10 mm proximal to the sciatic notch to the popliteal fossa, and then excised and frozen in less than 5 s on a flat surface of dry ice. The frozen nerve was cut into seven segments 5.7 mm long and desheathed under a dissecting microscope (×12).

It is important to define exactly what anatomic structure the sheath represents in our study. We removed the portion of connective tissue and neurovascular bundles around the nerve defined anatomically as the epineurium and the external portion of the perineurium; some of the deeper intraneural part of the perineurium probably remained.

Each dissected piece of nerve and, when noted, each piece of the surrounding sheath, was weighed on an analytical balance (±0.5 mg; Mettler AE 100, Hightstown, NJ) and then digested in a mixture of 0.5 ml tissue solubilizer (Solvable, Du Pont New England Nuclear) plus 0.1 ml distilled water at 50°C for 120 min. Five milliliters of Aquasol 2 (Du Pont New England Nuclear) liquid scintillation cocktail was added and radioactivity was assayed by liquid scintillation counting for 10 min.

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The specific radioactivity of the lidocaine solution was determined by adding 100 μl of the 1% [14C]lidocaine solution to Solvable (0.5 ml) and distilled H2O (0.1 ml) mixture and counted identically by liquid scintillation counting. Derived counts per minute (cpm) were divided by moles of lidocaine added to get the specific radioactivity. Efficiency of counting 14C was the same in all conditions.

The counts were corrected for sample background (10–15 cpm, n = 6), measured for a 5.7-mm piece of the contralateral sciatic nerve, unexposed to LA and removed at the time when the uptake was maximum in the injected nerve. The background nerve control showed no difference in counts compared with the background measured without nerve. The radioactivity was assumed to represent lidocaine HCl (product listed radiochemical purity > 98%) and tissue drug was expressed as nanomoles lidocaine per milligram wet weight of nerve, taking into account the specific radioactivity of the injected solution for each experiment. The total lidocaine was determined by summing the amount of lidocaine present in all segments counted. The “percentage uptake” was the ratio of the total moles of lidocaine present in the nerve over the total moles injected.

Statistical Analysis. Values of drug content were given as mean ± SE. Testing for the difference in lidocaine uptake in time and the difference in drug uptake at the three stages of functional block was achieved by Student’s t test. A P value < 0.05 constituted a statistical difference.

Lidocaine Uptake In Vitro

We determined the equilibrium uptake of lidocaine by an ensethral rat sciatic nerve exposed to different concentrations of radiolabeled lidocaine for 60 min. The purpose of these experiments was to correlate uptake in vitro with drug concentrations found in the nerve in vivo during full and partial block of function.

Radiolabeled Lidocaine Preparation. Lidocaine solutions were made in four concentrations (0.5, 1, 1.9, and 3.7 mM) by adding lidocaine HCl powder to modified Liley solution (NaCl 118 mM, KCl 5 mM, CaCl2·2 H2O 2 mM, NaHCO3 25 mM, NaH2PO4 1.2 mM, MgCl2·6 H2O 1 mM, Na2HPO4 3.6 mM, and glucose 10 mM). The solution was bubbled with a mixture of 95% O2/5% CO2 and its pH maintained between 7.4 and 7.5 at 37°C in a water bath. Ten microliters [14C]lidocaine was added per 5 ml lidocaine solution (1:500).

Sciotic Nerve Preparation. Male Sprague-Dawley rats (300 g) were killed by a lethal dose of pentobarbital administered intraperitoneally. The ensetheated sciatic nerves were immediately dissected as described in the in vivo experiments. Care was taken to ligate distal and proximal ends as well as the side branches of the nerve at the point where they exit the sheath to avoid uptake of lidocaine through the cut ends of the nerve.

The ensetheated nerves were incubated in the radiolabeled lidocaine solutions at 37°C for 60 min. At the end of the incubation the nerves were removed, placed on dry ice after excess solution was blotted off, de-seathed, weighed, and then processed for digestion and counting as described for the in vivo experiments.

