J Membrane Biol (2009) 229:1–9
DOI 10.1007/s00232-009-9170-8

ORIGINAL ARTICLES

State-Dependent Block of Na^+ Channels by Articaine Via the Local Anesthetic Receptor

Ging Kuo Wang · Joanna Calderon · Shiow-Jiin Jaw · Sho-Ya Wang

Received: 22 December 2008 / Accepted: 9 April 2009 / Published online: 6 May 2009

Abstract Articaine is widely used as a local anesthetic (LA) in dentistry, but little is known regarding its blocking actions on Na^+ channels. We therefore examined the state-dependent block of articaine first in rat skeletal muscle rNav1.4 Na^+ channels expressed in Hek293t cells. Articaine exhibited a weak block of resting rNav1.4 Na^+ channels at -140 mV with a 50% inhibitory concentration (IC_{50}) of 378 ± 26 μM ($n = 5$). The affinity was higher for inactivated Na^+ channels measured at -70 mV with an IC_{50} value of 40.6 ± 2.7 μM ($n = 5$). The open-channel block by articaine was measured using inactivation-deficient rNav1.4 Na^+ channels with an IC_{50} value of 15.8 ± 1.5 μM ($n = 5$). Receptor mapping demonstrated that articaine interacted strongly with a D4S6 phenylalanine residue, which is known to form a part of the LA receptor. Thus the block of rNav1.4 Na^+ channels by articaine is via the conserved LA receptor in a highly state-dependent manner, with a ranking order of open ($23.9\times$) > inactivated ($9.3\times$) > resting ($1\times$) state. Finally, the open-channel block by articaine was likewise measured in inactivation-deficient hNav1.7 and rNav1.8 Na^+ channels, with IC_{50} values of 8.8 ± 0.1 and 22.0 ± 0.5 μM, respectively ($n = 5$), indicating that the high-affinity open-channel block by articaine is indeed preserved in neuronal Na^+ channel isoforms.

Keywords Anesthetic action · Patch-clamp · Na channels · Na channel structure · Na channel properties

G. K. Wang · J. Calderon
Department of Anesthesia, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, USA

S.-J. Jaw (✉)
Department of Pediatric Dentistry, School of Dental Medicine, Tufts University, 1 Kneeland Street, Boston, MA 02111, USA
e-mail: wang@zeus.bwh.harvard.edu

S.-Y. Wang
Department of Biology, State University of New York at Albany, Albany, NY 12222, USA

Articaine (septocaine) is a local anesthetic (LA) used in dentistry that may also be used for infiltration anesthesia (Jeske and Blanton 2006; Schulze et al. 2006). Its use as a dental LA was first approved in the United States in 2000. Like traditional LAs, articaine blocks voltage-gated Na^+ channels of myelinated nerve fibers (Borchard and Drouin 1980). The estimated apparent dissociation constant of articaine was 65 μM for its block of peak Na^+ currents. Articaine is more appealing for dental use than other available LAs such as lidocaine and mepivacine because of its fast-acting nature. Unlike conventional LAs, articaine contains both ester and amide moieties (Fig. 1). In addition, the benzene group in conventional LAs is replaced with a thiophene group.

Voltage-gated Na^+ channels are responsible for the generation and propagation of action potentials on excitable membranes (Catterall 2000). During depolarization, resting Na^+ channels enter their open states and, subsequently, their inactivated states (Aldrich et al. 1983). These channels contain one large α subunit and one or two small β subunits (β1–β4). The α subunit has four homologous domains (D1–D4), each with six transmembrane segments (S1–S6), and the α subunit alone, when expressed in mammalian cells, can carry typical Na^+ currents. The actions of conventional LAs on voltage-gated Na^+ channels have been well characterized (Hille 2001; Catterall

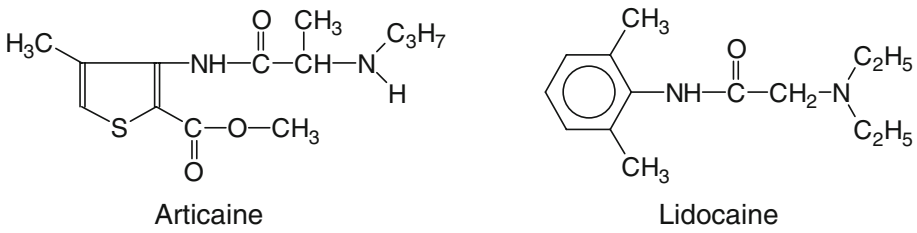

Fig. 1 Chemical structure of articaine and lidocaine. Articaine contains a secondary amine with a pK_a of 7.8, a value comparable to that of lidocaine with a tertiary amine ($pK_a = 7.9$)

and Mackie 2001). First, LAs elicit the tonic block of Na^+ currents when stimulated infrequently. Second, most LAs also produce additional use-dependent block of Na^+ currents during repetitive pulses. Third, LAs have higher affinities toward inactivated and open channels than their resting counterparts. Fourth, LAs bind to a single receptor within the inner cavity of the Na^+ channel.

Despite the widespread use of articaine to block the excitability of nerve and muscle cells in dentistry, little is known regarding its state-dependent actions on Na^+ currents or regarding its binding site within the Na^+ channel. We therefore investigated the binding affinities of articaine in the resting, open, and inactivated states of Na^+ channels. In addition, we tested whether articaine interacts with the Nav1.4 Na^+ channel via the conserved LA receptor. We chose Nav1.4 Na^+ channels in this study as a model system because this isoform has been used to study the LA block and the LA receptor extensively (Lipkind and Fozzard 2005; Nau and Wang 2004). Finally, we tested whether the open-channel block by articaine is preserved in neuronal Nav1.7 and Nav1.8 isoforms.

