Susceptibility to Lidocaine of Impulses in Different Somatosensory Afferent Fibers of Rat Sciatic Nerve

JASON HAITAO HUANG, JOHANN G. THALHAMMER, STEPHEN A. RAYMOND and GARY R. STRICHARTZ

Pain Research Group, Department of Anesthesia Research Laboratories, Harvard Medical School, Brigham and Women’s Hospital, 75 Francis Street, Boston, Massachusetts
Accepted for publication April 21, 1997

ABSTRACT
Mechanosensitive Aβ-fibers (n = 29) and nociceptive Aδ- (n = 6) and C-fibers (n = 10) of the rat sciatic nerve were superfused with lidocaine (LID, 0.1–1.4 mM) in vivo. The [LID] required to abolish single electrically stimulated impulses (tonic block) in axons was 0.2 to 0.8 mM for Aβ- and 0.1 to 1.4 mM for C-fibers. Within each of the fiber groups there was no dependence of blocking [LID] on conduction velocity; slower fibers were no more susceptible than faster ones. Mean block dependence of blocking [LID] on conduction velocity; slower fibers were no more susceptible than faster ones. Mean block [LID] equaled 0.28 mM (n = 17). Stimulation of nociceptive Aβ-fibers (n = 4) and C-fibers (n = 5) at 5 or 10 Hz for 10 pulses produced no phasic block at [LID]s (0.1–0.5 mM) below those required for tonic blockade. Uptake of 14C-lidocaine by the nerve, measured in vivo under conditions identical with those for electrophysiology, showed that: a) little drug was in the segments of nerve beyond the superfusion chamber, b) lidocaine was uniformly distributed in the nerve within the chamber, c) the intraneural lidocaine content was identical with that in nerves equilibrated in vitro. The results show a lack of monotonic dependence of sensitivity to local anesthetic on fiber diameter, but do suggest that mean susceptibility to nerve block by lidocaine differs for fibers grouped by, and perhaps according to, function.

It is a widespread belief in anesthesia and pharmacology that impulses in nerve fibers of smaller diameter are blocked by lower concentrations of local anesthetics (LA) than those of larger diameter (Catterall and Mackie, 1996). This historical hypothesis is based on studies of CAPs (Gasser and Erlanger, 1929; Heinbecker et al., 1934) that measured summed electrical elevations from axons of smaller diameter (slow CV) and showed that these elevations were decreased more by LA than those of larger diameter (fast CV) axons. This hypothesis, however, has been contradicted by results from single unit studies on mammalian fibers in vivo (Franz and Perry, 1974) and in vitro (Fink and Cairns, 1984, 1987). Some CAP studies have also found that fibers grouped by CV did not show a susceptibility to LA that always correlated with conduction speed (Heavner and de Jong, 1974; Gissen et al., 1980). On the other hand, a great deal of interfiber variation in LA sensitivity has been observed in axons of similar conduction velocity and, by inference, similar diameter (Fink and Cairns, 1987; Raymond, 1992). It has been suggested that the observed variations in LA sensitivity might be linked to differences in functional modalities of peripheral axons (Raymond and Gissen, 1987); this report is the first to investigate that hypothesis.

Local anesthetics have three important effects on axonal excitability. The first is a tonic reduction of excitability (measured at very low firing frequencies, e.g., < 0.1 Hz) which culminates in conduction failure. In vitro studies have shown that increase of LA at sub-blocking concentrations causes the conduction velocity of axons to decrease progressively until abolition of conduction (Fink and Cairns, 1984; Raymond, 1992). The second well-known effect of LA is its use-dependent action. During trains of stimuli in nerves exposed to LA in vitro, CAPs undergo progressive diminution, pulse by pulse, that increases with the frequency and number of impulses in the trains (Trubatch, 1972; Courtney et al., 1978). Voltage-clamp studies have revealed that this “use-dependent” action is caused by the increased affinity of LA for those configurations of Na+ channels that predominate during the membrane depolarization associated with the impulse, thus increasing the population of LA-bound channels and decreasing the capability of the axon to generate depolarizing currents needed to sustain propagation (see review, Butterworth).

ABBREVIATIONS: LID, lidocaine; CV, conduction velocity; LTM, light-touch mechanoreceptor; CAP, compound action potential; LA, local anesthetic; SA, slowly adapting mechanoreceptive afferents; RA, rapidly adapting mechanoreceptive afferents; M, muscle afferents.
and Strichartz, 1990). The third effect is the suppression of the activity-dependent aftereffects of axonal impulse conduction, which has been demonstrated in rat (Raymond et al., 1991) and frog (Raymond, 1992) sciatic nerve in vitro. To our knowledge, no in vivo single unit study on mammalian fibers has ever investigated these three effects of LA simultaneously.

