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Local Anesthetics Depress the Calcium Current of Rat Sensory Neurons in Culture

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Background: **Local anesthetics are known to inhibit the voltage-gated sodium current (I_{Na}) of the nerve membrane, but it has not been fully studied whether anesthetic concentrations of local anesthetics depress the voltage-gated calcium current (I_{Ca}) of mammalian neurons. The effects of local anesthetics on I_{Ca} evoked in cultured rat dorsal root ganglion cells were studied.**

Methods: **Whole cell patch clamp recordings were made from rat dorsal root ganglion cells cultured for 1–3 weeks. I_{Ca} was recorded using patch electrodes filled with Cs-aspartate in Na^+-free external solution containing 5 mM-Ba^{2+}. All drugs, including local anesthetics, were applied by miniperfusion from micropipettes by pressure ejection.**

Results: **Tetracaine (300 μM) depressed the peak amplitudes of high voltage-activated (HVA)-I_{Ca} to 22.6 ± 8.8% of control values (n = 14) without affecting the current-voltage relation. A tetracaine dose-response curve for HVA-I_{Ca} indicated an apparent dissociation constant of 79.5 μM. Tetracaine (30 μM) depressed nicardipine-sensitive HVA-I_{Ca} (L-type) to 14.3 ± 6.7% (n = 6), ω-conotoxin-sensitive HVA-I_{Ca} (N-type) to 81.6 ± 9.6% (n = 7), and low voltage-activated (LVA)-I_{Ca} (T-type) to 65.1 ± 11.1% (n = 6) of their respective controls. Local anesthetics other than tetracaine also depressed HVA-I_{Ca} but were of different potency; the rank sequence was dibucaine > tetracaine > bupivacaine ≫ procaine = lidocaine.**

Conclusions: **These results suggest that both HVA-I_{Ca} and LVA-I_{Ca} are depressed by tetracaine used at the concentrations required for spinal anesthesia and that the L-type Ca^{2+} channel among Ca^{2+} channel subtypes is the most susceptible to tetracaine. A good correlation between local anesthetic potencies to inhibit HVA-I_{Ca} and their anesthetic potencies implies that the inhibition of calcium influx through voltage-gated channels may contribute to spinal anesthetic mechanisms. (Key words: Anesthetic techniques: spinal. Anesthetics: local. Ions: calcium. Measurement techniques: whole cell patch clamp recording. Spinal cord: dorsal root ganglion.)**

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IT is well accepted that local anesthetics inhibit voltage-dependent sodium-current (I_{Na}) of nerve membrane and, as a result, block nerve impulses responsible for conducting sensory information such as pain or tactile sensation.[1,2] However, the depressant action of local anesthetics is not selectively exerted on I_{Na}. Several studies have shown that some local anesthetics also depress the voltage-dependent calcium-current (I_{Ca}) evoked in a number of tissues. For instance, lidocaine at a clinically relevant concentration of 100 μM inhibits I_{Ca} amplitude by 15% in Helix ganglionic neurons,[3] by 35% in frog dorsal root ganglionic cells (DRGs),[4] and by about 60% elicited in chick ventricular myocytes.[5] Tetracaine depresses slowly inactivating I_{Ca} evoked in guinea-pig cardiac myocytes with an apparent dissociation constant (K_d) of 80 μM,[6] which is within the range of cerebrospinal fluid (CSF) concentrations observed during spinal anesthesia. HS37, a derivative of bupivacaine, inhibits I_{Ca} evoked in frog DRGs with K_d of 30 μM, a concentration equivalent to that required to block I_{Na}.[7] Thus, it is likely that local anesthetics used in clinical concentrations may depress I_{Ca} elicited in mammalian neurons. However, this possibility has not been tested yet.

Three subtypes of Ca^{2+} channels, designated as T-type, N-type, and L-type, have been identified in chick DRGs: the low voltage-activated (LVA) I_{Ca}, T-type I_{Ca}, is activated by depolarizations more positive than −50 to −40 mV but is rapidly inactivated during sustained depolarization.[8,9] The high voltage-activated (HVA) I_{Ca} is activated by depolarizations more positive than −30 to −20 mV and inactivates at a slower rate than the LVA-I_{Ca}. The HVA-I_{Ca} is classified according to its pharmacologic properties: the L-type I_{Ca} is blocked by dihydropyridine (DHP) receptor antagonists such as nifedipine and nicardipine, whereas the N-type I_{Ca} is strongly inhibited by ω-conotoxin fraction GVIA (ω-CgTx). The existence of all the types of Ca^{2+} channels is demonstrated in rat DRGs.[10–13] Furthermore, a recent study suggests the existence of HVA-I_{Ca} insensitive to both DHP receptor antagonists and ω-CgTx in rat

Table 1. Ionic Composition of Internal and External Solution for Recording I_{Na} and I_{Ca} (mM)

	Internal Solution						
	CsCl	Cs-asp	TEA-Cl	$MgCl_2$	EGTA	HEPES	Na_2-ATP
I_{Na}	130	0	10	2	1.1	10	0
I_{Ca}	10	120	10	2	1.1	10	5
	External Solution						
	NaCl	Tris-Cl	TEA-Cl	KCl	$BaCl_2$	$MgCl_2$	HEPES	Glucose
I_{Na}	50	80	20	4.5	0	8	10	10
I_{Ca}	0	125	20	4.5	5	1	10	10

DRGs.[10] Differential effects of barbiturates[14,15] and dynorphin A,[16] an endogenous κ-opioid agonist, and nonselective depressant effects of volatile anesthetics[17] on different types of Ca^{2+} channel have been reported, but there are few studies to determine sensitivities of the Ca^{2+} channel subtypes to local anesthetics. The only available data show that DHP-sensitive HVA-I_{Ca} in skeletal muscle is more susceptible than LVA-I_{Ca} to tetracaine.[18]

The current study investigated the effects of tetracaine and other local anesthetics on I_{Ca} in cultured rat DRGs using the whole cell patch clamp technique. We show that tetracaine, when used in the concentrations required for spinal anesthesia, depresses both LVA- and HVA-I_{Ca} to below 50% of the respective controls. We also show that L-type I_{Ca}, among the four Ca^{2+} channel subtypes, is the most susceptible to tetracaine and that several local anesthetics inhibit HVA-I_{Ca} with different potencies. The rank order of inhibitory potency is dibucaine > tetracaine > bupivacaine ≫ procaine = lidocaine.