Materials and Methods

Cultures of HEK293 Cells Stably Expressing Na^+ Channels

Human embryonic kidney (HEK293) cell lines stably expressing rNav1.4 wild-type Na^+ channels and inactivation-deficient Na^+ channels (rNav1.4-L435W/L437C/A438W; $L_{435}L_{437}A_{438} \rightarrow W_{435}C_{437}W_{438}$ or WCW mutant) were re-established from frozen vials as described (Wang et al. 2004). The rNav1.4-WCW construct and its phenotypes have been detailed previously (Wang et al. 2003). The homologous inactivation-deficient rNav1.8-CW mutant channel and its stably transfected HEK293 cell line were likewise created. Expression of Na^+ currents in this cell line was found to be sufficient only when 1 mM lidocaine was added to the culture medium (Zhao et al. 2007). Cultured HEK293 cells were maintained at 37°C in a 5% CO_2 incubator in DMEM (Life Technologies, Inc., Rockville, MD) containing 10% fetal bovine serum (HyClone, Logan, UT), 1% penicillin and streptomycin solution (Sigma, St. Louis, MO), 3 mM taurine, and 25 mM HEPES (Life Technologies).

Na^+ Channel cDNA, Mutant Constructs, and Transient Transfection of HEK293t Cells

We created an additional S6 mutant channel construct (rNav1.4-WCW/F1579 K) in the rNav1.4-WCW cDNA background by site-directed mutagenesis as described (Wang et al. 2003). This specific rNav1.4 residue (F1579) is located in the middle of the D4S6 segment and appears to be critical for LA binding (Ragsdale et al. 1994; Nau and Wang 2004). A homologous inactivation-deficient hNav1.7-WCW mutant channel was likewise created. For transient transfection, HEK293t cells were grown to ~50% confluence and transfected by calcium phosphate precipitation. Transfection of mutant channels (5–10 μg) along with rat β1-pcDNA1 (10–20 μg) and reporter CD8-pih3 m (1 μg) was adequate for Na^+ current recording. The rat β1 subunit was included to increase the level of channel expression. Control experiments indicated that coexpression of the β1 subunit did not alter the binding affinities of articaine. Cells were replated 15 h after transfection in 35-mm dishes, maintained at 37°C in a 5% CO_2 incubator, and used after 1–4 days. Transfection-positive cells were identified with immunobeads (CD8-Dynabeads; Dynal, Inc., Lake Success, NY).

Electrophysiology and Data Acquisition

The whole-cell configuration of a patch-clamp technique (Hamill et al. 1981) was used to study Na^+ currents in HEK293 cells at room temperature (22 ± 2°C). Electrode resistance ranged from 0.5 to 1.0 MΩ. Command voltages were elicited with pCLAMP8 software and delivered by an Axopatch 200B (Axon Instruments, Sunnyvale, CA). Cells were held at −140 mV and dialyzed for 10–15 min before current recording. Most of the capacitance and leak currents were canceled with a patch-clamp device and by P/-4 subtraction. Liquid junction potential was not corrected. Peak currents at +30 mV were 2–20 nA for the majority of cells. Access resistance was 1–2 MΩ under the whole-cell configuration; series resistance compensation of >85%

typically resulted in voltage errors of ≤4 mV at +50 mV. Most dose–response studies were performed at +30 mV for outward Na^+ currents. Such recordings allowed us to avoid the complication of series resistance artifacts and to minimize inward Na^+ ion loading (Cota and Armstrong 1989). Curve fitting was performed by Microcal Origin (Northampton, MA). An unpaired Student's *t*-test was used to evaluate estimated parameters (mean ± SE or fitted value ± SE of the fit); p values <0.05 were considered statistically significant.

Solutions and Chemicals

Vials of 4% articaine HCl (124.7 mM) with epinephrine (1:100,000) were obtained from Septodont (1.7 ml per vial), France. Drugs were diluted in Ringer at 100 mM as stock solutions and stored at 4°C. Final drug concentrations (1 μM–1 mM) were made by serial dilution with the extracellular solution. Epinephrine alone at 1 μg/ml had no effects on Na^+ currents. Cells were perfused with an extracellular solution containing (mM) 65 NaCl, 85 choline-Cl, 2 $CaCl_2$, and 10 HEPES (titrated with tetramethylammonium-OH to pH 7.4). The pipette (intracellular) solution consisted of (mM) 100 NaF, 30 NaCl, 10 EGTA, and 10 HEPES (titrated with cesium-OH to pH 7.2).

Results

Resting and Inactivated Na^+ Channel Block by Articaine

Most LAs have relatively weak binding interactions with resting Na^+ channels. This appears to be true also for articaine. Figure 2a shows the block of Na^+ currents at various concentrations of articaine. Cells were held at −140 mV and depolarized to +30 mV for 5 ms. Peak currents were inhibited by articaine in a concentration-dependent manner. Less than 50% of peak currents were blocked at 300 μM articaine as shown in the dose–response curve (Fig. 2b; squares), with an estimated IC_{50} value of 378 ± 26 μM (Hille coefficient = 1.37 ± 0.14; $n = 5$).

In comparison, inactivated Na^+ channels appear to have a higher affinity for articaine. Cells were first depolarized by a conditioning pulse to −70 mV for 10 s and then returned to the holding potential (−140 mV) for 10 ms. A test pulse to +30 mV for 5 ms was applied to elicit the remaining Na^+ currents. The −70-mV conditioning pulse facilitated binding interactions between the drug and the inactivated Na^+ channel, whereas the drug-free inactivated Na^+ channels recovered rapidly, within 10 ms, at the holding potential. Figure 2c shows that most of the peak Na^+ currents are blocked at 300 μM articaine under such a pulse protocol.

