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Differential Peripheral Nerve Block by Local Anesthetics in the Cat

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Controversy still surrounds the differential susceptibility of nerve fibers to local anesthetic conduction block. In order to help resolve this controversy, we developed an *in vivo* model of peripheral nerve blockade in the cat that closely reproduced the clinical state. Using this model, differential rate of nerve blockade of A-alpha, A-delta, and C fibers by 2-chloroprocaine, lidocaine, bupivacaine, and etidocaine was observed and quantitated. C fibers were blocked first by 2-chloroprocaine, lidocaine and bupivacaine. Etidocaine blocked A-delta fibers first. A-alpha fibers always were blocked last. Of the four local anesthetics tested, 2-chloroprocaine produced the greatest differential rate of block of the nerve fibers, and etidocaine produced the least. (Key words: Anesthetics, local: bupivacaine; 2-chloroprocaine; etidocaine; lidocaine. Nerve: block, differential.)

ALTHOUGH DIFFERENTIAL NERVE BLOCK resulting from local anesthetics has been studied for many years, there are still controversies about the differential susceptibility of nerve fibers to local anesthetic conduction block. Some of the controversy stems from different approaches to the problem and different definitions of "differential block." Gasser and Erlanger[1] studied the effect of cocaine on dog saphenous nerve. Although they found that the compound action potential (CAP) of small nerve fibers disappeared before the CAP of large diameter fibers, some of the large fibers had been blocked before all of the small fibers had been blocked. This phenomena is called "a relative differential block" or "differential rate of block."[2] Nathan and Sears[2] applied local anesthetics to cat spinal roots. They found that there was a critical local anesthetic concentration that completely would block small myelinated fibers without blocking larger myelinated fibers. This they called an "absolute differential block" and required that equilibrium be established between the nerve and local anesthetic solution. Between small myelinated fibers and C fibers, they found a relative differential block; A-delta fibers being blocked first. Franz and Perry[3] obtained single unit recordings from the cat saphenous nerve and were able to produce an absolute differential block only when the local anesthetic was applied to less than 4 mm of nerve. When greater than 4 mm of nerve was bathed in a procaine solution, a differential rate of block was obtained with A-delta and C fibers being blocked before A-alpha. Gissen *et al.*,[4,5] using desheathed rabbit vagus and sciatic nerve preparations initially found a differential rate of block with C fibers being blocked before A-alpha fibers. However, when equilibrium was established between the nerve and local anesthetic solution, the A-alpha fibers were blocked more extensively than the C fibers.

All of the above studies were done either *in vitro* or with the nerve in some way isolated so that the local anesthetic could be applied to a well-defined segment of nerve. This study was designed to measure the local anesthetic action on a peripheral nerve in an *in vivo* model and to evaluate the differential blocking characteristics of four local anesthetics.

Material and Methods

Fifty-five cats were obtained from Kiser Lake, St. Paris, Ohio, and cared for by the Department of Laboratory Animal Medicine until used in this study. Anesthesia was induced with 40 $mg \cdot kg^{-1}$ ketamine administered intramuscularly and maintained with 0.1–0.6% methoxyfluorane, 67% N_2O, and O_2. Atropine, 0.1 mg, was given with the ketamine. Ventilation was controlled with a Harvard Model 66-I respirator via an indwelling endotracheal tube. The respiratory rate was started at 22 breaths/min. Catheters were inserted in the right common carotid artery and right internal jugular vein. Lactated Ringer's solution was infused at a rate of 5 $ml \cdot kg^{-1} \cdot h^{-1}$ via the intravenous line. The arterial catheter was attached to the pressure transducer for continuous recording of blood pressure on a Physiograph Model DMP-4B. Because muscle activity interfered with the recording of A-delta and C fiber action potentials, the cats were paralyzed with succinyldicholine. Succinyldicholine was added to the lactated Ringer's solution and infused at a rate of 10 $mg \cdot kg^{-1} \cdot h^{-1}$.

Cats were maintained at normal physiologic status by monitoring and maintaining normal blood pressure, pulse rate, esophageal temperature, urine output, and blood gases (table 1). Metabolic acidosis was corrected with iv sodium bicarbonate. Respiratory acidosis/alkalosis was corrected by adjusting the respiratory rate. Hypotension

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was controlled by administering fluid or reducing the concentration of methoxyfluorane. Hypertension was controlled by deepening the level of anesthesia. Esophageal temperature was controlled by warming the cat with a heating pad. It was possible to maintain all the cats within acceptable limits. There was a tendency for the cats to develop a metabolic acidosis as the experiment progressed, but this was controlled easily with bicarbonate.