Materials and Methods

Tissue Culture

Animal use in this study was approved by the Animal Care Committee of the Kurume University School of Medicine. The methods for preparing cultures of DRG neurons were similar to those previously described.[19] Briefly, 1- to 3-day-old Sprague-Dawley rat pups were decapitated under halothane anesthesia, and their dorsal root ganglia were rapidly removed and incubated at 37°C for 15 min in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline containing 0.05% collagenase (Sigma type 1) and 0.125% trypsin (GIBCO). The cells were then dissociated by trituration and plated onto 35-mm culture dishes. Cultures were maintained in a humidified incubator at 37°C with a 5% CO_2/95% air gas mixture, in Eagle's minimum essential medium supplemented with 5% horse serum (GIBCO), 30 ng nerve growth factor/ml, and some nutrient factors. After 4 or 5 days in culture, a mixture of 5-fluoro-2-deoxyuridine (15 μg/ml, Sigma) and uridine (35 μg/ml, Sigma) was added to the cultures for 2 days to suppress growth of nonneuronal cells, and, subsequently, the culture medium was changed weekly. Experiments were performed 1–3 weeks after plating.

Whole Cell Patch Recording

Electrophysiologic recordings were made on the stage of an inverted phase contrast microscope (Nikon) at room temperature (23–25°C). Basic techniques used for establishing tight-seal whole cell patch recordings were essentially the same as described previously.[19] Patch pipettes were sealed against the cell membrane by suction. The membrane patch was disrupted by further suction, after the tight-seal (>3 GΩ) between the patch pipette and the cell membrane was established. The neurons were then voltage-clamped using a single electrode voltage-clamp amplifier CEZ-3100 (Nihonkoden) in a discontinuous mode. The switching frequencies between passing current and sampling voltage were 10–12 KHz for recording I_{Ca} and 25–30 KHz for recording I_{Na}. Patch pipettes had a tip resistance of 2–4 MΩ for recording I_{Na} and 4–10 MΩ for I_{Ca}, when filled with their respective internal solutions (table 1). Ionic currents were digitized at a sampling rate of 20 KHz for I_{Na} and 1 KHz for I_{Ca} (512 samples for each current trace), using a PC-9801VM computer (NEC) with a computer-based program (DSS98-SV, Canopus). Digitized data were stored on a hard disk for further analysis and were plotted by a pen recorder (MP4400, Graphtec) for illustration. We did not digitally subtract leak

and capacitive current components from all records. However, leak currents were measured in 14 cells by a 10–20-mV hyperpolarizing voltage step from a holding potential of −80 mV. Because Ca^{2+} current was usually maximum at +10 mV and effects of drugs applied were tested at +10 mV in most experiments, leak current amplitudes at +10 mV were estimated by assuming a linear current-voltage relationship. The estimated leak current of the total inward current was 6.9 ± 2.2%.

The compositions of internal and external solutions used in the current study are listed in table 1. Ba^{2+} ions replaced Ca^{2+} in the medium when recording HVA-I_{Ca} because HVA-I_{Ca} runs down during a 15- to 20-min whole cell patch clamp in external solution containing Ca^{2+} ions. LVA-I_{Ca} (T-type I_{Ca}) was activated in a Ca^{2+}-medium because this current does not run down in this medium and because its amplitude is reduced by about 20% when Ca^{2+} ions in the external solution are replaced by equimolar concentrations of Ba^{2+} ions.[12] The *p*H of internal solutions was titrated to 7.2 with potassium hydroxide (KOH), and the osmolarity was adjusted to 310 mOsm with sucrose. The *p*H of external solutions was titrated to 7.35 with HCl, and the osmolarity was adjusted to 325 mOsm with sucrose.

Nicardipine was dissolved in dimethyl sulfoxide and diluted 1:2,000 in the external solution. Other drugs, including local anesthetics, were dissolved in distilled water as concentrated stocks and diluted (1:100 or more) in the external solutions for use in experiments. Micropipettes filled with test solutions containing a given concentration of drugs were positioned approximately 30 μM from the cell soma, and then drugs were delivered to the recording cell by pressure ejection at 7–10 psi. Our preliminary study showed that recovery of I_{Ca} amplitude usually remained 70% or less of the control when tetracaine (300 μM) was applied for longer than 60 s. Therefore, the application time of all local anesthetics tested in the current study were limited 60 s or less. Cells with the recovery amplitude less than 80% of the control were omitted from the current results.

A previous clinical study showed that the average concentration of tetracaine in CSF during spinal anesthesia was 12 mg/100 ml 5 min after the injection and was reduced to 1.5 mg/100 ml over the following 60 min.[20] Clinical concentrations of tetracaine, therefore, are in the range of 50–400 μM.

All quantitative values were expressed as mean ± SD. Paired Student's *t* test was used to evaluate the significance of differences between current amplitudes before and during a given concentration of drugs. Mann-Whitney-U test was used for evaluating the significance of the sensitivity differences of the Ca^{2+} channel subtypes to tetracaine. $P < 0.05$ was regarded as statistically significant. The number of neurons tested and *P* values are given in parentheses.