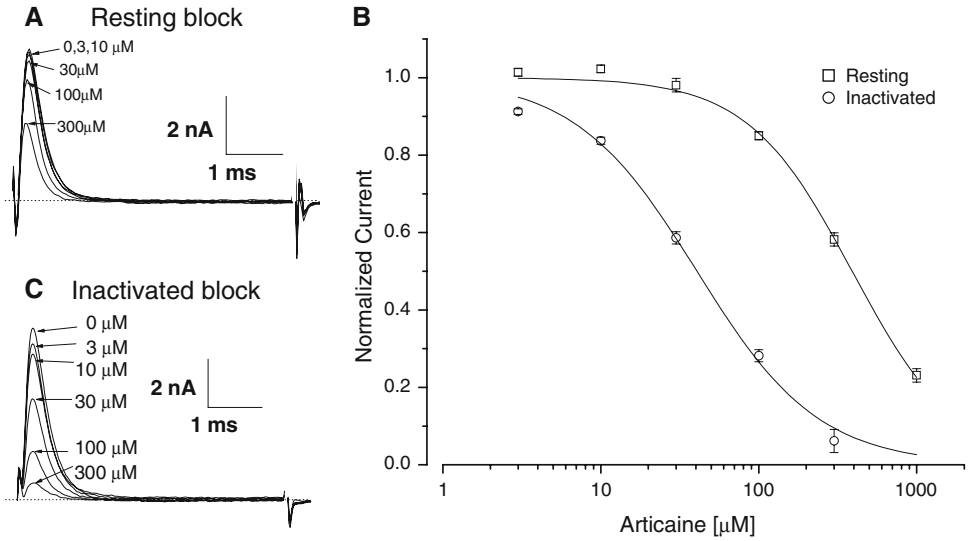

Fig. 2 Block of resting and inactivated wild-type rNav1.4 Na^+ currents at various articaine concentrations. **a** Na^+ currents were recorded and superimposed during the application of 0, 3, 10, 30, 100, and 300 μM articaine. Cells were held at −140 mV and received 5-ms test pulses of +30 mV at 30-s intervals. Initial capacitances were blanked for clarity. **b** A dose–response curve was constructed for resting (□) and inactivated currents (○). Peak currents were measured, normalized to the control (0 μM), and plotted against the articaine concentration. Plots were fitted with the Hill equation (solid lines), resulting in IC_{50} values of 378 ± 26 μM (Hill coefficient = 1.37 ± 0.14; $n = 5$) for the resting block and 40.6 ± 2.7 μM (Hill coefficient = 1.13 ± 0.08; $n = 5$) for the inactivated block. **c** Na^+ currents were recorded and superimposed during application of 0, 3, 10, 30, 100, and 300 μM articaine. Currents were evoked by a 5-ms test pulse of +30 mV following a −70-mV conditioning pulse lasting 10 s. An interpulse of 10 ms at the holding potential (−140 mV) was inserted to allow recovery of the drug-free inactivated Na^+ channels

The dose–response curve shown in Fig. 2b (circles) yielded an IC_{50} value of 40.6 ± 2.7 μM (Hill coefficient 1.13 ± 0.08; $n = 5$) for inactivated Na^+ channels. This result indicates that resting versus inactivated Na^+ channels differ by about ninefold in their affinities for articaine.

Most LAs induce a hyperpolarizing shift in the steady-state Na^+ channel inactivation curve (h_∞) (Hille 2001). We therefore investigated the possible h_∞ shift by articaine using a conventional two-pulse protocol (Hille 1977). A conditioning pulse ranging from −160 to −15 mV was applied for 100 ms before a brief test pulse. Figure 3a and b show traces of Na^+ currents in the absence and presence of 100 μM articaine, respectively, under this pulse protocol. The peak Na^+ currents were measured, normalized with respect to the control peak current at −160 mV, plotted against the conditioning voltage, and fitted with a Bolzmann equation (Fig. 3c). Without the drug, the $h_{0.5}$ value corresponded to −78.0 ± 0.3 mV ($n = 5$). Articaine at 100 μM significantly shifted the steady-state curve to the hyperpolarized direction, by 6 mV (−4.0 ± 0.3 mV; $n = 5$; $p < 0.05$) (Fig. 3c). This left shift in the h_∞ curve is similar to that induced by lidocaine and is consistent with the notion that articaine stabilizes the inactivated state of Na^+ channels (Hille 2001).

Use-Dependent Block of Na^+ Currents by Articaine During Repetitive Pulses

LAs, including lidocaine, are known to elicit use-dependent block of Na^+ currents during repetitive pulses (Schwarz et al. 1977; Hille 1977). Figure 4a shows that peak Na^+ currents are progressively reduced during repetitive pulses of +50 mV for 20 ms at 20 Hz. About 60% of peak currents were inhibited by 100 μM articaine after a total of 40 pulses were applied. We noticed that repetitive pulses with a duration as short as 1 ms also elicited a strong use-dependent block (Fig. 4b); up to 52.5% of peak currents were blocked. Maximal use-dependent block was achieved by repetitive pulses with a duration of 2–20 ms. This result implies that the use-dependent block induced by articaine requires channel

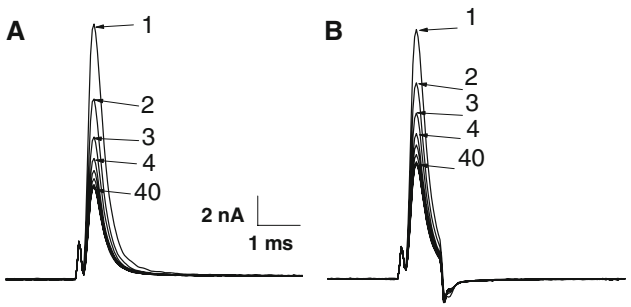

Fig. 4 Use-dependent block of Na^+ currents by 100 μM articaine during repetitive pulses. **a** Repetitive pulses at +50 mV for 20 ms were applied at 20 Hz for a total of 40 pulses in the presence of 100 μM articaine. Representative traces of Na^+ currents were recorded and superimposed. The number near the trace indicates the corresponding pulse number applied. **b** Repetitive pulses were identical to those in A except that the pulse duration was shortened to 1 ms. Note that more than 50% of peak Na^+ currents were blocked after 40 pulses

