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Frequency-dependent Conduction Block:

The Role of Nerve Impulse Pattern in Local Anesthetic Potency

Kenneth R. Courtney, Ph.D., Joan J. Kendig, Ph.D.,† Ellis N. Cohen, M.D.‡*

The depth of local anesthetic-induced conduction block is modified by the frequency of impulse traffic in the nerve (frequency-dependent conduction block). The present study was designed to compare the frequency-dependent characteristics of a number of local anesthetics of different lipid solubilities. Two antiarrhythmic drugs, quinidine and propranolol, were also included. Frequency dependence was assessed by measuring the height of the compound action potential of the frog sciatic nerve in response to single stimuli and to stimuli presented repetitively at different frequencies. All the drugs tested showed marked enhancement of block at 40 Hz. Nerves treated with highly lipid-soluble agents (bupivacaine, tetracaine, etidocaine), two experimental compounds of low and very low lipid solubility (GEA 968 and QX-572, respectively), and the antiarrhythmic agents took longer to develop and to recover from the effects of stimulation than those treated with drugs of moderate lipid solubility (procaine, lidocaine, prilocaine, mepivacaine, and benzocaine). The effects of repetitive stimulation were apparent at lower frequencies for drugs in the former group than in the latter. The results support an important role for frequency dependence in the antiarrhythmic and local anesthetic properties of these drugs. They also reveal unexpected similarities between drugs at the high and low extremes of lipid solubility with respect to the time course of frequency-dependent blocking actions. (Key words: Anesthetics, local; Heart, arrhythmia, antiarrhythmia; Nerve, conduction; Nerve, block; Nerve, refractory period.)

LOCAL ANESTHETICS block conduction by depressing the sodium conductance system, which is responsible for action potential generation.[1–5] It has long been recognized that local anesthetics block trains of action potentials more effectively than single action potentials.[4–6] This phenomenon has recently been labeled "frequency-dependent" (or "use-dependent") conduction block.[7] Most of the studies on the phenomenon of frequency dependence have been carried out under voltage-clamped conditions[7–11] using a limited number of local anesthetics to study their effects on sodium currents. Very little published information compares the many local anesthetics for frequency-dependent blocking actions, properties that may be of great importance to their apparent clinical potencies, and have also been suggested to be relevant to antiarrhythmic properties.[11,12] The present study was designed to compare the relative magnitudes of the frequency-dependent components of conduction block for a variety of local anesthetic and antiarrhythmic drugs of differing lipid solubilities. It is hoped that the results will add to the understanding of how local anesthetics can alter impulse traffic in nerves, and possibly cardiac muscle.

Methods

The sciatic nerves of 2–2½-inch male *Rana pipiens* frogs were removed, desheathed and split in half. Each bundle was mounted separately in a sucrose gap recording chamber (figure 1*A*). All experiments were carried out at room temperature. The Ringer's solution in the recording pool was of the following composition (in mM): NaCl 114, KCl 2.4, $CaCl_2$ 2.0, HEPES buffer 5, (titrated to *p*H 7.3 with 10 per cent tetramethylammonium hydroxide). In most experiments tetraethylammonium chloride (TEA) was present in a concentration of 7.5 mM to block potassium channels. This was done in order to identify the effects as specific sodium channel properties. The interpretation of changes in compound action potential amplitudes recorded by the sucrose gap technique, and controls for the effects of both sucrose and TEA, are discussed below.

Samples of the quaternary compound QX-572, and the tertiary compounds GEA 968 HCl, procaine HCl, lidocaine HCl, prilocaine HCl, mepivacaine HCl, bupivacaine HCl, tetracaine HCl, and etidocaine HCl were provided through the courtesy of Astra Pharmaceutical Company. Benzocaine and propranolol were kindly supplied by Ayerst Laboratories. Quinidine sulfate was obtained from Baker Chemical Company. Drug solutions were made up freshly each day in Ringer's solution and the *p*H checked. Measured *p*H was between 7.1 and 7.3 over all the experiments. The drug-containing Ringer's solution was flushed into the recording pool in volume sufficient to provide ten complete changes of the solution in the pool. Washout was carried out similarly. Frequency dependence was assessed at equilibrium,

* Research Associate.
† Assistant Professor.
‡ Professor.
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Address reprint requests to Dr. Kendig.