Results

Clinical Concentrations of Tetracaine Depress Ca^{2+} Current (I_{Ca})

An attempt was made initially to examine whether tetracaine can depress I_{Ca} when used at concentrations equivalent to those observed in the CSF during spinal anesthesia. Figure 1 shows effects of tetracaine (300 μM) on I_{Na} and I_{Ca} recorded under the respective ionic conditions (see methods). The neurons were voltage-clamped at V_H of −80 mV, and depolarizing voltage steps of 10 ms for I_{Na} and 200 ms for I_{Ca} were applied at 15-s intervals. Voltage steps, which were chosen to elicit the maximum peak amplitude, were −20 mV for I_{Na} and +10 mV for I_{Ca}. After stabilization of the current amplitudes, tetracaine (300 μM) was continuously applied to the neurons for 45 s by pressure ejection. Tetracaine abolished I_{Na} and also reduced the peak amplitude of I_{Ca} to approximately 20% of the control. The depressant action of tetracaine on I_{Na} and I_{Ca} was reversible, and the two currents recovered almost fully within a few minutes after removal of tetracaine.

Application of tetracaine (300 μM) for 45–60 s resulted in a reduction of the peak amplitude of I_{Ca} evoked at +10 mV to an average of 22.6 ± 8.8% of the control values (n = 14, $P < 0.00002$). The I_{Na} recorded at −20 to −10 mV was completely suppressed by the same concentration of tetracaine (n = 4). The I_{Na} was also abolished in the medium containing tetrodotoxin (TTX 1 μM) in all neurons tested (n = 11, data not shown). These results suggest that the I_{Ca}, like the TTX-sensitive I_{Na}, is markedly depressed by tetracaine at the concentration required for spinal anesthesia.

To determine whether tetracaine depresses the I_{Ca} by shifting the voltage dependence of activation of the current, current-voltage plots were constructed (fig. 2). Although the I_{Ca} with different amplitudes and time courses could be activated in response to depolarizing voltage steps more positive than −20 mV from V_H of −80 mV, the activated I_{Ca} could be contaminated by LVA-I_{Ca} (see below). Tetracaine (300 μM) substantially

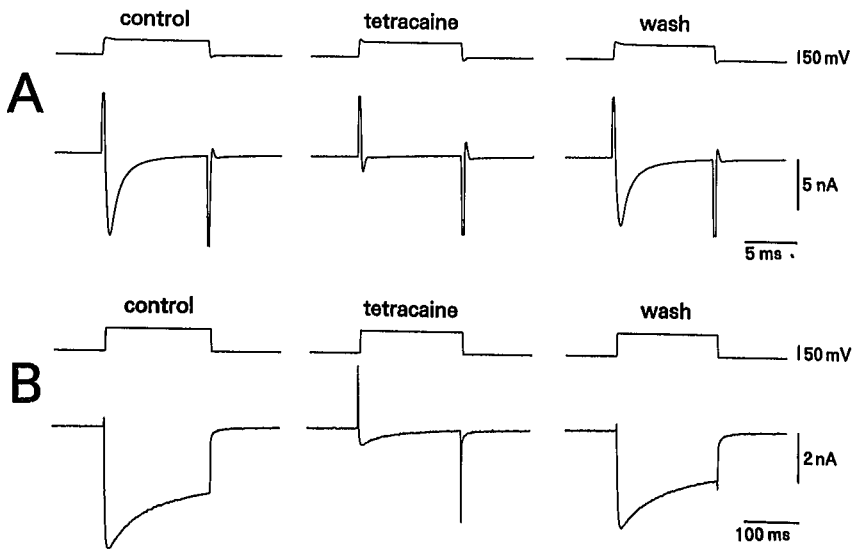

Fig. 1. Tetracaine strongly depresses calcium-current (I_{Ca}) as well as sodium-current (I_{Na}) of cultured rat dorsal root ganglion cell (DRG) neurons. Records displayed were obtained from two neurons (A and B), and each set shows the membrane potential (upper trace) and the clamp current (lower trace). In this and subsequent figures, the voltage protocol to elicit the I_{Na} or the I_{Ca} was the same unless otherwise described, and there was no correction for leak and capacitive currents. (*A*) Effect of tetracaine on the I_{Na}. The I_{Na} was evoked by depolarizing voltage steps of 10 ms to −20 mV from V_H of −80 mV. Capacity transients are seen at the make (upward deflection) and the break of pulses (downward deflection). Tetracaine (300 μM) applied for 45 s by pressure ejection blocked the I_{Na}. The trace denoted as wash was obtained 4 min after removal of tetracaine. (*B*) The I_{Ca} evoked by depolarizing voltage steps of 200 ms to +10 mV from V_H of −80 mV. Tetracaine (300 μM) applied for 45 s depressed the peak amplitude of the I_{Ca} to 20% of control. The trace denoted "wash" was obtained 1.5 min after removal of tetracaine.

