Anesthesiology
81:657–667, 1994
© 1994 American Society of Anesthesiologists, Inc.
J. B. Lippincott Company, Philadelphia

Concentration Dependence of Lidocaine-induced Irreversible Conduction Loss in Frog Nerve

Cedric R. Bainton, M.D.,* Gary R. Strichartz, Ph.D.†

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

Background: Concentration is a causal factor in local anesthetic nerve toxicity. Therefore, it is essential to define a concentration below which injury does not occur. We explored the relation of lidocaine concentration and nonreversible block after drug washout in frog sciatic nerve.

Methods: Frog sciatic nerve was mounted in sucrose-gap or extracellular recording chambers. The observed compound action potential in response to a supramaximal stimulus was used as a measure of nonreversible block after applying lidocaine in a range of concentrations (0.5–200 mM for 15 min) and then washing off (for as long as 180 min).

Results: Lidocaine causes a nonreversible block after washout that begins at concentrations as low as 40 mM and increases in a graded fashion with increasing concentrations to complete ablation of the compound action potential at 80 mM (~2%). Extended storage of frogs (5 weeks) at 4°C makes the nerves more resistant to the effects of lidocaine. The presence of nifedipine (10^{-5} M), an L-type calcium-channel blocker, makes the nerves more resistant to lidocaine as well.

Conclusions: Lidocaine induces a nonreversible loss of impulse activity in frog nerve in a progressive fashion with increasing drug concentration, beginning at 40 mM (~1.0%). The range of lidocaine that produces such changes in mammalian nerve awaits determination. (Key words: Anesthetics, local: lidocaine. Complications: nerve injury. Ions, calcium: nifedipine.

IN 1933, Lundy *et al.*[1] reported that 1.2 g procaine injected intrathecally in dogs caused irreversible paralysis when administered as a 25% solution, although 1.2 g administered as a 12.5% solution caused no injury. Thus, procaine, and perhaps all other local anesthetics, has the potential to be neurotoxic and the concentration of the drug is a critical factor. The role of concentration in nerve injury has subsequently been confirmed by several investigators.[2–5]

In 1991 Rigler *et al.*[6] and Schell *et al.*[7] described patients with cauda equina syndrome associated with the use of microcatheters for the continuous administration of spinal anesthetics. Additional studies, using models of cerebrospinal fluid,[8–10] suggested that local anesthetics, when injected slowly (as in some cases when microcatheters are used), were not dispersed in the mock spinal fluid; rather, the drugs layered to a dependent position, remained essentially unmixed, and contacted spinal nerves with minimal dilution. If undiluted drug is the cause of clinical neurotoxicity with the use of microcatheters, it follows that the concentrations of drugs as supplied by the manufacturers exceed a safe range.

Because anesthesiologists cannot predict how cerebrospinal fluid will dilute administered drugs, the appropriate modification to ensure safe practice would be to give concentrations of drugs that in themselves never exceed a safe concentration. To begin to define a safe concentration, Lambert *et al.*[11] examined the neurotoxic potential of local anesthetics used for spinal anesthesia as supplied by the manufacturers. They used frog sciatic nerve mounted in a sucrose-gap chamber as a model. The technique has the advantage that it can measure transmembrane potential as a measure of nerve viability. Local anesthetics were placed on the nerve for 15 min and then washed off for 2–3 h. They found that all of the undiluted anesthetics in concentrations supplied for clinical use (*e.g.*, 5% lidocaine, 0.5% tetracaine, or 0.75% bupivacaine) caused a nonreversible ablation of the stimulated compound action potential (CAP) after drug removal, whereas the membrane resting potential remained intact. Thus, local anesthetics had in some way made the nerves nonfunctional without

* Professor of Anesthesia, University of California, San Francisco.

† Professor of Anaesthesia (Pharmacology), Harvard Medical School.

Received from the Department of Anesthesia, University of California, San Francisco, California, and the Anesthesia Research Laboratories, Brigham and Women's Hospital, Boston, Massachusetts. Accepted for publication April 29, 1994. Supported in part by United States Public Health Service grant GM 35647 (to G.R.S.). Presented in part at the annual meeting of the Association of University Anesthetists, Winston-Salem, North Carolina, May 1993 and at the annual meeting of the American Society of Anesthesiologists, Washington, DC, October 1993.

Address reprint requests to Dr. Strichartz: Anesthesia Research Laboratories, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

destroying them. They also tested 7.5% dextrose and found no loss of CAP.

It remained to identify a concentration below which the nerve was not irreversibly affected. Thus we sought to measure a concentration–response relation for this nonreversible lesion. The obvious advantage of describing a concentration–response function is that the injury can be defined by absolute drug concentrations, which can then ultimately be compared for all relevant drugs. In addition, the response can be probed with a variety of agents to see whether the response can be perturbed, either increased or decreased, as a means to explore the mechanism of the nonreversible lesion.

Materials and Methods

Animals

The animals were cared for and treated according to the procedures approved by the Harvard Medical Area Guidelines for Animal Care and Use of Laboratory Animals. Bullfrogs (*Rana catesbeiana*) were anesthetized by hypothermia (−20°C, 30 min) before dissection, a method approved by the Harvard Committee on Animal Care. Animals were obtained from a single supplier (Charles D. Sullivan, Nashville, TN) and kept in a cold room at 4°C before use. Frogs were used within 2 weeks of delivery except during April and May when the supplier was prohibited by law from collecting frogs because of the natural breeding season. Thus, it was necessary to stock a supply of frogs on April 1 large enough to last 2 months. These frogs were kept in a cold room at 4°C with biweekly water changes. They showed absolutely no interest in feeder goldfish or crickets during this period. We mention these details because on or about May 8, approximately 5 weeks after the animals had been placed at 4°C (and 14 days since the previous stable experiments), frog nerves showed a distinct change in experimental response. We have called this an "adaptational" change, for want of a better term, suspecting that the 5-week semihibernation or the breeding cycle or both were causal factors.

Electrophysiologic Measurements

Sciatic nerves were excised from bullfrogs for mounting in sucrose-gap or extracellular chambers as a method to record CAPs.

