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The Mechanism of Action of Local Anesthesia by Tetraethylammonium Derivatives

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Tetraethylammonium (TEA^+) derivatives in which one of the ethyl groups is replaced by a longer chain alkane (C_4–C_{16}) were tested for their ability to block the compound action potential (CAP) of frog sciatic nerve. A parabolic relation between chain length and 100 per cent blocking dose was found, suggesting an optimal size of C_{12} for the inhibitory receptor. Two types of inhibition were observed. Type 1 was an inhibition of the CAP at all frequencies when the nerve was perfused with the TEA^+ derivative. Interaction with tetrodotoxin suggests this is a Na^+ channel effect. Type 2 was an irreversible (still present after nerve wash) frequency-dependent inhibition that is distinct from and synergistic with Na^+ channel blocking local anesthetics. From the kinetics of inhibition onset and transport studies, it is suggested that the ultralong action of the TEA^+ derivatives is mediated by binding to a receptor at the internal part of K^+ channels. (Key words: Anesthetics, local: tetraethylammonium derivatives.)

We have previously demonstrated that tetraethylammonium (TEA^+) derivatives (fig. 1) can induce local anesthesia of the rat infraorbital nerve with a duration proportional to the length of the long chain alkane substituted for one of the ethyl groups.[1] The mechanism and site of action of the TEA^+ derivatives in rat nerve are unknown; however, several hypothetical mechanisms are suggested by studies of these compounds on frog and squid nerve.

Using voltage clamp techniques, two different receptors for TEA^+ derivatives have been described. The first receptor type has been found in both squid giant axon[2,3] and node of Ranvier preparations from frogs.[4] It appears to be associated with the *internal* portion of ionic channels responsible for voltage dependent K^+ conductance.[5] The receptor consists of a region that requires the positive charge and 8 Å diameter of the TEA^+ moiety‡ (the same charge and dimension as a hydrated K^+ ion), as well as an adjacent hydrophobic binding site that demonstrates a 700-calorie increase in binding energy for each methyl group added from C_2 to C_{12}. The second type of TEA^+-derivative receptor (TEAR) has only been found on the *external* portion of the frog node of Ranvier.[3] The external TEAR also requires the TEA^+ configuration at one end of the ligand, but increasing the length of the hydrocarbon tail from C_4 to C_{12} *decreases* the affinity for the external receptor. Thus, it appears there is no hydrophobic binding site associated with the external TEAR. In addition, the external receptors appear to be associated with both Na^+ and K^+ channels (C_9 blocks 25 per cent of Na^+ channels at a concentration with blocks 50 per cent of the K^+ channels).[4] With these voltage clamp data in mind, we determined to elucidate the mechanism and site of action of anesthesia by externally applied TEA^+ derivatives using the frog sciatic nerve as an *in vitro* model system. As local anesthesia of peripheral nerves is directly related to a block of the high frequency axonal signals encoding pain,[6] we decided to examine the effects of external TEA^+ derivatives on axonal conduction at different frequencies, and by examining the interaction of TEA^+ derivatives with other local anesthetics with better defined sites of action, to suggest which TEAR and which ionic channels are involved in the ultralong anesthetic action of TEA^+ derivatives.

Materials and Methods

The TEA^+ derivatives were synthesized as described in another study.[1] All other chemicals were reagent grade.§

Transport of TEA^+ ^{14}C–C_{12}

The ability of ^{14}C-labeled C_{12} to transport across biological membranes was estimated using the partitioning procedure outlined by Green *et al.*[7] The aqueous phase contained 1 mM C_{12} (0.4 μCi) in 25 mM Tris Acetate *p*H 7.0 with 50 per cent ethanol. This was vortexed for 1 min with an equal volume of 9:1

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‡ Hille B: Quaternary ammonium ions that block the potassium channel of nerves. Abstracts, 11th meeting. *Biophysical Society 7:19, 1967.

§ Tetraethylammonium (C_2) was obtained from Aldrich Chemical Co; Carbon-14 labeled C_{12} (Ethyl-1-^{14}C, triethyldodecyl ammonium bromide) was synthesized by New England Nuclear; Dodecyltrimethyl ammonium bromide (TMA-C_{12}), tetrodotoxin, and L-αphosphatidyl choline from egg yolk were obtained from Sigma Chemical Co; Lidocaine and bupivacaine were provided by Astra Pharmaceuticals, and QX314 was a kind gift of Dr. Bertil Hille.

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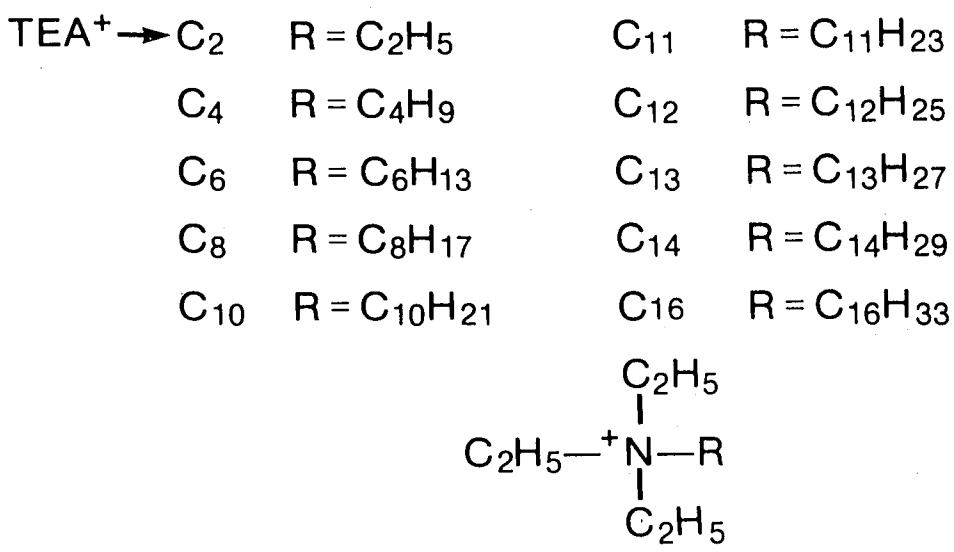