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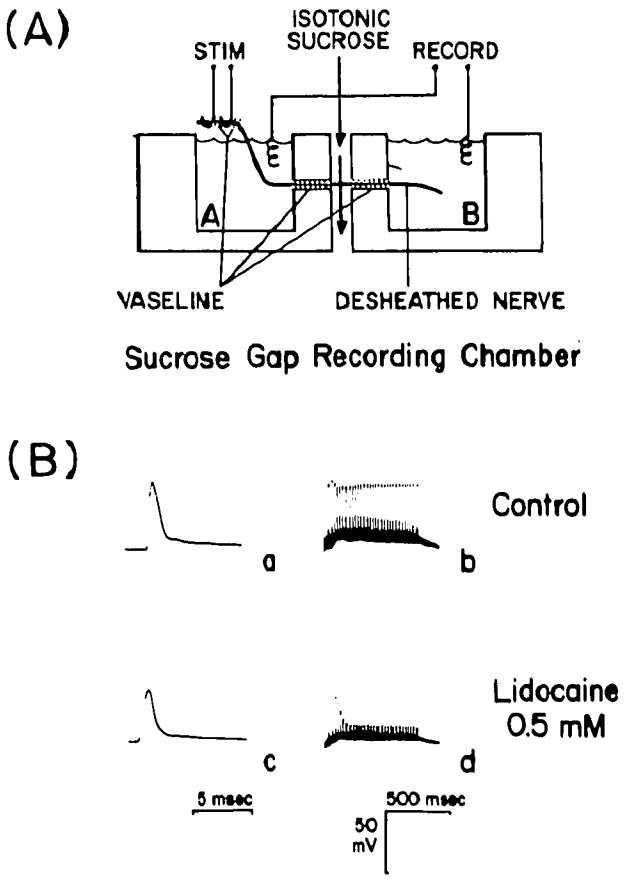

FIG. 1. *A*, Diagram of the sucrose gap recording technique. The desheathed frog sciatic nerve rests on a pair of stimulating electrodes insulated by vaseline seals from each other and from pool A, which contains a ground lead (not shown) and the reference recording electrode. Drugs diluted in frog Ringer's solution are applied to the 5-mm length of nerve in pool A. Isotonic sucrose solution, a nonelectrolyte, flows continuously through the 2.5-mm diameter channel indicated by the arrows, and together with vaseline seals (1.5 mm each) electrically insulates pool A from pool B. Pool B is filled with 120 mM KCl. The active electrode in pool B records, against the reference electrode in pool A, the electrical activity of the nerve segment in pool A.

B, Example of basal and frequency-dependent local anesthetic conduction blocks. *Upper row*: the normal (drug-free) nerve response (*a*) to a single stimulus and (*b*) to a 40-Hz train lasting 800 msec *Lower row*: 0.5 mM lidocaine produces a basal block of approximately 40 per cent (*c*); stimulation at 40 Hz for 800 msec intensifies the block to 80 per cent (*d*). Amplitude is measured from the baseline immediately preceding each response, in order not to include the after-depolarization. "Basal" block is defined as the decrease in amplitude of the response to single stimuli, calculated as percentage of control amplitude. Frequency-dependent block is the decrease in amplitude of the last impulse in a 40-Hz train compared with the same impulse under control conditions.

which was determined by identical records taken five minutes apart. For most drugs, equilibrium was attained in 15–25 minutes; the antiarrhythmic drugs required 30–35 minutes, and QX-572 almost an hour. Measurements were also made during drug equilibration and washout in experiments designed to provide dose–response curves for frequency-dependent versus basal block such as those shown in figure 4. Stimuli were single pulses or trains delivered from a programmable isolated stimulator to bipolar electrodes on a non-drug-treated section of the nerve. Stimulus duration was 0.1 msec and intensity twice that which evoked a maximum response. Nerve responses were displayed on an oscilloscope and photographed. Each drug was tested on at least two nerves taken from different animals.

Nerve conduction block was expressed as the percentage decrement in amplitude of the compound action potential. "Basal" (non-frequency-dependent) block was assessed by measuring the response to stimuli at frequencies so low that there was no detectable effect of preceding stimuli on the response to the test stimulus. Frequency-dependent block was assessed by measuring the decrement observed in the last response to an 800-msec 40-Hz train compared with the same response under control conditions (fig. 1*B*). This protocol was used to obtain the results shown in figures 2–4. Drugs were also compared over a range of stimulus frequencies, as shown in figure 5. Preliminary experiments were carried out to determine drug concentrations that produced approximately 25 per cent basal block, and the definitive experiments on frequency-dependent block were done at these concentrations. Basal blocks in those experiments, however, varied between 15 and 50 per cent, a not surprising incidence of variability among preparations.