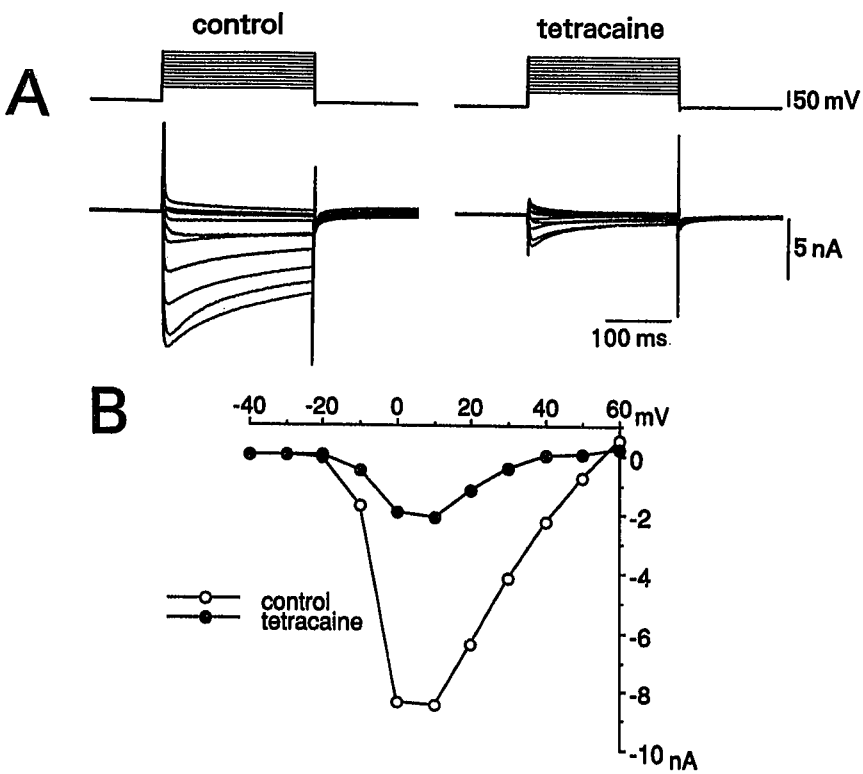

Fig. 2. Tetracaine inhibits I_{Ca} without affecting the voltage-current relationship. (*A*) Superimposed current traces are families of I_{Ca} recorded before (left) and during (right) applying tetracaine (300 μM). The V_H was −80 mV, and 200-ms steps were made in +10-mV increments from −40 to +60 mV at 15-s intervals. To reduce a cumulative depressant effect of tetracaine by a prolonged application (see text), the application of tetracaine was interrupted for a few seconds after each depolarizing voltage step. (*B*) Current-voltage plots for the I_{Ca} in the absence (open circle) and presence (filled circle) of tetracaine (300 μM). The peak amplitude of the I_{Ca} measured at each step potential is plotted as a function of membrane potential ranging from −40 to +60 mV. Leak current is included in the measurement of the peak current. Tetracaine inhibits the I_{Ca} with negligible effect on voltage-current relationships.

depressed I_{Ca} at all membrane potentials (fig. 2A). When the peak amplitude of I_{Ca} in the absence (open circle) and the presence (filled circle) of tetracaine was plotted against step potential as shown in figure 2B, the result showed that tetracaine reduced the peak current amplitude with little noticeable effect on I-V relation of HVA-I_{Ca}.

To compare the potency of tetracaine to depress the peak amplitude of HVA-I_{Ca} with that of TTX-sensitive I_{Na}, dose-response data were obtained (fig. 3). Both ionic currents were depressed by tetracaine in a concentration-dependent fashion, and the data points were fitted by the following equation:

$$I_T/I_C = K_d''/([\text{tetra}]'' + K_d''),$$

where I_C and I_T are the peak current amplitude before and during given concentrations of tetracaine, respectively; [tetra] is the concentration of tetracaine applied; K_d is the concentration of tetracaine that blocks 50% of the control response (I_C); and n is the slope factor describing the steepness of the curve. The dose-response curves thus obtained had the n and K_d values of 1.2 and 37.5 μM for I_{Na} and 1.0 and 79.5 μM for HVA-I_{Ca}, respectively.

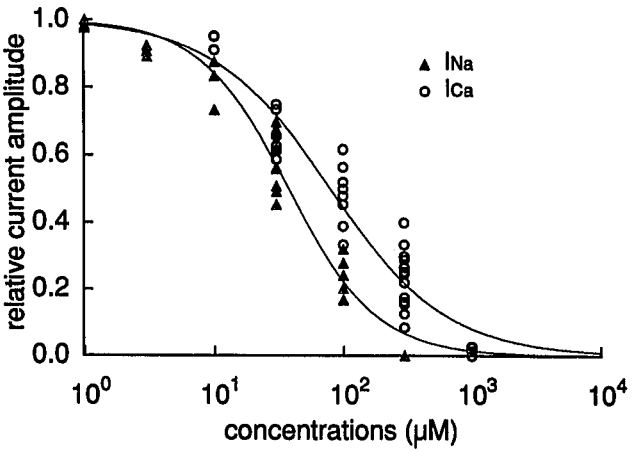

Fig. 3. Concentration-dependent depressant effect of tetracaine on I_{Na} and I_{Ca}. Current amplitude of either the I_{Na} or the I_{Ca} measured in the presence of tetracaine is normalized to the respective control amplitude in the absence of tetracaine, and thus normalized current amplitudes of the I_{Na} (filled triangle) or the I_{Ca} (open circle) are plotted as a function of tetracaine concentration. Dose-response curves for both the I_{Na} and I_{Ca} are fitted with the single binding-site model described in the text. The apparent dissociation constant and the slope factor, respectively, were 37.5 μM and 1.2 for the I_{Na} and 79.5 μM and 1.0 for the I_{Ca}.

Effect of Tetracaine on Different Types of Calcium Currents

We next examined the four types of I_{Ca} to determine their sensitivity to tetracaine. Depolarizing voltage steps of −30 to −40 mV from V_H of −80 mV or more negative elicited small transient I_{Ca} in 16% (14 of 86) of neurons tested. The transient I_{Ca} had the peak amplitude of 100–400 pA and usually was inactivated within 100 ms. The transient I_{Ca} was abolished by shifting V_H to more positive than −60 mV, while it slightly increased in amplitude by shifting V_H to more negative than −80 mV (up to −100 mV). These characteristics of the transient I_{Ca} are consistent with those of LVA-I_{Ca} (T-type I_{Ca}) previously reported in mouse or rat DRGs in culture.[11,12] Therefore, the transient I_{Ca} was identified as the LVA-I_{Ca}. Figure 4 shows the effects of tetracaine on LVA-I_{Ca} and HVA-I_{Ca} evoked in the same neuron: the peak amplitude of the LVA-I_{Ca} was reduced to 68% of the control by 30 μM tetracaine and to 51% of the control by 100 μM tetracaine (fig. 4A). Thirty and 100 μM tetracaine also reduced the peak amplitude of the HVA-I_{Ca} to 66% and 43% of the respective control (fig. 4B). Table 2 summarizes the concentration-dependent depressant effect of tetracaine on the LVA-I_{Ca} and HVA-I_{Ca} obtained from three to 14 cells. These data suggest that tetracaine causes a similar degree of inhibition on both the HVA-I_{Ca} and LVA-I_{Ca}.