To study differential nerve block, the anesthetized and paralyzed cats were prepared as follows. The saphenous nerve was exposed at the groin and placed carefully on stainless steel stimulating electrodes. Seven centimeters distal to the proximal exposure, another cut was made over the saphenous nerve and the isolated nerve was placed on the stainless steel recording electrodes. The exposed nerve was bathed in mineral oil to keep it from drying out. A 2-inch 22-gauge B-D spinal needle was positioned so that the tip touched the nerve between the two electrodes. Visualization of both the nerve and the needle was accomplished by making a small slit in the skin above the nerve where the injection was to be given (fig. 1). The nerve was stimulated at 0.0167 Hz supramaximally by a Grass S88 stimulator. The stimulating pulse for A-alpha and A-delta fibers was a square pulse 4–10 V by 0.1 ms. The stimulating square pulse for C fibers was 10–20 V by 1 ms. The A-alpha, A-delta, and C nerve fiber action potentials were identified by the following latencies: A-alpha at 1 ms, A-delta at 5–6 ms, and C at 50–70 ms. The nerve action potential was recorded and measured on a Tektronix 564B storage oscilloscope. A maximum of two nerve blocks (one in each hind leg) was done in each cat. Figure 2 shows typical recordings before, during, and after a block.

TABLE 1. Summary of Physiologic State of the Cats

	Experimental Cats (Observed Values)	Awake, Resting Cats (Normal Values)
Respiratory rate (breaths/min)	2–24	20–30*
Temperature (°C)	36.5–40.5	38.9 ± 0.3†
Blood pressure (mmHg)	90/60–200/140	120/75*
Heart rate (beats/min)	165–200	110–240*
Pa_{O_2} (mmHg)	125–170	
Pa_{CO_2} (mmHg)	25–46	29.9 ± 0.6,† 36*
*p*H	7.30–7.47	7.46,† 7.35*
Base excess (mEq/l)	−2 to −6	
Urine output (ml)	5–250	
iv fluids (ml)	80–225	

* From Green.[7]
† From Herbert and Mitchel.[6]

When the nerve action potential was stable, 0.3 ml of the local anesthetic solution was injected via the spinal needle. In order to examine the differential blocking characteristics of a local anesthetic, a concentration of local anesthetic was used that was low enough to cause a slow onset but high enough to insure that at least one fiber type would be blocked totally. The concentrations were 2-chloroprocaine, 0.15% (4.9 mM) and 0.30% (9.8 mM); lidocaine, 0.1% (3.7 mM); etidocaine, 0.1% (3.2 mM); and bupivacaine, 0.025% (0.77 mM) and 0.050% (1.5 mM). Changes in the amplitude of the nerve action potential were recorded at 30s, 60s, and then every minute until onset of block was complete. The time required for the first fiber type to be blocked 100% (usually the C fiber) varied from about 1 to 35 min. The nerve then was stimulated once every 10–15 min until the CAPs for all fiber types reappeared. The nerves were not followed