The primary aim of the present work was to examine LA blockade of functionally characterized sensory axons. We focused on primary afferents of rat sciatic nerve conducting in the Aβ-, Aδ- and C-fiber ranges. Lidocaine, a well-studied and widely used clinical LA, was used. We measured the range of lidocaine concentrations that was required to abolish single impulses induced by infrequent electrical stimulation (tonic blockade). For low threshold mechanosensitive (LTM) Aβ-fibers, we also studied the use-dependent action of lidocaine as well as its effects on activity-dependent conduction latency changes with trains of high-frequency electrical stimulation. Additionally, we determined the net uptake of radiolabeled lidocaine to assess the concentrations and spatial distribution of lidocaine along the nerve.

An abstract reporting part of this work has been published (Huang et al., 1995).

Materials and Methods

Animal preparation. Male Long-Evans rats weighing 500 to 550 g (Charles River Laboratories, Wilmington, MA) were initially anesthetized with an intraperitoneal injection of 50 mg kg⁻¹ sodium pentobarbital (Abbott Laboratories, North Chicago, IL). The jugular vein was cannulated for intravenous administration of sodium pentobarbital (5–6 mg kg⁻¹ h⁻¹), which maintained general anesthesia throughout the experiment; supplementary boli were administered as necessary, with use of absence of both the corneal reflex and the increase of heart rate during electrical stimulation as the endpoint for adequate anesthesia. The heart rate was monitored with a Tektronix 408 EKG monitor (Tektronix, Beaverton, Oregon). Core body temperature was maintained at 38.0 ± 0.5°C with a water-circulated heating pad. At the end of an experiment rats were sacrificed by an overdose of sodium pentobarbital (100 mg kg⁻¹). Animal treatment for these experiments was approved by the Harvard Medical Area Standing Committee on Animals.

A longitudinal skin incision was made in the hind leg and the skin and muscle freed up to expose the sciatic nerve and its posterior tibial branch. The skin at the incision was then sown around a metal ring which thus formed a pool to hold mineral oil, and the dorsum of the foot was glued to a Plexiglas platform. A section of the freed nerve was placed in a plastic chamber 22 mm in length, and bathed in a physiological solution containing lidocaine at various concentrations. The bathing chamber was sealed with a mixture of magnesium silicate, silicone oil and a hardener which was generously provided to us by Dr. Sandkühler (Universität Heidelberg, Heidelberg, Germany). The sheath of the nerve inside the chamber was kept intact and care was taken to avoid tension, rotation or constriction of the nerve. Outside the proximal end of the chamber the nerve was placed in the groove of a dissection mirror where the nerve sheath was cut open and nerve fibers were pulled from the nerve trunk to the proximal end. Fine dissection of nerve bundles was done on the mirror, and small nerve fascicles were wrapped around a platinum wire electrode through which the signals were recorded. During the experiment, the oil pool temperature was maintained at 35.0 ± 0.5°C by a water-circulated, feedback-controlled, heating pad placed underneath the rat body and its hind leg. In some of the experiments, an oil circulation chamber built in our laboratory was also used to keep the nerve-containing oil pool temperature constant, as measured by a thermocouple placed close to the nerve trunk in the oil pool.

Solutions. The lidocaine solutions were made by adding lidocaine HCl powder (Sigma Chemical Co., St. Louis, MO) to modified Liley solution (NaCl, 118 mM; KCl, 5 mM; CaCl₂·2H₂O, 2 mM; NaHCO₃, 25 mM; NaH₂PO₄, 1.2 mM; MgCl₂·6H₂O, 1 mM; Na₂HPO₄, 3.6 mM; glucose, 10 mM). The solution was warmed to 35 ± 0.5°C and bubbled with a mixture of 95% O₂/5% CO₂ to maintain the pH between 7.4 and 7.5 before perfusing it through the bathing chamber.

Identification of single units. Single units were isolated and characterized by natural stimulation, applying innocuous and noxious mechanical stimuli to various sides of the glabrous skin of the foot. The receptive field, modality and location were identified conventionally (Sanders and Zimmerman, 1986; Leem et al., 1993). A unit was characterized as nociceptive when it responded to either noxious mechanical pinching with a serrated forcep tip or the hard squeezing of a skin fold by the fingers of the experimenter. A unit was characterized as low-threshold mechanoreceptive when it responded to light touch by the tip of a glass probe. Three subtypes of low-threshold mechanoreceptive afferents were sought in this study: slowly adapting mechanoreceptive (SA), rapidly adapting mechanoreceptive (RA) and muscle (M) afferents. Rapidly adapting units reduce discharge rates quickly after stimulus onset (<4 s) and also fire when the stimulus is released. Slowly adapting units respond for a longer time (>4 s) and, as the name implies, take longer to reduce firing rates under maintained stimulation. Muscle afferents responded to any mechanical stimulus that altered muscle tension (e.g., stretching by lateral displacement) and continued to discharge with little or no adaptation.