Excised nerves were stored at 4°C in a frog Ringer's solution composed of 110 mM NaCl, 2.5 mM KCl, and 2.0 mM $CaCl_2$ and buffered with 5.0 mM 3-[N-morpholino]-propanesulfonic acid. The *p*H was adjusted to 7.2 with 1 N NaOH. All measurements were made at room temperature.

The nerves were used within 72 h of dissection. Stored nerves with sheaths intact remain fully viable for this time period. Nerves were desheathed just before they were mounted in a sucrose-gap chamber.[12] Mounting consisted of placing the nerve across a series of wells separated from each other by petroleum jelly seals. The proximal end of the nerve segment was placed into two stimulating wells and laid distally across a "test" well for drug testing and an "intracellular" well for recording. These last two wells were separated by a sucrose gap (3 mm in diameter) through which the nerve was gently threaded. The open wells were filled with frog Ringer's solution. Stimulating electrodes of Ag–AgCl were placed in the two proximal wells, recording electrodes across the sucrose gap in the test and intracellular pools and a ground electrode in the test pool. The nerve was stimulated (SD-9 stimulator, Grass Instruments, Quincy, MA) supramaximally (70 V) with 50-μs square-wave pulses at both 1- and 40-Hz frequency. The CAP was measured across the sucrose gap through which 0.18 M sucrose flowed continuously (0.2–0.3 $ml \cdot min^{-1}$). CAPs were recorded on a storage oscilloscope (Tektronix, Beaverton, OR), which permitted superimposition of the 40- and 1-Hz stimulation. The recorded 1-Hz CAP was the average response resulting from five or more consecutive stimuli. The 40-Hz CAP was measured as the minimum amplitude reached after a continuous stimulus (approximately 5 s). The 40-Hz response was invariably less than the 1-Hz response. Measurements were made at 5-min intervals during control and drug washout periods until the CAP was stable for at least 15 min. Polaroid photographs (Cambridge, MA) were taken as indicated in results.

In general, 1 h was required for the CAP to reach a stable maximum. Nerves suitable for study were selected during this interval. Criteria for selection, before the application of test drugs, are derived from the data as outlined in figure 1. (1) Preparations should be observed for at least 1 h before the application of test drugs; (2) CAP should have reached a stable maximum; and (3) CAP should demonstrate less than 5% decay per hour after reaching maximum. In figure 1, 8 of 11 preparations satisfied these selection criteria. In these eight the average decay rate in CAP after the stable maximum was reached was 3%/h. The ratio of the control CAP amplitude at 3 h to that at 1 h was 0.94 ±

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

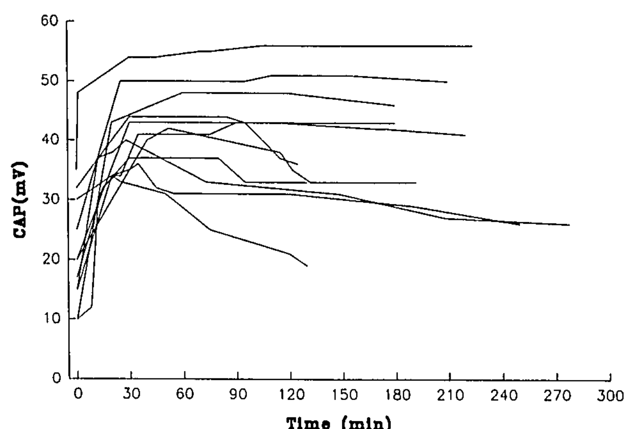

Fig. 1. Compound action potentials (CAP) from 11 frog sciatic nerves were observed for stability with time. Nerves were mounted in sucrose gap chambers (GAP) and stimulated once per second with a supramaximal stimulus (70 V). The CAPs took as long as 60 min to mature (reach a maximum). The CAPs for 8 nerves are essentially stable thereafter. Three show rapid decay beginning within 60 min. These data were used to define selection criteria for usable nerve preparations.

0.04 (mean ± SE, n = 8) This 2-h period is approximately equal to the time when drug was applied and washed out. Thus each nerve was observed for as long as 3 h to complete a test. No preparation was observed for more than 3 h. To speed the collection of data, five identical chambers were set up and run in parallel sharing stimulating and recording equipment through a multiplexing switch.

Nerves were also mounted in an "extracellular" recording chamber by draping a nerve sequentially across electrodes in three pools arranged in series. The first pool contained two stimulating electrodes, a middle pool contained a ground electrode and a final pool held two recording electrodes. Lateral pools were emptied of Ringer's solution and filled with mineral oil. The central pool was filled with frog Ringer's solution. It was in this central pool that drugs were applied and washed off. Pools were separated by petroleum jelly dams. Nerves were stimulated as in the sucrose gap (see above).

When the CAP had stabilized during the control period of both sucrose-gap and extracellular setups (30–60 min), plain frog Ringer's solution was removed from the test pool and replaced with solutions of varying concentrations of lidocaine (0.5–200 mM) dissolved in frog Ringer's solution, *p*H-adjusted to 7.2. Lidocaine was applied for 15 min and then removed. The test pools were actively washed for 10 min by frequently replacing the contents with fresh Ringer's solution through a Pasteur pipette. This was followed by a continuous superfusion of fresh Ringer's solution through a tubing and needle at 1 ml/min for the subsequent hours of washing.

Source of Drugs

Nifedipine and lidocaine were obtained from Sigma Chemical (St. Louis, MO) and 3-[N-morpholino]-propanesulfonic acid sodium buffer from Calbiochem (a Jolla, CA).

Data Treatment

Data are presented as direct values of CAP or as residual CAP expressed as a percentage of control values.

Although this approach disregards the spontaneous decay in CAP (approximately 6% over the 2-h test period; fig. 1) we elected not to apply a correction because times for drug washout were essentially identical for all nerve preparations, such that the error was constant. Differences between CAPs in control and lidocaine were evaluated by Student's two-tailed *t* tests. *P* values are reported in the text. Data are reported as means ± SEM.

Concentration–response curves were calculated with a Logistics Plot (MicroCal Origin, Northampton, MA), with the following equation for residual CAP (percentage of control CAP [%CAP]):

$$\%CAP = 100/(1 + [\text{lidocaine}]/EC_{50})^n).$$

Midpoints of the curves (concentrations producing nonreversible block in 50% of nerve preparations: [EC_{50}]) and slope characteristics were derived from this equation. Statistical significance of the difference of EC_{50} and slope characteristics were derived from this equation. Statistical significance of the difference of EC_{50} or slope for the different experimental conditions was established at $P < 0.05$ by an unpaired *t* test[13] (table 1).