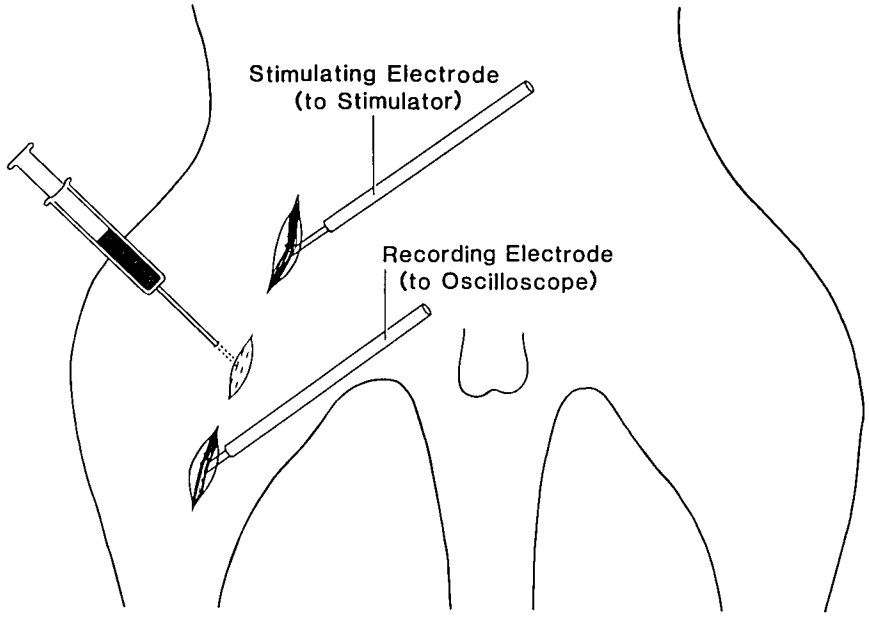

FIG. 1. Diagram of the positioning of the recording and stimulating electrodes and the injection site.

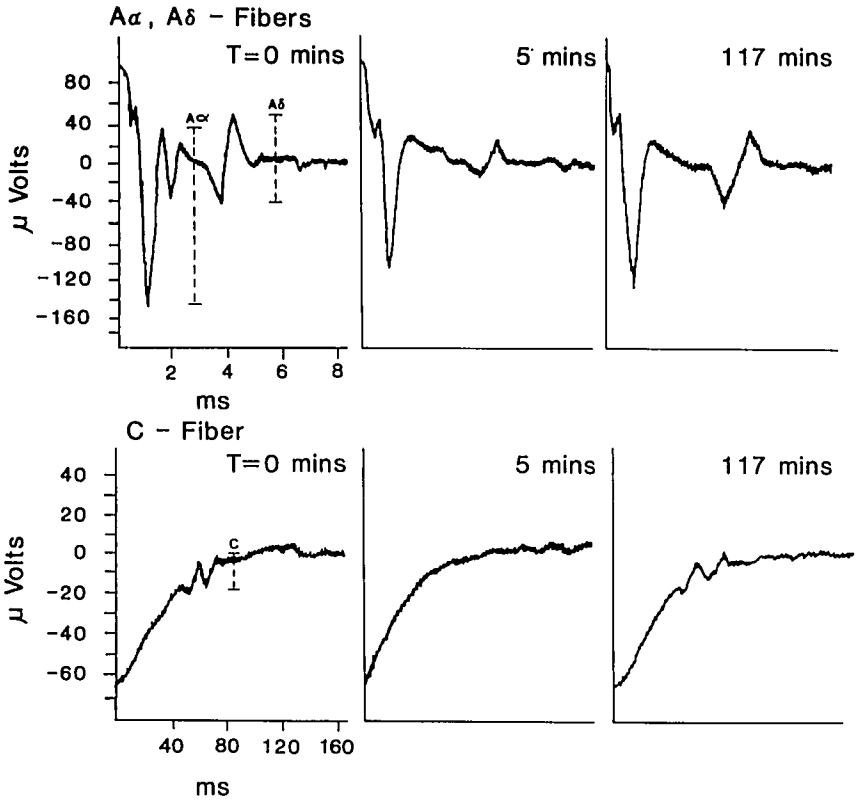

FIG. 2. Compound action potentials before and during a block with 0.3 ml, 0.15% lidocaine. The top panels are A-alpha and A-delta fiber action potentials, and the bottom panels are C fiber action potentials. The dashed vertical lines represent the amplitudes of the compound action potentials. In this instance, a small correction was applied to the C fiber CAP, since the baseline was curved. The time of the recordings are shown on the panels.

to complete recovery, and the experiments typically lasted 5–7 h. The percentage block achieved during onset was calculated for A-alpha, A-delta, and C fibers and for each local anesthetic according to the formula:

$$\% \text{ block} = \frac{\text{preblock amplitude} - \text{amplitude after local anesthetic injection}}{\text{preblock amplitude}} \times 100$$