Electrical stimulation (amplitude 1.5 times the threshold; 5–10 V and 0.2 ms for A-fibers and 50–70 V and 0.5 ms for C-fibers) was delivered to isolated units by a Grass S44 stimulator through E2 subdermal recording electrodes (Grass Instrument Co., Braintree, MA) placed subcutaneously and proximal to the receptive field. The resting CV of fibers was determined by measuring the distance from the stimulation site (1.2–1.5 cm) to the recording site and dividing it by the resting response latency to electrical stimulation. Fibers with conduction velocities below 2.0 m s⁻¹, between 2.5 and 15 m s⁻¹ and above 15 m s⁻¹ were considered C-, Aδ- and Aβ-fibers, respectively.

The "normalized latency increase" was calculated according to the following formula, to account for the variations in conduction length of fibers:

\[
\text{Normalized latency increase (\%)} = \frac{\text{Latency change (ms)} - \text{Resting latency (ms)}}{\text{Chamber length (mm)}} \times 100
\]

The chamber exposure length was constant at 22 mm and the conduction length, 120 to 150 mm, was the distance between stimulating and recording electrodes.

Both the collision method (Iggo, 1958) and cross-coupled physiological and electrical latency changes (Thalhammer et al., 1994) were used to ensure that an electrically activated unit was identical with the unit activated by natural stimulation of its receptive field.

Measurement of tonic and phasic block. Tonic block was measured by increasing the concentration of lidocaine in the nerve chamber by steps of 0.1 mM until impulse conduction was fully abolished. All latency measurements were obtained at steady state after 20 min of lidocaine application. At steady state, each unit was stimulated by infrequent stimuli (1 Hz for Aβ-fibers and 0.2 Hz for Aδ and C-fibers) to determine the tonic action of lidocaine.

The use-dependent and activity-dependent actions of lidocaine were studied in LTM Aβ-fibers with use of a preset stimulation profile that was developed to reveal the various phases of changes in latency that usually occur during repetitive stimulation. The profile consists of three discrete phases: 1) a 1-Hz stimulus period, lasting 30 s, to assess base-line conduction latency and latency fluctuations under conditions when no cumulative effects were noticeable; 2) a
period of repeated short bursts (20 trains, presented at 1.3 Hz, of 10 stimuli at 200 Hz), for measuring cumulative changes during each short burst and during the entire period of repeated bursting; 3) a 1-Hz recovery period lasting 60 s. The activity-dependent measurements consisted of burst supernormality and depression, which were derived from the response latencies to the above profile, as will be explained below. Phasic block was quantified as the percent of impulses that failed to conduct during the 200-Hz stimulation in the above profile.

Phasic block was examined in Aδ- and C-fibers by stimulating these fibers with 10 to 20 pulses at 5 and 10 Hz, frequencies well within their natural discharge pattern (Burgess and Perl, 1967).

The response signals were amplified by a differential electrometer preamplifier (MetaMetrics, Cambridge, MA) and filtered with an adjustable, high-frequency single pole filter (1–5 kHz). Filtered signals were further amplified and visualized on a Tektronix 5103N oscilloscope (Tektronix, Inc., Beaverton, OR) and relayed to a differential amplitude discriminator designed and built in our laboratory. Response latency data were obtained through our “Hunter” system, which times both stimuli and responses (±1 μs resolution) and stores each event to disk. The data were then graphed and analyzed by MicroCal Origin (Microcal Software, Northampton, MA) and Quattro Pro (Borland International, Scotts Valley, CA).

Stability of preparation. The whole procedure required observation of a given unit for periods as long as 6 h. Control experiments showed that resting conduction latency of three individual tactile units remained quite stable, increasing by less than 3% when continuously monitored for as long as 6 h. In consideration, the background increase of the conduction latency was not taken into account in the measurements reported in this study. All electrophysiological measurements in this study were done within 6 h after a unit was isolated.

In vivo uptake of lidocaine. Lidocaine uptake was studied using the 14C-lidocaine solution prepared by adding 10 μl 14C-lidocaine (DuPont-NEN, Boston, MA; 0.1 mCi/ml ethanol) to each 5 ml of 0.4 mM lidocaine solution that was superfused through the chamber. After in vivo exposure times of 7 min, 20 min or 60 min, four pieces of the nerve were excised: one distal to the chamber (7 mm), one within the bathing chamber (22 mm) and two adjacent pieces proximal to the chamber (7 mm each). The nerve pieces were quickly frozen on a flat surface of dry ice. The nerve segment from within the bathing chamber was further cut into three segments of equal length (7.3 mm each) using a surgical blade. All excised nerve segments were then desheathed under a dissecting microscope. Each desheathed piece of nerve was weighed (±0.1 mg) and then digested at 50°C for 2 h in a 5-ml scintillation vial containing a mixture of 0.5 ml of tissue solubilizer (Solvable, DuPont-NEN) plus 0.1 ml of deionized water. Five milliliters of Aquasol 2 (DuPont-NEN) liquid scintillation cocktail was added and radioactivity was assayed by liquid scintillation counting for 5 min. The specific radioactivity of the lidocaine solution was determined by adding 10 μl of the 14C-lidocaine solution to Solvable (0.5 ml), deionized H2O (0.1 ml) and Aquasol 2 (5.0 ml) mixture and counting identically by liquid scintillation counting. Derived counts per minute were divided by moles of lidocaine added to define the specific radioactivity. Efficiency of counting 14C was the same in all conditions and the radioactivity was assumed to represent lidocaine HCl. Tissue drug was expressed as nanomoles lidocaine per mg wet weight of nerve (Popitz-Bergez et al., 1995).