FIG. 1. Structure of TEA^+ derivatives. All the quaternary ammonium (QA) compounds tested consisted of TEA^+ (C_2) which has had one of its ethyl groups replaced by a longer carbon chain (R group). For example, in C_{12} one ethyl was replaced by $C_{12}H_{25}$. Their length in Å is as follows: C_2 6.6; C_4 9.1; C_6 11.6; C_8 14.1; C_{10} 16.5; C_{11} 17.8; C_{12} 19.0; C_{13} 20.3; C_{14} 21.5; and C_{16} 24.0.

(V:V) heptane/chloroform with and without 140 mM egg yolk lecithin. The phases were separated by centrifuging at 1000 × *g* for 10 min, and aliquots counted in dimilume 30 on a Packard Prias® liquid scintillation counter. The partition coefficient was then calculated from the mean distribution of four experiments.

In Vitro Block by TEA^+ Derivatives

Northern Rana pipiens (College Biological Supply, Bothell, Washington) were maintained in tap water at 4° C. Frogs were pithed and the sciatic nerve carefully dissected out of adjoining muscle and connective tissue from the entry into the spinal cord to the lower part of the leg. Nerves were kept moist by application of frog Ringer's solution (110 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 111 mM glucose, and 10 mM Na^+-phosphate buffer *p*H 7.2). The outer sheath of connective tissue (epineurium) was removed with fine needles under a dissecting microscope whose field was illuminated by indirect flourescent light. Nerves were placed in a sucrose-gap nerve chamber containing five 9-mm sections separated by Vaseline® seals. Ringer's solution was placed in each section, except the section between the recording electrodes which contained isotonic sucrose. The drug in Ringer's solution was perfused through the chamber between stimulating and recording electrodes (platinum) at a

TABLE 1. Effect of Quaternary Ammonium Compounds on Compound Action Potentials of Frog Sciatic Nerve

Nerves	Drug	Concentration (mM)	Per Cent Block	Per Cent Recovery*	Maximum Frequency Transmitted† (Hz)	Conduction Velocity‡ (m/s)			Absolute Refractory Period‡ (ms) at 1 Hz		
						Control	Drug	Washed	Control	Drug	Washed
4	*TEA–C_2	20	0	——	500	16 ± 3	14 ± 2	14 ± 2	1.2 ± .2	1.4 ± .2	1.3 ± .2
4	C_4	20	0	——	500	18 ± 2	16 ± 3	16 ± 2	1.0 ± .1	1.2 ± .2	1.2 ± .3
5	C_6	20	0	——	500	18 ± 3	14 ± 3	14 ± 2	1.0 ± .1	1.3 ± .2	1.3 ± .3
4	C_8	15	100	0	——	19 ± 3	10 ± 2	10 ± 3	1.1 ± .1	2.5 ± .2	2.9 ± .2
6	C_{10}	5	100	63	50	18 ± 2	10 ± 1	13 ± 2	1.2 ± .1	4.1 ± .3	2.7 ± .3
4	C_{11}	1	100	100	60	17 ± 2	7 ± 1	11 ± 3	1.0 ± .2	4.4 ± .2	1.1 ± .2
5	C_{12}	0.5	100	86	68	21 ± 2	13 ± 1	19 ± 2	1.2 ± .1	4.1 ± .3	1.3 ± .2
4	C_{13}	1	100	10	65	21 ± 3	13 ± 2	15 ± 3	1.0 ± .2	2.2 ± .2	1.7 ± .3
6	C_{14}	2	100	0	——	21 ± 2	14 ± 2	16 ± 1	1.3 ± .2	2.3 ± .3	1.8 ± .2
4	C_{16}	20	0	——	500	17 ± 3	16 ± 2	16 ± 2	1.0 ± .2	1.0 ± .2	1.0 ± .2
3	QX314	20	63	0	50	18 ± 2	14 ± 3	14 ± 2	1.1 ± .3	1.4 ± .2	1.2 ± .2
3	Lidocaine	1.5	100	100	500	17 ± 2	14 ± 2	17 ± 3	1.2 ± .1	2.8 ± .3	1.2 ± .2
3	Bupivacaine	0.4	100	100	500	20 ± 3	12 ± 2	20 ± 3	1.2 ± .1	2.4 ± .2	1.2 ± .2
4	TTX	0.03	100	100	500	22 ± 2	0	22 ± 2	1.2 ± .2	1.2 ± .2	1.2 ± .2
4	TMA–C_{12}	0.5	100	100	500	19 ± 2	17 ± 2	19 ± 3	1.0 ± .2	2.5 ± .3	1.1 ± .1

* Per cent recovery of CAP at 1 Hz after 100 per cent block is washed off.