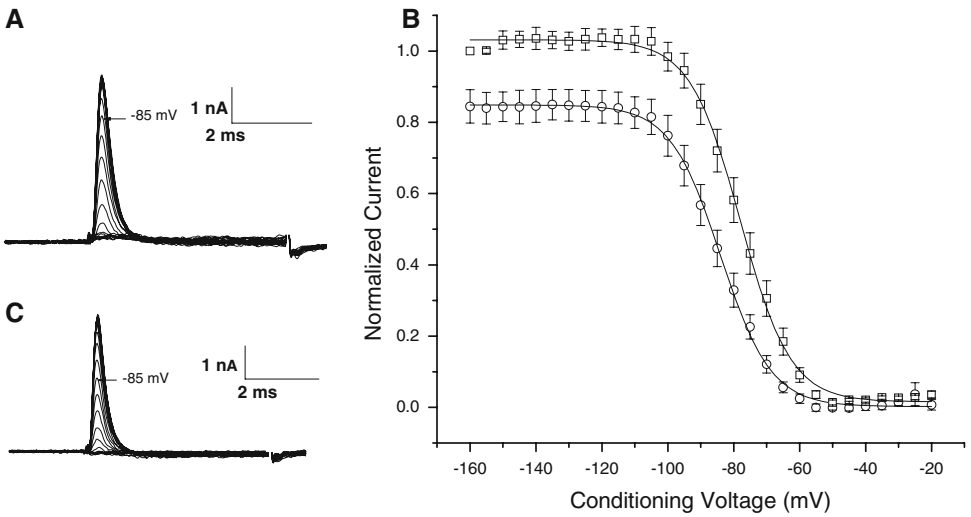

Fig. 3 Steady-state inactivation curve (h_∞) of rNav1.4 wild-type Na^+ channels. **a** Superimposed traces of Na^+ currents were evoked by a +30-mV test pulse lasting 5 ms. Each test pulse was preceded by a 100-ms conditioning pulse which ranged from −160 to −15 mV, increasing in 5-mV increments. The pulse protocol is shown in the inset. **b** Superimposed traces of Na^+ currents were evoked as in A in the presence of 100 μM articaine. **c** The resulting peak currents were measured, normalized to within cell control currents, and plotted against the conditioning voltage. The plots were then fitted with a Boltzmann function. The average midpoint ($V_{0.5}$) and slope (k) for the control were −78.0 ± 0.3 and 7.6 ± 0.3, respectively (□; $n = 5$). In the presence of articaine the average midpoint ($V_{0.5}$) and slope (k) for 100 μM articaine were −84.0 ± 0.3 and 7.7 ± 0.2, respectively (○; $n = 5$)

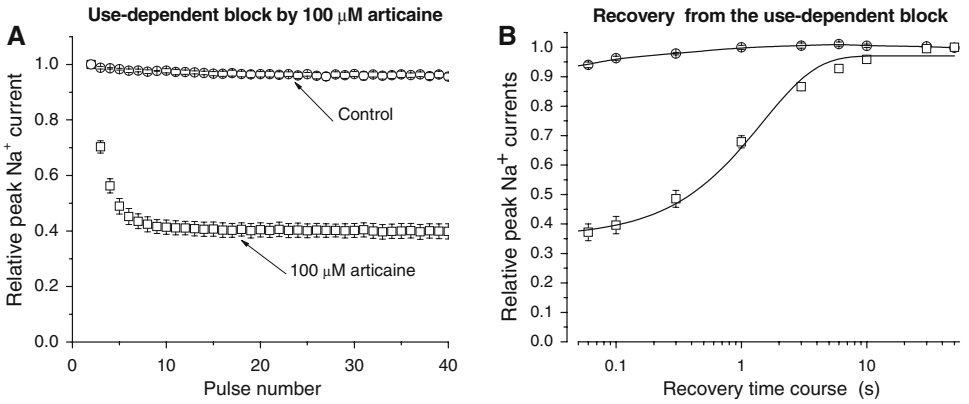

Fig. 5 Development of use-dependent block and its recovery at 100 μM articaine. **a** Peak currents were measured in the presence of 100 μM articaine, normalized with respect to the peak amplitude at 1 P, and plotted against the corresponding pulse (□). The curve (solid line) was best fitted by an exponential function with a time constant of $\tau = 1.58 \pm 0.13$ pulse ($n = 5$). Little or no use-dependent block was found in control currents (○). **b** Recovery from the use-dependent block was measured at various intervals after 40 repetitive pulses (+50 mV for 20 ms) were applied. The recovery time course at 100 μM articaine (□) could be fitted by a single exponential function with a time constant of 1.47 ± 0.16 s ($n = 5$). Control peak currents without articaine were measured, normalized, and plotted against time under identical conditions (○)

activation/opening and is likely due to the open-channel block of this drug.

The time course of this use-dependent block was quite rapid, with a time constant of 1.58 ± 0.13 pulse^{-1} (Fig. 5a, squares; $n = 5$). The steady-state block reached $60.3\% \pm 0.3\%$. Little or no use-dependent block was found in control currents (Fig. 5a, circles; $n = 5$). The recovery from the use-dependent block was measured after repetitive pulses at various intervals. The recovery time course (Fig. 5b, squares) could be fitted by a single exponential function with a time constant of 1.47 ± 0.16 s ($n = 5$).

Open Na^+ Channel Block by Articaine Via the LA Receptor

To study the open-channel block by articaine directly, we used inactivation-deficient mutant Na^+ channels. Figure 6a shows the current traces at +50 mV before and after various concentrations of articaine. Without the drug, the currents decayed slowly; most of the currents remained at the end of a 50-ms pulse. With 100 μM articaine, a rapid time-dependent block was evident and more than 85% of the maintained currents were blocked at the end of the pulse. The dose–response curve (Fig. 6b) indicates an IC_{50} value of 15.8 ± 1.5 μM (Hill coefficient, 1.08 ± 0.10; $n = 5$; filled squares) for the block of maintained Na^+ currents and 212 ± 11 μM (Hill coefficient, 1.16 ± 0.06; $n = 5$; open squares) for the block of peak Na^+ currents. We interpreted the results to mean that the open-channel block corresponds to the block of maintained currents, whereas the resting-channel block corresponds primarily to the block of peak currents. However, since the open-channel block occurs rapidly during channel opening, the block of peak currents likely overestimates the resting-channel block. This notion is supported by extrapolating the exponential fits of the open-channel block back to the rising phase of the control current trace. The interception points were measured, normalized to the peak amplitude of the control current, and plotted against the concentration. The dose–response curve yields an IC_{50} value of 352 ± 21 μM ($n = 5$), which is not significantly different from that of the resting block shown in Fig. 2b ($IC_{50} = 378 \pm 26$ μM; $n = 5$).

