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Tetraethylammonium Derivatives:

Ultralong-acting Local Anesthetics?

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Derivatives of tetraethylammonium ion (TEA^+) were synthesized in which one ethyl group was replaced by a C_6, C_8, C_{10}, C_{12}, C_{14}, or C_{16} side chain. These TEA^+ derivatives were tested for duration of sensory block of the rat infraorbital (trigeminal) nerve. The duration of sensory anesthesia increased exponentially from 1.2 hours to 388 hours as chain length increased from C_2–C_{12}, while C_{12}, C_{14}, and C_{16} all produced a similar reversible block of 17–20 days. The block duration of C_{12} was correlated with C_{12} bound to the infraorbital nerve; C_{12} not bound by the nerve was quantitatively excreted by the kidneys. These data, along with the lack of observable microscopic toxicity, suggests that TEA^+ derivatives may be a useful new class of ultralong-acting local anesthetics. (Key words: Anesthetics, local: tetraethylammonium derivatives.)

ARMSTRONG[1] (1975) has demonstrated that derivatives of the tetraethylammonium ion (TEA^+) bind, as does TEA^+, in a one-to-one fashion to K^+ channels and block the delayed outward (voltage dependent) K^+ current of the action potential in squid axon without affecting the resting membrane potential.

These compounds also block Na^+ currents[7] by an undefined interaction with the Na^+ channel; either of these effects could block repeated action potential generation and produce local anesthesia. As the binding energy to one of these sites, the K^+ channel, was strongly related to the length of an alkane chain attached to the TEA^+ molecule, we decided to synthesize a series of TEA^+ derivatives in which one of the ethyl groups of TEA^+ was replaced by a larger alkane (fig. 1), and then test these compounds as possible local anesthetics on the rat infraorbital nerve preparation.[2]

Materials and Methods

Tetraethylammonium was obtained from Aldrich Chemical Co. The TEA^+ derivatives C_4, C_6, C_8, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, and C_{16} were synthesized as follows: a 1:1 molar ratio of triethylamine (Sigma Chemical Co.) and the appropriate N-bromoalkane (Aldrich, Sigma, and Eastman Organic Chemicals) were refluxed at 60° C in acetone for 2 hours and excess acetone was distilled off. The quaternary ammonium salt was crystallized out of the remaining acetone by cooling to 0° C, the product collected on filter paper, and washed with petroleum ether (50–110° C) to remove excess reactants. Yield was generally 10 per cent. After drying to remove the petroleum ether, the crystals were dissolved in water to make appropriate solutions for experiments.

Purity was tested by thin layer chromatography on silica gel (Merck). Fifty μg was spotted and chromatographed in chloroform:methanol:ammonium hydroxide (30:15:1, volume/volume). Spots were visualized using 18 N H_2SO_4 and oxidation at 300° C. Analysis indicated that 90 per cent of the organic material was concentrated in a single component. C_{12} was also synthesized by Biosearch, Inc. (San Rafael, Ca.), and they confirmed the purity of C_{12} synthesized in our laboratory by thin layer chromatographic methods.

Male Sprague-Dawley rats were obtained from a local animal supply house at 300–400 g body weight, and fed Purina® rat chow and tap water *ad libitum.* After light ether anesthesia, the rats were injected with sodium pentobarbital, 10 mg, ip, and given additional pentobarbital, if necessary, until the animal lost righting reflex and allowed injection of the TEA^+ derivatives. Each compound (1.0–5.0 mg), in 0.2 ml of normal saline, was injected onto the trigeminal nerve through a 30-gauge needle guided to the nerve by a metal jig positioned on the roof of the mouth by the incisors and molars.[2] The contralateral trigeminal nerve was used as a control throughout the experiment. Anesthesia was tested by stimulating the lip/whisker area innervated by the maxillary nerve on experimental and control sides and observing whether the stimulus produced a reflex contraction of abdominal muscles (unanesthetized) or not (anesthetized). Stimulation consisted of 1–10V, 6-ms square wave pulse trains (2 pulses/s for 5 s) produced by a Grass S48 stimulator triggering a stimulus isolation unit (DS-2, Digitimer Ltd®.), and were delivered through bipolar electrodes (two 30-gauge needles, 2 mm apart). The muscle twitch response was quantitated by recording the EMG from muscle of the abdominal wall, and displaying it on a Tektronix® 5111 oscilloscope after amplification (Tektronix® AM502 differential