Statistics. The results are reported here as means ± S.E.; the numbers of independent observations are also included. One-way ANOVA test and unpaired two-tail Student’s t test (on normally distributed data) were used to evaluate the difference of mean tonic blocking concentrations of lidocaine among functional groups of axons, and the difference of mean normalized latency increases (see “Results”) before block. P < .05 was considered to be statistically significant.

Results

Tonic blocking of conduction by lidocaine. From about 100 experiments (1 unit per rat per experiment), 45 units were selected that fulfilled the following criteria: 1) receptive fields were all in the glabrous skin; 2) no cutaneous injury had been made within or close to (<10 mm) the receptive field; 3) the shape and amplitude of the unit action potential remained relatively stable during the whole experiment; 4) after conduction block by lidocaine the response of a unit to both natural and electrical stimulation returned within 30 to 45 min after washout with Liley solution.

Axons of sensory afferents differed in their tonic sensitivity to lidocaine. Traces from a nociceptive afferent C-fiber (resting CV = 1.05 m s−1) in which infrequently stimulated impulse conduction (0.2 Hz) was blocked by 0.8 mM lidocaine are shown in figure 1. At incrementally increasing concentrations of lidocaine the conduction latency increased progressively, reaching steady state after 15 to 20 min at each concentration until, at 0.8 mM lidocaine, conduction failure occurred.

The concentration-response curves of lidocaine for the three fiber groups were expressed as a percentage of fibers...
eventually blocked at a given concentration (fig. 2). Lidocaine concentrations necessary to block nociceptive C-fibers spread over a wide range, 0.1 to 1.4 mM, whereas the lidocaine concentration to block Aδ- or Aβ-fibers had a narrower range: 0.1 to 0.6 mM for nociceptive Aδ-fibers and 0.2 to 0.8 mM for LTM Aβ-fibers. The median blocking sensitivity for different functional groups differed (table 1). Nociceptive C-fibers had the highest median blocking concentration, 0.80 ± 0.32 mM, which is significantly higher than that of nociceptive Aβ-fibers (P < .0001) and LTM Aβ-fibers (P < .02). Nociceptive Aδ-fibers tended to be blocked at a lower concentration (0.32 ± 0.18 mM) than that for blocking the LTM Aβ-fibers (0.41 ± 0.15 mM), although the difference was not statistically significant (P = .21). Furthermore, nociceptive fibers as a group (including both Aδ- and C-fibers) showed a significantly higher median blocking concentration (0.62 ± 0.36 mM) than the median for LTM Aβ-fibers (P < .01).

Lidocaine also produced a reversible, monotonic decrease in the conduction velocity. For example, for the C-nociceptor shown in figure 1, the conduction slowing before impulse blockade was quantitated by the increase in conduction latency; the largest measured latency before failure was 144% of the control latency. The normalized latency change for this unit, which estimates the percentage of impulse slowing that would occur if the entire conduction length of the axon were to be exposed to lidocaine, was 248%, (see “Materials and Methods”; resting conduction latency = 118 ms, conduction length = 124 mm and latency change = 52 ms before blockade). Latency measurements reported in table 1 were taken after exposure to a particular sub-blocking lidocaine solution for 20 min, a time which was adequate to achieve a steady state of lidocaine uptake (see below). In the experiment shown in figure 1, action potential propagation through the chamber was restored by washout with Liley solution for 30 min. At that time the conduction latency of the unit had recovered to just slightly above the control latency (within 10%).

Within each functional fiber group, there was no correlation between resting conduction velocity and lidocaine blocking concentrations (fig. 3A): the calculated linear correlation coefficients were 0.16, 0.36 and 0.06 for nociceptive C-fibers, Aδ-fibers and LTM Aβ-fibers, respectively. Similarly, there was no correlation between the normalized latency increase that occurred just before conduction block and the resting CV (fig. 3B) for nociceptive Aδ-fibers and LTM Aβ-fibers (correlation coefficients = 0.49 and 0.09, respectively). However, a significant correlation was found between the normalized latency increase and the resting CV for nociceptive C-fibers (correlation coefficient = 0.82). Furthermore, on average, in these most LA-resistant axons, conduction also slowed more before the impulse was abolished than in the myelinated fibers (table 1).

Comparison was made within the Aβ-fiber group to see

![Fig. 2. The concentration-failure curve of lidocaine for tonic block of different fiber groups.](image)

![Fig. 3. Scatter plot of lidocaine-blocking concentration and normalized latency increase as a function of resting CV. (A) lidocaine-blocking concentrations vs. resting CV; (B) normalized latency increase just before conduction block (calculated by the formula described in the text) vs. resting CV. △, RA, n = 10; •, SA, n = 11; □, M, n = 8; ○, nociceptive Aδ-fibers, n = 6; ■, nociceptive C-fibers, n = 10.](image)
whether or not the lidocaine susceptibility was related to the functional submodalities. Table 2 shows that there was no significant difference among the mean tonic blocking concentrations for the three functional subgroups of the mechanosensitive Aβ-fibers: RA, SA and M. Among the three subgroups, M axons showed the smallest mean latency increase before conduction block, but this was not significantly lower than those for axons of the other two subgroups.