The sucrose gap technique reduced the potency of local anesthetic drugs, presumably by holding the membrane potential at a slightly hyperpolarized level. As a control for this effect, standard killed-end recordings without sucrose were done for four drugs: lidocaine, GEA 968, etidocaine, and benzocaine.

TEA was used in these experiments to enable the drugs to be compared for their effects on sodium channel properties. TEA, a specific potassium channel-blocking drug, prevents the increase in potassium conductance that contributes to the repolarization phase of the action potential, and may also depolarize the nerve by decreasing resting potassium conductance, thus enhancing local anesthetic potency. Control experiments without TEA were therefore carried out on the same group of four drugs used in the sucrose controls, namely lidocaine, GEA 968, etidocaine, and benzocaine. Controls were also done with neither TEA nor sucrose present.

Results

All local anesthetics and antiarrhythmic drugs tested showed increased potencies when the nerve was

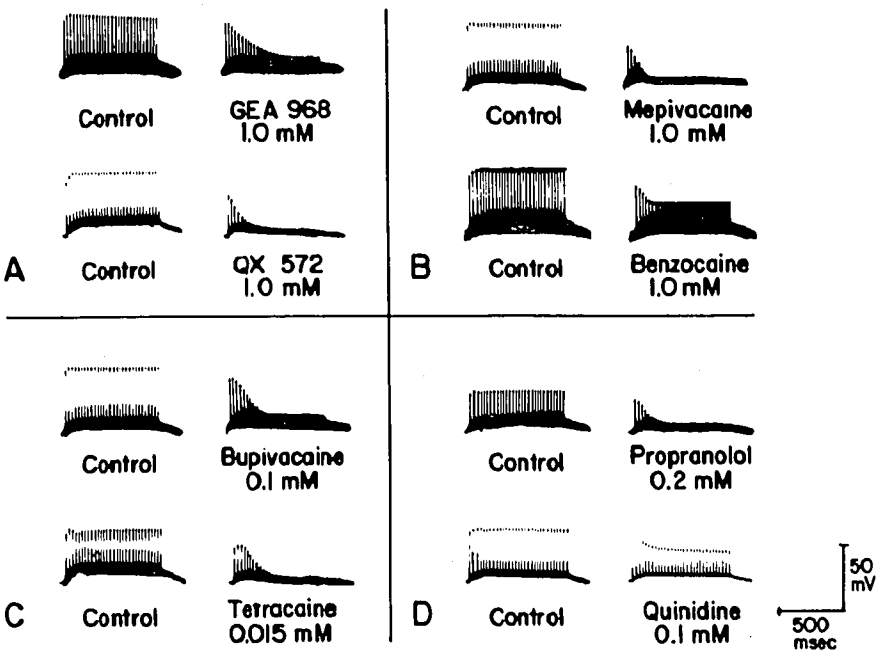

FIG. 2. Examples of frequency-dependent effects of four groups of drugs. *A*, QX-572 and GEA 968 are experimental local anesthetics of low lipid solubility. *B*, mepivacaine and benzocaine represent local anesthetics of intermediate lipid solubility, as does lidocaine in figure 1. *C*, tetracaine and bupivacaine are highly lipid-soluble and thus very potent local anesthetics. *D*, propranolol and quinidine are antiarrhythmic agents. Although all drugs have frequency-dependent blocking properties, there are differences in patterns of onset of the frequency-dependent components. Onset is relatively rapid with drugs of intermediate lipid solubility, maximum frequency-dependent block being achieved after 4–8 impulses at 40 Hz. Drugs in the other groups require 12–18 impulses to achieve the maximum effect of 40-Hz stimulation.

stimulated repetitively. Possible artifacts due to the use of the sucrose gap technique and blockade of potassium channels by TEA were excluded in control experiments. Without sucrose, the baseline offset (accumulating afterpotentials) seen during stimulation (figs. 1 and 2) disappeared, and both basal and frequency-dependent blocks were enhanced. The ratio of frequency-dependent block to basal block was diminished for all drugs in the absence of sucrose. However, the temporal characteristics of frequency-dependent block remained the same for each drug, and the reported differences among the four drugs were maintained. In the absence of sucrose, TEA had no effect on either basal or frequency-dependent block with GEA 968, enhanced both slightly with lidocaine and benzocaine, and enhanced both markedly, while reducing the frequency-dependent/basal block ratio somewhat, with etidocaine. TEA did not alter the temporal characteristics of frequency-dependent block or change the relationships among drugs. When sucrose was used, TEA had no effect on either basal or frequency-dependent block.