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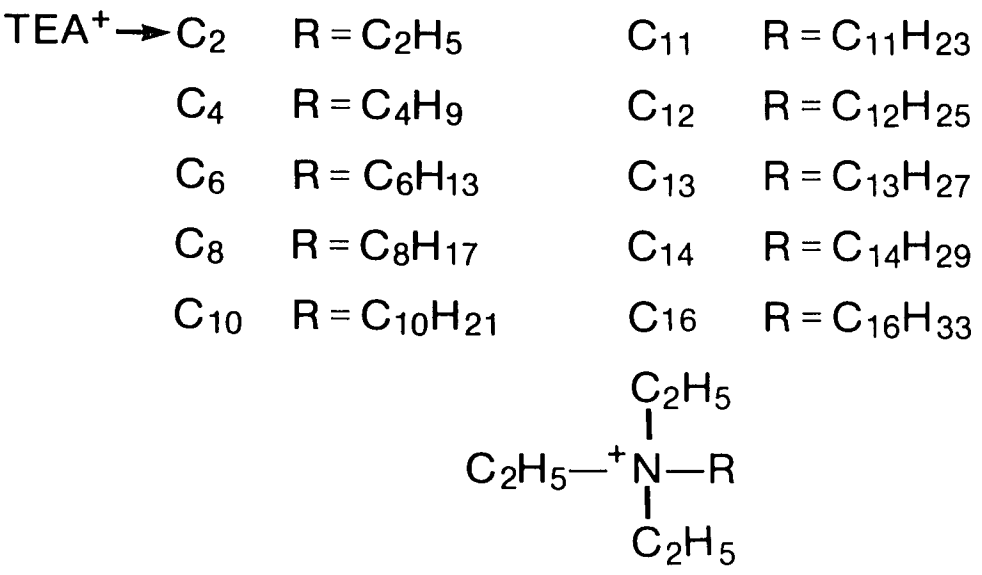

FIG. 1. Structure of TEA^+ derivatives. TEA^+ derivatives were synthesized as described in the text. Their length calculated from bond length addition is as follows: C_2 6.6 Å; C_6 11.6 Å; C_8 141. Å; C_{10} 16.5 Å; C_{12} 19.0 Å; C_{14} 21.5 Å; C_{16} 24.0 Å.

amplifier). Comparison of the EMG response produced by stimulating the control and treated side allowed an accurate determination of the presence or absence of trigeminal nerve block. For C_2 through C_{10}, the duration of block was measured from 100 per cent block of the EMG response of the treated side to 100 per cent recovery of EMG response compared to control. For C_{12}, C_{14}, and C_{16}, the gradual loss of block made us choose 80 per cent recovery of the EMG response as the endpoint for analgesia as the animals were sensitive to the painful stimulus at this time, and 100 per cent recovery of the EMG required additional days.

Dose-response curves were estimated by injecting 0.5 mg of the TEA^+ derivatives in 0.1 ml, and then injecting another 0.5 mg every 10 min until a complete block was obtained. The dose-response curves were universally very steep with minimal effect seen below the blocking dose, and no incremental increase in block duration above the blocking dose (see Discussion). The blocking dose determined using sequential injection was verified by injecting a 1–5 mg bolus of TEA^+ derivative in 0.2 ml. There was good agreement between these two techniques.

TABLE 1. Sensory Analgesia by Quaternary Ammonium Compounds C_2–C_{16}

Compound	No. of Animals	Dose (mg)	Onset of Block* (min)	Duration of Block* (hours)
C_2	4	5.0	19 ± 2	1.2 ± 0.6
C_6	4	2.5	18 ± 6	6 ± 1
C_8	4	1.7	10 ± 5	21 ± 2
C_{10}	4	2.0	6 ± 3	80 ± 8
C_{12}	5	1.0	3 ± 2	388 ± 96
C_{14}	4	1.0	11 ± 6	402 ± 72
C_{16}	4	2.3	24 ± 7	480 ± 96

* Mean ± SD.