We also estimated the on- and off-rate constants for articaine using the τ value determined from Fig. 6a. The plot of $1/\tau$ versus articaine concentration (Fig. 7) yields a linear fit with an intercept value (corresponding to off-rate, k_{-1}) of 115 ± 13 s^{-1} and a slope (corresponding to on-rate, k_1) of 5.3 ± 0.7 μM^{-1} s^{-1}. The calculated dissociation constant (k_{-1}/k_{-1}) for articaine binding with open Na^+ channels is 21.6 μM, which is close to the estimated IC_{50} value of 15.8 μM.

A mutation at the critical LA binding site, F1579 K, in the inactivation-deficient Na^+ channel drastically reduced the open-channel block induced by articaine. Figure 6c shows the mutant current traces at various articaine concentrations. The IC_{50} for the open mutant channels was estimated to be 238.9 ± 9.8 μM (Hill coefficient $= 1.06 \pm 0.04$; $n = 5$; Fig. 6b, filled circles). The IC_{50} for the resting mutant channels was estimated to be 712.0 ± 19.1 μM (Hill coefficient $= 1.16 \pm 0.04$; $n = 5$; Fig. 6b, open circles). The reduction in binding for resting and for open channels after F1579 K substitution was about 3.3-fold and 15.1-fold, respectively. Another mutation, F1579A, also reduced the block induced by articaine, although to a lesser extent. The IC_{50} values for the resting and open mutant channels were estimated to be 338.1 ± 7.7 μM (Hill

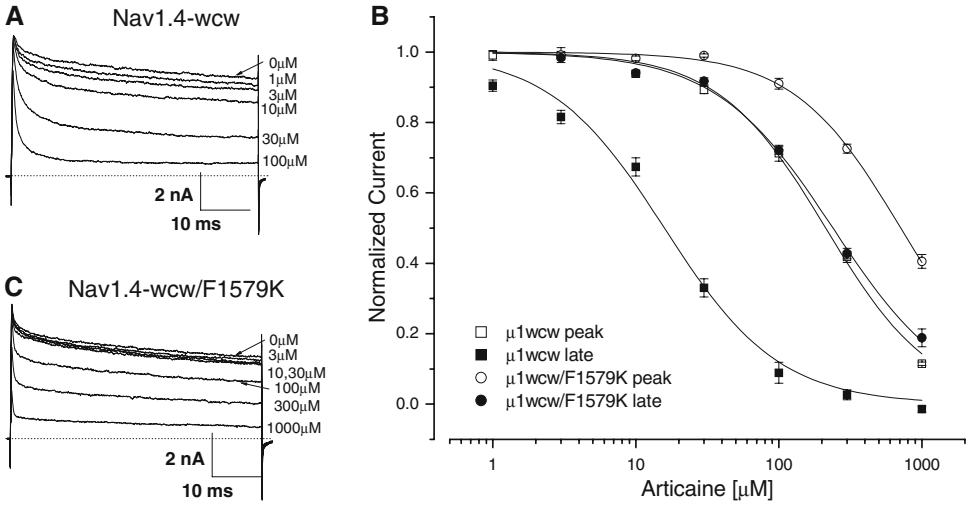

Fig. 6 Block of inactivation-deficient rNav1.4-WCW and rNav1.4-WCW/F1579 K Na^+ channels at various articaine concentrations. **a** Representative traces of Nav1.4-WCW Na^+ currents were recorded and superimposed during the application of 0, 1, 3, 10, 30, and 100 μM articaine. Cells were held at −140 mV and received 50-ms test pulses of +30 mV at 30-s intervals. **b** A dose–response curve was constructed from the currents as presented in A. Peak and late currents for inactivation-deficient rNav1.4-WCW and rNav1.4-WCW/F1579 K Na^+ channels were measured, normalized to their respective control (0 μM), and plotted as a function of articaine concentration. The resulting plots were fitted with the Hill equation (solid lines). The IC_{50} value for the rNav1.4-WCW peak current block was 212.6 ± 10.5 μM (Hill coefficient = 1.16 ± 0.06; $n = 5$) and the IC_{50} value for the late current block was 15.8 ± 1.5 μM (Hill coefficient = 1.08 ± 0.10; $n = 5$). The IC_{50} for the rNav1.4-WCW/F1579 K peak current block was 712.0 ± 19.1 μM (Hill coefficient = 1.16 ± 0.04; $n = 5$) and the IC_{50} value for the late current block was 238.9 ± 9.8 μM (Hill coefficient = 1.06 ± 0.04; $n = 5$). **c** Representative traces of Nav1.4-WCW/F1579 K Na^+ currents were recorded and superimposed during the application of 0, 3, 10, 30, 100, 300, and 1,000 μM articaine. Cells were held at −140 mV and received 50-ms test pulses of +30 mV at 30-s intervals