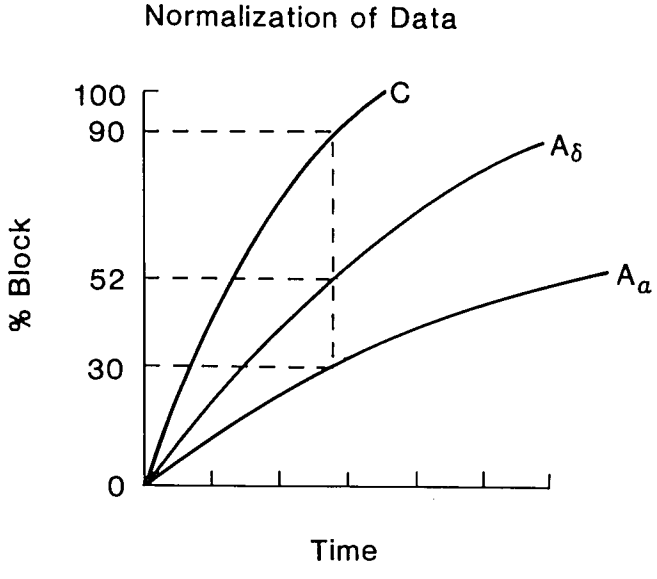

FIG. 3. Idealized set of curves showing how the data were normalized to the C fiber block. The % A-alpha and % A-delta block were determined at 25%, 50%, 75%, 90%, and 100% C fiber block.

Because the time of onset of the blocks varied greatly, an absolute rate of block had no meaning. Therefore, the data were normalized to the extent of C-fiber block (fig. 3). This normalization had the effect of emphasizing the differential rate of blocking of the local anesthetics. The results were analyzed using Student's *t* test (figs. 4 and 5) and one-way analysis of variance followed by Scheffe's critical difference test (table 2 and figure 6).

Results

Every local anesthetic tested showed a differential rate of block. Table 2 compares the percent of A-alpha fibers and A-delta fibers blocked when C fibers were blocked 90%. The end point of 90% of C fibers blocked was chosen arbitrarily as a point at which to make statistical comparisons.

Figure 6 shows the percentage block of A-alpha fibers (4A) and A-delta fibers (4B) as a function of percentage C fiber block for each local anesthetic. The dotted line represents the curve that would result if no differential

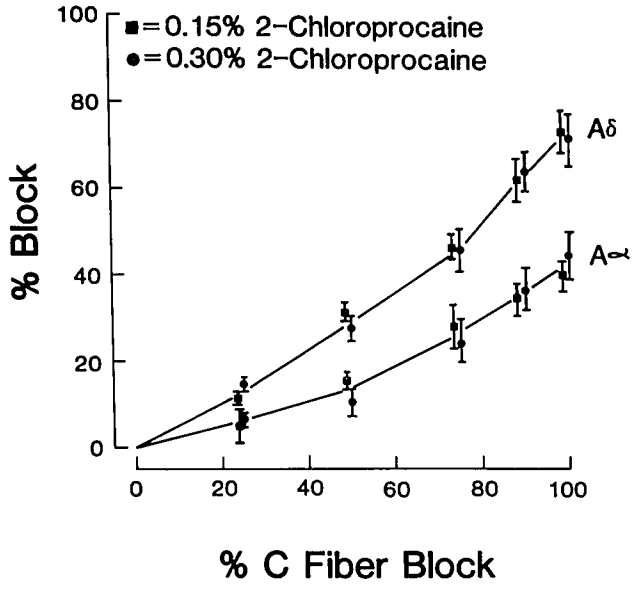

FIG. 4. Concentration dependence of differential blocking for 2-chloroprocaine. Each point is the average ± SEM of five blocks. The dotted line is the reference line for no differential block.

block occurred. It is clear that for A-alpha *versus* C fibers, etidocaine produces the least differential rate of block, while 2-chloroprocaine produces the greatest differential rate of block. At 75%, 90%, and 100% C fiber block, 2-chloroprocaine was significantly different ($P < 0.05$) from etidocaine. The differential block observed for A-delta *versus* C fibers was less pronounced. Etidocaine actually blocked the A-delta fibers before the C fibers, although the difference at 90% C fibers block was not significant. Lidocaine, bupivacaine, and 2-chloroprocaine produced about the same differential rate of block. Lidocaine and 2-chloroprocaine were significantly different ($P < 0.05$) from etidocaine at all levels of C fiber block.