Use-dependent block by lidocaine. Lidocaine induced a use-dependent failure of conduction during high frequency stimulation of mechanoreceptor afferents. At 0.1 mM lidocaine, no impulse conduction failures were observed in any LTM Aβ-units (n = 17: 7 RA, 4 SA, 6 M) during the 10-pulse, 200-Hz stimulation train used to induce phasic blockade. The phasic blocking concentration of lidocaine that caused either 10% or 50% failure of impulse conduction in fibers that were not already tonically blocked ranged from 0.2 to 0.6 mM and 0.2 to 0.7 mM, respectively. The use-dependent blocking effect, assessed as the ratio of phasic block/tonic block, was greatest at lidocaine concentrations of 0.2 to 0.3 mM (fig. 4A).

We calculated the weighted failure (the number of failed impulses over total number of stimulated impulses) during burst stimulation at different lidocaine concentrations for 17 LTM Aβ-units and plotted a concentration-response curve for weighted failure (fig. 4B). Whereas the IC_{50} of lidocaine for tonic block was 0.41 mM (table 1), a 50% phasic block at 200 Hz could be produced by 0.28 mM lidocaine (fig. 4B). This reflects an effective increase of 46% in lidocaine’s potency for blocking impulses in Aβ afferents.

The use-dependent block of LTM Aβ-fibers produced by lidocaine did not correlate with resting CV (fig. 5). The correlation coefficients for the 10% or 50% phasic blocking concentrations against the resting CV were 0.16 and 0.24, respectively. Furthermore, the use-dependent block susceptibility was not related to the functional submodalities of the LTM Aβ-fibers (data not shown).

Four nociceptive Aδ-fibers and five C-nociceptors were studied for use-dependent block using a stimulation profile consisting of 5-Hz and 10-Hz bursts (10 stimuli in each burst) separated by a 30-s, 0.5-Hz recovery period. These are impulse frequencies consistent with discharge to a natural noxious stimulus in small diameter nociceptive fibers (Burgess and Perl, 1967; Perl, 1968; Raymond et al., 1990). None of these fibers showed any phasic conduction failure during the bursts at lidocaine concentrations that were below their tonic blocking concentrations (0.1–0.7 mM). The results indicate that the frequency or the number of burst stimuli used here

![Fig. 4. Phasic effect of lidocaine. LTM Aβ-units were stimulated with 200-Hz burst stimulation. Total number of units: 17, including 7 SAs, 4 RAs, and 6 Ms. (A) phasic blocking effect of lidocaine. The y-axis represents (in percentage) the number of Aβ-fibers phasically blocked. (B) phasic dose-response curve of lidocaine where the y-axis represents (in percentage) the number of failed impulses over total number of stimulated impulses, i.e., adds both tonically and phasically blocked fibers. The smooth curve in B is the best-fitting third-order polynomial to the hatched bars, and the dashed line indicates the lidocaine concentration (0.28 mM) which intercepts the 50% level on this curve.

The effects of lidocaine on such activity-dependent changes in latency were examined in 17 LTM Aβ-units (7 RA, 4 SA...
and 6 M) with a preset stimulation profile (see "Materials and Methods"). Under control conditions, not all units have a burst supernormality. Lidocaine changed burst supernormality in a dose-dependent manner in these axons: at 0.1 mM lidocaine, the burst supernormality decreased by an average of 198 ± 88% from the drug-free values; at 0.2 mM lidocaine, the burst supernormality decreased by an average of 443 ± 127% (n = 16: 6 RA, 4 SA, 6 M). As a result, at 0.2 mM lidocaine positive supernormality was not observed in any of the units that showed this behavior under control conditions (n = 10: 4 RA, 2 SA, 4 M); in fact, only further slowing of conduction was present as a result of previous impulse activity during burst stimulation. As shown in figure 6B, lidocaine at 0.4 mM produced tonic slowing of the resting conduction and an even greater slowing plus occasional failures during the burst, and at 0.5 mM lidocaine (fig. 6C) no impulses in the burst were conducted after the first one, reflecting use-dependent block (see fig. 4A above). Supernormal behavior returned after lidocaine washout, although some tonic conduction slowing persisted (fig. 6D).

After prolonged repetitive impulse conduction in LA-free conditions, axons show a long-lasting increase in conduction latency which is termed “depression” (Raymond, 1979; Carley and Raymond, 1987). In the present study most Aβ-units (14 of 17) showed some degree of depression after the 200-Hz burst stimulation (fig. 7A). Among these fibers, depression was reduced by lidocaine in a concentration-dependent manner in 11 (4 RA, 4 SA, 3 M; fig. 7, B and C). In general, lidocaine induced a slowing of conduction in Aβ-fibers during a burst but suppressed the endogenous slowing that otherwise follows prolonged bursts in the absence of lidocaine, as shown by the altered patterns of dots and hatching in figure 7.