Results

Comparison of Reversible and Nonreversible Lidocaine Block of Frog Sciatic Nerve

The effect of 0.5 mM lidocaine on CAPs of a frog sciatic nerve mounted in a sucrose-gap chamber was to reduce the CAP amplitude at rates of stimulation of 1 and 40 Hz (fig. 2). This concentration of lidocaine degraded the CAP response to both 1- and 40-Hz stimu-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

Table 1. Parameters for the Fitted Curves in Figure 9

Experimental Condition	*n*	EC_{50}	±S.E.	*P* value Compared to GAP J-A	Slope	±S.E.	*P* Value Compared to GAP J-A
GAP (Jan–Apr)	63	50.45	1.93	—	4.854	0.838	—
Nifedipine	48	60.40	6.79	<0.2	2.133	0.542	<0.02*
GAP (May)	58	82.89	6.73	<0.001*	2.997	0.887	<0.2
Extra (May)	23	64.50	8.05	<0.001*	2.748	0.866	<0.2

* Significantly different, $P < 0.05$.

EC_{50} and slope are derived from the logistics equation, see methods.

lation, but the 40-Hz response was much more sensitive to lidocaine's inhibition. Alternatively, stated as ratios, the 40-Hz CAP, which was 86% of its companion 1-Hz CAP before lidocaine (because of normal refractory behavior) was now only 46% of its companion 1-Hz CAP. Thus, the absolute 40-Hz response and the ratio of 40-Hz/1-Hz responses were better measures of existing lidocaine effect. This observation exemplified the well-documented, use-dependent aspect of reversible inhibition by local anesthetics.[14] Both responses reverted to control levels within 15–20 min of washing.

In contrast, the effect of 40 mM (~1%) lidocaine on 1- and 40-Hz CAPs of a frog sciatic nerve was complete ablation of the CAP while present on the nerve (fig. 3). On washing, the CAP returned in 45 min to a new level diminished from the control. This new level remained stable for the subsequent 2 h of observation. The residual CAP at 1 Hz was only 65% of the original 1-Hz CAP, a 35% decrement. By comparison, the 40-Hz response, which was 86% of the companion 1-Hz response at control onset, was now 74% of its companion 1-Hz response after lidocaine, only a 12% decrement.

Forty millimolar lidocaine clearly degraded the residual CAP response to 1-Hz stimulation after the drug had been washed off for 1–2 h but had only a minor effect on the 40-Hz/1-Hz ratio. Although the cause of this degradation is unclear, residual lidocaine in the nerve is possible. To test this possibility, we compared the differential effects of 0.5 mm lidocaine on 1- and 40-Hz stimulus responses to the 1- and 40-Hz stimulus responses after washout of 40 mM lidocaine. Reversible

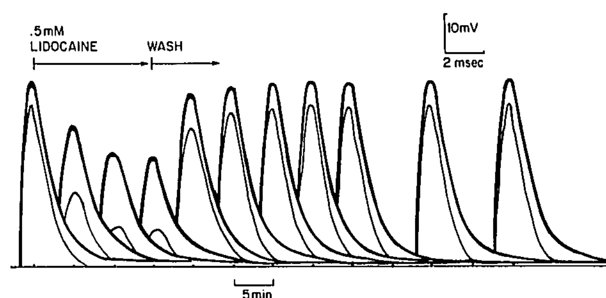

Fig. 2. The reversible effect of a given concentration of lidocaine on the compound action potential (CAP) of frog sciatic nerve. Lidocaine 0.5 mM was applied to a stable nerve preparation for 15 min and then washed off for 45 min. Photographs of the CAPs recorded on a storage oscilloscope were taken before drug application and at 50-min intervals thereafter. These images were traced from the originals and superimposed on each other at the indicated time intervals. There are two CAPs on each tracing, one resulting from a 1-Hz stimulus (heavy line) and one resulting from a 40-Hz stimulus (thin line). When 0.5 mM lidocaine is applied to the nerve, the 1-Hz CAP falls to approximately 50% of control, whereas the 40-Hz CAP is more sensitive, falling to 20% of control. Both 1- and 40-Hz CAPS revert to normal after 15–20 min of wash with frog Ringer's solution and are stable thereafter.

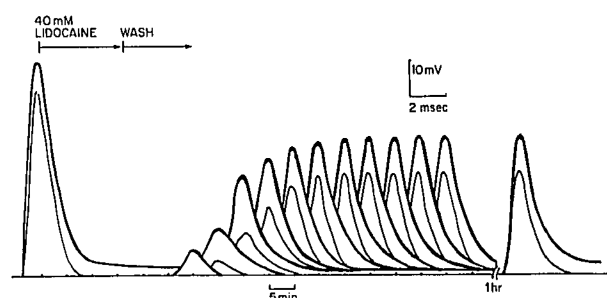

Fig. 3. The nonreversible effect of a given concentration of lidocaine. Forty millimolar lidocaine is applied to a stable nerve preparation for 15 min and then washed with frog Ringer's solution for 2 h. Photographs of compound action potentials (CAPs) were traced and redrawn in time as in figure 2. As expected, 40 mM lidocaine completely ablated the CAP when applied to the nerve. The 1-Hz CAP response began to return after 10–15 min of washing and reached a new level in 45 min, where it was stable for the subsequent 2 h of observation. The recovered 1-Hz CAP is only 65% of the original. In contrast, the 40-Hz response recovers with only a small decrement in the relation to its companion 1-Hz CAP.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