For purposes of comparison, the drugs were divided into antiarrhythmics (propranolol and quinidine) and three groups of local anesthetics: those with low lipid solubility (QX-572, GEA 968), those with moderate or intermediate lipid solubility (procaine, lidocaine, benzocaine, prilocaine, mepivacaine), and those with high lipid solubility (bupivacaine, tetracaine and etidocaine).[13] In all groups a 40-Hz stimulus train enhanced conduction block. However, differences among drugs appeared in the relative rapidities of onsets of frequency-dependent block during a train of stimuli (Fig. 2). At the end of an 800-msec train, response amplitude approached a plateau level; when impulses between train onset and achievement of a plateau were enumerated, clear differences among drugs emerged. Drugs of low lipid solubility

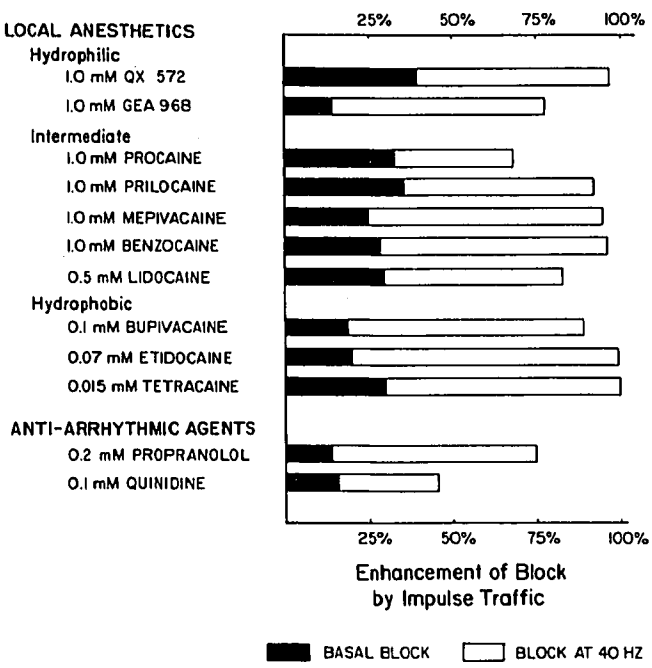

FIG. 3. Enhancement of block by repetitive stimulation. Local anesthetics are arranged in order of increasing potency and (roughly) increasing lipid solubility[13]; quinidine and propranolol are grouped separately. Bars represent an average of 2–3 nerves with each agent. Because of the small numbers, estimates of variability are not given. The shaded area represents basal block, the clear area the incremental block produced by 40-Hz stimulation. All agents tested block nerves by a mechanism sensitive to stimulus frequency.