Regan *et al.* have shown that the HVA-I_{Ca} in rat DRGs consists of three components: DHP-sensitive L-type, ω-CgTx-sensitive N-type, and ω-CgTx-insensitive N-type.[10] The current study confirmed three components of HVA-I_{Ca}. The peak amplitude of HVA-I_{Ca} evoked at +10 mV from V_H of −80 mV was decreased by 26.6 ± 8.4% of the control by nicardipine (10 μM, n = 6, $P < 0.0005$), a DHP receptor antagonist, and by 53.9 ± 6.8% of the control by ω-CgTx (3 μM, n = 7, $P < 0.0003$). The combined application of nicardipine (10 μM) and ω-CgTx (3 μM) left 13.9 ± 8.5% of HVA-I_{Ca} unblocked (n = 11, $P < 0.000001$). Tetracaine applied at high concentrations (*e.g.*, 300 μM) blocked both the L-type and N-type I_{Ca}. However, when it was applied at relatively low concentrations (*e.g.*, 30 μM), a marked difference in sensitivity to tetracaine is evident between the L-type and N-type I_{Ca} ($P < 0.003$). Figure 5 shows a typical example. Tetracaine (30 μM) caused roughly a 30% reduction in the amplitude of HVA-I_{Ca} in the two neurons (Aa, Ba). The components of HVA-I_{Ca} inhibited by tetracaine were non-inactivating currents (Ac, Bc). After the HVA-I_{Ca} had recovered to its original amplitude by tetracaine wash, the neurons were exposed to either

nicardipine (10 μM, Ab) or ω-CgTx (3 μM, Bb), and then tetracaine was applied a second time. The HVA-I_{Ca} that remained in the presence of nicardipine was reduced only slightly by tetracaine (Ab). Thus, the current amplitude depressed by tetracaine in the presence of nicardipine (Ad) was much smaller than that of HVA-I_{Ca} in the absence of nicardipine (Ac). In contrast, the HVA-I_{Ca} insensitive to ω-CgTx was further depressed by tetracaine (Bb). The current amplitude depressed by tetracaine in the presence of ω-CgTx (Bd) was nearly identical to that in the absence of ω-CgTx (Bc).

Tetracaine (30 μM) decreased the amplitude of HVA-I_{Ca} evoked in a medium containing nicardipine (10 μM) to an average of 81.6 ± 9.6% of the control (n = 7, $P < 0.02$), whereas the same concentration of tetracaine reduced the amplitude of HVA-I_{Ca} evoked in a medium containing ω-CgTx (3 μM) to an average of 14.3 ± 6.7% of the control (n = 6, $P < 0.01$). In three other neurons, the amplitude of HVA-I_{Ca} insensitive to both Ca channel blockers was little affected by tetracaine (30 μM): the average amplitude of the HVA-I_{Ca} was 84.0 ± 6.7% of the control ($P < 0.1$). These results suggest that the L-type I_{Ca} is the most susceptible to tetracaine among the four Ca channel subtypes.

Effect of Other Local Anesthetics on HVA-I_{Ca}

The potency of other local anesthetics to depress HVA-I_{Ca} also was examined. Figure 6 illustrates typical examples of effects of dibucaine, bupivacaine, lidocaine, and procaine at the concentration of 300 μM on the HVA-I_{Ca} and dose-response curves for the inhibitory actions of five local anesthetics including tetracaine on the HVA-I_{Ca}. Dibucaine completely suppressed HVA-I_{Ca}, and bupivacaine decreased HVA-I_{Ca} to 45% of the control amplitude, whereas procaine and lidocaine reduced HVA-I_{Ca} amplitude by only 10–15% compared with those of their respective controls (fig. 6A). Each of the local anesthetics depressed the HVA-I_{Ca} in a concentration-dependent fashion, and the data points were fairly well fitted with curves estimated from the single binding-site model equation described above (fig. 6B). However, the potency to block the HVA-I_{Ca} was quite different among the local anesthetics: K_d was 34.5 μM for dibucaine, 79.5 μM for tetracaine, 156 μM for bu-

Table 2. Concentration Dependence of the Effect of Tetracaine on LVA-I_{Ca} and HVA-I_{Ca}

Concentration (μM)	LVA-I_{Ca} (n)	HVA-I_{Ca} (n)
30	65.1 ± 11.1 (6)	66.2 ± 6.0 (6)
100	40.8 ± 8.4* (3)	48.2 ± 9.2† (8)
300	23.9 ± 9.1 (4)	22.6 ± 8.8‡ (14)

Values (mean ± SD) indicate the percentage reductions in the peak current amplitudes of the LVA-I_{Ca} and HVA-I_{Ca} produced by tetracaine. The number of neurons in each group is indicated in parentheses. Tetracaine causes a similar degree of inhibition on both the LVA-I_{Ca} and HVA-I_{Ca}.

* $P < 0.05$ significant difference between 30 and 100 μM tetracaine.

† $P < 0.003$ significant difference between 30 and 100 μM tetracaine.

‡ $P < 0.003$ significant difference between 100 and 300 μM tetracaine.

