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Kinetics of Uptake and Washout of Lidocaine in Rat Sciatic Nerve *In Vitro*

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Abstract

Background—The potency and efficacy of local anesthetics injected clinically for peripheral nerve block depends strongly on the rate of neural drug uptake. However, since diffusion into surrounding tissues and removal by the vascular system are major factors in the overall distribution of lidocaine *in vivo*, true kinetics of drug/neural tissue interactions must be studied in the absence of those confounding factors.

Methods—Uptake: Ensheathed or desheathed isolated rat sciatic nerves were exposed to [¹⁴C]-lidocaine for 0–180min and then removed and the lidocaine content of nerve and sheath analyzed. Washout: Isolated nerves were soaked in [¹⁴C]-lidocaine for 60min and then placed in lidocaine-free solution for 0–30min, with samples removed at different times to assess the drug content. Experimental variables included the effects of the ensheathing epineurium, lidocaine concentration, pH, presence of CO₂-bicarbonate, and incubation duration.

Results—The equilibrium uptake of lidocaine increased with incubation time, concentration and the fraction of molecules in the non-ionized form. The uptake rate was unaffected by drug concentration, but was about halved by the presence of the epineurial sheath, with the washout rate slowed less. Slight alkalization, from pH 6.8 to pH 7.4, by bicarbonate-CO₂ buffer or a non-bicarbonate buffer, enhanced the neural uptake, and to the same degree. The washout of lidocaine was faster after shorter incubations at high concentrations than when equal amounts of lidocaine were taken up after long incubations at low lidocaine concentrations.

Conclusion—Lidocaine enters a nerve by a process other than free diffusion, through an epineurial sheath that is a slight obstacle. Given the rapid entry *in vitro* compared to the much

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smaller and transient content measured *in vivo*, it seems highly unlikely that lidocaine equilibrates with the nerve during a peripheral blockade.

Introduction

In the performance of a peripheral nerve block, local anesthetic (LA) is deposited near the nerve using anatomical landmarks, often abetted by ultrasound guidance and nerve stimulator techniques. Drug molecules diffuse through the surrounding tissues to reach the peripheral nerve and interrupt nerve conduction. Deficits in sensory and motor function result when the intraneural content of LA reaches a particular level, L_c ,¹ and when the longitudinal spread is sufficient for a critical distance of exposure, d_c .² Both L_c and d_c may be functional or anatomical characteristics of each particular class of nerve fiber.³

After a bolus injection the LA concentrations around the nerve are varying continually and equilibrium may not be reached at any time during a procedure. Not only is the surrounding concentration of LA changing due to tissue binding, vascular uptake and diffusion, but the pH varies starting from that of the typically somewhat acidic injectate, pH ~6.5,⁴ and moving towards the normal pH, ~7.4, buffered by the local tissue and fluids. Further complicating the dynamics of LA distribution, the LA itself, and some of the adjuvants, including added bicarbonate:CO₂, modify local vascular tone and thus influence the drug's removal.⁵

Functional nerve blocks characterized by deficits in sensory, skeletal motor and autonomic functions result when the blockade of conducted action potentials reaches such a degree that weakness and partial numbness occur, followed by paralysis and total anesthesia. Within the nerve, different modalities of nerve function are blocked depending on the fibers that support those functions and their conduction sensitivity to LAs. The most susceptible fibers are the small myelinated fibers, A-gamma, that account for rapid motor block, and A-delta, that obtund fast pain and cold sensation. These are followed by impulse blockade in the larger myelinated A-beta and A-alpha fibers, accounting for loss of touch and a secured motor block. Against traditional teaching, but supported by the overwhelming majority of experimental studies, the least susceptible to LA block are the small non-myelinated C-fibers that carry slow pain and warm sensation, and among these fibers it is the slowest conducting that are the most resistant to block.^{1,6}

Thus, the overall time course of functional nerve block that occurs clinically is a complicated phenomenon that depends on the pharmacodynamics and complex pharmacokinetics of LA action. Earlier studies on neurologically characterized blocks by lidocaine in rat sciatic nerve⁷, and the corresponding kinetics of lidocaine's neural distribution *in vivo*⁸, showed that after the minimally effective concentration of 1% (37 mM) injected as a 0.1 mL bolus, lidocaine was rapidly but inefficiently taken up; less than 5% of the injected dose actually was detected in the nerve during the block. Manipulations of the local vascular flow, with epinephrine,^{9,10} and of the solution pH and composition,¹¹ were able to modify the block duration and the uptake, altogether demonstrating the critical role of lidocaine's local pharmacokinetics in determining nerve block.

Since multiple factors influence these kinetics *in vivo*, it is desirable to conduct an *in vitro* study that simplifies the *in vivo* situation by excluding extraneural anatomical and vascular factors and permits the direct observation of neural uptake while under controlled conditions. In the isolated nerve, uptake of LA is unaffected by diffusion into adventitia, fat and muscle. The effect of the epineural sheath on LA distribution can be observed by comparing uptake and washout between ensheathed and desheathed nerves. The concentration of LA can be preset and remains unchanged for the duration of the experiment

while the duration of incubation in LA is controlled. Composition of the bathing solution can be adjusted and the effect of drug concentration and pH can be determined. Finally, the distribution of LA within the nerve can be implied by observing the washout of LA after different intervals of incubation.

Methods

Nerve preparation

Male Sprague-Dawley rats weighing 250-300 gm were euthanized by a lethal dose of sodium pentobarbital (Nembutal, Sigma-Aldrich, St. Louis MO, 50mg/rat i.p.), in a procedure approved by the Harvard Committee on Animals. Both sciatic nerves were then dissected from the hip to the ankle within 10 minutes postmortem. Both ends of the nerve as well as the side branches were ligated with silk 5-0 sutures to avoid uptake of lidocaine through the cut ends of nerve. The segment of nerve removed was about 30-35 mm long, and effort was made to use the segments with the fewest branches.