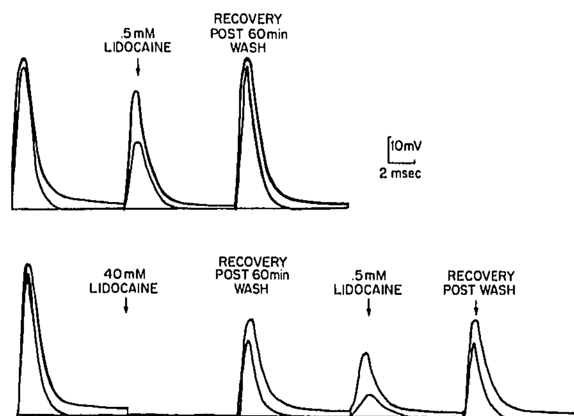

Fig. 4. Reversible (0.5 mM) and nonreversible (40 mM) effects of lidocaine compared on a single nerve preparation. The experiment is shown sequentially across (*top left* to *bottom right*). The results are presented vertically, with 0.5 mM over 40 mM lidocaine, for ease of comparison of control, drug application, and washout states. The full time course of change in compound action potential (CAP) is not shown; rather, the stable end result of that change is depicted as a single tracing of CAPs for 1- and 40-Hz stimulation. The recovery CAP after a 60-min wash (*top right*) is repeated (*bottom left*). Lidocaine 0.5 mM when applied to the nerve reduced both the 1- and the 40-Hz CAPs. The effects were completely reversed on washing. In contrast, 40 mM lidocaine abolished the CAP when applied. The 1-Hz CAP that returned after washing was only 70% of control, but the ratio of 40-Hz/1-Hz CAP was essentially unchanged. These new, stable CAP levels responded to the reapplication of 0.5 mM lidocaine in even more pronounced fashion, effects that were completely reversed with washing.

(0.5 mM) and nonreversible (40 mM) effects of lidocaine, as demonstrated in figures 2 and 3 on separate nerves, were thus compared on the same nerve (fig. 4).

As noted in figures 2 and 4, the 40-Hz CAP response was always less than the 1-Hz response and also was more sensitive to the presence of 0.5 mm lidocaine than is the 1-Hz CAP response. Thus, the ratio of the 40-Hz/1-Hz responses that resulted from 0.5 mM lidocaine, determined for a nerve preparation before the application of 40 mM lidocaine, could be used as a semiquantitative measure of residual lidocaine that persisted after washout. As shown in figure 4, the 40-Hz/1-Hz ratio that persisted after washout of 40 mM lidocaine was 0.76 (bottom traces) compared with a ratio of 0.57 caused by the direct application of 0.5 mM lidocaine (top traces). Thus, any lidocaine that remained in the nerve after the washout of 40 mM lidocaine must have be less than that when the nerve was equilibrated with 0.5 mM.

With this evidence the degraded 1-Hz CAP response after washout of 40 mM lidocaine could be interpreted. Because any residual lidocaine present after 40 mM lidocaine washout was less than 0.5 mM, it was thought that the 1-Hz CAP response after 40 mM lidocaine washout should lie somewhere between the control CAP and that caused by the direct application of 0.5 mM lidocaine. However, the degradation of the 1-Hz CAP response (bottom trace, fig. 4) was greater than that caused by the direct application of 0.5 mM lidocaine (top trace). Thus, residual lidocaine could explain part but not all of this degraded response; an additional process must have been operating. Reapplication of 0.5 mM lidocaine caused a more pronounced decrement of CAPs, particularly the 40-Hz response, and thus provided further evidence that this nerve was now altered after the application of 40 mM lidocaine.

Figure 5 compares the shape of CAPs stimulated at 1-Hz in the control state with those after the washout of 60 mM lidocaine and during the direct application of 0.5 mM lidocaine during these two states. The residual CAP after washout of 60 mM lidocaine was a scaled-down version of the control CAP with the same time to peak height and width of base. In contrast, when 0.5 mM lidocaine was applied directly during these two conditions, the peak height was delayed and the CAP was widened. These results are further evidence

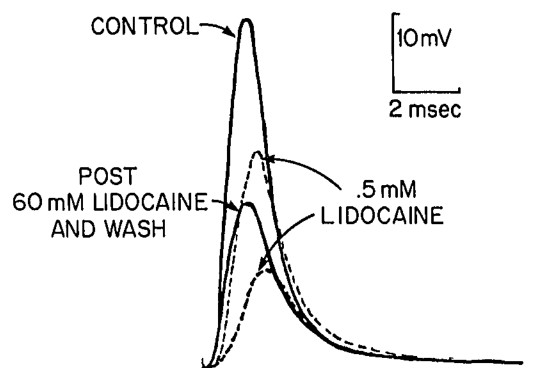

Fig. 5. Comparison of the shape of compound action potentials (CAPs) in four states: control, after washout of 60 mM lidocaine, with direct application of 0.5 mM lidocaine during control, and with direct application of 0.5 mM lidocaine after washout of 60 mM lidocaine. CAPs traced from photographs are redrawn over the points of stimulus artifact for each condition. Solid lines = CAPs for control and after application and washout of 60 mM lidocaine; dotted lines = CAPs that result from direct application of 0.5 mM lidocaine during control (taller CAP) and after 60 mM lidocaine (shortest CAP). The degraded response after 60 mM lidocaine is a scaled-down version of the control, with identical time for peak height (1.3 ms) and width of base (4.7 ms). In contrast, peak height and base are delayed during the direct application of 0.5 mM lidocaine.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

that the degraded CAP after application and washout of 60 mM lidocaine was not the result of residual lidocaine.

Repeated Application of the Same Lidocaine Concentration

The effect of repeated application of 60 mM lidocaine on a single sciatic nerve preparation was not cumulative (fig. 6). A nonreversible degradation of the CAP occurred after washout of the initially applied 60 mM lidocaine. Subsequent applications and washouts of 60 mM lidocaine did not further degrade the CAP; rather, the CAP recovered each time to the same level. This result indicates that the state of degraded CAP was dependent on concentration but independent of the time, beyond 15 min, that a given concentration was applied to the nerve. After each application of 60 mM lidocaine a subsequent application of 0.5 mM lidocaine showed a greater 40-Hz sensitivity to the applied drug than in the control state; this test also was constant after each exposure and washout of high-concentration lidocaine. This finding provides further evidence that the nerve was in an altered yet stable state.