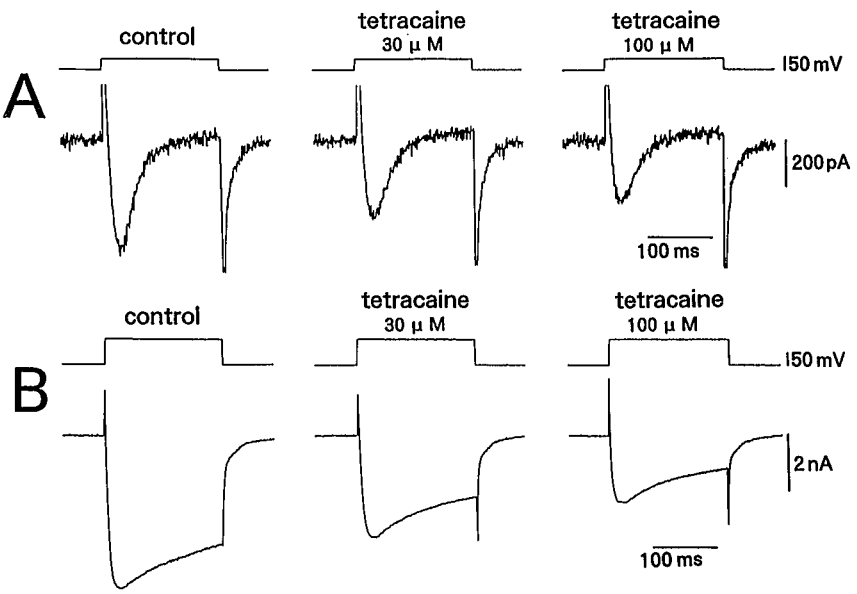

Fig. 4. Concentration-dependent effects of tetracaine on LVA-I_{Ca} and HVA-I_{Ca}. All records displayed were obtained from the same neuron, and each set shows the membrane potential (upper trace) and the clamp current (lower trace). (*A*) Effect of tetracaine on the LVA-I_{Ca}. The LVA-I_{Ca} was activated by depolarizing voltage steps of 200 ms to −40 mV from V_H of −80 mV. (*B*) Effect of tetracaine on the HVA-I_{Ca}. The HVA-I_{Ca} was activated by depolarizing voltage steps of 200 ms to +10 mV from V_H of −80 mV.

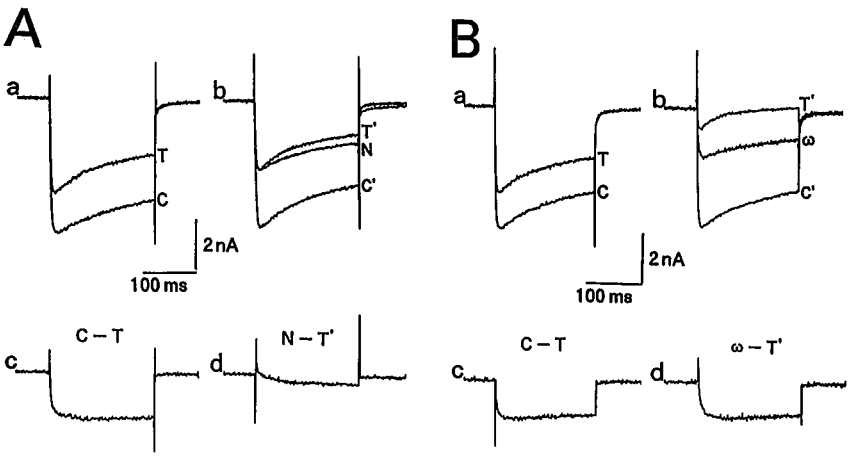

Fig. 5. Differential depressant effects of tetracaine on L-type and N-type HVA-I_{Ca}. (*A*) Effect of tetracaine on the HVA-I_{Ca} evoked in the absence and the presence of nicardipine. (a) Current traces obtained before and during application of tetracaine (30 μM) are denoted on the right as C and T, respectively. (b) Current traces were obtained after wash of tetracaine applied a first time (C'), during an exposure to nicardipine (10 μM) for 4 min (N), and during an exposure to tetracaine, which was applied a second time immediately after stopping the application of nicardipine (T'). (c) Current difference obtained by subtracting trace T from trace C in a. (d) Current difference obtained by subtracting trace T' from trace N in b. Note that the inhibition of the HVA-I_{Ca} by tetracaine in the presence of nicardipine is much less than that in the absence of nicardipine. (*B*) Effect of tetracaine on the HVA-I_{Ca} evoked in the absence and presence of ω-CgTx. The notations on the right of the current traces in *B* are the same as in *A* except ω instead of N. The current trace (ω) was obtained during an exposure to ω-CgTx (3 μM) for 3 min. The inhibition of the HVA-I_{Ca} by tetracaine in the absence (c) and the presence (d) of ω-CgTx is nearly identical.

pivacaine, 2,640 μM for procaine, and 2,790 μM for lidocaine.

Discussion

The Depressant Action of Tetracaine on Calcium Currents

The current study showed that tetracaine at a concentration of 300 μM depressed the peak amplitude of HVA-I_{Ca} elicited in cultured rat DRGs to 23% of the control without affecting voltage-current relation. The K_d of tetracaine for blocking HVA-I_{Ca} was 79.5 μM and was twice as large as the K_d for blocking the TTX-sensitive I_{Na} (37.5 μM). Tetracaine at 100 and 300 μM also depressed the peak amplitude of LVA-I_{Ca} to 41% and 24% of the respective control. A previous clinical study showed that the concentrations of tetracaine in CSF during spinal anesthesia were in the range of 50–400 μM.[20] Tetracaine is highly protein-bound, but the CSF protein concentration is normally 0.5% or less than the serum protein concentration. One report in the literature indicates that no significant binding of bupivacaine was observed in human CSF.[21] Because the protein-binding affinity of bupivacaine is comparable with that of tetracaine, the amount of tetracaine bound by proteins in CSF should be negligible. Some amounts of tetracaine injected into the subarachnoid space would be taken up by both neuronal and non-neuronal elements before reaching the nerve membranes of DRGs by diffusion, and the resultant concentration of tetracaine reaching the DRG nerve membranes may be lower than that in CSF. However, our study shows that both the LVA-I_{Ca} and HVA-I_{Ca} of cultured rat DRGs are markedly depressed by tetracaine at concentrations less than the maximum CSF concentration during spinal anesthesia. The results are consistent with those obtained from previous studies that the block of HVA-I_{Ca} is less potent than that of I_{Na}[4–7] and occurs in a voltage-independent manner.[4,5,7,22] The blocking potency of tetracaine for the HVA-I_{Ca} is apparently consistent with that obtained from other preparations: tetracaine at 400 μM completely blocks HVA-I_{Ca} of skeletal muscle cells,[18] and the K_d of tetracaine is 80 μM in cardiac myocytes.[6] A similar degree of inhibition by tetracaine of both the LVA-I_{Ca} and HVA-I_{Ca} is also comparable with nonselective depressant action of volatile anesthetics on LVA-I_{Ca} and HVA-I_{Ca} in cardiac cells.[17] However, the HVA-I_{Ca}