Solutions of Lidocaine

A 37 mM (1%) stock solution of lidocaine-HCl was made by dissolving 10mg lidocaine hydrochloride powder (MW 270.8, Sigma-Aldrich Chemical Co, St Louis, MO.) per 1.0 ml of 154 mM (0.9%) NaCl buffered with 5mM PIPES (Piperazine-N,N'-bis[2-ethanesulphonic acid], Sigma, St Louis, MO). The pH of this stock solution was adjusted at 22°C to 6.9 with NaOH.

Three incubation solutions of lidocaine were composed to explore the role of different factors in uptake kinetics. **1.** Lidocaine solutions were prepared by diluting the stock solution into *PIPES Buffered Saline, PBS*, composed of (in mM): NaCl 154, PIPES 5, adjusted to pH= 6.8 - 6.9 at 37° C with NaOH.

2. To more closely approximate the *in vivo* conditions, when a complex ionic solution fills the interstitial space, lidocaine was diluted in *Liley's* physiological buffer composed of (in mM): NaCl 118, KCl 5, CaCl₂·2H₂O 2.0, NaHCO₃ 25, NaH₂PO₄ 1.2, Na₂HPO₄ 3.6, MgCl₂·6H₂O 1.0, glucose 10.0. The solution was bubbled with 95% O₂/5% CO₂ and maintained at 37° C in a water bath. Stock solution of lidocaine was added to this Liley's solution to reach final concentrations, as noted. The final, "physiological" pH equalled 7.4 - 7.5.

3. In order to separate the complex effects of the physiological solution from the effects of pH buffering *per se*, as represented by solutions **1.** and **2.**, lidocaine solutions were made by adding lidocaine hydrochloride to *Bicarbonate Buffered Saline, BBS*, composed of (in mM): NaCl 150, NaHCO₃ 15, and bubbled with 95% O₂/5% CO₂; final pH=7.4-7.5 at 37°C. This *BBS* solution had an ionic composition close to that of *PBS* but with a pH and buffer identical to *Liley's*.

Concentrations of Lidocaine

The lidocaine concentration bathing the nerves was initially set at 1% (37 mM), as used for published *in vivo* experiments in rats.^{8,9,12,13} However, the amount of lidocaine taken up into the nerve *in vitro* was so far in excess of the amount found *in vivo* that the lidocaine concentrations were adjusted to: 0.5mM (0.0135%), 1mM (0.027%), 1.9mM (0.05%); 3.7mM (0.1%); 7.4mM (0.2%) and 19mM (0.5%). Radio-labelled [¹⁴C]-lidocaine (Dupont-New England Nuclear, Boston MA, 0.1mCi/mL ethanol) 5μL was added to 10 mL of the lidocaine solutions to give a final radiochemical activity of 5×10⁻⁵ mCi/mL. This dilution results in ~1% of the lidocaine molecules carrying the radioactive ¹⁴C nucleus, but these

molecules are identical in chemical reactivity to those with ^{12}C at the same position on the molecule. The ratio of labelled to unlabelled lidocaine molecules remains the same in all experiments and consequently, the radiolabel serves as a tag to follow the total lidocaine uptake into tissues. In the following text the final radioactive lidocaine is abbreviated **LID*.

Uptake procedures

The sciatic nerve (composed of surrounding epineurial sheath and inner nerve fibers, perineurial barriers and intraneural blood vessels ¹⁴⁻¹⁷) had its cut ends tied off, by 5-0 silk suture, to prevent **LID* diffusion into the ends. Nerves were immersed in lidocaine solution for periods of 5, 10, 30, 60 and sometimes 120 and 180 minutes. At the end of the specified time, nerve was removed from the lidocaine solution, excess solution was blotted on Whatman No.1 filter paper and the nerve frozen immediately on a small block of dry ice, preventing any redistribution of the LA.

The frozen nerve was then “desheathed” by plucking off the outer, epineurium sheath with No. 6 jeweler’s forceps (Dumont Instruments), under a dissecting microscope at 12X magnification (Wild Heerbrugg M5, Switzerland). The nerve and the epineurium were separately weighed (1 to 10mg, Mettler Balance, Model AE 100) and placed in a vial containing 0.1ml double deionized water (DDW) and 0.5ml of tissue solubilizer (Solvable R, DuPont, NEN Research Products, Boston MA).

Two other vials were made up and managed in the same way; one contained only 0.1ml DDW and 0.5ml Solvable to determine background radiation whereas the second vial contained, in addition, 0.1ml of the incubating **LID*, to determine the specific radioactivity of the lidocaine solution.

The tightly capped vials were then placed in a heated oven at 60°C for 90 minutes. At the end of this period, the vials were checked to confirm that the tissue had been digested. Five mL of liquid scintillation fluid (Aquasol 2, NEN-DuPont) was added to each vial and radioactivity was assayed by liquid scintillation counting for 10 min. The radioactivity observed was assumed to represent lidocaine HCl (chromatographically determined to be 99.8% radiochemically pure by the supplier) and the derived counts-per-minute were divided by the total moles of added lidocaine to calculate the specific radioactivity. Intraneural content of drug was expressed as nmoles lidocaine per mg wet weight of nerve. Efficiency of counting ^{14}C was the same in all conditions.

Effects of the epineurial sheath

The effect of the superficial sheath (epineurium) on the uptake and washout of lidocaine was measured to determine its influence on lidocaine’s penetration into nerve. The ensheathed nerve was treated as described above. For the desheathed nerve, the circumferential epineurium was removed by dissecting forceps and No. 4 iridectomy scissors under 12X magnification (Wild Herrbrugg M5 dissecting microscope) before immersing the nerve in the **LID* solution. The nerve, ensheathed or desheathed, was removed at specified times and the intraneural lidocaine content estimated as described above.