Characteristics of Lidocaine Concentration versus *Degraded CAP*

Figure 7 is a plot of residual CAP amplitudes after the application and washout of varying concentrations of lidocaine. The residual CAP amplitude, plotted as a percentage of the control CAP, was strongly dependent on concentration, decreasing as the applied lidocaine concentration increased. The decrease was insignificant at 20 mM lidocaine (0.96 ± 0.03, n = 4) relative to control (0.94 ± 0.04, n = 8) (see Materials and Methods). At concentrations at or greater than 40 mM the irreversible decrease (CAP 0.74 ± 0.07 of control, n = 8) was significantly greater than the spontaneous loss of impulse amplitude in control nerves (fig. 7, inset). The fitted curve had a midpoint at 50 mM (1.4%), and degradation was essentially complete at 80 mM (2.2%) lidocaine. These data indicate that the response was reproducible over two periods because data points for January were virtually superimposable on the March–April points.

Adaptational Variations in Sensitivity to Lidocaine

On May 8, several weeks after the nerve-donating frogs had been maintained at 4°C, we began to observe that residual CAPs after application and washout of 80–100 mM lidocaine were dramatically increased. This change was confirmed by repeated measurement with freshly made lidocaine solutions. Thus it was necessary to repeat the tests of concentration–response relations for the residual CAP. We also believed it necessary to substantiate this change by using an entirely different method: the mounting of nerves in an extracellular recording chamber.

The extracellular chamber was used here for two reasons. First, the conditions of sucrose-gap recording involve both threading a bent nerve through a narrow orifice, thereby potentially injuring the nerve, and flowing ion-free sucrose through the nerve, producing a local hyperpolarization[15] and thereby altering concentration–inhibition relations for reversible block by local anesthetics.[16] In the extracellular chamber neither of these conditions occurs.

Second, the nerve is placed only superficially on the electrodes in the extracellular chamber so that its position can be adjusted with minimal manipulation. In several experiments we pulled nerve regions that originally lay underneath the bordering petroleum jelly seals into the central test pool during the postexposure perfusion period. This technique permitted the washout of marginal parts of the nerve into which high concentrations of lidocaine may have diffused and remained, a possibility suggested by tracer studies with methylene blue dye. That situation also theoretically

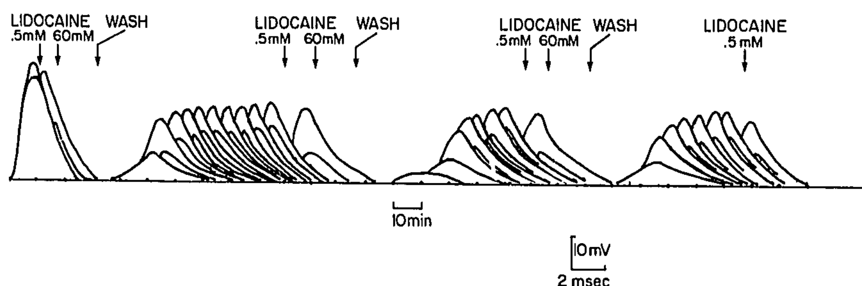

Fig. 6. The effect of repeated application of 60 mM lidocaine on a single nerve preparation. Compound action potentials (CAPs) were traced from photographs and redrawn in real time. Each tracing consists of two CAPs, the tallest in response to 1-Hz stimulation and below this the response to 40 Hz. Arrows = the application 0.5 mM and 60 mM lidocaine and onset of washing.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

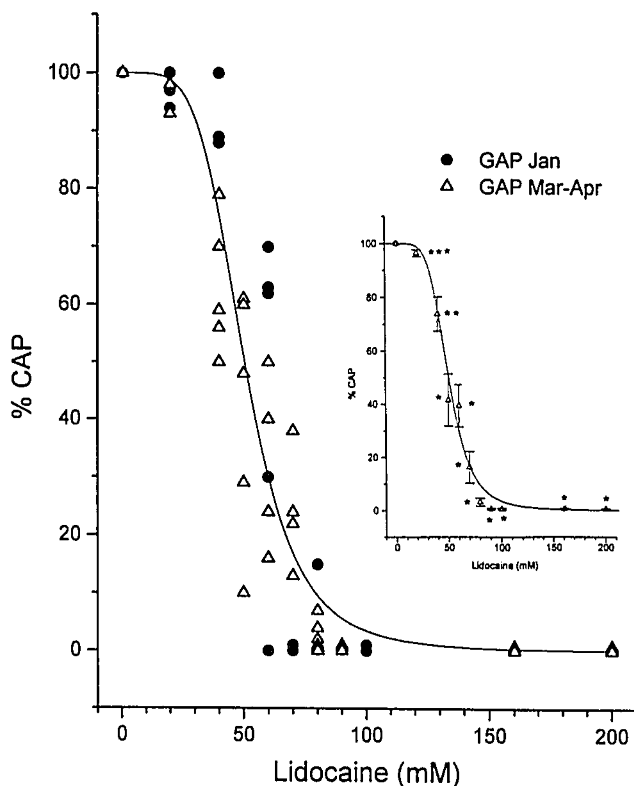

Fig. 7. Residual 1-Hz compound action potentials (CAPs) that result after the application and subsequent washout of lidocaine plotted against the concentration of lidocaine applied. Residual CAPs are calculated as a percentage of control CAP before application of lidocaine. Each point represents a single nerve preparation with a single drug application. The n for the zero values graphed at 70, 80, 90, 100, 160, and 200 mM lidocaine are 2, 10, 4, 4, 4, and 5, respectively. Experiments were done during January and repeated during March and April. The data points for these separate intervals were closely superimposed, and thus a single curve was fit to all points. (*Inset*) The mean ±SE values from these individual points. The CAP after lidocaine exposure significantly differed from the CAP after 3 h in Ringer's solution: *$P < 0.005$; **$0.025 > P > 0.01$; ***$P > 0.1$.

could have occurred with the sucrose-gap method. However, in these experiments we observed no better recovery of the CAP when the marginal regions of nerve were superfused than when the nerve was left in its original position. Accumulation of lidocaine in these marginal regions does not appear to explain irreversible impulse blockade. We did use the extracellular chamber, along with the sucrose-gap chamber, to explore the aforementioned change in sensitivity.