† Maximum stimulus frequency transmitted by nerve after wash.

‡ See text: conduction velocity and absolute refractory period measured before application of drug, just before 100 per cent block, and after wash ± SEM.

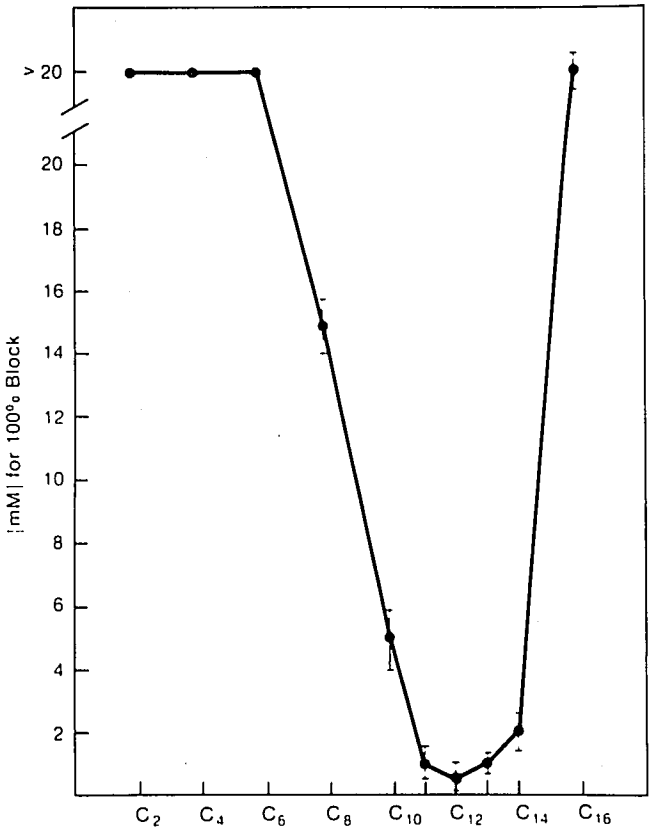

FIG. 2. Effect of chain length on blocking potency of externally applied QA compounds. The minimum concentration ± SEM of a given compound that produces 100 per cent block of the CAP is plotted *vs.* the chain length of the substituted side chain. C_2, C_4, C_6 and C_{16} require a concentration in mM for 100 per cent block > 20 mM.

rate of 20 ml/hr, and the drug concentration was increased until a 100 per cent block of nerve conduction at all stimulation frequencies was achieved within one hour, or until a concentration of 20 mM was reached.

The nerve was then washed with drug-free Ringer's solution until the CAP had recovered at all stimulation frequencies, or until it was decided that the remaining nerve block was irreversible with washing (after 1 hr of washing).

A frequency dependent block was defined as one in which the magnitude of the compound action potential decreased with increasing frequency. In order to quantitate the magnitude of the frequency dependent block a computer (Digital Declab® 11/03) was programmed to deliver a train of supramaximal stimulation pulses which increased logarithmically from 1 Hz to 100 Hz in 10 s. Output pulses were stretched by a pulse shaper and used to trigger a stimulus isolation unit (Digitimer LTD®, Model DS2) which controlled the stimulus amplitude (0.5–1.0 V) and duration (0.1 ms). Compound action potentials (CAP) were amplified (Tektronix® AM502), and displayed along with the stimulus train on an oscilloscope (Tektronix® 5111). Trains of CAP were stored and photographed (C5B oscilloscope camera, Tektronix®). The maximal slope of this train of CAP (dmV/d frequency) was taken as an estimation of the magnitude of frequency dependence.

To determine conduction velocity and absolute refractory period, a Grass S48 stimulator delivered pulses to the stimulus isolation unit and a trigger to the oscilloscope. The distance between the stimulus electrode and first recording electrode divided by the time from stimulus artifact to maximum CAP gave the conduction velocity. The maximum time between twin stimulus pulses where no second CAP was seen defined the absolute refractory period. Conduction velocity and the absolute refractory period were measured before drug application, just before the nerve was 100 per cent blocked by drug, and after a long (30 min) wash with drug free Ringer's solution.

Results

Characteristics of the TEA-derivative Block

The results of nerve block by TEA^+ derivatives are summarized in table 1. It was found that con-