Washout experiments

The rate at which lidocaine exited the nerve was determined in washout experiments into lidocaine-free solutions. At the end of a controlled uptake period, the sciatic nerves were placed in a large reservoir (500ml) of drug-free *Liley’s* solution which was bubbled with 95% O_2 /5% CO_2 at 37°C for 0, 2, 5, 10, 30 or 60 minutes. At specified times, 3-4 nerve segments were removed, the external fluid gently blotted off on Whatman No.1 paper and the nerve placed on dry ice, desheathed, weighed, digested and the content of lidocaine

remaining in the nerve determined by liquid scintillation counting, as for the uptake experiments.

The influence of the epineurium on washout was studied by comparing washout in ensheathed nerves, with epineurium left intact during the washout, and only removed after the nerve was taken from the washout reservoir, with washout in desheathed nerves from which the epineurium was removed before the initial uptake procedure.

In addition, the washout kinetics of lidocaine were compared after two nerve incubation procedures, one of long duration and the other of short duration but in a higher *LID concentration so as to achieve a similar intraneural content before washout. From the preceding uptake experiments it was estimated that similar intraneural levels of lidocaine resulted by incubating the nerve in either *LID 1.9 mM (0.05%) for 60 minutes or in 7.4 mM (0.2%) *LID for 5 minutes, both yielding an uptake of 7-9 nmol/mg wet tissue. Sciatic nerve was incubated in lidocaine 7.4mM (0.2%) for 60 minutes.

The radioactivity of the solution in the washout reservoir was measured by placing 0.1ml of this solution in a vial with 0.1ml DDW and 0.5ml Solvable and treating it identically to the other vials. These “reservoir counts” ranged from 11.3 to 20.1 cpm, not different from background (with no added radioactivity), 9.4 to 17.2 cpm, indicating that the eluted lidocaine was diluted by the washout solution to undetectably low levels.

Wet vs Dry Weights

The intraneural content of lidocaine might be affected by the intracellular uptake or efflux of water and nerve swelling or shrinking after prolonged incubation. To test for this possibility sciatic nerves were incubated in 37mM lidocaine for 30, 60, 90 and 180 minutes and then desheathed and weighed wet. The desheathed nerves were then placed in a hot air oven and dried for 90 minutes and reweighed. The wet weight was compared with the dry weight for each incubation period and the ratio was found to be not different for the various incubation times. The wet/dry ratio for 30, 60, 90, and 120 min, was 3.54, 3.20, 3.54, and 3.64, respectively, indicating that swelling by water uptake did not occur during the incubations.

Analysis

Data on lidocaine content were collected and averaged for each time and condition for 4-6 separate pieces of nerve, derived from 3-4 rats in each experiment. Each experimental condition was examined in 4-5 separate experiments (except for the washout experiment shown in Figure 6); the mean $\pm$ SD for lidocaine content among these 4-5 independent experiments is reported here. Lidocaine nerve content, symbolized by L_N , is shown as a function of *LID concentration, [*LID], after long (60 min) incubations, and assumed to be in equilibrium with free *LID at concentration [*LID]_s. Such equilibrium data were fit by a simple binding curve with a derived maximum binding capacity (L_{max}) and an equilibrium dissociation constant (K_L), by equation 1:

$$L_N = L_{max} \frac{[*LID]_s}{[*LID]_s + K_L} \quad (\text{Eq. 1})$$

Uptake kinetics were determined by fitting the nerve (or sheath) content versus incubation time (t) by a single exponential function:

$$L_N = L_{\infty} [1 - \exp(-t/\tau)] \quad (\text{Eq. 2})$$

where L_N is the nerve (or L_E the sheath) content of *LID at time t , L_∞ the “equilibrium” content at infinity, and τ (tau) the uptake time-constant that best fits the data.

Washout kinetics were fit by a single decaying exponential function:

$$L_N = L_0 \exp(-t/\tau) + L_i \quad (\text{Eq. 3})$$

where L_N is the neuronal content after washout time t , τ is the washout time constant, L_0 is the nerve content that is removable during the washout period, and L_i is the “irreversible” (tightly bound) content that is only very slowly washed out and so remains at the end of washout. In a preliminary stage of analysis a double exponential function was fitted, but the added variance with the additional degrees of freedom did not improve the goodness of fit so this method was not pursued.

Data are graphed and fitted by the corresponding equations above, using GraphPad Prism software (San Diego, CA). Fitted parameters with their means and standard deviations generated by the fitting program are given in the Results and, when appropriate, values are compared for significant differences using ANOVA.

Results

Lidocaine uptake at clinical concentrations

The first experiments were conducted to simulate the conditions for the *in vivo* uptake of lidocaine.^{8,9,13} The sciatic nerve with epineurial sheath intact was exposed to the “clinical” concentration of 37 mM (1%) lidocaine in buffered saline solution (pH 6.8) for up to 3h. At selected times the nerve was removed and desheathed and the sheath and nerve separately assayed for lidocaine content. Figure 1 shows that the surrounding sheath equilibrates more rapidly than its contained nerve, although the final *LID contents are equal. The time constant for uptake by the sheath is 6.7 ± 2.7 min (mean $\pm$ SD) whereas that for nerve uptake is 27.4 ± 15.9 min ($P < 0.01$); the respective equilibrium uptakes (L_∞) are 49.0 ± 2.9 nmoles/mg wet for the sheath and 43.8 ± 7.2 nmoles/mg wet for the nerve ($P > 0.05$). Since the wet mass of epineurium equals ~ 0.45 times the mass of the desheathed nerve, it contained 30% of the “total neural” lidocaine and the nerve contained $\sim 70\%$ when equilibrium was reached.

The wet/dry mass ratio of the ensheathed nerve remained constant (3.5 ± 0.1) over 3 hrs of incubation under physiological conditions, showing that neither swelling nor shrinking occurred.