Data are presented as lidocaine uptake (nanomoles per milligram wet tissue) (mean ± SE) versus lidocaine concentration (millimolar).

Results

All animals awoke rapidly after exposure to the general anesthetic, permitting an evaluation of the neurologic functions as early as 1 min after injection of LA.

Evolution of the Functional Block in Time

Proprioception, motor, and nociceptive functions were fully blocked within 5 min of the LA injection (3–5 min, n = 17). Functions started to recover at about 35 min (range 32–40 min, n = 5), at which point the sensation to deep pain recovered first, followed by motor function and superficial pain, and finally by proprioception at 65 min. All functions tested were fully recovered at 70 min (n = 5).

Total Lidocaine Uptake in the Nerve over Time

The time course of total lidocaine uptake by the nerve consisted of a phase of rapid increase (from 0 to 5 min), a period of relatively constant concentration (from 5 to 15 min) and a period of decline (from 20 to 60 min) (fig. 1). During the first 3 min, the intraneural uptake increased very rapidly. Then the intraneural content of lidocaine remained within the range of 1.5 ± 0.5% (n = 11 at 3 min) to 1.9 ± 0.17% of the injected mass (n = 3, at 15 min). Over those first 15 min the mean lidocaine concentration was 14.3 ± 1.5 nmol/mg wet nerve (n = 30). These values of uptake at the different times to 15 min after lidocaine injection.

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Lidocaine Distribution at Different Stages of Functional Block

During the times when all functions were blocked (5 to 35 min) the total lidocaine present in the nerve averaged 61 ± 4.1 nmol (n = 13), or 1.6 ± 0.12% of the total injected dose. The longitudinal spread of lidocaine (quantified below) extended over five or six segments (28–34 mm). The concentrations in the segments with the highest lidocaine content were respectively 6.8 ± 0.4, 6.8 ± 0.8 and 6.8 ± 0.9 nmol/mg wet nerve at 10, 15, and 30 min (n = 5, 4, 4) (figs. 2A–2C).

At the return of deep pain (32–40 min) the total amount of lidocaine present in the nerve had decreased to a mean of 12 ± 1.3 nmol (n = 5), or 0.33 ± 0.035% of the total injected dose. The extent of longitudinal spread was four or five segments (22.8–28.5 mm). Lidocaine content in the segment with the highest concentration was 1.6 ± 0.15 nmol/mg wet nerve (fig. 2D).

When all functions were fully recovered a minimal amount of lidocaine was still present in the nerve, about 2.3 ± 1.3 nmol (or 0.065 ± 0.035% of the injected dose). The longitudinal spread of lidocaine was 2–3 segments (11.4–17.1 mm). Lidocaine content in the segment with the highest concentration was 0.18 ± 0.01 nmol/mg wet nerve (fig. 2E).

Over the course of the block the injected LA solution may spread along the outside of the nerve and intraneural LA molecules might also diffuse longitudinally. These processes were assessed quantitatively by graphically analyzing the longitudinal distribution of lidocaine.

The distribution of lidocaine over the different longitudinal segments was fit regressively by a Gaussian function with specified central amplitude L(0) and width (σ):

\[ L(x) = L(0) \times \exp[-2((x - x_c)/\sigma)^2] \]

where \( L(x) \) = the intraneural lidocaine content at a distance \( x \) from the center of the distribution \( x_c \), and \( L(0) \) = the intraneural lidocaine content at \( x_c \). This continuous function fits well the measured, quantized distribution in discrete segments, as seen by the dotted lines in figure 2, and provides an accurate estimate of lidocaine spread within the nerve at different times.

Early after the onset of full block (10 min), σ equaled 9.4 mm and maintained this value throughout the full block period (8.6 mm at 15 min, 8.9 mm at 30 min), expanding only slightly (11.6 mm) during partial re-
covery (return of deep pain). Detectable concentrations of lidocaine were spread over all segments in nerves taken at the time of full functional recovery (fig. 2E), but the distribution was obviously asymmetric and could not be fit by the Gaussian function. The actual concentrations of lidocaine were also very low and difficult to assess quantitatively with much confidence.