**In vivo uptake of 14C-lidocaine.** The time course of lidocaine uptake by the nerve during superfusion in vivo had a phase of rapid uptake from 0 to 7 min; a steady state was reached in about 20 min (fig. 8, inset). Further exposure for 60 min did not result in a significantly greater uptake of lidocaine by the nerve than that at 20 min (P > .10).

The longitudinal distribution of lidocaine in the nerve after 20 min of superfusion is shown in figure 8. The nerve segments inside the bathing chamber had a mean lidocaine content of 1.0 ± 0.2 nmol/mg of wet nerve. The nerve segments outside the bathing chamber contained very little lidocaine: there was less than 0.05 nmol/mg of wet nerve in segments 1 and 5, and no lidocaine was detectable in segment 6. Uptake of lidocaine into these nerves in vivo was very close to the uptake measured in isolated, ensheathed nerve incu-

---

Fig. 5. Phasic blocking concentration vs. Aβ resting CV. The y-axis plots the lidocaine concentrations at which all studied LTM Aβ-fibers, ordered on the x-axis by their resting conduction velocity, demonstrated impulse failure during high-frequency stimulation; ■ fibers that had more than 10% of failures during 200-Hz bursts. ○, fibers that had more than 50% of failures during 200-Hz bursts. The fibers in the slower conducting range (15–27 m·s⁻¹) were no more susceptible to phasic block than those in the intermediate (30–35 m·s⁻¹) or higher (35–45 m·s⁻¹) ranges.

Fig. 6. An example of the effect of lidocaine on activity-dependent changes of impulse conduction. (A) Without lidocaine, the axon (RA, resting CV = 33.7 m/s) showed a modest supernormal conduction, the second impulse having a shorter latency than the first. (B) At 0.4 mM lidocaine, the unit still conducted but was subnormal and there were some conduction failures during bursts, e.g., discontinued traces beyond the 6th stimulus. (C) At 0.5 mM lidocaine, only the first impulse of each burst was conducted and its latency was increased over control (panel A). (D) After 30 min of washout in Liley solution, the unit became supernormal again and there were no conduction failures during bursts, although some conduction slowing remained. The separate symbols identify the different trains of high frequency (200 Hz, 10 stimuli) stimulation.
bated for 60 min in 0.4 mM 14C-lidocaine at 35°C (Popitz-Bergez et al., 1995).

**Discussion**

**Lidocaine susceptibility and functional modalities.** With regard to diameter, the *in vivo* results on rat sciatic nerve reported here are consistent with prior *in vitro* findings in frog sciatic nerve (Raymond, 1992) and rabbit vagus nerve (Fink and Cairns, 1984), as well as *in vivo* work in cat sciatic nerve (Franz and Perry, 1974), all of which showed that nerve diameter, as estimated by conduction velocity, does not predict the tonic anesthetic susceptibility for conduction failure of individual fibers.

As for function, although we did not observe a tight clustering of tonic lidocaine blocking concentrations according to a fiber’s functional modality, the LA susceptibility was not randomly distributed for different functional groups. Low-threshold mechanoreceptor Aβ-fibers were blocked over a relatively narrow range of lidocaine concentrations (0.2–0.8 mM), whereas nociceptive C-fibers tended to be blocked over a wider range (0.1–1.4 mM). When characterized by their median blocking concentrations (IC50s), nociceptive Aδ-fibers were blocked at the lowest concentration, 0.32 mM, compared with 0.41 mM for LTM Aβ-fibers and 0.80 mM for nociceptive C-fibers.

The difference in tonic lidocaine susceptibility observed between nociceptive C-fibers and Aδ-fibers was not altogether surprising. These two groups of fibers subserve two distinct types of pain perception. Nociceptive C-fibers innervating the glabrous skin have been studied extensively in rat, monkey and human, where they are believed to be involved in the conduction of burning pain or “slow pain” (LaMotte and Campbell, 1978; Fleischer et al., 1983; Campbell et al., 1989), and are not easily sensitized to heat (Campbell and Meyer, 1983; LaMotte et al., 1983). Nociceptive Aδ-fibers, also called high-threshold mechanoreceptive Aδ-afferents (Burgess and Perl, 1967; Perl, 1968), are probably similar to the type I nociceptive A-fibers found in human and monkey, fibers which are responsible for the primary hyperalgesia after injury to glabrous skin (Campbell and Meyer, 1986) and which have lower thresholds to mechanical stimuli than nociceptive C-fibers (Campbell et al., 1989). Pricking pain or “fast pain” is conducted by Aδ-fibers. The different functional roles and impulse codes for nociceptive C-fibers and Aδ-fibers could be associated with the physiological attributes of axons, and in turn, result in a difference in impulse conduction safety (Raymond et al., 1990) and hence, susceptibility to lidocaine.