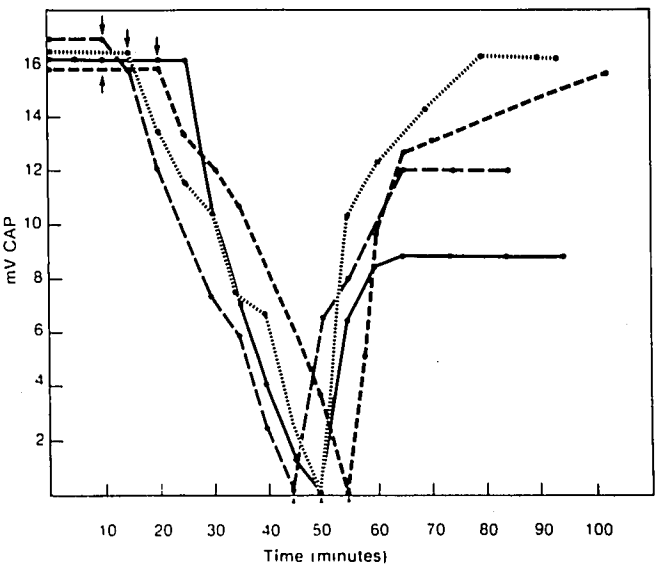

FIG. 3. Block of CAP by QA compounds C_{10}–C_{13}. After a control value for the CAP at 1 Hz was established, the nerve was perfused with drug (solid arrows) and the CAP recorded at 5-min intervals. When the CAP became 0 mV, the nerve was washed (interrupted arrows) with drug-free Ringer's solution until a maximum recovery was seen. Nerves that had similar initial CAP were selected and each point represents data from one nerve. C_{10} (—-●-—); C_{11} (·····●·····); C_{12} (– – –●– – –); C_{13} (—●—).

centrations of a given TEA^+ derivative below the 100 per cent blocking concentration ([100 per cent block]) either decreased the size of the CAP very slowly or not at all. Since the resulting blocks were irreversible, the dose/response curve was very steep and [100 per cent block] was used as an appropriate point of comparison for the compounds. There was an extremely strong relationship between chain length of the TEA^+ derivatives and their [100 per cent block] (fig. 2), but when other data from table 1 were analyzed, the TEA^+ derivatives were found to fall into three distinct groups.

C_2 through C_6, and C_{16} did not produce any significant effect on the CAP at 20 mM and their [100 per cent block] is significantly greater than the other TEA^+ derivatives tested. C_8 and C_{14} gave 100 per cent block at concentrations less than 20 mM, but these blocks were completely irreversible at any frequency, and showed no frequency dependence. Finally, C_{10} through C_{13} produced partially irreversible, frequency dependent inhibition at the lowest concentration (fig. 2) and since C_{10} to C_{13} represented the most interesting and potent compounds both *in vivo* and *in vitro*, we decided to concentrate on their mechanism of action and extend our results to the other derivatives.

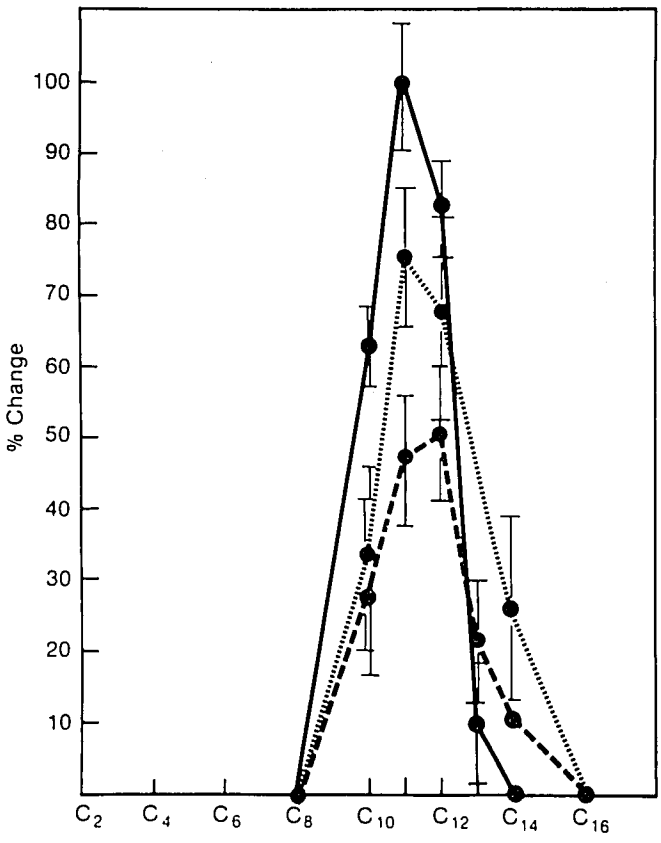

FIG. 4. Recovery of CAP after 100 per cent block by QA compounds. The percent recovery of the compound action potential at 1 Hz after washing with drug-free Ringer's solution (—●—) correlated well with the percent recovery of the refractory period (·····●·····) and the conduction velocity (– – ● – –).