Figure 8 compares the results of May experiments, measured by the sucrose-gap and extracellular-chamber methods, with the pervious results of January–April done only by sucrose gap. All three data sets showed a graded decline in residual CAP with increasing lidocaine. In each data set, 20 mM lidocaine caused a minor decrement in CAP, and lidocaine at or greater than 40 mM caused a significant decrease ($P < 0.05$). Curves for May, independent of method, were essentially the same. These curves were shifted to the right, with midpoints (EC_{50}) now at 65 and 83 mM lidocaine compared with 50 mM lidocaine in the January–April data points. These EC_{50} differences were significant (table 1). Zero points occurred at approximately 150 mM lidocaine for the May data compared with 80 mM for January–April data. All data sets showed considerable scatter in the midrange, made more noticeable in May because the curves were extended to the right. However, all data sets were consistent in showing a zero point.

The cause of this variation in concentration dependence is not known. We note only that it occurred at a time of semihibernation and during the mating season. We called this change an adaptational variation.

Effect of Nifedipine on Lidocaine Residual Compound Action Potential Dose Response

The mechanism of lidocaine's ability to cause nonreversible degradation in the CAP is not known. One possibility is that intracellular calcium increases to a toxic level, and therefore we hypothesized that blocking calcium channels might thus have a protective effect. Ten micromolar nifedipine was added to lidocaine solutions to test this possibility in L-type calcium channels.

The effect of 10 μM nifedipine was to shift the response curve to the right and elevate the zero point of complete degradation (80 *vs.* 150 mM). In addition, the slope was significantly shallower (fig. 9) than the sucrose-gap (January–April) slope, $P < 0.02$ (table 1). These data looked remarkably similar to the data from May (fig. 8), which showed an adaptational displacement of the response curve. However, nifedipine data were collected April 11–24 and spanned by stable controls during this period. The dramatic adaptational change was first observed on May 8.

Discussion

This work confirms the observation[11] that transient exposure to concentrated lidocaine completely ablates the CAP of excised, desheathed frog sciatic nerve. This ablation occurred in all conditions of measurement,

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

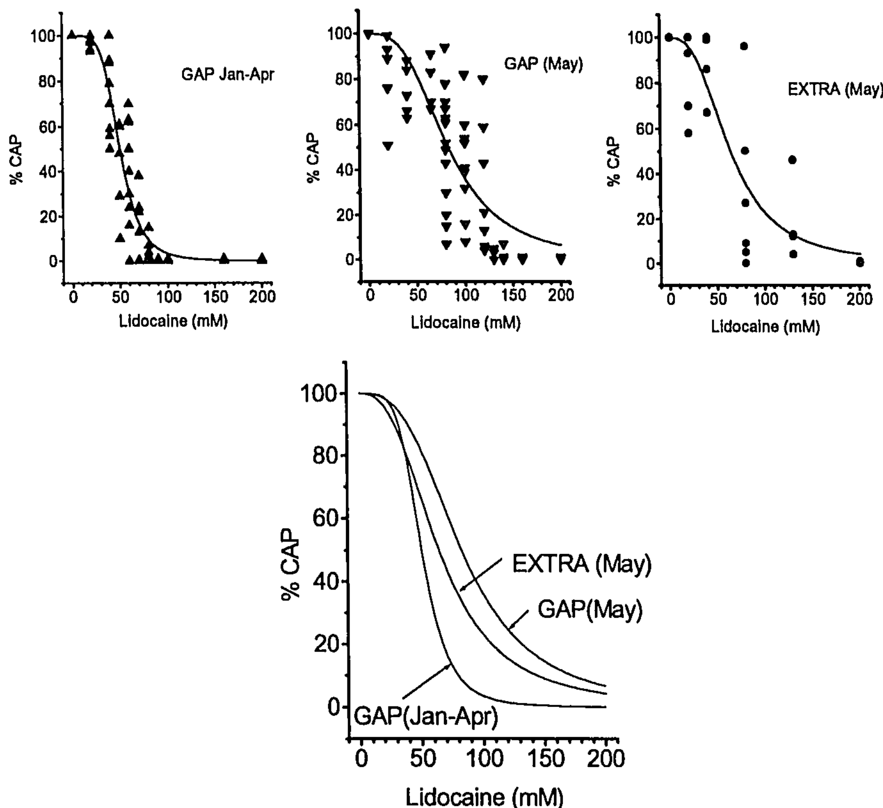

Fig. 8. Three data sets of residual compound action potential (CAP) in response to increasing lidocaine concentrations. % CAP = residual CAP expressed as a percentage of control CAP. (*Top left*) Figure 7 shown again for comparison. (*Top middle*) Sucrose-gap determinations (GAP) done in May. (*Top right*) Extracellular-chamber (EXTRA) determinations done in May. Each point is a single nerve preparation with a single drug application. Derived curves are superimposed below for comparison. The displacement of May CAP curves from January–April CAP curves is significant: $P < 0.001$ (table 1).

whether by sucrose gap or extracellular chamber, and whether affected by adaptational change or the addition of nifedipine.

In addition, the current work demonstrates that the degradation is a continuously graded response. It became significant at a concentration of 40 mM (1.0%) lidocaine and increased in graded fashion with increasing concentrations of lidocaine. There may be displacements in this curve caused by adaptational factors or by nifedipine but the overall pattern was consistent.

Lambert *et al.*,[11] using radiolabeled tracer drug, directly measured neural lidocaine after drug washout and concluded that residual lidocaine alone could not explain the complete loss of impulse activity. We support that view, based on the stable nature of degraded responses in time and the observation that both the 40-Hz stimulus responses and the waveforms of degraded CAPs differed from those caused by low-concentration lidocaine. Thus we believe the degraded response results from an injury to the nerve, yet to be identified.

The appearance of the degraded CAP as a scaled-down version of the control CAP, without other changes in shape, suggests to us that the degraded response results from a progressive loss of individual functional axons rather than a simultaneous graded effect on all axons.

The observed adaptational changes to this response remain unexplained. Those who do continued studies in the frog are cautioned that frequent controls are necessary to identify these events.