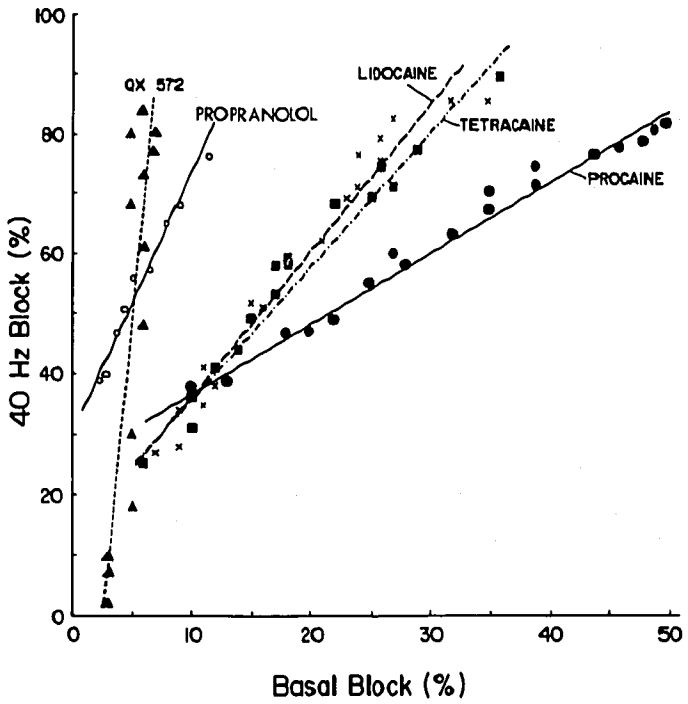

FIG. 4. Relationship between basal and frequency-dependent conduction blocks. Measurements were made during drug equilibration and washout. Frequency dependence was measured at 40 Hz; data points represent one nerve for each agent. Replications of the experiments produced similar results. Curves for 40-Hz block versus basal block could be approximated by a straight line for values of 40 Hz block between 20 and 80 per cent. Slopes for most local anesthetics were approximately 2. QX-572 produced a very steep slope; the antiarrhythmic agent propranolol was intermediate between QX-572 and most of the local anesthetics, while procaine had a somewhat less steep slope than most local anesthetics.

(the permanently charged quaternary compound QX-572 and GEA 968) and high lipid solubility (bupivacaine, tetracaine and etidocaine) required relatively large numbers of impulses (12–18) to reach the maximum effect of use. Quinidine and propranolol also fall in this group. The other drugs, including all the commonly used local anesthetics of intermediate lipid solubility, required only 4–8 impulses to achieve maximum frequency effects. This temporal property was relatively dose-independent for each drug at levels of basal block of 40 per cent or less.

When the frequency-dependent and basal components of block were measured, there was no clear difference among drugs when repetitive activity at 40 Hz for 800 msec was used as the test (fig. 3). Nerves blocked by all drugs showed a two- to fourfold frequency-dependent intensification of basal block at 40 Hz.

When frequency-dependent block was plotted against different depths of basal block produced by varying drug concentration during equilibration and washout, some differences among drugs appeared (fig. 4). Although the number of nerves tested with each drug (2–3) was too small for statistical treatment, differences among drugs were reproducible on replication. The experimental cationic blocking agent QX-572 showed an exceedingly steep slope for the relationship between basal block and frequency-dependent block produced by varying anesthetic concentrations: at levels of basal block of less than 10 per cent, stimulation of 40 Hz increased the block to nearly 100 per cent. The slope of the curve of frequency-dependent versus basal block was about 2 for a large group of drugs, including lidocaine, benzocaine, and tetracaine, and was somewhat less for procaine. The antiarrhythmic drug propranolol produced a steeper slope than most local anesthetics, but it was not as steep as that of QX-572.

When impulse frequency was varied, as shown in figure 5, the least lipid-soluble and most lipid-soluble of the drugs showed similarities. A normal nerve can carry 100 impulses per second with little impairment of action potential amplitude. All the drugs tested sharply reduced the maximum following frequency. Examples of selected drugs are shown in figure 5. QX-572, GEA 968, tetracaine, bupivacaine, etidocaine, and the antiarrhythmic drugs quinidine and propranolol were most potent in this respect. These drugs caused the nerve to show a very long "memory" for recent depolarizations; stimuli applied at frequencies as low as 0.5 Hz after a period of rest produced accumulation of block. The remaining local anesthetics of intermediate lipid solubility showed memory of less than 2 seconds, requiring stimulus frequencies higher than 2 Hz before any significant incremental block developed. It should be emphasized that this phenomenon (memory) is highly dose-dependent.

Discussion

The interpretation of results obtained by the sucrose gap technique requires comment, as does the use of TEA. In the sucrose chamber employed, the compartment that contained the reference electrode was traversed by approximately 5 mm of active nerve, and drugs were applied to this compartment. A 5-mm segment of myelinated nerve would be expected to include two or three nodes of Ranvier. When a conduction-blocking drug was applied to this segment of nerve, some axons continued to generate action potentials, probably in some cases