Effects of the incubating solution composition on uptake

When the pH was increased to the physiological range and bubbled with CO_2 , as in the *Liley's* solution, 1% lidocaine becomes insoluble and precipitates.⁴ Therefore, in the remaining experiments we studied much lower concentrations of lidocaine. Incubations to equilibrium (2 h) in the physiological *Liley's* solution, buffered with bicarbonate: CO_2 at pH=7.4, result in a slightly smaller total but tighter affinity uptake than that for incubations in the PBS at pH=6.8 (Figure 2). Fitting these data by Eq. 1 (see Methods) yields L_{max} values of 55.9 ± 8.8 nmoles/mg wet for *Liley's* solution and 79.8 ± 19.0 nmoles/mg wet for PBS ($P < 0.05$). The affinity is indicated by the inverse of the equilibrium dissociation constant, K_L , which is 12.2 ± 3.8 mM for *Liley's* and 41.6 ± 19.4 mM for PBS ($P < 0.001$), showing about a 3-fold higher affinity for lidocaine in the *Liley's* solution. When the simple saline solution is buffered by bicarbonate: CO_2 , BBS, instead of PIPES, and the pH equals that of *Liley's* solution, pH 7.4, the saturating uptake $L_{\text{max}} = 85.9 \pm 62.7$ nmoles/mg wet and $K_L = 22.0 \pm 25.6$ mM, differing insignificantly from the L_{max} in PBS ($P > 0.05$) and

significantly from that in Liley's ($P < 0.05$), and also differing in K_L values from the other two solutions ($P < 0.01$ for both). Thus the uptake capacity of the nerve is relatively dependent on ionic composition, being lower in the more physiological solution, but is not affected by the difference in pH. The apparent affinity is greatest in the physiological solution and is least in the slightly acidic simple salt solution, a condition where more of the free lidocaine in solution is in the charged cationic rather than the neutral base form.¹⁸

Concentration-independence of uptake kinetics

The concentration dependence of the uptake should be studied at lidocaine concentrations below saturation, i.e., well below the K_L values at equilibrium. Towards this end the ensheathed nerves were incubated for times up to 1 hr in 1.85 mM (0.05%) or 7.4 mM (0.2%) lidocaine in Liley's solution (Figure 3). The uptake kinetics were fit by a single exponential (Eq. 2, Methods) with time constant of 8.8 ± 1.5 min and L_∞ values of 6.6 ± 0.4 nmoles/mg wet for 1.85 mM [*LID], and, $\tau = 9.1 \pm 4.7$ min ($P > 0.05$) and $L_\infty = 21.6 \pm 3.6$ nmoles/mg wet ($P < 0.005$) for 7.4 mM. In other words, the rate at which lidocaine was taken up by the nerve was constant and thus independent of the bathing concentration, although the final content differed and was approximately proportional to that concentration. This suggests that the rate-limiting step for uptake is not diffusion of free drug into the nerve, which would depend on concentration, but must be found in some other process.

Uptake kinetics of ensheathed vs desheathed nerves

One candidate for the rate-limiting factor is certainly the penetration through the surrounding epineurial sheath.¹⁴ To test this hypothesis we examined the uptake kinetics of 0.2% (7.4 mM) lidocaine in PBS into ensheathed and desheathed nerves. The former had their epineuria removed at the end of the incubation whereas the latter were micro-surgically stripped of their epineuria before the incubation began. Analysis of these uptake data, shown in Figure 4, reports about the same equilibrium uptakes, $L_\infty = 21.6 \pm 3.6$ mg wet tissue in ensheathed nerves and 17.2 ± 2.8 mg/wet tissue ($P > 0.05$) in desheathed nerves. However, the uptake was about half as fast in the ensheathed nerve, with time constant 9.1 ± 4.7 min, than in the desheathed nerve, with time constant 5.3 ± 2.9 min ($P < 0.02$), results consistent with the epineurial sheath providing some obstacle to the entry of lidocaine into the nerve.

Influence of the sheath on washout kinetics

If the circumferential sheath slows the uptake of lidocaine from the bathing solution, is it also an impediment to the removal of lidocaine from the nerve? We addressed this question by pre-incubating ensheathed and desheathed nerves for 60 min in 0.2% lidocaine dissolved in Liley's solution, to load both preparations equally (Figure 3), and then placed the nerves in a large reservoir of drug-free Liley's, removing nerve sections and assaying their *LID content at various times to measure the washout kinetics (Figure 5). For a clearer estimate of the kinetics from 4-5 separate experiments, each uptake was normalized to its equilibrium uptake value at zero time, just before washout began. Analysis of the averaged normalized data using Eq. 3 (see Methods) shows a pool of drug that is rapidly washed out, L_W , as a fraction of the total content (0.81 ± 0.15 in the ensheathed nerve and insignificantly larger, 0.92 ± 0.07 , in the desheathed nerve [$P > 0.05$]). This more rapidly removable component is washed out with a somewhat slower rate from the ensheathed than the desheathed nerve (respective time constants = 8.3 ± 3.6 min and 5.2 ± 2.2 min, $P < 0.05$), suggesting that the rate of washout from this pool is slightly influenced by the sheath. There is an "irreversible" component of neural lidocaine that is only slowly removed during the washout period, L_i , but it is only a small fraction, ~10%, of the total lidocaine equilibrium content. Within the limits of this method, this slow reversing fraction differs insignificantly between the two preparations, being 0.18 ± 0.12 in the ensheathed nerve and 0.08 ± 0.05 in the desheathed nerve.

Effect of the loading procedure on washout kinetics

In clinical use LA are administered as bolus injections of high concentrations and as continuously delivered solutions of lower concentration.^{19,20} We investigated the washout kinetics of lidocaine after these two types of exposure as a surrogate for examining the actual duration of nerve block *in vivo*. From the uptake kinetic studies at different lidocaine concentrations (cf. Figure 3 and above Results) it appears that approximately the same net uptake resulted from a brief exposure to a high concentration (5 min in 0.2%) as that from a long exposure to a lower concentration (60 min in 0.05%), so these conditions were used to load separate ensheathed nerve preparations in Liley's solution. However, since the uptake between the two conditions was not identical (the final uptake ranging from 4.6 to 6.8 nmoles/mg wet weight determined just before washout began), these "initial" values were used to normalize these starting values for each of the 3 experiments done at the two conditions, and the *averaged normalized* values for the washout calculated (Figure 6). As with the other washout study, the washout kinetics were fit by the sum of a virtually constant component, L_i , equal to 0.05 ± 0.06 in the rapidly loaded nerves and 0.04 ± 0.06 in the slowly loaded nerves ($P > 0.05$), plus a removable component fit by a single exponential function (Eq. 3) with identical rapidly removable fractions for the rapidly loaded, 0.94 ± 0.09 , and the slowly loaded, 0.94 ± 0.07 , nerves. The time constants for this component's washout were slower in the slowly loaded nerves, 12.6 ± 3.1 min, than in the rapidly loaded nerves, 8.3 ± 1.8 min ($P < 0.05$), showing that a long incubation at low concentrations takes longer to wash out than a fast incubation at high concentrations.