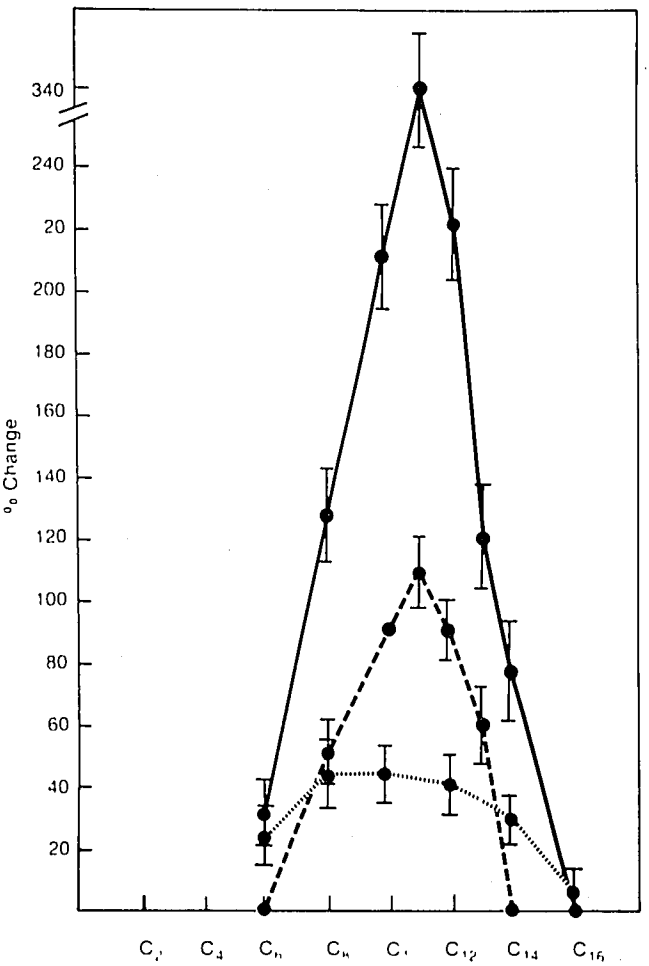

FIG. 5. Frequency dependence of QA compounds. Frequency dependence of the block of the CAP (– – ● – –) was measured as the maximum slope of the CAP amplitude versus log frequency of stimulation (d mv of CAP/d log Hz) after washing off of excess drug/non-specific binding. This correlated well with the percent increase relative to control of the absolute refractory period (—●—) just before 100% block. The maximum percent decrease in conduction velocity (·····●·····) did not show a good correlation to either determination.

Figure 3 shows the kinetics of onset and recovery of CAP block by C_{10} through C_{13} at 1 Hz stimulation frequency. To achieve 100 per cent block, 30 min of drug perfusion were required. During this period, the nerves demonstrated frequency dependent inhibition. After washing for 30 min with drug-free Ringer's solution, the CAP at 1 Hz, the conduction velocity, and the absolute refractory period of the nerve, had recovered in a parallel fashion, and the extent of their recovery was determined by the chain length of the TEA^+ derivative (fig. 4). However, there remained an irreversible frequency dependent inhibition. The

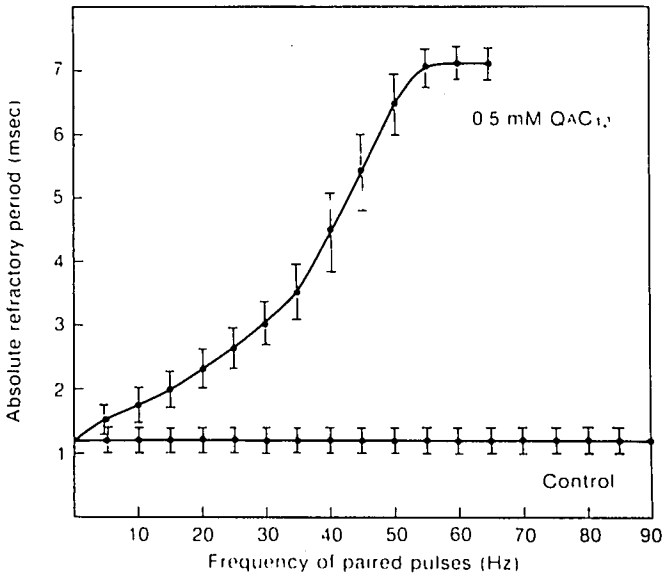

FIG. 6. Effect of frequency on the absolute refractory period. The untreated nerve did not show any effect of the frequency of paired test pulses on the absolute refractory period. The nerve treated with TEA–C_{12}, however, showed a marked increase in the absolute refractory period with increasing frequency of stimulation by paired pulses that plateaued at 7.0 ms at the same frequency the size of the CAP reached the minimum value.

magnitude of the frequency dependence was also a function of the TEA^+-derivative chain length, and correlated with the per cent *decrease* in conduction velocity and *increase* in the absolute refractory period measured just before 100 per cent block (fig. 5). This correlation between the irreversible frequency dependence after wash and the refractory period appeared to have simple mechanistic implications. We measured the absolute refractory period as a function of frequency of stimulation after treatment with C_{12}, and found a dramatic increase in the refractory period with increasing frequency up to 60 Hz where the CAP becomes negligible and the refractory period reaches a maximum (fig. 6).

In order to examine the effect of the ethyl groups in the action of TEA^+ derivatives, we tested the trimethyl-dodecyl ammonium derivative (TMA-C_{12}). The 100 per cent block for TMA-C_{12} was the same as the TEA^+ derivative (table 1) and the development of block took place during the same time course as seen in figure 3. When the nerve was washed, the frequency-dependent block completely reversed over a 25-min period.