Figure 4 shows the percentage block of A-alpha and A-delta fibers as a function of percentage C fiber block for two concentrations of 2-chloroprocaine. The same degree of differential rate of block was produced with 0.15% and 0.3% 2-chloroprocaine. This lack of sensitivity to concentration was not true for bupivacaine. Figure 5 shows the differential rate of block for 0.025% and

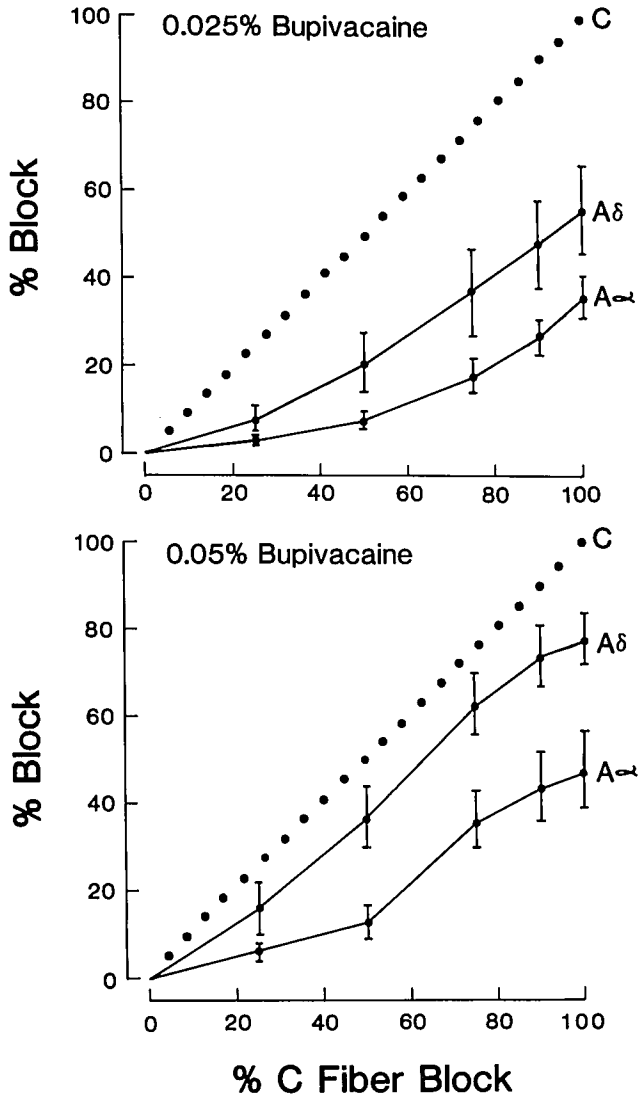

FIG. 5. Concentration dependence of differential blocking for bupivacaine. Each point is the average ± SEM of five blocks. The dotted line is the reference line for no differential block.

0.050% bupivacaine. At the higher concentration, the curves are shifted toward the zero-differential block reference line. When the A-alpha fiber block, using 0.05% bupivacaine, was compared with the A-alpha fiber block

TABLE 2. Relative Depression of Compound Action Potentials by Local Anesthetics

Local Anesthetic	Fiber Type (%)			Statistical Significance		
	Aα	Aδ	C	Aα vs. Aδ	Aα vs. C	Aδ vs. C
0.15% 2-chloroprocaine	35.4	61.2	90	$P < 0.01$	$P < 0.001$	$P < 0.001$
0.3% 2-chloroprocaine	36.2	63.3	90	$P < 0.01$	$P < 0.001$	$P < 0.01$
0.1% lidocaine	52.0	69.8	90	$P < 0.02$	$P < 0.001$	$P < 0.01$
0.1% etidocaine	65.6	91.5	90	$P < 0.01$	$P < 0.02$	NS
0.025% bupivacaine	28.2	48.2	90	NS	$P < 0.001$	$P < 0.05$
0.050% bupivacaine	44.4	74.2	90	$P < 0.02$	$P < 0.001$	NS