Discussion

The objective of this study was to determine the kinetics of lidocaine's uptake and release by isolated peripheral nerve, for comparison with the published neuronal pharmacokinetics *in vivo*. The current results show that lidocaine is taken up by the nerve with a slight delay after uptake by the epineurial sheath, that the equilibrium uptake level is proportional to the drug's free concentration, until saturation occurs, but that the rate of the uptake is independent of concentration, showing that the process of neuronal uptake is not free diffusion. Equilibrium partitioning of lidocaine into nerve is greater at the slightly alkaline physiological pH of 7.4 than at the somewhat acidic pH of pharmaceutical formulations, e.g., 6.5-6.8, consistent with the higher partitioning of the neutral base species that is favored by the higher pH. Removal of the outer, epineurial sheath speeds lidocaine's uptake into the nerve, with a smaller effect on its washout. Finally, longer incubations resulted in a slower rate of overall removal than briefer incubations, even when the concentrations were adjusted to give the same net uptake at the start of washout.

In vivo versus *in vitro* uptake. The neural content of lidocaine increases proportionately to its concentration in the incubation solution, up to ~5-10mM (0.14 - 0.28%), when uptake begins to saturate. In the linear range the neural uptake corresponds to a partitioning with coefficient (slope) equal to ~3.5 nmoles/mg rat nerve/mM lidocaine in the bathing solution. Others have reported comparable results from studies on isolated nerve. Rosenberg et al. found that ensheathed rat sciatic nerve exposed to 1mM lidocaine took up 1.4 nmoles/mg wet nerve²¹ and Strobel and Bianchi measured 1.5 nmoles/mg wet of toad sciatic nerve incubated in 0.5 mM lidocaine.²² Comparing the nerve mass with the volume of bathing solution gives a nerve:buffer distribution coefficient of ~3-4.^{10,21,23}

Given the relatively large hydrophobicity of lidocaine, with octanol:buffer distribution coefficient of 110 at 37° and pH 7.4^{18,24} and high fat: buffer partition coefficient,²¹ the most likely tissue location of the drug molecules is in the membranes, absorbed at the lipid aqueous interface.^{25,26} Although the conduction blocking actions of LAs occur through their binding to discrete sodium channels in the axon membranes²⁷, these sites are far too sparse

to be detected within the total absorption to all the bilayer membranes, e.g., myelin, in the nerve.²⁸

By comparison, neural lidocaine content in sciatic nerve in vivo measured during blocking procedures is far lower. In the segment of the nerve that is closest to the locus of injection of 0.1 mL containing 1% (37mM) lidocaine, pH=6.8, Popitz-Bergez et al. measured 8-10 nmoles/mg wet nerve at the time of maximum local anesthesia (motor and nociceptive block), 15 min after the injection⁸. Nakamura et al. detected neural contents of 1-6 nmoles/mg wet after in vivo blocks with 0.05-0.2 mL volumes (pH=6.8), respectively, using volume - dose (mass) combinations that gave the same effective concentration, 5-6 mg/mL (equal to ~0.5%), adequate to fully block 50% of the rats, i.e., a population-based EC50.¹³ Likewise, Sinnott et al. found a peak lidocaine content in nerves taken 4 min after injection of 0.5% lidocaine, pH=7.8, of 10-11 nmoles/mg wet, higher than the other two values and consistent with greater penetration of drug from the more alkaline solution.⁹ The important point here is that these values are well below the ones from equilibrium uptake of the isolated nerve in physiological conditions, 33 or 40 nmoles/mg wet at 0.5% and 1% lidocaine, respectively (Figure 2).

These peak in vivo uptakes correspond instead to equilibrium levels found in isolated nerve bathed in 2-3 mM, not the 18-37 mM that was injected. Further analyses show that when nociceptive responses begin to return, 30-40 min after the injection, the neural content equals ~2 nmoles/mg wet^{9,12} which corresponds to the in vitro content from equilibration with 1mM lidocaine. This is also the minimum concentration of lidocaine that was required to block impulse conduction in all types of nerve fibers in vivo when the sciatic nerve was equilibrated with lidocaine solutions delivered through a nerve-encompassing perfusion chamber,¹ showing a good correspondence between the effective conditions/content for block when a bolus injection was delivered in vivo or when equilibrium could be achieved.

The current findings emphasize the inefficiency of peripheral nerve block by showing the discrepancy between the minimum effective lidocaine concentration for block in vivo, 0.5-1% or 18-37 mM¹³ and the effective block in vitro, 1mM.¹ There are several reasons for this inefficiency. One is the limited mass/dose of the drug that is delivered in vivo. Unlike the virtually infinite reservoir of drug in which the nerve was equilibrated for the present in vitro experiments, functional block of rat sciatic nerve was efficiently effected at 0.1-0.2 mL, corresponding to a total dose of 1-2 mg.¹³ The measured nerve:buffer distribution coefficient of 3-4 for lidocaine in vitro would predict that 67-75% of the delivered dose would equilibrate into the nerve if no other factors interfered. Instead, however, the injected solution is rapidly removed by surrounding tissues, the local circulation, and also spreads longitudinally along the nerve axis such that it is distributed along a length of ~3 cm of nerve, which contains only ~2% of the total injected dose at the time of maximum block.^{8,12,13}