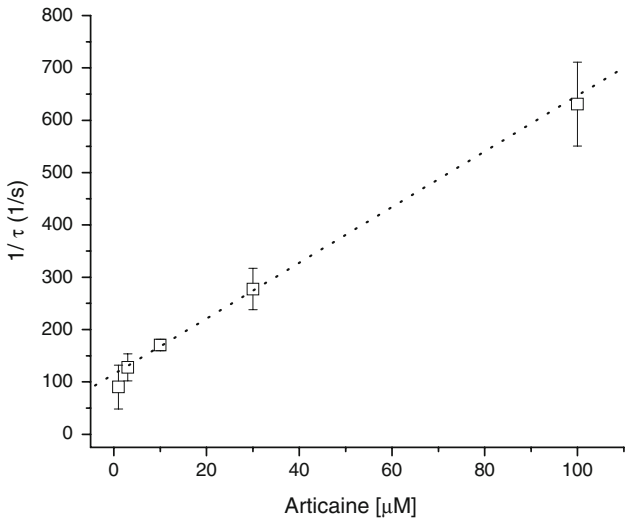

Fig. 7 Time-dependent block of inactivation-deficient rNav1.4-WCW Na^+ currents at various articaine concentrations. The decaying phase of the Na^+ currents in the presence of various articaine concentrations, as shown in Fig. 6a, were each normalized to the control (0 μM) and the resulting plot was fitted by a single exponential function. The normalization was applied to remove the slow-decaying component found in the control. The inverse of the time constant, τ, was plotted against the articaine concentration (y intercept = 114.7 ± 12.8 s^{-1}; slope = 5.3 ± 0.7; $n = 5$; linear correlation coefficient $r = 0.993$)

coefficient = 1.14 ± 0.02; $n = 5$) and 73.7 ± 3.8 (0.97 ± 0.04; $n = 5$), respectively, corresponding to about a 1.6- and 4.7-fold reduction in IC_{50} values. The stronger effect of F1579 K substitution in articaine binding is consistent with the electrostatic interaction between the F residue and the positive charge present in the amine group of LAs (Ahern et al. 2008). On the other hand, since F1579A substitution increases IC_{50} of the open state by only about fourfold, it would appear that other forces, such as hydrophobic interactions between articaine and the local anesthetic receptor, are also important.

Open-Channel Block of Inactivation-Deficient Neuronal Nav1.7 and Nav1.8 Isoforms by Articaine

Among many neuronal Na^+ channel isoforms, articaine may also target neuronal Nav1.7 and Nav1.8 channels for in vivo analgesia since these isoforms are found in tooth pulp (Luo et al. 2008; Warren et al. 2008). We therefore tested whether the strong open-channel block by articaine is preserved in these isoforms. Figures 8a and b show that the maintained currents in the inactivation-deficient hNav1.7-WCW and rNav1.8-CW current traces are blocked by articaine in a concentration-dependent manner.

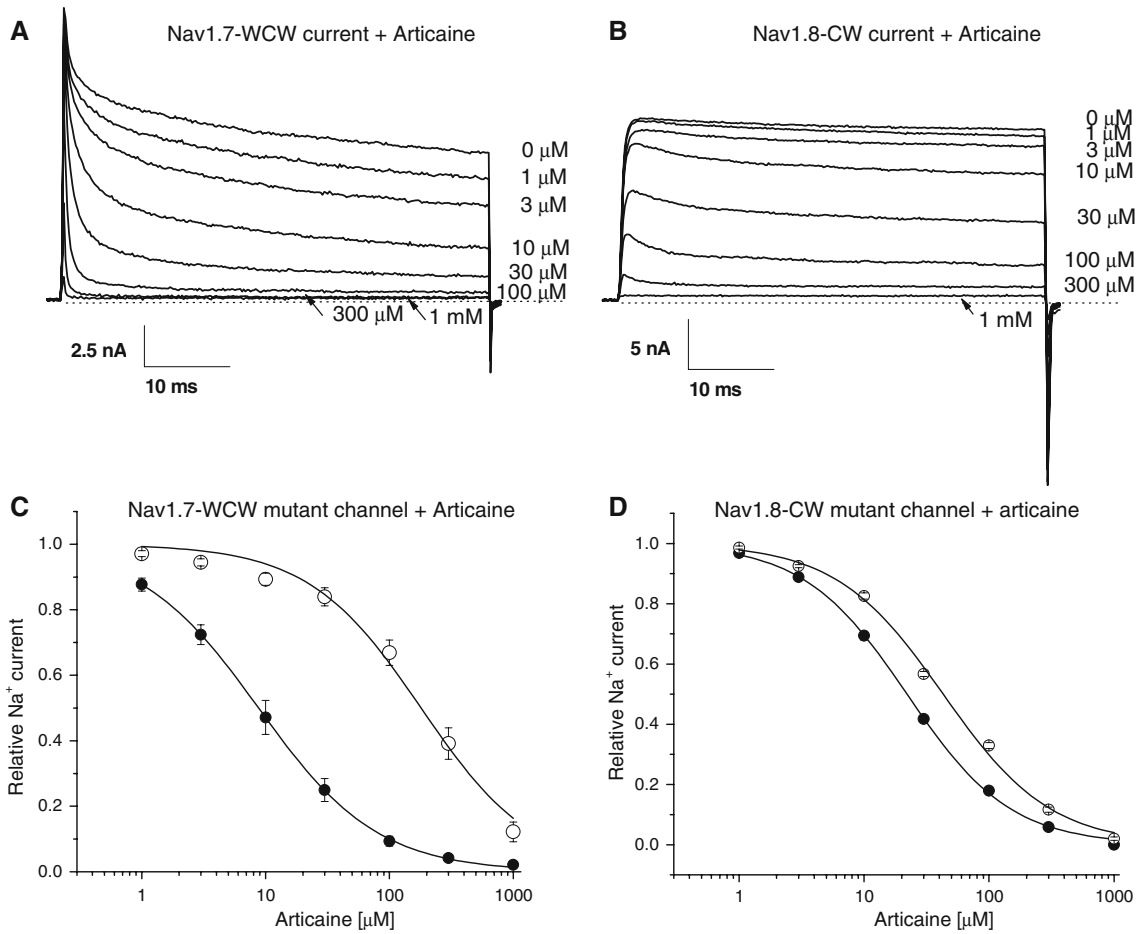

Fig. 8 Block of inactivation-deficient hNav1.7-WCW and rNav1.8-CW Na^+ channels at various articaine concentrations. Superimposed current traces of hNav1.7-WCW (**a**; top) and rNav1.8-CW (**b**; top) mutants were recorded at various articaine concentrations. Time-dependent block is apparent for hNav1.7-WCW but less evident for rNav1.8-CW mutant channels. Peak and maintained late currents were measured, normalized with respect to the control values without drug, and plotted against the corresponding articaine concentration. The resulting plots were fitted with the Hill equation (**c**, **d**; solid lines). The IC_{50} values and Hill coefficients for the open-channel block of hNav1.7-WCW and rNav1.8-CW (•) and the block of their peak currents (○) are given in the text