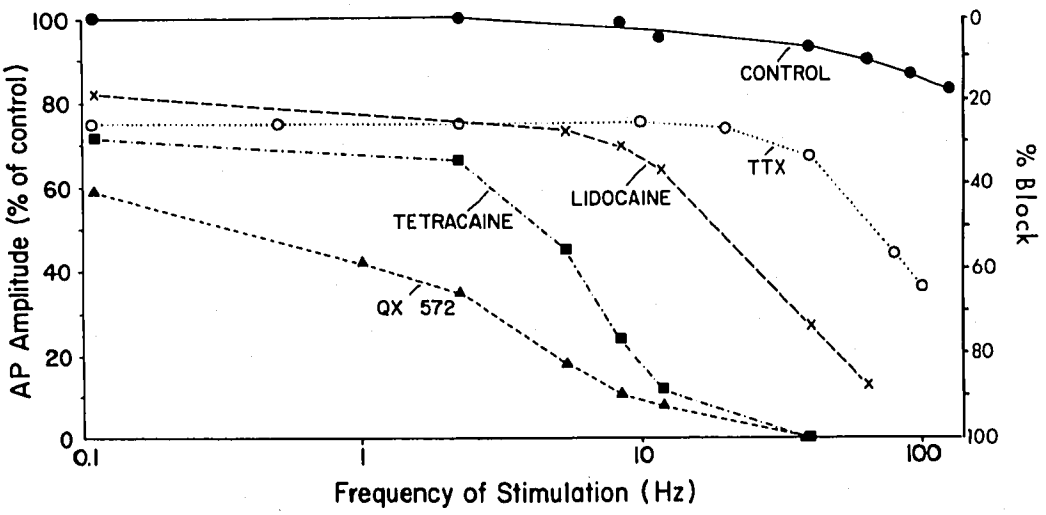

FIG. 5. Effects of stimulus frequency on response amplitude. At frequencies to 5 Hz, stimuli were applied until amplitude was stable. Above 5 Hz, 800-msec trains were used. Normal (drug-free) nerves respond at 100 Hz or less without significant decrement. The data for tetrodotoxin (TTX) from an earlier study are included to show that tetrodotoxin does not act at a site that produces frequency-sensitive conduction block. Very lipid-soluble drugs (e.g., tetracaine) and lipid-insoluble drugs (QX-572) show the effects of repetitive impulse traffic at lower frequencies than drugs of intermediate solubility (lidocaine).

of reduced amplitude, at the nodes in the drug pool. Others were blocked completely at some point within the drug compartment, and their only contribution to the recorded potential was the depolarization spreading electrotonically from the last active node in the stimulating compartment. Contributions to the recorded potential attributable to active response within the stimulating compartment must have been small, since it was possible to abolish the recorded response completely with drug overdoses. Changes in compound action potential amplitude therefore reflected both changes in the amplitude of action potentials in individual axons and changes in the number of axons contributing actively to the response.

It is clear that all the drugs tested block conduction in a manner strongly dependent on the frequency of impulses in the nerve. There are differences among drugs in frequency-dependent blocking potencies at 40 Hz, propranolol and the experimental drug QX-572 appearing more potent than most local anesthetics in this respect, and procaine slightly less potent (fig. 4). There are, moreover, differences among drugs in the rates of development of frequency-dependent block during a train of stimulation and in the time courses of recovery to basal blocking levels following stimulation. Drugs of intermediate lipid solubility, including procaine, prilocaine, mepivacaine, benzocaine, and lidocaine, showed maximum frequency-dependent block after 4–8 impulses at 40 Hz. Nerves treated with these drugs also recovered relatively quickly to basal block levels following rapid use, and required stimulus frequencies of more than 2 Hz to produce significant frequency-dependent block. The most and least lipid-soluble drugs, however, developed frequency-dependent block relatively slowly during stimulation, requiring 12–18 impulses to reach maximal effects. The time constants for recovery were also long, with effects of stimulation appearing at very low frequencies and lasting several seconds. The antiarrhythmic drugs quinidine and propranolol also fall in this second group.

It should be emphasized that in these studies benzocaine, although uncharged, acts like a typical local anesthetic in the intermediate lipid-solubility range. Frequency-dependent blocking actions can apparently be produced by both neutral (benzocaine) and charged (QX-572) species of the drug.