One explanation for the adaptational change lies in the acclimatization of frogs to low temperatures. Frogs, as poikilotherms,[17] alter their physiologic and biochemical characteristics[18] in response to prolonged changes in ambient temperature.[19] The lipid content of cellular membranes[18,19] and perhaps even the density of ionic channels are modified in response to a marked drop in temperature.[20,21] Cold adaptation leads to a greater membrane fluidity, particularly at low temperatures.[19,20,22] Overall, the physiologic features of axonal impulses in frogs are demonstrably altered by cold and adaptation.[22–24] Therefore, the nerves we studied in frogs kept at 4°C for 5 weeks probably differed chemically and physiologically from those kept for 3 weeks or less. Whether these changes altered the membrane uptake of lidocaine[25] or the ability of the nerve to recover from lidocaine's insult by virtue of an altered

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

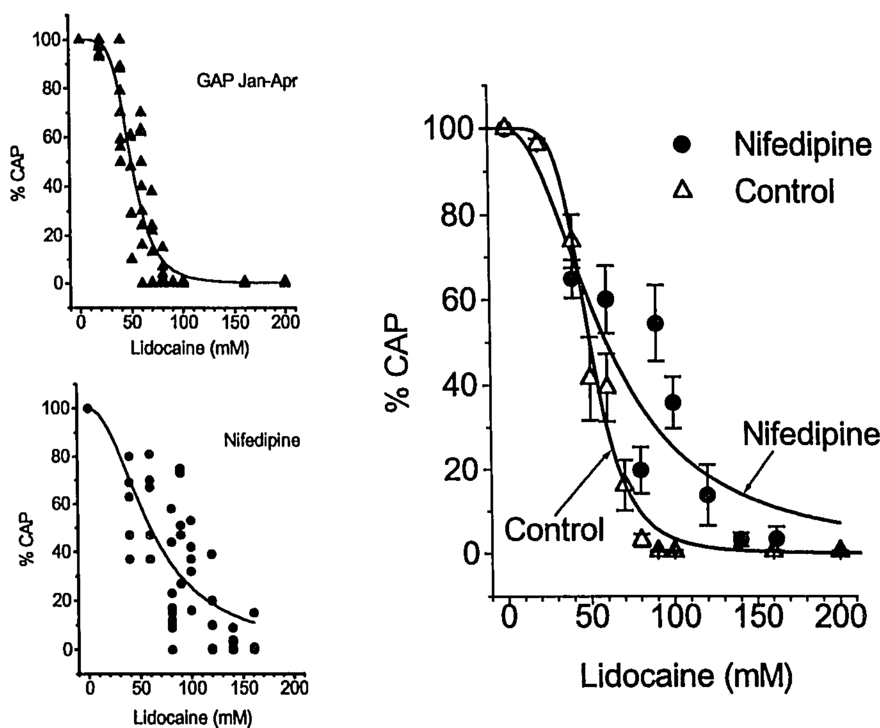

Fig. 9. The effects of 10 μM nifedipine combined with lidocaine on the residual compound action potential (CAP) of frog nerve mounted in a sucrose gap chamber. % CAP = residual CAP expressed as a percentage of control CAP. (*Left*) Each point represents a single nerve preparation and single drug application. The plot of control data (*top left*) is figure 7 shown again for comparison. (*Right*) The means ± SE of these single points, superimposed. Nifedipine lifted the response curve to the right, with a slight displacement of midpoint (60 mM lidocaine) and a large displacement of the zero point of complete degradation (approximately 150 mM lidocaine). The slope of nifedipine responses is significantly different from control: $P < 0.02$.

density of ion channels or ion pumps was not determined. Understanding this phenomenon may be a clue to understanding the nature of the injury itself or may suggest ways to make local anesthetics safer in the future.

Nifedipine is a selective inhibitor of L-type calcium channels, blocking almost all these channels at 10 μM.[26] In frog node of Ranvier, however, all detectable ionic currents can be ascribed to sodium[27] or potassium channels,[28] and there is no electrical contribution from any type of calcium channel. Interestingly, glial cells do express calcium channels[29] and the possibility arises that the direct insult on conduction is through an action on the myelinating Schwann cells, leading, for example, to an acute and persistent paranodal demyelination. Such an injury has been reported from histologic examination of peripheral nerves exposed *in vivo* to high concentrations of local anesthetics.[3]

It is not unreasonable to consider that frog nerve, which demonstrates all the mammalian properties of reversible anesthetic block, might show the mammalian properties for nonreversible block as well. Indeed, the concentration of lidocaine that caused complete ablation of CAP, 80–150 mM, is identical to that predicted to have caused injury in humans.[8]

The desheathed frog sciatic nerve resembles the mammalian spinal roots in that for both preparations nerve fibers are exposed directly to the drugs at the concentration present in the bathing solution. Differences in these two tissues are many, including the temperature of exposure and assay, presence of protein in cerebrospinal fluid and not in Ringer's solution, spinal circulation that will take up and reduce local anesthetic in cerebrospinal fluid and the intrinsic differences between biochemical constituents of mammalian and amphibian nerves. Which of these differences is an important factor in the determination of susceptibility to irreversible nerve injury is not known, but we believe that the general phenomena we describe qualitatively here occur in all nerve fibers.

White matter of the mammalian optic nerve suffers anoxic injury resulting in irreversible impulse loss, an injury that depends on the presence of extracellular calcium[30] but that is insensitive to nifedipine up to 10^{-5} M.[31] It remains to be shown whether the protective actions of nifedipine against local anesthetic-induced injury that we observe in frog nerves will also occur in mammalian nerve or whether the ineffective role of these drugs against anoxic injury in mammals also extends to the injury from local anesthetics.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

The description of this injury in frog nerve may bring us closer to defining a critical concentration of lidocaine that is safe for humans. These results raise the possibility that injury to mammalian nerve may possess the same properties of progressive degradation beginning at a surprisingly low concentration of lidocaine. Thus, those conducting *in vivo* mammalian studies should consider testing for effects in these low concentration ranges.

In summary, lidocaine caused a nonreversible degradation in CAP in stimulated, excised frog sciatic nerve. This degradation was concentration-dependent beginning at 40 mM (1.0%) lidocaine and increasing with increasing concentration to complete ablation at 80 mM lidocaine. The degraded response was a new steady state and not the result of residual lidocaine.

These findings are best explained by an increasing loss of functional axons rather than a simultaneous graded effect on all axons. These results also suggest the presence of unknown adaptational factors that can make the nerves more resistant to degradation. In addition, nifedipine can make nerves more resistant to degradation.