Dose–response analyses (Fig. 8c, d) indicate that the open-channel block by articaine yields an IC_{50} value of 8.8 ± 0.1 μM for hNav1.7-WCW channels (Hill coefficient $= 0.90 \pm 0.01$; $n = 5$) and 22.0 ± 0.5 μM for rNav1.8-CW channels (Hill coefficient $= 1.05 \pm 0.02$; $n = 5$). The IC_{50} value for rNav1.8-CW is significantly higher ($p < 0.05$) than that of hNav1.7-WCW and rNav1.4-WCW mutants. Unexpectedly, the block of the peak currents in rNav1.8-CW traces yielded an IC_{50} value of 42.9 ± 2.7 μM (Hill coefficient $= 1.01 \pm 0.06$), which was significantly lower than that in hNav1.7-WCW (180 ± 18 μM; Hill coefficient $= 0.95 \pm 0.09$; $n = 5$). As a result, the time-dependent block by articaine in inactivation-deficient rNav1.8-CW Na^+ currents is less evident compared with that of hNav1.7-WCW counterparts.

Discussion

We have investigated the actions of articaine on rNav1.4 Na^+ channels expressed in Hek293t cells. Like lidocaine, articaine produced tonic block of Na^+ currents when the cell was stimulated infrequently and shifted the steady-state inactivation curve (h_∞) to the hyperpolarizing direction. A significant use-dependent block was observed when the cell was stimulated repetitively at 20 Hz. We also measured the open-channel block by articaine directly using inactivation-deficient mutant Na^+ channels. These blocking actions are discussed below in the context of binding interactions between articaine and its receptor within the rNav1.4 Na^+ channel. Block of inactivation-deficient Nav1.7 and Nav1.8 neuronal isoforms was also determined for comparison.

Articaine Block of rNav1.4 Na^+ Channels Is Highly State Dependent

According to Hille's (1977) modulated receptor hypothesis, traditional LAs have higher affinities for open and inactivated states than the resting state of Na^+ channels. Our results show that the binding affinities of articaine with rNav1.4 Na^+ channels are highly state dependent, with IC_{50} values of 15.8, 40.6, and 378 μM for the open, inactivated, and resting states of Na^+ channels, respectively, with a rank order of open (23.9×) > inactivated (9.3×) > resting (1×) state. In comparison, the IC_{50} values for lidocaine in the open, inactivated, and resting states of rNav1.4 Na^+ channels under identical conditions are 20.9, 19, and 491 μM, respectively, with a rank order of inactivated (25.8×) ≈ open (23.5×) > resting (1×) state (Wang et al. 2004). Interestingly, the binding affinity of articaine for inactivated Na^+ channels (40.6 μM) is much lower than that of lidocaine (19 μM). This potency difference in inactivated Na^+ channels may explain why different concentrations of articaine (4% or ~125 mM) versus lidocaine (2% or ~74 mM) cartridges are used in dentistry.

The Use-Dependent Block by Articaine Is Mainly Due to the Open-Channel Block

One of the hallmarks of LAs is their ability to elicit use-dependent block during repetitive pulses (Hille 2001). Likewise, articaine is able to produce strong use-dependent block during repetitive pulses. We have also found that brief pulses, with a duration as short as 1–2 ms, induce use-dependent block almost as efficiently as pules with 20-ms duration (Fig. 3). This phenomenon suggests that the use-dependent block is not directly via the inactivated block since an increase in pulse duration will enable the Na^+ channels to enter their inactivated states and to enhance the use-dependent magnitude. Indeed, inactivated Na^+ channels may not interact with LAs rapidly if the LA receptor is guarded by the inactivation gate (Starmer et al. 1984). Our results instead indicate that the use-dependent block induced by articaine is largely attributable to the open-channel block. This is also consistent with the fact that the IC_{50} value of articaine for open Na^+ channels is 2.5-fold lower than that for inactivated Na^+ channels (i.e., 15.8 and 40.6 μM, respectively).

Open-Channel Block by Articaine Is Preserved in Neuronal Nav1.7 and Nav1.8 Na^+ Channel Isoforms

Neuronal Na^+ channel isoforms such as Nav1.7 and Nav1.8 are clearly involved in nociception. For example, Nav1.7 channels are an essential and nonredundant requirement for nociception in humans (Cox et al. 2006) whereas Nav1.8-expressing neurons are essential for mechanical, cold, and inflammatory pain (Abrahamsen et al. 2008). The open states of Nav1.4, Nav1.7, and Nav1.8 isoforms all appear to be sensitive to articaine block, with IC_{50} values ranging from 8.8 to 22.0 μM. These values are significantly lower than the value found for the resting and inactivated state of the rNav1.4 wild type (40.6 and 378 μM, respectively). The strong time-dependent block of inactivation-deficient Na^+ currents was found in both the hNav1.7 and the rNav1.4 isoforms but was less evident in the rNav1.8 counterparts (Fig. 8). The reason for this phenomenon found in rNav1.8 Na^+ channels is unclear. Nonetheless, such a phenotype could be due to its higher IC_{50} value (i.e., lower binding affinity for the Nav1.8 open state). Binding of articaine to slow inactivated states (Leffler et al. 2007) could also contribute to the rather narrow difference in the IC_{50} of the Nav1.8-CW resting (43 μM) and open (22 μM) states. Alternatively, rapid interactions between articaine and the intermediate closed states of Nav1.8 Na^+ channels could occur during activation gating as suggested previously for lidocaine and benzocaine (Wang et al. 2004). This would mask the early phase of the time-dependent block of inactivation-deficient Nav1.8 Na^+ currents induced by articaine.