Carbon-14 labeled C_{12} (Ethyl-1-^{14}C, triethyldodecyl ammonium bromide) was synthesized by New England Nuclear. To determine the binding of C_{12} to the infraorbital nerve, and its mode of excretion, rats were injected with a 1-mg dose of C_{12} containing 0.5 μCi ^{14}C, and placed in an animal metabolism unit. Feces and urine were collected daily. Feces were dissolved in Soluene 350 at 50° C, and after decolorization with hydrogen peroxide and isopropyl alcohol, were counted in Dimilume® 30 liquid scintillation cocktail (Packard Instruments); urine was added directly to cocktail. At the end of the binding experiments, rats were sacrificed and the infraorbital nerve dissected free, washed, divided into five 4-mm sections, and dissolved in Soluene prior to counting in Dimilume on a Packard® PRIAS liquid scintillation counter.

Electron microscopy was performed by the pathology department of the Virginia Mason Hospital. The infraorbital nerve was dissected from the treated animals 7–10 days after injection and fixed in Karnovsky's fixative (1 per cent paraformaldehyde, 1 per cent gluteraldehyde in 0.1 M Na-cacodylate buffer, *p*H 7.4) overnight, post-fixed in osmium, dehydrated in alcohol, and embedded in Epon prior to sectioning.

Electron micrographs of 20–30 myelinated and unmylelinated axons from three C_{12} treated and three control infraorbital nerves were evaluated in a blind fashion by the investigators for axonal swelling, myelin ultrastructure, and general integrity on a 1–5 scale. After evaluation, scores for treated and control infraorbital nerves were compared.

Results

As the length of the carbon side chain was increased from C_2 to C_{12}, the duration of sensory analgesia increased exponentially, and thereafter C_{12}–C_{16} remained relatively constant (table 1 and fig. 2). The curve shows a major change in the action of these compounds, as blocking duration increased from hours to days, with C_{10} being an intermediary point. In addition, while the blocking duration for C_2–C_{10} had a distinct end point, C_{12}, C_{14}, and C_{16} showed a slow decay of the sensory block over a period of several days.

As the duration of block increased from C_2 to C_{12}, the minimal dose for anesthesia decreased, as did the onset time of nerve block. From C_{12}–C_{16}, the onset time for block increased substantially and the blocking dose for C_{16} also showed an increase.

The majority of C_{12} injected onto the infraorbital nerve was transported into the systemic circulation and excreted by the kidneys; 70 per cent of the drug appeared in the urine within 24 hours after injection (fig. 3). Some C_{12} (29.8 per cent) was excreted along with the feces 2–3 days after injection. The remaining C_{12} (0.2 per cent) remained bound to the infraorbital nerve, and had a half-life of approximately 18 days *in vivo* (fig. 4). The gradual loss of sensory block between 17 and 20 days correlates well with the loss of C_{12} bound to the infraorbital nerve during the period. Infraorbital nerves taken from the side contralateral to the injection site did not contain bound C_{12} at any time after injection.

Analysis of electron micrographs of C_{12} treated and control infraorbital nerves revealed no significant effect of C_{12} on axonal ultrastructure. Generalized appearance, myelin sheath integrity, and microtubule number appeared identical for the two groups.