The data on tonic susceptibility of single units to lidocaine reported here and in other studies have been understood to imply that intrinsic differences in conduction safety exist among fibers (Raymond and Gissen, 1987). Prior studies
strongly suggest that such differences do not originate solely on the basis of fiber size (Fink and Cairns, 1984; Raymond, 1992). Conduction safety depends on several features of axonal electrophysiology other than size, including the density and gating behavior of particular ion channels, the locus and degree of myelination, the density of Na⁺/K⁺ or other electrogenic ion pumps, etc. Grouping fibers by function in this study has, to a certain extent, revealed such a difference, with the assumption that lidocaine is equally intrinsically potent in inhibiting the Na⁺ channels in these different primary afferents.

It is important to point out that in this study we did not attempt to differentiate functional subgroups within Aβ- or C-nociceptive fibers based on coding or firing pattern. It is possible, therefore, that the wide range of tonic lidocaine-blocking concentrations observed in nociceptive C-fibers reflects a variation in lidocaine sensitivity among different subgroups of nociceptive axons.

The length of nerve segment exposed to LA. Many studies of differential block with local anesthetics, in which nerve trunks were exposed to LA, were done with exposure lengths of 30 mm or less (see review, Raymond and Gissen, 1987). Studies with longer nerve regions exposed (Fink and Cairns, 1984; Raymond, 1992) do not support the historically familiar finding that nerve axon diameter governs the susceptibility to blockade. How does impulse conduction proceed in regions exposed to near blocking anesthetic concentrations? Such conduction is “decremental” (Lorente de Nó and Condouris, 1959; Raymond et al., 1989), decreasing in velocity continuously through the exposed axons’ length, a fact that contradicts the traditional idea (Franz and Perry, 1974; Nathan and Sears, 1961) that an exposure length sufficient that contradicts the traditional idea (Franz and Perry, 1974; Nathan and Sears, 1961) that an exposure length sufficient to cover three successive nodes in the largest fibers (Takeuchi and Tsasaki, 1942) ensures that length is not a factor in potency or in differential blockade. Because the effect of a change in exposure length on tonic LA susceptibility is smaller at long initial lengths, being minimal above 15 mm exposure in large myelinated fibers (Raymond et al., 1989), in this study the bathing chamber was made as long as was anatomically congruent (22 mm).

The results from 14C-lidocaine uptake experiments reported here demonstrate that the part of nerve exposed to lidocaine was indeed restricted to the 22 mm of length inside the chamber: little drug was present in the nerve at the margins beyond the chamber. The lidocaine content of nerve within the chamber was uniformly distributed, being similar at the ends and in the middle of the exposed segment. This content is almost identical with lidocaine levels found after long soaks of ensheathed nerves in vitro and supports the assumption that during superfusion the anesthetic equilibrates across the sheath and within the nerve during steady-state blockade. The small amount of lidocaine detected at the margins outside the chamber was probably the result of the diffusion of the drug in the space between the individual nerve axons as well as the effect of blood circulation via the small blood vessels inside the nerve.

Triple actions of lidocaine: tonic, use-dependent and activity-dependent effects. The dynamic effects of tonically sub-blocking concentrations of lidocaine during impulse discharge are complex and will depend on the pattern of impulses contained within a burst. For the LTM Aβ-fiber population that was examined carefully here, the overall slowing in CV and the increase in failure probability during 200-Hz burst stimulation was large at concentrations (>0.4 mM) where half the Aβ-fibers were already tonically blocked (fig. 6). At lower lidocaine concentrations (0.2–0.3 mM), phasic block at 200 Hz accounted for a relatively greater increase in failures, even though the overall failure rate was less than at 0.4 mM. Lidocaine’s phasic impulse blocking action is determined by its intrinsic dissociation rate from “activated” axonal sodium channels (Chernoff, 1990) as well as by its concentration-dependent association rate. The effective potency for impulse failure is thus frequency dependent (fig. 4A) in a way that will be more pronounced for the more slowly dissociating LAs, such as bupivacaine (Chernoff, 1990).

Conduction slowing is also a frequency-dependent phenomenon which is altered by LAs and which may be consequential for sensory processing in the spinal cord. At the sub-blocking concentration of 0.2 mM, lidocaine reverses the transient supernormality, a large enough effect in the exposed area to offset the supernormal conduction that was present in the rest of the conduction path not exposed to lidocaine. Lidocaine also inhibits the depression that accumulates with repeated impulse activity. The residual depression (fig. 7C) probably reflects the depression present in the region of the nerve unexposed to LA. By both actions, the LA reduces the endogenous dynamic changes in CV while it imposes its own use-dependent slowing.