Articaine Targets the Local Anesthetic Receptor Within the Inner Cavity

The receptor for LAs has been modeled within the inner cavity of rNav1.4 Na^+ channels (Lipkind and Fozzard 2005; Tikhonov et al. 2006). A cluster of residues within D1S6, D3S6, and D4S6 are found to form the LA receptor. The most critical amino acid that is involved in LA binding is a phenylalanine residue within the D4S6 segment (Catterall and Mackie 2001). In particular, this F1579 residue appears to interact with the positive charge at the amine group of LAs by charge-π electron interactions (Ahern et al. 2008). Articaine apparently also targets the LA receptor for its actions since the mutation of the D4S6 phenylalanine residue reduces its binding significantly. This is particularly the case for F1579 K substitution, which introduces a positive charge via the lysine residue at the LA receptor site (Fig. 6). Our results therefore suggest that despite significant differences in its structure from lidocaine (Fig. 1), articaine operates like traditional LAs to block the Na^+ channel permeation pathway via the same LA receptor within the inner cavity.

In summary, articaine acts as traditional LAs and blocks Na^+ channels in a use-dependent manner via the LA receptor within the inner cavity. Articaine has the highest affinity for the open state, an intermediate affinity for the inactivated state, and the lowest affinity for the resting state

of Na^+ channels. The use-dependent block is largely due to the open-channel block induced by articaine during repetitive pulses.

Acknowledgments This work was supported by National Institutes of Health Grant GM48090. We are grateful to Dr. Edward Moczydlowski for the HEK293 cell line expressing wild-type rNav1.4 channels and to Drs. James Trimmer, Norbert Klugbauer, and John Wood for the rNav1.4, hNav1.7, and rNav1.8 cDNA clones, respectively.

References

Abrahamsen B, Zhao J, Asante CO et al (2008) The cell and molecular basis of mechanical, cold, and inflammatory pain. Science 321:702–705

Ahern CA, Eastwood AL, Dougherty DA, Horn R (2008) Electrostatic contributions of aromatic residues in the local anesthetic receptor of voltage-gated sodium channels. Circ Res 102:86–94

Aldrich RW, Corey DP, Stevens CF (1983) A reinterpretation of mammalian sodium channel gating based on single channel recording. Nature 306:436–441

Borchard U, Drouin H (1980) Carticaine: action of the local anesthetic on myelinated nerve fibres. Eur J Pharmacol 62:73–79

Catterall WA (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26:13–25

Catterall WA, Mackie K (2001) Local anesthetics. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (eds) Goodman & Gilman's The pharmacological basis of therapeutics. Macmillan, New York, pp 367–384

Cota G, Armstrong CM (1989) Sodium channel gating in clonal pituitary cells: the inactivation step is not voltage dependent. J Gen Physiol 94:213–232

Cox JJ, Reimann F, Nicholas AK et al (2006) An SCN9A channelopathy causes congenital inability to experience pain. Nature 444:894–898

Hamill OP, Marty E, Neher ME, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100

Hille B (1977) Local anesthetics: hydrophilic and hydrophobic pathways for the drug receptor reaction. J Gen Physiol 69:497–515

Hille B (2001) Classical mechanisms of block. In: Ion channels of excitable membranes. Sinauer Associates, Sunderland, MA, pp 503–536

Jeske AJ, Blanton PL (2006) Selection of local anesthetics in dentistry: clinical impression versus scientific assessment. J Am Coll Dent 73:21–24

Leffler A, Reiprich A, Mohapatra DP, Nau C (2007) Use-dependent block by lidocaine but not amitriptyline is more pronounced in tetrodotoxin (TTX)-resistant Nav18 than in TTX-sensitive Na^+ channels. J Pharmacol Exp Ther 320:354–364

Lipkind GM, Fozzard HA (2005) Molecular modeling of local anesthetic drug binding by voltage-gated Na channels. Mol Pharmacol 68:1611–1622

Luo S, Perry GM, Levinson SR, Henry MA (2008) Nav17 expression is increased in painful human dental pulp. Mol Pain 4:16

Nau C, Wang GK (2004) Interactions of local anesthetics with voltage-gated Na^+ channels. J Membr Biol 201:1–8

Ragsdale DS, McPhee JC, Scheuer T, Catterall WA (1994) Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. Science 265:1724–1728

Schulze KE, Cohen PR, Nelson BR (2006) Articaine: an effective adjunctive local anesthetic for painless surgery at the depth of the muscular fascia. Dermatol Surg 32:407–410

Schwarz W, Palade PT, Hille B (1977) Local anesthetics: effect of pH on use-dependent block of sodium channels in frog muscle. Biophys J 20:343–368

Starmer CF, Grant AO, Strauss HC (1984) Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. Biophys J 46:15–27

Tikhonov DB, Bruhova I, Zhorov BS (2006) Atomic determinants of state-dependent block of sodium channels by charged local anesthetics and benzocaine. FEBS Lett 580:6027–6032

Wang S-Y, Bonner K, Russell C, Wang GK (2003) Tryptophan scanning of D1S6 and D4S6 C-termini in voltage-gated sodium channels. Biophys J 85:911–920

Wang S-Y, Mitchell J, Moczydlowski E, Wang GK (2004) Block of inactivation-deficient Na^+ channels by local anesthetics in stably transfected mammalian cells: evidence for drug binding along the activation pathway. J Gen Physiol 124:691–701

Warren CA, Mok L, Gordon S, Fouad AF, Gold MS (2008) Quantification of neural protein in extirpated tooth pulp. J Endod 34:7–10

Zhao J, Ziane R, Chatelier A, O'leary M, Chahine M (2007) Lidocaine promotes the trafficking and functional expression of Nav18 sodium channels in mammalian cells. J Neurophysiol 98:467–477