Definition of this concentration-dependent response for lidocaine permits a similar comparison for all local anesthetics. Probing of this concentration-dependent response may permit exploration of the mechanism of this injury.

The authors thank Rachel Abrams and Ellen Jacobson for their expert typing of the manuscripts and of abstracts. The authors also thank Dr. Stephen Raymond, Dr. Neil Castle, Dr. Douglas Wingrove, Dr. Kin Wong, and Dr. G. Richard Arthur for their critiques both scientific and literary.

References

1. Lundy JS, Essex HE, Kernohan JW: Experiments with anesthetics: IV. Lesions produced in the spinal cord of dogs by a dose of procaine hydrochloride sufficient to cause permanent and fatal paralysis. JAMA 101:1546–1550, 1933

2. Skou JC: Local anesthetics: II. The toxic potencies of some local anesthetics and of butyl alcohol, determined on peripheral nerves. Acta Pharmacol Toxicol 10:292–296, 1954

3. Kalichman MW, Powell HC, Myers RR: Quantitative histologic analysis of local anesthetic-induced injury in rat sciatic nerve. J Pharmacol Exp Ther 250:406–413, 1989

4. Adams HJ, Mastri AR, Eicholzer AW, Kilpatrick G: Morphologic effects of intrathecal etidocaine and tetracaine on the rabbit spinal cord. Anesth Analg 53:904–908, 1974

5. Ready LB, Plumer MH, Haschke RH, Austin E, Sumi SM: Neurotoxicity of intrathecal local anesthetics in rabbits. ANESTHESIOLOGY 63:364–470, 1985

6. Rigler ML, Drasner K, Krejcie TC, Yelich SJ, Scholnick FT, DeFontes J, Bohner D: Cauda equina syndrome after continuous spinal anesthesia. Anesth Analg 72:275–281, 1991

7. Schell RM, Brauer FS, Cole DJ, Applegate RL II: Persistent sacral nerve root deficits after continuous spinal anaesthesia. Can J Anaesth 38:908–911, 1991

8. Ross BK, Coda B, Heath CH: Local anesthetic distribution in a spinal model: A possible mechanism of neurologic injury after continuous spinal anesthesia. Reg Anesth 17:69–77, 1992

9. Rigler ML, Drasner K: Distribution of catheter-injected local anesthetic in a model of the subarachnoid space. ANESTHESIOLOGY 75: 684–692, 1991

10. Lambert DH, Hurley RJ: Cauda equina syndrome and continuous spinal anesthesia. Anesth Analg 72:817–819, 1991

11. Lambert DH, Lambert LA, Strichartz GR: Irreversible conduction block in isolated nerve by high concentrations of local anesthetics. ANESTHESIOLOGY 80:1082–1093, 1994

12. Schneider M, Datta S, Strichartz G: A preferential inhibition of impulses in C-fibers of the rabbit vagus nerve by veratridine, an activator of sodium channels. ANESTHESIOLOGY 74:270–280, 1991

13. Glantz SA, Slinker BK: Primer of Applied Regression and Analysis of Variance. New York, McGraw-Hill, 1990, p 502

14. Courtney KR, Kendig JJ, Cohen EN: Frequency-dependent conduction block: The role of nerve impulse pattern in local anesthetic potency. ANESTHESIOLOGY 48:111–117, 1978

15. Julian FJ, Moore JW, Goldman DE: Current-voltage relations of the lobster giant axon membrane under voltage clamp conditions. J Gen Physiol 45:1217–1238, 1962

16. Courtney KR, Strichartz GR: Structural elements which determine local anesthetic activity, Handbook of Experimental Pharmacology: Local Anesthetics. Edited by Strichartz G. Berlin, Springer-Verlag, 1987, pp 53–93

17. Prosser CL, Nelson DO: The role of nervous systems in temperature adaptation of poikilotherms. Ann Rev Physiol 43:281–300, 1981

18. Cossins AR, Friedlander MJ, Prosser CL: Correlations between behavioral temperature adaptations of goldfish and the viscosity of fatty acid composition of their synaptic membranes. J Comp Physiol 120:109–121, 1977

19. Cossins AR, Prosser CL: Evolutionary adaptation of membranes to temperature. Proc Natl Acad Sci USA 75:2040–2043, 1978

20. Ritchie JM, Rogart RB: The binding of labeled saxitoxin to the sodium channels in normal and denervated mammalian muscle, and in amphibian muscle. J Physiol (Lond) 269:341–354, 1977

21. Friedlander MJ, Kotchabhakdi N, Prosser CL: Effects of cold and heat on behavior and cerebellar function in goldfish. J Comp Physiol 112:19–45, 1976

22. Schwarz W: Temperature experiments on nerve and muscle membranes of frogs. Pflügers Arch 382:27–34, 1979

23. Engelhardt A: Die temperaturabhängigkeit der erregunsleitungs-geswindigkeit in kalt-und warmblüternerven. Z Vergl Physiol 33:125–128, 1951

24. McDonald HS, Chiaramonte G, Tarnowski LE: Influence of temperature acclimation on conduction velocity in sciatic nerves of *Rana pipiens*. Am Zool 3:547–548, 1963

25. Korten K, Sommer TJ, Miller KW: Membrane composition modulates thiopental partitioning in bilayers and biomembranes. Biochim Biophys Acta 599:271–279, 1980

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023

26. Plummer MR, Logothetis DE, Hess P: Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. Neuron 2:1453–1463, 1989

27. Hille B: Pharmacological modifications of the sodium channels of frog nerve. J Gen Physiol 51:199–219, 1968

28. Dubois JM: Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane. J Physiol (Lond) 318:297–316, 1981

29. Barres BA, Chun LLY, Corey DP: Ion channels in vertebrate glia. Ann Rev Neurosci 13:441–474, 1990

30. Stys PK, Ransom BR, Waxman SG, Davis PK: Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc Natl Acad Sci USA 87:4212–4216, 1990

31. Stys PK, Ransom BR, Waxman SG: Effects of polyvalent cations and dihydropyridine calcium channel blockers on recovery of CNS white matter from anoxia. Neurosci Lett 115:293–299, 1990

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/81/3/657/328132/0000542-199409000-00020.pdf by guest on 26 March 2023