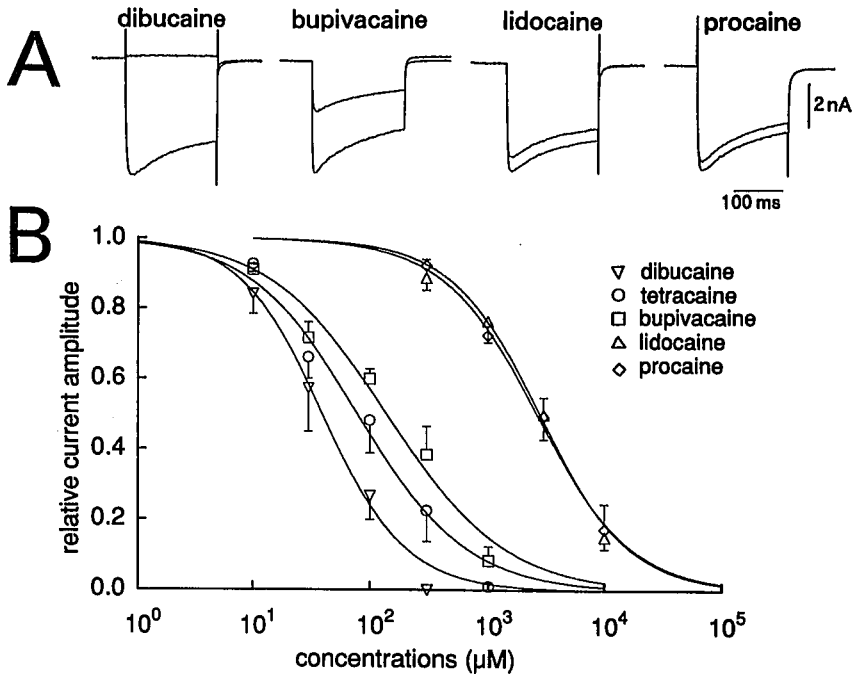

Fig. 6. Effects of several local anesthetics on HVA-I_{Ca}. (*A*) Pairs of current traces were obtained before and during application of 300 μM each of dibucaine, bupivacaine, lidocaine, and procaine. In each pair of traces, the control record is always the larger of the two. (*B*) Concentration-dependent depressant effects of five local anesthetics on HVA-I_{Ca}. The amplitude of the HVA-I_{Ca} measured in the presence of a given concentration of local anesthetics is normalized to the respective control amplitude measured in the absence of local anesthetics, and thus obtained relative current amplitudes of HVA-I_{Ca} (ordinate) are plotted as a function of local anesthetics concentration (abscissa). Each data point represents the mean ± SD obtained from 4–14 cells. The continuous curves through the data points were obtained by the same procedure described in figure 3. The apparent dissociation constant and the slope factor, respectively, were 34.5 μM and 1.2 for dibucaine, 79.5 μM and 1.0 for tetracaine, 156 μM and 0.9 for bupivacaine, 2,640 μM and 1.1 for procaine, and 2,790 μM and 1.1 for lidocaine.

demonstrated in muscle tissues is presumably reflecting an activation of L-type I_{Ca}, because, so far, N-type I_{Ca} has not been reported in nonneuronal cells. Our study showed that there was a marked difference in sensitivities of L-type Ca^{2+} channel and other Ca^{2+} channel types to tetracaine. Tetracaine (30 μM) depressed the HVA-I_{Ca} evoked in the presence of ω-CgTx (3 μM) to 14% of the control amplitude, whereas the same concentration of tetracaine inhibited the HVA-I_{Ca} evoked in the presence of nicardipine (10 μM) to 82% of the control. Regan *et al.* have shown that ω-CgTx (3 μM) causes saturable depression of N-type I_{Ca} without affecting L-type I_{Ca} in rat DRGs.[10] Thus, HVA-I_{Ca} in the presence of ω-CgTx consists of L-type I_{Ca} and HVA-I_{Ca} insensitive to both the Ca^{2+} channel blockers. Because the latter component usually represented 10–20% of the total amplitude of HVA-I_{Ca} and was depressed to 84% of the control by tetracaine (30 μM), the current that remained during exposure to ω-CgTx and tetracaine mainly was due to ω-CgTx-insensitive, nicardipine-insensitive HVA-I_{Ca}. Therefore, it seems evident that tetracaine at a concentration of 30 μM would cause further stronger inhibition on the pure L-type I_{Ca}. These results suggest that the L-type I_{Ca} is the most susceptible to tetracaine among the four types of Ca^{2+} channels including LVA-I_{Ca} (T-type), although the sensitivity rank of the three Ca^{2+} channel subtypes other than the L-type was not determined. The results also suggest that neuronal L-type I_{Ca} may be more susceptible to tetracaine than nonneuronal L-type I_{Ca}. Furthermore, our results suggest that the high potency of tetracaine to block the L-type I_{Ca} may exceed its potency to cause a tonic inhibition on TTX-sensitive I_{Na}; tetracaine (30 μM) reduced the peak amplitude of TTX-sensitive I_{Na} to 58% of the control, when it was activated at 15-s intervals.