Interaction with Na^+ Channel Blocking Anesthetics

Lidocaine and bupivacaine had a markedly different nerve blocking action than the TEA^+ or TMA derivatives. Onset of block was almost immediate after the perfusion of the drugs, and the inhibition of the CAP was proportional to the concentration of drug in the media rather than the all-or-none inhibition of the TEA^+ derivatives. At 0.4 mM bupivacaine or 1.5 mM lidocaine, 100 per cent block is achieved. At less than 100 per cent block, both compounds demonstrated frequency-dependent inhibition, but unlike the TEA^+ derivatives, the inhibition was completely removed by washing off the anesthetic. To look for possible interaction between the TEAR and tertiary amine anesthetic site, we tested the effect of bupivacaine and lidocaine on a nerve blocked with 0.5 mM C_{12} and then washed, leaving the nonreversible frequency-dependent component. To achieve 100 per cent block, one-

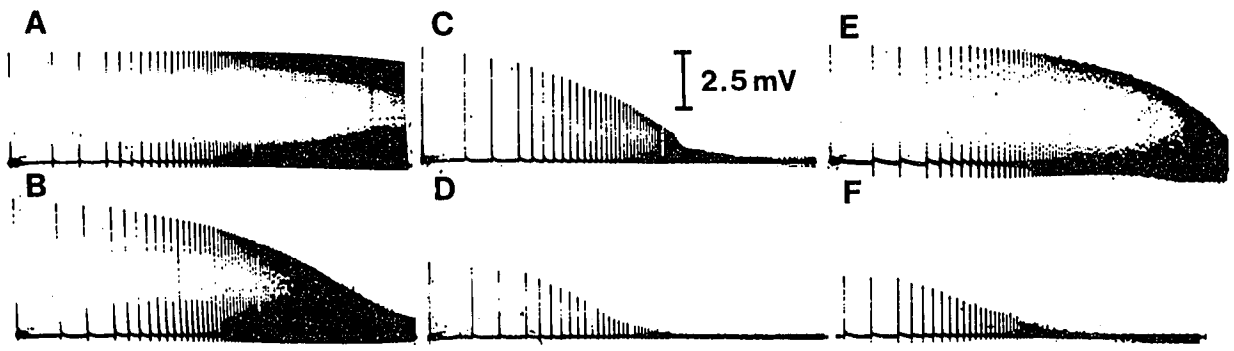

FIG. 7. Synergistic block by C_{12} combined with other local anesthetics. All nerves had approximately the same control CAP amplitude (16 mV) before treatment. *A*. CAP of untreated nerve given a series of supramaximal stimuli of 1–100 Hz over a 10 sec period. *B*. Same nerve treated with 0.5 mM TEA–C_{12}, and then washed. Note frequency dependent Type 2 inhibition. *C*. 20 mM QX314 after 40 min treatment and washing. Note sharp frequency dependent inhibition and decrease of CAP at 1 Hz relative to control. *D*. Treated with 0.5 mM TEA–C_{12}, washed, and then treated with 20 mM QX314 for 20 min. Notice greatly decreased amplitude and lower frequency cut off compared to either compound above. *E*. Nerve treated with 0.1 mM lidocaine. Note slight frequency dependence at relatively high frequency. *F*. Nerve blocked with 0.5 mM QAC_{12} and washed, then tested with 0.1 mM lidocaine. Note synergistic effect of both compounds on frequency dependence and amplitude.

half of the usual dose of bupivacaine or lidocaine was needed, and at lower doses the tertiary amine anesthetics increased the frequency dependence and magnitude of inhibition (fig. 7).

The quaternary analog of lidocaine, QX314, did not produce 100 per cent block even at 20 mM, although at this concentration there was a complete block of CAP conductance above 50 Hz (fig. 7). This frequency dependent inhibition had an onset time similar to C_{10}–C_{12} (~40 min), and like the TEA^+ derivatives, was irreversible with washing. To test for binding site interaction between QX314 and TEA^+ derivatives, a nerve was blocked with C_{12} and washed. Addition of QX314 produced further frequency-dependent inhibition noted by the sharper decline of the CAP and a decrease in the maximum frequency transmitted from 68 to 20 Hz (fig. 7).

Tetrodotoxin (TTX) at 30 nM produced a complete block of CAP at all frequencies within 2 min, and at low doses demonstrated no frequency-dependent inhibition. The TTX block was completely reversible with washing in 13 min. When TTX was added to a nerve previously blocked with C_{12} and then washed, there was no effect on the onset time or reversal of the TTX block (table 2), but when TTX was added while the C_{12} block was being established (C_{12} still in the perfusate) and before the nerve was washed, the TTX block was not reversible after 2 hours. During the same blocking period, TMA-C_{12} also interacted with TTX to produce a 2-hour reversal time for TTX block. Lidocaine increased the recovery time from TTX block to 70 min, and at 20 mM, QX314 approximately doubled the recovery time of the TTX block (table 2).

Internal or External Localization

To test the significance of an external proteinaceous TEAR for TEA^+ derivative pharmacology, we used a mild tryptic digestion (0.1 per cent trypsin in Ringer's solution for 25 min) which had no effect on the conduction of the nerve, but which decreased the sensitivity of the nerve to TTX (in agreement with Baumgold's[8] finding with STX). This procedure, however, had no effect on the 100 per cent block, onset, or frequency dependence of block by C_{12}.

Finally, partitioning experiments as outlined by Green *et al.*[7] were used to estimate the ability of C_{12} to move across the membrane by phospholipid mediated transport. C_{12} did not partition into the organic phase without phosphatidylcholine, while lidocaine had a partition coefficient of 2.1 (heptane/water) in this system. With the addition of phosphatidylcholine, a significant movement of C_{12} into the organic phase was observed, giving a partition coefficient of 0.2.