Of note, in 14 animals nerve length first measured in situ was compared with the length of the nerve after rapid freezing on dry ice. A “shrinkage factor” of 8.1 ± 2.7% (mean ± SE) was found. This represented a maximum difference of 7.9 mm or a mean difference of 2.7 mm in length of the nerve in situ compared with frozen. Although this factor would alter the estimate of true spatial profile of lidocaine in vivo, it would be applied uniformly to all samples and would not affect the measured time course of uptake or the actual drug content.

In Vitro Lidocaine Uptake

What concentration of lidocaine in a solution bathing the nerve to equilibrium would produce the intraneural drug contents that we assayed during the various phases of block? We answered this question experimentally by incubating ensheathed, isolated nerves in different lidocaine solutions under physiologic conditions. Lidocaine uptake at equilibrium in the nerve in vitro increased proportionally to increasing lidocaine concentration in the bathing solution (fig. 3). Intraneural drug concentrations after exposure for 60 min to 0.5, 1, 1.9, and 3.7 mM lidocaine were, respectively, 2.19 ± 0.058 (n = 3), 4.42 ± 0.147 (n = 3), 6.93 ± 0.277 (n = 9), and 12.11 ± 1.14 (n = 4) nmol/mg wet. (Sixty minutes was found to be sufficient for lidocaine uptake to reach its equilibrium value in these ensheathed nerves at 37°C [data not shown]).

An “average” value of lidocaine in the whole nerve can be estimated by the concentration of lidocaine at the regions that are ±2° from the peak concentration (fig. 2). These values of lidocaine were assessed during complete block and partial recovery and are indicated by arrows on the y axis of figure 3. Their intersection with the experimental uptake line demarcates a range of corresponding free drug concentrations on the x axis.
The lidocaine content in the sheath has a different relation to the intraneural lidocaine content at different phases of block. During onset the mean of the ratios of sheath to nerve lidocaine (each expressed as nanomoles per milligram wet tissue) equaled 1.92 ± 0.23 (n = 7), at full block this ratio equaled 1.57 ± 0.19 (n = 4) and at partial recovery it equaled 0.54 (0.77 and 0.31, n = 2). These changing ratios reflect a gradient of drug from the sheath to the nerve that declines over the course of the block.

**Discussion**

In the past, clinical investigations have primarily explored the relation between physicochemical properties\(^6\) or dose\(^1\) of the administered LA and the functional aspects of nerve block, whereas neurophysiological consequences of LA action have been the primary concern of in vitro studies of CAP\(^1,3\) or single nerve impulses.\(^15\) Measurement of drug uptake, behavioral effects, and electrophysiologic changes all in one species would be useful for the assessment of differential nerve block and its underlying mechanisms. In the current experiments, we determined values of lidocaine uptake in vitro during functional loss and recovery from sciatic nerve block in awake animals, as well as equilibrium uptake in vitro. This discussion compares these results with previously published results and relates the data on content and behavior to reported electrophysiologic effects of lidocaine.

The onset and duration of neural function blockade in our model differed in certain aspects from those in other models studying pharmacokinetics of lidocaine in the rat. In studies on pharmacokinetics of lidocaine in the rat intraorbital nerve, using 0.2 ml 1% lidocaine, onset of block was observed within 1 min of LA injection and sensory block lasted about 100 min.\(^19,20\)

In our study, where half that dose was injected, block appeared within 1–2 min and was complete by 5 min and all functions had recovered in about 60 min. In another study of pharmacokinetics of lidocaine for sciatic nerve block in the rat, recovery from motor block was observed 56 min after injection with 0.2 ml 1% lidocaine.\(^21\) Despite the smaller dose of LA used in the current study, we observed about the same duration of motor block. To explain some of the differences in block evolution and duration between the studies one must consider that different response criteria and stimuli were used while evaluating the functional block. Furthermore, the presence of different levels of general
anesthesia has to be considered as a potential contaminant of the functional evaluation.