Single unit vs. CAP data. The sensitivity of Aβ-fiber impulses to lidocaine can be compared with data from Rosenberg and Heavner (1980) in which inhibition of the A-fiber CAP was studied in vitro in rat sciatic nerve. The median value for tonic block of Aβ-fibers from the present study was 0.41 mM (table 1), substantially higher than the 0.19 mM IC₅₀ value from CAP inhibition found by Rosenberg and Heavner (1980). The difference suggests that the suppression of CAP amplitudes by low concentrations of lidocaine (<0.2 mM) is not caused by the abolition of impulse conduction in fibers so much as it is caused by the differential slowing of impulse conduction and/or the decrease in the impulse currents in single fibers. Neither of these two factors presages a change in perception to the extent that abolition of impulses does, although both may contribute to altered burst patterns.

The correlation of impulse blockade with behavioral modifications. Impulse blockade can be correlated with behavioral changes assessed neurologically in the rat after injection of lidocaine near the sciatic nerve (Popitz-Bergez et al., 1995). Within 5 to 10 min after injection of 0.1 ml of 38 mM (1%) lidocaine (pH = 6.8), a steady state of intraneuronal lidocaine is reached. This plateau level, averaging about 4 nmol/mg wet nerve and maintaining a constant longitudinal, Gaussian-like spread with σ ~ 10 mm, persists for 10 to 15 min and then declines to control levels over the next 40 to 50 min. The plateau value of intraneuronal lidocaine from such injections can also be reached by equilibrium in vitro incubation in bathing concentration of 0.8 mM lidocaine (Strichartz, G. R. and Leeson, S., unpublished observation), a concentration which in the present study blocked impulses in 100% of both LTM Aβ-fibers and nociceptive Aδ-fibers and in 65% of the nociceptive C-fibers. During this plateau period, neurological examination showed that motor function and proprioception in regions innervated by the sciatic nerve
were also fully blocked and that nociception was greatly reduced (Thalhammer et al., 1995).

Differential behavioral deficits were observed at the onset and during the regression of block (Thalhammer et al., 1995) and might be correlated with lidocaine’s differential impulse blockade. Proprioception was the first activity to show impairment, followed by motor function and nociception. This finding is consistent with the dependence of proprioception on mechano-sensitive Aβ-fibers and the greater mean vulnerability of such fibers to impulse blockade by lidocaine (table 1). During regression of the block at 30 to 40 min there was an early, graded recovery of nociception; withdrawal to deep digit pinch, mediated by proximal thigh and hip muscles, returns at a time when pinching only the skin produces no withdrawal response. Intraneural lidocaine assessed at this stage corresponds to an equilibrium bathing concentration of 0.2 to 0.3 mM, for which tonic block of nociceptive Aδ-fibers is 50 to 65%, of LTM Aβ-fibers is 10 to 40% and block of nociceptive C-fibers is 10 to 15% (cf. fig. 2). This partial impulse blockade is thus consistent with the observation of partial anesthesia in vivo.

Activation of Aδ-fibers may be more prominent during skin pinch compared with a relatively greater recruitment of C-fibers when deeper tissue structures of the digit are squeezed, which could account for the differential behavioral response on the basis of differential tonic block. However, until we know the afferent discharge patterns in the various fiber groups for these different stimuli and the overall phasic failure properties under these conditions, we cannot predict the integrated actions of LAs on peripheral afferent impulse propagation. Given the current tools and techniques, an integrated approach should be capable of resolving this correlation under the dynamic conditions of in vivo local anesthesia.

One caution is in order. The small number of Aδ and C-nociceptors in this study may not be representative of the overall population of nociceptors, because only robust axons with persistent conduction properties fit our selection criteria. Possibly, fibers with more marginal conduction properties are important contributors to nociceptive input in intact animals and some care should be used in extending these results to the behavior of all nociceptors in conscious, responding animals.

In conclusion, the results reported here support prior single unit studies showing the lack of correlation of LA sensitivity with fiber diameter. The separation of nociceptors in Aδ- and C-fibers on the basis of CV shows that these two classes, despite serving the same general sensory modality, are blocked at about 4-fold different anesthetic concentrations. Therefore, function per se does not determine susceptibility to block, assuming that pinching pain, burning pain and aching pain are the same modality (Campbell and LaMotte, 1983). On the basis of the apparent differential sensitivity to lidocaine of Aδ- over C-fibers, during peripheral nerve block an anesthetist might presume from stimuli that selectively induce first pain (e.g., pin prick) the existence of a degree of analgesia that is in actuality insufficient to block second pain (e.g., burning, aching). Which of these forms of pain accompanies surgical incision and its sequelae is an important question for establishing anesthesia adequate for surgery and sufficient to preempt postoperative hyperalgesia (Kissin and Raja, 1995).

Acknowledgments

We wish to thank Dr. S. Leeson and Dr. F. A. Popitz-Bergez for help with the lidocaine uptake experiments, D. S. Chang for collecting some of the data and S. S. Waikar for writing a computer program for data analysis. Excellent secretarial support by Ms. Ellen Jacobson is gratefully regarded.

References


Send reprint requests to: Gary R. Strichartz, PhD, Department of Anesthesia Research Laboratories, Brigham and Women’s Hospital, 75 Francis Street, Boston, MA 02115.