Vascular removal. Vascular removal is an important sink for the injected LA and is a larger factor for the less hydrophobic LAs, such as lidocaine, which become less absorbed by surrounding fat, muscle and the neural membranes per se, than for the more hydrophobic drugs such as bupivacaine. Co-injections of epinephrine (1:100,000) with lidocaine during rat nerve blocks have shown that the critical action of the vasoconstrictor in prolonging the block is at the initial uptake phase into the nerve,^{9,10} where the most intense vasoconstriction, countering the vasodilatory action of the lidocaine⁵, resulted in a larger initial loading of the LA into the nerve.⁹ The measured rate of removal of lidocaine from the nerve in vivo is unaffected by the co-injection of epinephrine, probably because by the time the block regresses much of the vasoconstrictor has been removed or metabolized.²⁹

The limited importance of CO₂:bicarbonate buffer. Bicarbonate has been traditionally used with LAs as an adjuvant to alkalinize the somewhat acidic pharmaceutical formulations.⁴ The uptake studies here showed that weak alkalization with bicarbonate-buffered Liley's solution did enhance lidocaine's neural uptake, but no more than the same degree of alkalization using nonbicarbonate buffer. The presence of CO₂, at 5%, and its associated HCO₃⁻, did not increase the octanol:buffer partitioning of lidocaine²⁴ yet CO₂:bicarbonate buffered solutions increase the depth of epidural blockade³⁰ and enhance the conduction blocking actions of lidocaine on isolated peripheral nerve.^{31,32} Analysis of various LAs and of the role of pH changes per se have lead to the conclusion that CO₂:bicarbonate acts by acidifying the cytoplasm, and favoring the more potent, charged species of intracellular LAs, and/or by altering the drug binding sites on the nerve membrane.³² However, although such enhancement of lidocaine block has been demonstrated in isolated nerve, neurobehavioral measures of nerve block reveal that its duration is significantly shorter when lidocaine is injected in a bicarbonate buffer.¹¹ This shortening is probably due to the vasodilatory actions of bicarbonate on the peripheral vasculature, because it does not occur when the bicarbonate is used in an epinephrine-containing solution.

A model for LA distribution in peripheral nerve blocks. The distribution of LAs within the peripheral nerve is key to understanding the overall time-course of a nerve block. Three factors are critical for this understanding: 1. the delivered dose is rapidly removed from its injected locus by vascular removal and by uptake into surrounding tissues, of which the nerve is usually the smallest structure (Figure 7). 2. LA molecules do not diffuse freely into the nerve but are slowed slightly by the barrier of the epineurium, probably more severely by the epithelial-like perineurium^{15,16}, and partition reversibly to all of the membranous structures in the nerve, including the extensive myelin surrounding the larger nerve fibers (Figure 7). 3. During the "steady state" period of total anesthesia/numbness during clinical blockade, the LA distribution is changing within the nerve and in the surrounding structures. These factors can be best appreciated by consideration of the schematic of nerve anatomy in Figure 7 and by the hypothetical graphs of lidocaine in the three "compartments" shown in Figure 8.

LA molecules partition strongly (hydrophobic adsorption) into the perineural fat and less into the more massive (and less lipid-like) muscle surrounding the nerve. The initial uptake into these tissues, immediately after the drug solution's delivery, will rapidly deplete the free concentration of anesthetic. Drug molecules may dwell in these tissues for a long time, depending on their hydrophobicity and the intra-tissue vascular supply, serving as an effective buffer to release drug for neural uptake long after a bolus injection. One can appreciate that a small nerve with limited adjacent tissue and a high vascularity, such as the intraocular nerve, will have a different relationship between delivered and neurally absorbed dose than will a large nerve with massive surrounding tissues, such as the femoral nerve.

LA molecules are amphipathic, having both hydrophobic moieties and polar, and sometimes charged, hydrophilic ones. As a consequence, LAs are bound at the aqueous surface of the negatively charged lipid bilayer membranes,^{25,26} with affinities that are determined by both ionic and hydrophobic properties.³³ Passage into the nerve is not by a process of free diffusion, but rather by a dynamic series of adsorptions to and desorptions from all the membrane structures in the nerve, including passing through the epineurial structure and the more formidable and multicellular perineurium.^{16,17} This explains why the rate of anesthetic uptake is independent of the bathing concentration, since free diffusion is not the rate-limiting step for uptake. It also explains why the LA removal during washout from isolated nerve is slower after long incubations, since longer incubations allow the LA to penetrate deeper into the nerve and require that during its removal, in the absence of vascular flow, it must encounter more adsorbing membranes in its egress to the outside than if it had been

more superficially adsorbed into the mantle of the nerve. Although a LA molecule must permeate the axonal plasma membrane to reach the cytoplasmic compartment of a cell and the blocking site on the sodium channel ³⁴, this permeation is fast compared to the slow migration through the nerve bundle by adsorption-desorption.

The reaction of LAs with the effector compartment that contains the axons of the nerve is correctly considered as a rate competition reaction. Uptake into adjacent tissue and removal by vasculature rapidly deplete the supply of delivered dose and compete with the nerve for the free LA molecules. The resulting neural content is remarkably transient, reaching its peak value early after injection and then slowly declining as drug is removed by the vasculature while it is also being slowly released from the adjacent tissues, even during an apparently constant nerve block. The overall duration of an effective block, during which the neural content exceeds the critical content for conduction block (given an adequate length of drug exposed nerve²), is largely dependent upon the initial loading into the nerve but also depends on the ability of the adjacent tissues to “buffer” the neural concentration, as shown by the parallel slow decay of L_T and L_N (Figure 8). Of course, if the initial delivery is too far from the nerve, no release from the adjacent tissues can result in a clinically effective neural content, regardless of the pharmacological manipulation of kinetic factors.