The amount of drug injected in our experiments is close to the minimal amount that would produce a complete functional block, as an incomplete and much shorter functional block was achieved with 0.1 ml 0.75% lidocaine. In other unpublished experiments conducted in our laboratory we have observed significant differences in duration of functional loss accompanying sciatic nerve block when the same dose of LA was injected at different pHs. This raises a further caution in comparing the results among different studies: injected solutions should be as similar as possible if the drug, not the formulation, is of primary interest. We chose to buffer our solution at pH 6.9 because we wanted injections of a constant, predictable pH close to physiologic conditions but still capable of containing 1% lidocaine in solution. We were not able to dissolve 1% lidocaine at pH 7.4 and 36°C, however, so we chose the highest pH at which this dose was soluble. We assumed that during the period of the block in vivo, the rat’s endogenous buffering capacity, from interstitial fluid and tissues, would eventually dominate the region exposed to the lidocaine solution, raising the pH near to 7.4; but we do not know how rapidly this occurs.

In the in vitro studies the pH and buffer system were chosen to sustain neuronal physiologic functions and metabolism at near-physiologic conditions over the incubation period of 1 h, simulating the situation in vivo.

It is interesting that the minimal ratio of dose to body weight producing a full block of function seems to be the same for rats and humans. In humans successful blockade of the sciatic nerve has been achieved with 10 ml 2% lidocaine although, traditionally, larger doses are injected; this corresponds to a dose-weight ratio of 2.86 × 10⁻³ g/kg for a 70-kg person. A fortuitously identical value of 2.86 × 10⁻³ g/kg calculated for a 350 g rat is shown in this study to provoke a complete functional block. Despite this agreement, analogies of scale must be made cautiously because the nerve fibers and their physiologic parameters are dimensionally identical in large and small mammals and are not scaled to length or mass. The spread of lidocaine that we measure in the rat is over a length (1–2 cm) where decremental conduction block occurs; these small changes in the length of axons containing the LA can produce decisive differences in impulse propagation or failure. In humans, the spread of lidocaine from the 100-fold larger volume probably extends over 5–10 cm, a length for which small variations in exposure length probably are much less important for determining impulse blockade.

The mean amount of lidocaine, 6.2 nmol/mg wet, found here in the nerve segment of highest concentration at 30 min of complete functional block was half of that reported by Fink et al. in the rat infraorbital nerve in vivo, a value of about 11 nmol/mg wet at the site of injection 30 min after injection with 0.2 ml 1% lidocaine. Grossly, it appears that intraneural drug content is proportional to injected dose (volume) and not concentration. Scurluck and Curtis found a value of about 7.4 nmol/mg wet using the same infraorbital technique, but they washed the nerve with normal saline to remove drug not bound to tissue, a procedure that may account for this lower value.

In the current study the total amount of lidocaine in the nerve when response to deep pain recovered was 4 nmol/mg wet, about one third of the lowest amount at which full block was observed. The total amount in the rat infraorbital nerve at the end of behavioral block was a comparable 3.7 nmol/mg wet, despite the higher maximum value. Similarly, the mean amount of drug we found at partial recovery in the segment with the highest concentration, 1.73 nmol/mg wet, was close to the maximum amount of lidocaine (1 nmol/mg wet) present in the rat infraorbital nerve at the return of the response to pinching of the upper lip.

Residual lidocaine could be measured over a short distance (11.4 mm) when all functions had recovered at about 1 h. Our mean total residual concentration of 0.67 ± 0.41 nmol/mg wet is twice the value (but within the SE) of 0.33 nmol/mg wet described by Ackerman et al. when motor paralysis subsided 56 min after injection of the LA.

By comparing uptake results in the rat sciatic nerve in vivo and in vitro we were able to relate the amounts of lidocaine obtained at the different behavioral end points to those achieved by incubation to equilibrium. The minimal total amount of intraneural lidocaine that induced a complete block of function was 33 nmol (8.7 nmol/mg wet), which corresponds to an exposure of the enmeshed nerve in vitro to a lidocaine concentration of 2.25 mm for 60 min (fig. 3). To achieve the content of 1.6 nmol/mg wet present in the most concentrated segment when deep pain returns one would have to equilibrate the nerve in vitro with a 0.5 mm lidocaine solution.