Table 2. Effect of Anesthetics on Recovery from Tetrodotoxin Nerve Block

Anesthetic Agent	Recovery Time (min)
TTX 30 nM*	13 ± 3 (4)§
0.5 mM TEA–C_{12} + TTX†	17 ± 4 (4)
20 mM QX314 + TTX	30 ± 5 (4)
1.0 mM Lidocaine + TTX	70 ± 10 (3)
TTX + 0.5 mM TEA–C_{12}‡	120 (3)
TTX + 0.5 mM TMA–C_{12}	120 (3)

* TTX used at 30 nM in all cases.
† TTX added after nerve block with TEA–C_{12} and washed.
‡ TTX added as nerve was blocked with TEA–C_{12} or TMA–C_{12}.
§ Time ± SD. The number of trials in parentheses.

Discussion

Specificity of the TEA^+-derivative Block

Our data on the specificity of the TEA^+-derivative nerve block are in good agreement with Armstrong's[3] data on the block of K^+ currents by *internal* TEA^+ derivatives. In both cases, the internal TEAR showed an increase in binding from C_2 to C_{12}; however, by extending past C_{12} (19.0 Å) we have apparently found the upper limit for the hydrophobic binding region of the TEAR. We also found that changing the remaining ethyl groups to methyl groups decreased the inhibition of K^+ channels. There was a specificity in site and conformation of ligands that suggested a specific TEAR.

Na^+ and K^+ Channel Components of TEA^+-Derivative Block: Type 1 and Type 2 Inhibition

We have demonstrated that TEA^+ derivatives can produce two distinct types of inhibition of neural conduction. The first is an initial block of the CAP at all frequencies with a slow onset (Type 1) in the presence of TEA^+ derivatives; the second is an irreversible frequency dependent inhibition present after removal of the external drug solution (Type 2).

The first type of inhibition is dependent on the chain length of the hydrophobic component of the TEA^+ derivative, with a maximal effectiveness at C_{12} (19.0 Å). The Type 1 inhibition does not require the triethyl configuration in the polar head group, and is mimicked by trimethyl derivatives in both time and magnitude of dose response.

Cahalan and Almers[9] have recently shown that QX314, and presumably the charged form of tertiary amine local anesthetics, synergistically facilitate the binding of TTX by producing a Na^+ channel "plugged" at both ends and protecting TTX and the local anesthetic from dissociation by Na^+ ions. This provides a mechanistic interpretation for results ob-

tained by Adams[10] and ourselves[11] that demonstrated a synergistic effect of TTX on blocking duration of local anesthetics *in vivo*. We have seen in this study that QX314, lidocaine, and the Type 1 C_{12} inhibition prolong TTX block *in vitro*. This suggests that the Type 1 block has a TTX-sensitive Na^+ channel component similar to other local anesthetics. The fact that external QX314 (which has a structure identical to TEA^+ derivatives except for the hydrophobic group) reaches its internal sodium channel site within the same time course as C_{12} Type 1 inhibition, also suggests the possibility of Na^+ channel block by TEA^+ derivatives. This is in agreement with Armstrong and Hille's[4] observation that TEA^+ derivatives block some Na^+ conductance when applied externally to frog node of Ranvier.

The second type of inhibition, exemplified by TEA^+–C_{12}, is an irreversible frequency dependent inhibition present after washing the axon. The magnitude of the frequency effect is also related to chain length, with a maximal effect with C_{12}. This type of inhibition does not prolong TTX binding, implying that it is not associated with the Na^+ channel. In addition, there is a synergistic relation between the Type 2 block by C_{12} and QX314, bupivacaine, and lidocaine, both in the magnitude and frequency dependence of block. This again suggests that the Type 2 block is associated with a site distinct from conventional local anesthetic sites (Na^+ channels). The Type 2 site also requires the triethylammonium conformation, which mimics a hydrated K^+ ion in size and charge. In summary, the Type 2 inhibition site appears to be due to a specific TEAR interaction which mediates a frequency dependent increase in the absolute refractory period (fig. 6). This in turn is responsible for a frequency dependent inhibition that appears distinct from the Na^+ channel,[12] and as shown by Armstrong's[3] data, is most probably associated with K^+ channel block.

Though voltage dependent K currents are not considered to be important in myelinated mammalian nerve repolarization, recent evidence[13–15] demonstrates they are a significant factor in mammalian C-fiber function. This suggests that the ultralong duration pain block by TEA^+ derivatives in the rat infraorbital nerve is a result of Type 2 inhibition in unmyelinated C fibers, and that in both our *in vivo* and *in vitro* preparations, we observed primarily a block of voltage dependent potassium channels.

Internal *vs.* External Localization

Several lines of evidence suggest that both the Type 1 and Type 2 sites are internal receptors. The long onset time of TEA^+ derivatives and the negative effect of external proteolytic digestion, as well as, the long recovery period of Type 1 inhibition (compared to the tertiary amine local anesthetics) suggests a diffusional barrier to these sites of action. The ability of phosphatidylcholine to move the C_{12} derivative into an organic phase indicates that phospholipid mediated micelles can transport these amphiphilic substances across the axolemma. This would also explain the lack of a graded dose dependence to external TEA^+ derivative application. A certain minimal dose is required by the transport system which is several orders of magnitude greater than what is needed when TEA^+ derivatives are applied internally.[3] QX314 which is known to act only internally demonstrates similar behavior, requiring a much higher dosage and a much longer time to produce inhibition when applied externally.