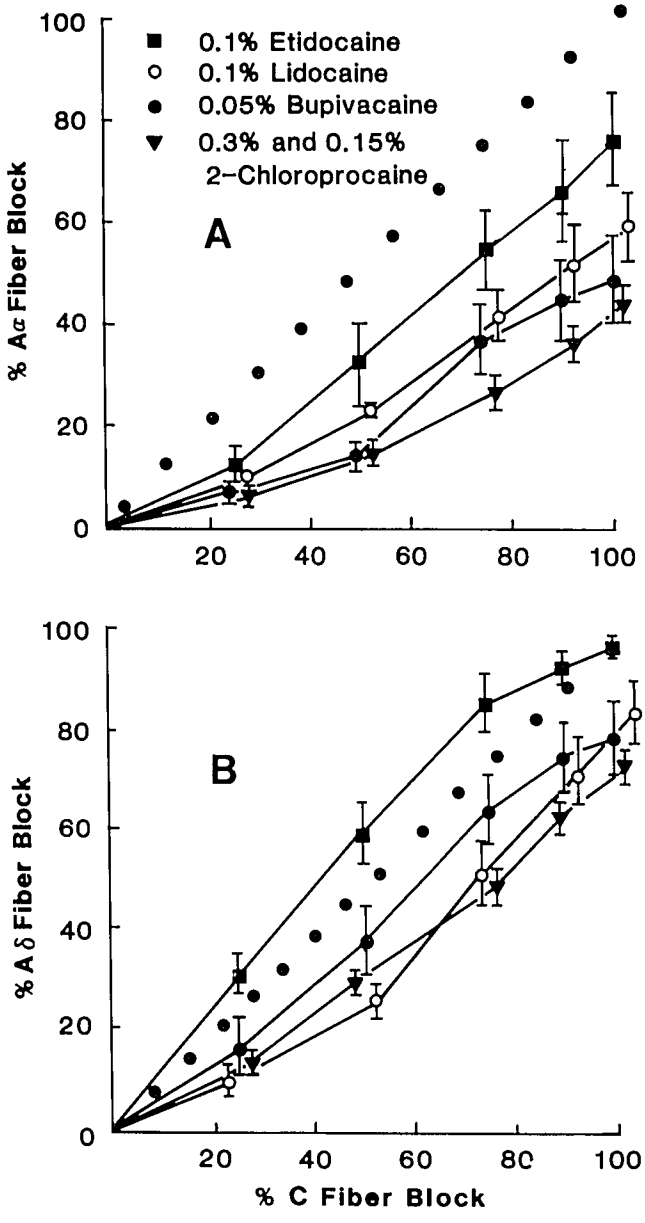

FIG. 6. Relation of C fiber block to A-alpha fiber block (*A*) and A-delta fiber block (*B*). Each point is the average ± SEM of at least five blocks. The dotted line is the reference line for no differential block.

using 0.025% bupivacaine, the difference was not significant using Student's *t* test ($P > 0.05$). The same was true for A-delta fibers at the two bupivacaine concentrations. However, when the data were analyzed using a two-way analysis of variance (fiber type and drug concentrations), both factors were found to make a significant contribution ($P < 0.01$) at 90% C fiber blockade.

Discussion

This study examined differential rate of blocking by local anesthetics of peripheral nerves in an *in vivo* model. The model was constructed to be reasonably similar to the human clinical state. In achieving this similarity, other desirable features were sacrificed necessarily. First, the tested animal in this model is a mammal and therefore more likely to resemble human nervous tissue than an amphibian. However, the cat is more costly and more difficult to manage than, for example, a frog. Second, the anesthetic solution in this model was injected into intact tissue so that adsorption, distribution, and elimination of the local anesthetic from the site of injection was as close to the clinical state as possible. Unfortunately, by injecting into intact tissue, knowledge of the exact position of the local anesthetic relative to the nerve is lost. Also undeterminable is the amount of local anesthetic that reaches the nerve. Part of this uncertainty was eliminated by one's being able to see the nerve and tip of the needle through the transparent subcutaneous tissue. Third, because nerve action potentials were measured instead of the animal's overt physical response resulting from the activation of the nerve, objective (and not subjective) measurements are taken from the model. This objectivity was obtained at the expense of the more clinically relevant phenomena of pain, movement and autonomic nervous system responses. Furthermore, in order to measure the nerve action potentials, the cat was anesthetized and paralyzed, clearly not an ideal situation. Nevertheless, the effect of succinyldicholine, nitrous oxide, and methoxyfluorane on peripheral nerve conduction is minimal.