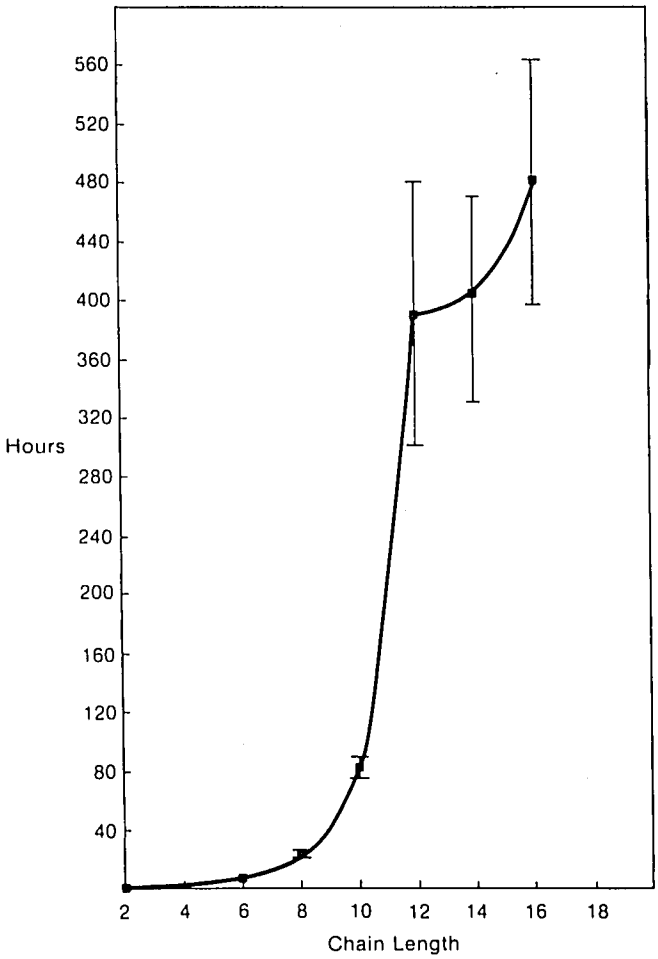

FIG. 2. Blocking duration of TEA-derivatives compounds C_2–C_{16}. The duration of sensory analgesia was determined by evaluating the response to electrical shock to the area innervated by the infraorbital nerve after injection of C_2–C_{16}. Animals were monitored after injection until the control and experimental sides were identical (C_2–C_{10}) or until 80 per cent of the block had worn off (C_{12}, C_{14}, and C_{16}).

Discussion

Hundreds of local anesthetics have been synthesized and tested over the past century. Only a few, however, have proven to be clinically useful. Most of the drugs tested conformed to Lofgren's scheme.[3] This scheme consists of an intermediate chain separating a lipophilic end from a hydrophobic end. The intermediate chain is typically 6–9 Å in length, and contains a carbonyl group. The lipophilic end is an aromatic residue, and the hydrophilic end is an amino group. These components have appeared to be essential to a clinically useful local anesthetic. The compounds tested here have only two of these characteristics and therefore may indicate that the "essentials" can possibly be reduced even further. These compounds have an amino group (hydrophilic) and a carbon chain (lipophilic group). There is no carbonyl group, no aromatic group, and the amino group is an ethylated quaternary rather than the usual tertiary amine which has a pK_a in the physiologic range. But the remaining similarities seem to be enough to produce a potent family of local anesthetics. There is still a hydrophilic end, a lipophilic end, and a comparable molecular weight and length. The useful range of lengths for conventional local anesthetics runs from about 13 Å (lidocaine) to about 18 Å (tetracaine). The same is true for these quaternary compounds. The useful range as far as potency, penetrance, and block duration appears to be in the range from C_8 (14.1 Å) to C_{12} (17.8 Å). Also, like conventional local anesthetics, the potency and block duration increase with increasing compound lengths.