In Vitro vs. In Vivo Inhibition

There is good agreement between our results using the frog and rat nerve preparations. There are, however, some discrepancies that occur as a result of dose selection. We established the blocking dose as that which produced a 100 per cent block in 10 min *in vivo* while *in vitro*, the 100 per cent blocking dose was the minimal dose that produced nerve block in one hour. This selection was based upon the practical evaluation of the onset of a successful injection *in vivo* and evaluation of the characteristics of onset of block *in vitro*. Not surprisingly, up to thirty times the concentration was needed to block in less than 10 min *in vivo* than in 1 hour *in vitro*. Similarly, at much higher concentrations, C_2 through C_6 and C_{16} produced block *in vivo*, while at lower concentrations, no block was seen *in vitro*.

Conclusion

There are two mechanisms involved in anesthesia by TEA^+ derivatives. Type 1 inhibition appears to have a Na^+ channel component in common with conventional local anesthetics. Type 2 inhibition, however, appears unique and specific to the TEA^+ derivatives and involves a nonreversible, frequency-dependent inhibition, produced by increasing the refractory period of the nerve. Our data suggest that the TEAR responsible for Type 2 inhibition is on the inner surface of the axolemma. The transport process to the internal TEAR and then the ligand-receptor affinity appear to be related to chain length. Using voltage clamp studies, Armstrong[3] has localized an internal TEAR at the mouth of voltage dependent K^+ channels. His receptor shows the same ligand specificity and high

binding energy for TEA^+ derivatives as we have observed, as well as interference in K^+ conduction that could lead to an increase in the refractory period of the nerve. Although frequency dependent inhibition also occurs at Na^+ channel sites,[12] and TEA^+ derivatives appear to have a percentage of Na^+ (Type 1) and K^+ (Type 2) components of block related to their chain length, the relatively "pure" Type 2 blocks seen with C_{11} and C_{12} are best explained as predominantly K^+ channel effects.

As there are presently no drugs which bind with a high binding energy to K^+ channels, C_{11} and C_{12} should be of considerable interest to neurobiologists as potential tools for studying K^+ channels. C_{11} and C_{12} also have the characteristics of ultralong acting local anesthetics. Their profound frequency effect indicates a highly dissociative motor *vs.* sensory block.[16] The TEA^+ derivatives block at relatively low doses and, therefore, should have a favorable therapeutic to toxic ratio. Also, they will not easily cross the blood-brain barrier as they are permanently charged, and thus they cannot easily get to the CNS sites where conventional local anesthetics produce their primary toxicity. The potential of TEA^+ derivatives as a new class of local anesthetics certainly warrants further investigation, as they appear to have a unique set of pharmacological properties that provide onset of anesthesia through Na^+ channel effects and an ultralong duration of action by K^+ channel interaction.

References

1. Scurlock JE, Curtis EM: Tetraethylammonium derivatives: ultralong-acting local anesthetics? ANESTHESIOLOGY 54: 265–269, 1981
2. Tasaki I, Hagiwara S: Demonstration of two stable potential states in squid giant axon under tetraethylammonium chloride. J Gen Physiol 40:859–885, 1975
3. Armstrong CM: Potassium pores of nerve and muscle membrane, Membranes, A Series of Advances, Volume 3. Edited by Eisenman G. New York, Marcel Dekker, Inc., 1975, pp 325–358
4. Armstrong CM, Hille B: The inner quaternary ammonium ion receptor in potassium channels of the node of ranvier. J Gen Physiol 59:388–400, 1972
5. Hille B: The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. J Gen Physiol 50:1287–1302, 1967
6. Price DD, Mayer DJ: Neurophysiological characterization of the anterolateral quadrant neurons subserving pain in *M. mulatta.* Pain 1:59–72, 1975
7. Green DE, Fry M, Blundin GA: Phospholipids as the molecular instruments of ion and solute transport in biological membrane. Proc Natl Acad Sci USA 77:257–261, 1980
8. Baumgold J: ^{3}H-Saxitoxin binding to nerve membranes: inhibition by phospholipase A^2 and by unsaturated fatty acids. J Neurochem 34:327–334, 1980
9. Cahalan MD, Almers W: Interactions between quaternary lidocaine, the sodium channel gates, and tetrodotoxin. Biophys J 27:39–56, 1979
10. Adams HV, Blair MR, Takman BH: The local anesthetic activity of tetrodotoxin alone and in combination with vasoconstrictors and local anesthetics. Anesth Analg (Cleve) 55:568–573, 1976
11. Scurlock JE, Curtis BM: The clinical potential of tetrodotoxin. ANESTHESIOLOGY 51:S232, 1979
12. Courtney RK: Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. J Pharmacol Exp Ther 195:225–236, 1975
13. Sheratt RM, Bostock H, and Sears TA: Effects of 4-aminopyridine on normal and demyelinated mammalian nerve fibers. Nature 283:570–572, 1980
14. Chiu ST, Ritchie JM: Potassium channels in nodal and internodal axonal membrane of mammalian myelinated fibers. Nature 284:170–171, 1980
15. Kocsis JD, Waxman SG: Absence of potassium conductance in central myelinated axons. Nature 287:348–349, 1980
16. Scurlock JE, Meymaris E, and Gregus J: The clinical character of local anesthetics: a function of frequency-dependent conduction block. Acta Anaesthesiol Scand 22: 601–608, 1978