Difference in the Blocking Potency of Local Anesthetic Agents for HVA-I_{Ca}

A marked difference in the potency to inhibit the HVA-I_{Ca} could be detected among local anesthetic species; the sequence of the potency was dibucaine > tetracaine > bupivacaine ⩾ procaine = lidocaine. This sequence is generally consistent with that of anesthetic potencies of these local anesthetics. A recent biochemical study shows that lidocaine, procaine, and bupivacaine at concentrations of 10–100 μM occasionally augment binding of nitredipine to DHP receptor sites in brain and cardiac tissues.[23] Nevertheless, we observed no increment in the amplitudes of HVA-I_{Ca} by these local anesthetics in the current study. The mechanism responsible for the potency difference among local anesthetics is not clear, but possession of either an ester (tetracaine,

procaine) or amide function (dibucaine, bupivacaine, lidocaine) does not determine the inhibitory potency of local anesthetics for the HVA-I_{Ca}. pKa, which determines the relative proportion of uncharged base to charged cation forms of local anesthetics, has no influence on the potency either, because the potency of tetracaine (pKa 8.4) is greater than that of either procaine (pKa 8.9) or lidocaine (pKa 7.8). Previous studies have suggested lipid solubility of local anesthetics to be primarily responsible for the potency to block TTX-sensitive I_{Na}.[1,2,24] The sequence of lipid solubility of local anesthetics, which is determined by the octanol/buffer partition coefficient, is dibucaine > tetracaine > bupivacaine ≫ lidocaine > procaine[24] and is in good agreement with the potency sequence to block the HVA-I_{Ca}. It appears that lipid solubility of local anesthetics may be a primary determinant of the blocking potency for the HVA-I_{Ca}. A reciprocal relationship between the lipophilicity of local anesthetics and the blocking potency for the potassium channel of squid axon has been reported: the more lipophilic local anesthetic agents (*e.g.*, dibucaine, tetracaine) exert less of the effect on the potassium current, whereas the less lipophilic local anesthetic agents (*e.g.*, procaine, lidocaine) produce the more potent inhibition on this current.[2]

Calcium Current Blockage and Spinal Anesthesia

Dorsal root ganglion cells are considered one of the most suitable preparations for investigating the mechanisms underlying spinal anesthesia, because DRGs are located inside the dura, such that they are easily accessible to local anesthetics injected intrathecally. The inhibition of the I_{Na} in the segment of primary sensory afferents between spinal roots and the spinal cord has been thought of as the primary mechanism underlying spinal anesthesia.[1,2] The current study showed that tetracaine, at concentrations required for spinal anesthesia, depressed the HVA-I_{Ca}, LVA-I_{Ca}, and TTX-sensitive I_{Na}. These results raise the possibility that the inhibition of Ca^{2+} influx through the voltage-gated Ca^{2+} channels and of subsequent inhibition of Ca^{2+}-dependent biochemical processes might contribute to the mechanisms of spinal anesthesia. Ca^{2+} influx through the voltage-gated Ca^{2+} channels is essential for neurotransmitter release.[8,9,25] Pharmacologic studies have shown that ω-CgTx depresses neurotransmitter release, whereas DHP antagonists do not affect synaptic transmission in most cases.[8,9,14,25–28] These studies suggest the N-type Ca^{2+} channel to be primarily responsible for releasing neurotransmitters. Tetracaine at concentrations of 100–300 μM depressed the N-type I_{Ca}, but the blocking potency for the N-type I_{Ca} was far less than that for the TTX-sensitive I_{Na}. If Ca^{2+} influx through N-type Ca^{2+} channel predominantly controls neurotransmitter release from DRG nerve terminals and tetracaine depresses presynaptic N-type Ca^{2+} channel with a potency comparable with that for somatic N-type Ca^{2+} channel, tetracaine-induced inhibition of synaptic transmission in the spinal cord through the blockage of presynaptic N-type Ca^{2+} channel may partly but not mainly act as the mechanism of spinal anesthesia. However, several studies have shown that high potassium-induced release of substance-P from cultured DRGs is strongly inhibited by DHP antagonists.[29,30] There are also several reports that electrically evoked substance-P release is enhanced by Bay K 8644, a DHP agonist, and partially inhibited by γ-amino butyric acid and norepinephrine, both of which reduce the L-type I_{Ca} of DRGs.[29,31,32] These results suggest that Ca^{2+} influx through the L-type Ca^{2+} channels may be involved in releasing substance-P from DRG nerve terminals. Substance-P release usually requires high-frequency trains of impulses at A_δ and C fibers. It seems likely that repetitive firings produce sustained depolarizations of A_δ- and C-fiber terminals and result in an activation of the L-type Ca^{2+} channel. Substance-P is presumably a neurotransmitter or neuromodulater of the nociceptive information.[33] The blocking potency of tetracaine for the L-type I_{Ca} was similar to or even stronger than that for the TTX-sensitive I_{Na}. Taking all these results together and assuming that tetracaine depresses presynaptic L-type Ca^{2+} channel with a high potency comparable with that for somatic L-type Ca^{2+} channels, the inhibition of presynaptic L-type Ca^{2+} channel by tetracaine may participate in a preferential impairment of the pain transmission during spinal anesthesia.

Based on the results obtained in ours and other studies discussed above, we conclude that the inhibition of the voltage-dependent Ca^{2+} current, especially L-type Ca^{2+} current, probably occurs during spinal anesthesia by tetracaine and propose that the inhibition of calcium influx through voltage-dependent Ca^{2+} channels and of subsequent Ca^{2+}-dependent biochemical processes contributes, at least partially, to the mechanisms of spinal anesthesia.

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