This study also demonstrates differential rate of nerve blocking of A-alpha, A-delta and C fibers at low concentrations by all four local anesthetics tested. In general, C fibers were blocked first, followed by A-delta, and then A-alpha fibers. However, with etidocaine, A-delta fibers were blocked before C fibers. Even though all the local anesthetics tested produced a differential block, not all of them developed the same degree of differential rate of block. Etidocaine produced the least and 2-chloroprocaine produced the greatest differential rate of block.

The concentrations of local anesthetics used in this study were about $^1/_{10}$ of clinically used concentrations. These low concentrations required accurate placement of the anesthetic solution to produce a block. Although we could see both the needle and nerve, it was not possible to make all injections identically. At the low concentrations used, small variations in needle placement produced considerable variation in the block achieved. This variability is reflected in the large SEM values seen in Figure 6 and the variability of the time required to block one fiber type completely (1–35 min). Although statistical significance is lacking in many of the comparisons, the trends are clear and we feel the conclusions drawn from these trends are valid.

These results corroborate and extend a mechanism of differential block proposed by Franz and Perry.[3] They

used procaine which, like 2-chloroprocaine, has a low lipid solubility. Proteins and extracellular markers tend to penetrate the nerve irregularly. A local anesthetic of low lipid solubility, such as procaine, also would tend to be distributed irregularly throughout the nerve trunk.[3] In order to block myelinated fibers, about three successive nodes must be blocked. Since the nodes of A-alpha fibers are farther apart than the nodes of A-delta fibers, an irregular local anesthetic distribution would be less likely to cover the critical length of the three consecutive nodes of an A-alpha fiber than an A-delta fiber. Hence, A-delta fibers are blocked before A-alpha.

Etidocaine, on the other hand, is very lipid soluble, and the membranes of the nerve trunk are not as great a barrier to diffusion into the axons. Therefore, it is reasonable to assume that etidocaine would be distributed more evenly and the critical lengths of all the axons would be exceeded at about the same time. Any differential block would be the result of the different concentration of local anesthetic required to block a fiber. Our results suggest that when the local anesthetic is distributed evenly in the nerve trunk, A-delta fibers are blocked at the lowest anesthetic concentration, followed by C fibers and A-alpha fibers blocked at the highest local anesthetic concentration.

Anesthetics that have an intermediate lipid solubility (bupivacaine, lidocaine) will produce a differential rate of block by a combination of both mechanisms (distribution of the local anesthetic and intrinsic susceptibility of the nerve fiber to the local anesthetic). The effect of concentration of bupivacaine and 2-chloroprocaine on differential block fits this general scheme. Bupivacaine is less lipid soluble than etidocaine but much more lipid soluble than 2-chloroprocaine. The effect of raising the concentration from 0.025% to 0.050% reduced the irregularity of the distribution, and so it behaved more like etidocaine. Because 2-chloroprocaine is so lipid insoluble, raising the concentration from 0.15% to 0.3% did not improve the distribution. This model would predict that had we looked at much higher concentrations of 2-chloroprocaine, we would probably have seen a trend toward etidocaine also. In the same way, this model predicts that etidocaine would have shown an effect of concentration only at very low concentrations.

These results are in partial agreement with the *in vitro* work reported by Gissen *et al.*[4,5] Both this study and Gissen's showed a tendency for the differential rate of block to disappear with increasing local anesthetic concentrations. Both studies also showed A-alpha fibers to be structurally more resistant to local anesthetic block than C fibers either because the critical length is larger (this article) or because the myelin sheath presents a barrier to local anesthetic diffusion.[5] Therefore, both studies show a differential rate of block with C fibers and A-delta or B fibers being blocked before A-alpha fibers. However, in this study, we never observed a crossover between the extent of C fiber block and A-alpha fiber block. This difference could be due to the fact that in Gissen's *in vitro* preparation, the nerve and local anesthetic solution had time to reach equilibrium. In this *in vivo* preparation, equilibrium never is reached.

In summary, we have introduced a new model of peripheral nerve blockade in the cat. Using this model, we have demonstrated differential peripheral nerve blockade by 2-chloroprocaine, lidocaine, bupivacaine, and etidocaine. Of the four local anesthetics tested, 2-chloroprocaine produced the greatest differential rate of block, and etiodocaine produced the least. The results are consistent with and extend a model of differential blockade proposed by Franz and Perry[3] and are in partial agreement with the work of Gissen *et al.*[4,5]

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