In summary, peripheral nerve block by LAs is a highly inefficient process due to the rate competition nature of the drug distribution. There is no way to change the anatomical organization around the peripheral nerve, but block efficiency can be improved by increasing the membrane-penetrating species by neutralizing, or slightly alkalinizing the solution, by the use of vasoconstrictors and by applying the drug as close as is safely possible to the target nerve. Although we knew all this from clinical practice, the present paper provides a theoretical framework for its integrated understanding.

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References

1. Huang JH, Thalhammer JG, Raymond SA, Strichartz GR. Susceptibility to lidocaine of impulses in different somatosensory afferent fibers of rat sciatic nerve. *J Pharmacol Exp Ther*. 1997; 292:802–11. [PubMed: 9262344]
2. Raymond SA, Steffensen S, Gugino LD, Strichartz GR. The role of length of nerve exposed to local anesthetics in impulse blocking action. *Anesth Analg*. 1989; 68:563–70. [PubMed: 2785778]
3. Raymond, SA.; Gissen, AJ. Mechanisms of differential nerve block. In: Strichartz, GR., editor. *Local Anesthetics*. Springer-Verlag; Berlin, New York: 1987. p. 95-164.
4. Peterfreund RA, Datta S, Ostheimer GW. pH adjustments of local anesthetic solutions with sodium bicarbonate: Laboratory evaluation of alkalinization and precipitation. *Reg Anesth*. 1989; 14:265–70. [PubMed: 2562097]
5. Johns RA, DiFazio CA, Longnecker DE. Lidocaine constricts or dilates rat arterioles in a dose-dependent manner. *Anesthesiology*. 1985; 62:141–4. [PubMed: 3970363]
6. Gokin AP, Philip B, Strichartz GR. Preferential block of small myelinated sensory and motor fibers by lidocaine: in vivo electrophysiology in the rat sciatic nerve. *Anesthesiology*. 2001; 95:1441–54. [PubMed: 11748404]
7. Thalhammer JG, Vladimirova M, Bershadsky B, Strichartz GR. Neurological evaluation of the rat nerve block with lidocaine. *Anesthesiology*. 1995; 82:1013–1025. [PubMed: 7717536]
8. Popitz-Bergez FA, Leeson S, Strichartz GR, Thalhammer JG. Relation between functional deficit and intraneural local anesthetic during peripheral nerve block. A study in the rat sciatic nerve. *Anesthesiology*. 1995; 83:583–592. [PubMed: 7661359]

9. Sinnott C, Cogswell LP III, Johnson A, Strichartz GR. On the mechanism by which epinephrine potentiates lidocaine's peripheral nerve block. *Anesthesiology*. 2003; 98:181–188. [PubMed: 12502995]
10. Fink BR, Aasheim G, Kish SJ, Croley TS. Neurokinetics of lidocaine in the infraorbital nerve of the rat in vivo: Relation to sensory block. *Anesthesiology*. 1975; 42:731–6. [PubMed: 1130741]
11. Sinnott CJ, Garfield JM, Thalhammer JG, Strichartz GR. The addition of sodium bicarbonate to lidocaine decreases the duration of peripheral nerve block in the rat. *Anesthesiology*. 2000; 93:1045–1052. [PubMed: 11020760]
12. Popitz-Bergez FA, Leeson S, Thalhammer JG, Strichartz GR. Intraneural lidocaine uptake compared to analgesic differences between pregnant and non-pregnant rats. *Reg Anesth*. 1997; 22:363–371. [PubMed: 9223204]
13. Nakamura T, Popitz-Bergez F, Birknes J, Strichartz GR. The critical role of concentration for lidocaine block of peripheral nerve in vivo: Studies of function and drug uptake in the rat. *Anesthesiology*. 2003; 99:1189–1197. [PubMed: 14576558]
14. Franco CD. Connective tissues associated with peripheral nerves. *Reg Anesth Pain Med*. 37:363–5. [PubMed: 22683708]
15. Stolinski C. Structure and composition of the outer connective tissue sheaths of peripheral nerve. *J Anat*. 1995; 186(Pt 1):123–30. [PubMed: 7649808]
16. Low, RN. The perineurium and connective tissue of peripheral nerve. In: Landon, DN., editor. *The Peripheral Nerve*. Chapman & Hall; London: 1976. p. 159-187.
17. Thomas PK. The connective tissue of peripheral nerve: an electron microscope study. *J Anat*. 1963; 97:35–44. [PubMed: 13981107]
18. Sanchez V, Arthur GR, Strichartz GR. Fundamental properties of local anesthetics. I. The dependence lidocaine's ionization and octanol:buffer partitioning on solvent and temperature. *Anesth Analg*. 1987; 66:159–165. [PubMed: 3813059]
19. Richman JM, Liu SS, Courpas G, Wong R, Rowlingson AJ, McGready J, Cohen SR, Wu CL. Does continuous peripheral nerve block provide superior pain control to opioids? A meta- analysis. *Anesth Analg*. 2006; 102:248–57. [PubMed: 16368838]
20. Capdevila X, Pirat P, Bringuier S, Gaertner E, Singelyn F, Bernard N, Choquet O, Bouaziz H, Bonnet F. Continuous peripheral nerve blocks in hospital wards after orthopedic surgery: a multicenter prospective analysis of the quality of postoperative analgesia and complications in 1,416 patients. *Anesthesiology*. 2005; 103:1035–45. [PubMed: 16249678]
21. Rosenberg PH, Kytta J, Alila A. Absorption of bupivacaine, etidocaine, lignocaine and ropivacaine into n-heptane, rat sciatic nerve, and human extradural and subcutaneous fat. *Br J Anaesth*. 1986; 58:310–4. [PubMed: 3947493]
22. Strobel GE, Bianchi CP. The effects of pH gradients on the action of procaine and lidocaine in intact and desheathed sciatic nerves. *J Pharmacol Exp Ther*. 1970; 172:1–17. [PubMed: 5416944]
23. Bianchi CP, Strobel GE. Modes of action of local anesthetics in nerve and muscle in relation to their uptake and distribution. *Transactions of the New York Academy of Sciences*. 1968; 30:1082–1092. [PubMed: 5250162]
24. Strichartz GR, Sanchez V, Arthur GR, Chafetz R, Martin D. Fundamental properties of local anesthetics. II. Measured octanol:buffer partition coefficients and pKa values of clinically-used drugs. *Anesth Analg*. 1990; 71:158–170. [PubMed: 2375517]
25. Zhang J, Hadlock T, Gent A, Strichartz GR. Tetracaine-membrane interactions: effects of lipid composition and phase on drug partitioning, location and ionization. *Biophys J*. 2007; 92:3988–4001. [PubMed: 17351014]
26. Watts A, Poile T. Direct determination by 2H-NMR of the ionization state of phospholipids and of a local anesthetic at the membrane surface. *Biochim Biophys Acta*. 1986; 861:368–72. [PubMed: 3019404]
27. Strichartz G. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol*. 1973; 62:37–57. [PubMed: 4541340]
28. Ritchie JM, Rogart RB. The binding of saxitoxin and tetrodotoxin to excitable tissue. *Rev Physiol Biochem Pharmacol*. 1977; 79:1–50. [PubMed: 335473]