The “average” uptake at a distribution that is 2.8 wide of 5–4 nmol/mg wet obtained during full block of function would result from equilibration with a
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bathing solution of 0.7–0.9 mm lidocaine. At the return of deep pain at least 1 nmol/mg wet was contained in a 2 g length of nerve, a concentration that would be obtained in vitro after equilibration with 0.25 mm lidocaine. These equilibrating concentrations can be compared with lidocaine concentrations causing different degrees of CAP inhibition, as previously described by Rosenberg and Heavner. They showed that the A-fiber CAP recorded from a desheathed rat sciatic nerve was reduced about 50% after 30–45 min in a 0.25 mm lidocaine solution and by about 75% after exposure to 0.4 mm for the same time. Stys et al., using the area of the A-fiber CAP in the isolated rat optic nerve, reported that 1.0 mm lidocaine completely abolished the signal and 0.1 mm reduced it by about 10%. The findings of both of these studies are plotted in figure 4. Of course, these considerations apply only to the A-fiber elevation of the CAP, and no direct extension can be made either to the C-fiber signal or to individual nerve fibers, nor is the effect of repetitive stimulation accounted for.

With these caveats it is still noteworthy that the intraneural lidocaine at full functional block corresponded to equilibrium uptake from a solution of drug known to inhibit the A-fiber CAP completely, and that the content at partial functional block corresponded to a concentration that only partially (50%) inhibited the A-fiber CAP.

One additional comment on correlating the in vivo and in vitro observations is necessary. In vitro conditions were chosen to yield equilibrium uptake but the maximum uptake in the most concentrated segment in vivo, about 9 nmol/mg wet, is only 25% of the 36 nmol/mg wet that is found in the ensheathed nerves equilibrated in vitro with 1% lidocaine. Clearly, even the maximum uptake from the nerve at the injection site in vivo is far from equilibrium with the injected solution. As further evidence for equilibrium in vitro, the specific lidocaine uptake (nanomoles per milligram wet) of the sheath equalled that of the nerve after 60 min incubation (data not shown). By comparison, the ratio of sheath lidocaine to neural lidocaine is unequal and progresses from greater than one to less than one through the course of functional block. This implies that a gradient of lidocaine concentration exists at the mantle region of the nerve and that both functional block and intraneural lidocaine content, although they reach a steady state from 10–30 min, never achieve a condition of LA equilibration.

A longitudinal gradient of lidocaine was present throughout the block and, surprisingly, the extent of LA spread within the nerve did not change much from onset to recovery. It seems that hydrodynamic flow at and just after the injection distributes the drug around and along the sciatic nerve and that subsequent intraneural migration and diffusion of the drug is exclusively along a radial direction. The drug appears to move little along the longitudinal direction, which has a distribution over 35 mm in a nerve 2 mm in diameter. The ratio of longitudinal spread to radial penetration may be an important parameter in defining the pharmacokinetics of clinical nerve block and may, because of anatomic dimensions, differ substantially between humans and rats.

In this study we have shown the relation between intraneural lidocaine and the degree of blockade of neural function. Intraneural lidocaine at full block is consistently about three times that when sensation to deep pain returns while other neurologic functions are still blocked. Residual lidocaine was detected in the nerve after all neurologic function had returned but the effects on impulse activity are not known. The relation of intraneural lidocaine to applied concentration and the varying ratio of sheath:nerve lidocaine indicate

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the presence of a gradient of lidocaine radially, into the nerve, in addition to the persistent longitudinal gradient that was measured throughout the block.

The awake, unrestrained rat is reliable for study of the pharmaco logic characteristics of LAs in vivo and the behavioral correlates of peripheral neural blockade. Determination of the amount of LA present during the full course of a nerve block in vivo is crucial to a study of the action of LAs on clinically relevant impulse conduction in isolated whole nerves and single nerve fibers.

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