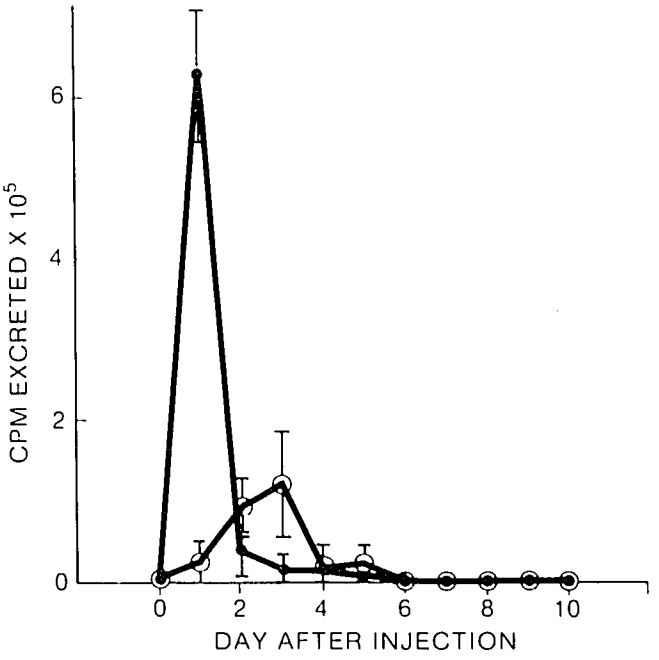

FIG. 3. Excretion of ^{14}C-triethyldodecyl ammonium bromide (C_{12}). The total CPM excreted in the urine (—●—) and the total CPM excreted in the feces (—○—) of rats injected with 0.5 μCi (1×10^6 CPM) of ^{14}C–C_{12} was determined daily. Each point is the mean of three determinations.

Quaternary derivatives of conventional local anesthetics, such as QX314 (lidocaine with three ethyls on the amino group), act at the same site on the axon as lidocaine, with approximately the same potency and specificity for the sodium channel.[4] They appear to be effective as a local anesthetic when applied to the exterior of the axon, provided time is allowed for the diffusion of the charged molecule through the membrane to the internal Na^+ channel site. This transport may be accomplished by proposed phospholipid mediated transport systems.[5] TEA^+ derivatives have a Na^+ channel blocking component that changes with the length of the attached side chain. TEA^+ (C_2) gives very little sodium channel block,[6] while C_9 blocks 25 per cent of Na^+ channels when 50 per cent of the K^+ channels are blocked,[7] and TEA^+ derivatives may accomplish this Na^+ channel block by interaction at the same site accessible to external QX314. However, external block by the larger TEA^+ derivatives (greater than C_9) may be due to a nonspecific detergent effect rather than interaction with Na^+ channels or K^+ channels.[7]

Thus, there are at least three possible sites for the local anesthetic action of TEA^+ derivatives. They may bind to K^+ channels and slow axon repolarization, block Na^+ channels at the site of action of tertiary local anesthetics, or perturb the function of either channel or other membrane processes by a nonspecific detergent interaction. The plausibility of these mechanisms is evaluated by Curtis and Scurlock.[8] But regardless of the site of action proposed, no anesthetic agent and few drugs bind to their site of action for more than a period of hours, and it was suspected that these TEA^+ derivatives might not be acting as local anesthetics, but as neurolytic agents, and that the three-week blocks produced by C_{12} to C_{16} were the result of recovery of function after neural damage. However, our analysis of electron micrographs indicated no obvious ultrastructural damage, and more importantly the loss of sensory analgesia was well correlated with the level of C_{12} bound to the infraorbital nerve. Thus, the presence of the TEA^+ derivative on the axon is correlated with analgesia and this makes it unlikely that we are observing recovery from an initial neurolytic trauma.

The 18-day half-life of C_{12} on the axon also correlates well with the turnover of various nervous system proteins (which have a half-life of approximately