29. Fink BR, Aasheim GM, Levy BA. Neural pharmacokinetics of epinephrine. *Anesthesiology*. 1978; 48:263–6. [PubMed: 637334]
30. Curatolo M, Petersen-Felix S, Arendt-Nielsen L, Lauber R, Högström H, Scaramozzino P, Luginbühl M, Sieber TJ, Zbinden AM. Adding sodium bicarbonate to lidocaine enhances the depth of epidural blockade. *Anesth Analg*. 1998; 86:341–7. [PubMed: 9459246]
31. Bokesch PM, Raymond SA, Strichartz GR. Dependence of lidocaine potency on pH and pCO₂. *Anesth Analg*. 1987; 66:9–17. [PubMed: 3099602]
32. Wong K, Strichartz GR, Raymond SA. On the mechanism potentiation of local anesthetics by bicarbonate buffer. Drug structure-activity studies on isolated peripheral nerve. *Anesth. Analg*. 1993; 76:131–143. [PubMed: 8418714]
33. Desai S, Hadlock T, Messam C, Chafetz R, Strichartz GR. Ionization and adsorption of a series of local anesthetics in detergent micelles: Studies in model membranes using in drug fluorescence. *J Pharmacol Exp Therap*. 1994; 271:220–28. [PubMed: 7965718]
34. Hille B. Local anesthetics: Hydrophilic and hydrophobic pathways for the drug receptor interaction. *J Gen Physiol*. 1977; 69:497–515. [PubMed: 300786]

Figure 1.

The time-course of uptake of lidocaine from a 1% (37mM) Liley's solution, pH 7.4, 37° C, measured in the epineurium-free nerve (●) and in the epineurium (sheath, ○), which was removed at the end of each incubation period. The curves are fits of Equation 2 (see Methods) to the data from the separated tissues.

Figure 2.

Equilibrium uptakes of lidocaine by intact, ensheathed sciatic nerves incubated in Liley's physiological solution, pH 7.4 (●) or in saline buffered with PIPES, pH 6.8 (○). (Lidocaine was precipitated in the Liley's solution at 37mM, accounting for the abbreviated dataset.) The initial slopes of the curves, at the low drug concentration, are equal to the nerve:buffer distribution coefficients, and are about twice as high in Liley's solution as in PIPES saline. Curves fit to Equation 1.

Figure 3.

Time-course of the uptake of lidocaine at 2 different concentrations into the neural compartment of ensheathed nerve incubated in Liley's solution at 37° C. Curves are fits to the data of Equation 2. Although the final equilibrium levels are approximately proportional to the concentration, the rate constants for the uptake kinetics are the same.

Figure 4.

Uptake time-course of lidocaine into the neural compartment of sheathed (●, epineurium intact during incubation) or desheathed (○, epineurium removed before incubation) sciatic nerve. The curves are fits of Equation 2 to the data points.

Figure 5.

Kinetics of release of lidocaine from the sciatic nerve during washout in Liley's solution from sheathed (●) or desheathed (○) nerve. The epineurial sheath was removed before the loading step, and the data, from 3 independent experiments for the two experimental conditions, was normalized to the very similar tissue levels measured just before the washout began. Curves are fits of Equation 3 to the data.

Figure 6.

Kinetics of the release of lidocaine from ensheathed sciatic nerve incubated in Liley's solution during the loading phase under two conditions: a brief incubation at a higher concentration (●) and a longer incubation at a lower concentration (○). Data are normalized to the lidocaine content measured at the end of the loading phase, and are combined from 3 independent experiments. Curves are fits of Equation 3 to the data.

Figure 7.

A schematic of the sciatic nerve (*N*) showing fascicles of myelinated and non-myelinated nerve fibers encased in the perineurium, a structure composed of tightly connected epithelial cells. The perineurium and the inner endoneurium of connective tissue are all enclosed by the epineurium. Blood vessels (*V*) course inside and outside the nerve and in the adjacent tissue (*T*), which is typically much more massive than the nerve. Injected solutions of local anesthetic (*L_s*) surround the nerve from which drug is taken up by it and by the adjacent tissue (fat and muscle) and removed by the vasculature.

Figure 8.

Theoretical curves describe the kinetics of lidocaine's distribution during a nerve block *in vivo*. Lidocaine is injected, in an ideal situation, immediately adjacent to the nerve in a solution with concentration L_s which decreases rapidly. The tissues surrounding the nerve take up lidocaine, L_T , as it diffuses from the injected solution, and then more slowly release it, providing a reservoir that delivers drug for an extended period of time. Lidocaine in the nerve, L_N , is taken up rapidly from the injected solution and then is removed with fast and slow decay phases, the fast phase from the intraneural vascular removal and the uptake back into adjacent tissues, and the slow phase from the exchange from the high-capacity adjacent tissues. Effective conduction block occurs when L_N exceeds the critical blocking content, L_c .