Propranolol, and quinidine to a lesser extent, appear to exert frequency-dependent effects similar to those of the local anesthetic drugs. Frequency dependence is particularly marked in the case of propranolol, which is frequency-sensitive at much lower rates of stimulation than most drugs, and which blocks strongly at 40 Hz at concentrations where basal block is negligible (fig. 4). The present results are in accord with recent studies that support a role for frequency-dependent conduction block in the cardiac antifibrillatory effects of propranolol and other antiarrhythmic drugs.[12,14,15] Although some of the drugs found in the present study to have the most marked frequency-dependent blocking characteristics have not been used clinically as antiarrhythmic drugs, at least one, QX-572, has been studied for antiarrhythmic effects.[16]

There is not yet a molecular model that completely accounts for the properties of frequency-dependent conduction block. In the nerve, sodium conductance kinetics are described by the classic Hodgkin-Huxley equation[17]; sodium channels may be resting, open, or inactive. A channel in the resting state responds to

an adequate depolarizing stimulus by opening to permit sodium ions to pass through the membrane for a brief period, then reverting to the closed or inactive state. In the inactive state the channel no longer responds to a stimulus by opening. Normally, sodium channels recover from the inactive state with a time constant of 5–10 msec, which accounts for the normal refractory period following an impulse. It is strongly suggested that the frequency-dependent component of conduction block is due to an alteration in the process of sodium channel inactivation by local anesthetic drugs. It is known that there is a shift in voltage dependence of the inactivation process in the drug-altered fraction of the sodium channels, indicating that the inactive state is altered by the presence of an anesthetic drug.[7,11,12,18,19]

In the present experiments the use of TEA to block potassium channels permits the conclusion that all drugs were exerting frequency-dependent effects through an action on the sodium channel. This, however, does not imply that the mechanisms of frequency dependence at the molecular level are precisely the same for all drugs. Previous studies have shown that for the extremely hydrophilic drugs, opening of the sodium channel is necessary to permit the drug to bind to and unbind from the receptor.[8,11,18]

This requirement, added to the alteration of inactivation, may be the basis for the particularly marked frequency dependence shown by these drugs. Since the drugs of intermediate lipid solubility showed faster rates of onset of frequency-dependent block, and shorter memories for accumulated block, than drugs of low lipid solubility, one might have expected even faster rates of onset and shorter memories for highly lipid-soluble drugs. This was not the case. The similarity between drugs of low and high lipid solubilities, as opposed to those of intermediate lipid solubility, was an unexpected finding of this study. The unexpectedly marked frequency-dependent behavior of benzocaine was also surprising. In voltage-clamped studies this drug has been shown to shift the voltage dependence of inactivation, but not to show enhancement of block on repetitive stimulation.[18] It is possible that frequency-dependence is strongly a function of after-potentials following the action potential. Its extent in active nerves may therefore not be readily predictable from studies in voltage-clamped preparations where the membrane potential is held constant between clamp pulses.

The phenomenon of frequency-dependent conduction block may require a careful redefinition of local anesthetic potency. Future studies of comparative potencies will have to describe much more completely the conditions under which measurements are made. In particular, apparent local anesthetic potency varies with the rate of impulse traffic in each nerve. All drugs selectively block nerves firing at high rather than low frequencies. Bupivacaine, tetracaine and etidocaine extend this selective block to much lower frequencies of impulse traffic than other local anesthetics. The phenomenon of frequency dependence is related to concentration in the sense that the time constant for recovery from frequency-dependent increments of block is longer at lower concentrations. (The rationale for this statement is given by the kinetics of blocking and unblocking described in other studies[11,12] and in the footnote§). In view of this relationship, the frequency-dependent blocking phenomenon may partially account for the long durations of action of these drugs.

Frequency-dependent conduction block may offer a basis for selective block of different sensory nerve types. An extremely phasic sensory nerve cell, firing very short widely-spaced bursts, will be relatively resistent to block. This relative resistance will be greatest to the more potent, more lipid-soluble drugs, which require many impulses to demonstrate maximal frequency dependence. The nerve of a tonic receptor firing at very low frequencies will also be relatively unimpaired. Nerve cells that fire in a pattern of 10–50 impulses/sec in bursts lasting half a second or longer will have their normal burst length curtailed by all drugs at concentrations lower than those associated with complete basal block. The pattern of impulses arriving centrally from these sensory neurons may be expected to be very different from the patterns generated peripherally. It is suggested that the phenomenon of frequency-dependent conduction block may thus play a considerable role in modulating anesthetic-associated analgesia.

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§ This time constant can be derived from an equation governing the first-order binding (k) and zero-order unbinding (l) reactions.

$$db/dt = kD(1 - b) - lb$$

where D is the concentration of local anesthetic near the receptor site and b is the fraction of channels in the blocked state. The relaxation time constant is $(kD + l)^{-1}$.

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