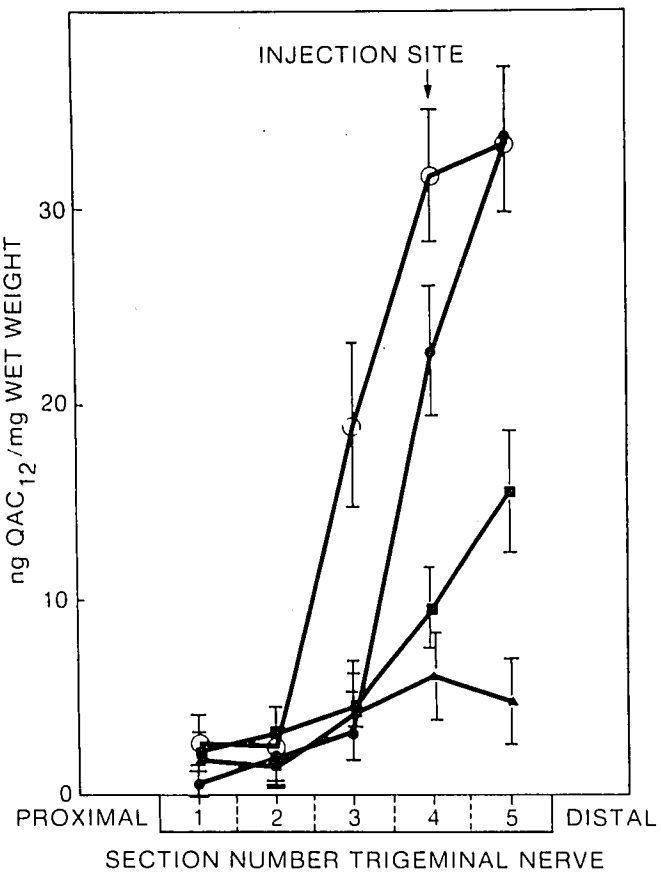

FIG. 4. Turnover of ^{14}C-triethyldodecyl ammonium bromide (C_{12}) in the rat intraorbital nerve *in vivo*. Rats treated with ^{14}C–C_{12} were sacrificed 3 days (—○—), 8 days (—●—), 18 days (—■—), and 25 days (—▲—) postinjection and the bound C_{12} quantitated in order to determine its half-life in the axon. Each curve was constructed with data from three animals.

20 days[9,10]). Thus one could postulate that one-half of the Na^+ and K^+ channels will turnover in about 20 days. If C_{12} is bound irreversibly to some components of the channels after about 20 days, one-half of the blocked channels would be replaced, and one would see a very slow return of axonal conduction and sensation, as we have observed.

Lipids also turnover in the axonal membrane, and as it appears that lipid-protein membrane units are synthesized and inserted into the membrane rather than individual components, it is impossible to differentiate between C_{12} that is turned over with a lipid *vs.* protein component. It is clear, however, that the binding energy of TEA^+ derivatives to some "receptor" is a function of the length of their side chain and that side chains greater than twelve carbons in length result in a binding duration that can best be accounted for by intergal association and turnover with membrane constituents rather than a reversible binding interaction which may be present in the shorter acting C_2 to C_{10} derivatives. The increase in onset of block time and blocking dose from C_{12} to C_{16} (table 1) suggests that C_{12} is the optimal size for these receptors hydrophobic binding region.

In addition to ultralong action, the permanent charge of these compounds provides them with some interesting and clinically useful properties. The charge should hinder them from crossing the placental or blood-brain barrier, thus dramatically improving the therapeutic to toxic ratio. This also accounts for their quick and quantitative excretion by the kidneys, and minimizes the chances for cumulative toxicity or enzymatic breakdown. Contrarily, the charge makes it more difficult to cross connective tissue barriers to reach *in vivo* axons, and 30 times the concentration needed for *in vitro* block of desheathed nerves[8] was necessary *in vivo*. Furthermore, Armstrong[1] found concentrations of C_{10} one thousand times less than we used effective when applied intraaxonally, and thus the lack of dose-response to external TEA^+ derivatives may simply reflect the transport barriers between the injection site and receptor site, *i.e.*, a certain minimal dose of TEA^+ derivative is necessary to facilitate transport and above this concentration the transport system saturates or achieves a steady state.

In conclusion, we have synthesized a series of compounds based on the addition of a long hydrocarbon tail to the tetraethylammonium ion. These TEA^+ derivatives produce local anesthesia of 6 hours to 20 days simply by changing the length of the alkane side chain. These compounds do not appear to act as neurolytic agents but block axonal conduction by interfering in some fashion with the function of the axonal membrane. As it appears that these compounds have little apparent neurotoxicity, we feel they may represent a new generation of local anesthetics that may be particularly useful in the management of chronic pain, and long-term postoperative analgesia.

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