Local Anesthetic Inhibition of Baseline Potassium Channels with Two Pore Domains in Tandem

Christoph H. Kindler, M.D.,* C. Spencer Yost, M.D.,† Andrew T. Gray, M.D., Ph.D.‡

Background: Recently, a new structural family of potassium channels characterized by two pore domains in tandem within their primary amino acid sequence was identified. These tandem pore domain potassium channels are not gated by voltage and appear to be involved in the control of baseline membrane conductances. The goal of this study was to identify mechanisms of local anesthetic action on these channels.

Methods: Oocytes of Xenopus laevis were injected with cRNA from five cloned tandem pore domain baseline potassium channels (TASK, TKEK-1, TOLK-1, ORK-1, and TWK-1), and the effects of several local anesthetics on the heterologously expressed channels were assayed using two-electrode voltage-clamp and current-clamp techniques.

Results: Bupivacaine (1 nM) inhibited all studied tandem pore potassium channels, with TASK inhibited most potently. The potency of inhibition was directly correlated with the octanol-buffer distribution coefficient of the local anesthetic, with the exception of tetracaine, for which TASK is relatively insensitive. The approximate 50% inhibitory concentration for TASK were 709 μM mepivacaine, 222 μM lidocaine, 51 μM R(−)-ropivacaine, 53 μM S(−)-ropivacaine, and 608 μM tetracaine; 41 μM bupivacaine, and 39 μM etidocaine. Local anesthetics (1 nM) significantly depolarized the resting membrane potential of TASK cRNA-injected oocytes compared with saline-injected control oocytes (tetracaine 22 ± 6 mV, 7 ± 1 mV, respectively, and bupivacaine 31 ± 7 mV, 6 ± 4 mV).

Conclusions: Local anesthetics inhibit tandem pore domain baseline potassium channels, and they could depolarize the resting membrane potential of cells expressing these channels.

Whether inhibition of these channels contributes to conduction blockade or to the adverse effects of local anesthetics remains to be determined. (Key words: Baseline conductance; stereoselectivity; voltage-clamp; Xenopus oocytes.)

LOCAL anesthetics block propagation of compound action potentials by binding to voltage-gated sodium (Na+) channels. However, increasing evidence suggests that local anesthetics also potently block potassium (K+) currents. For example, it has been shown that 20 μM bupivacaine inhibits delayed rectifier and baseline K+ currents in frog atrial myocytes, and a 50% inhibitory concentration (IC50) of 22 μM has been established for bupivacaine for the transient outward K+ current (Ito) in rat myocytes. The Ito current, which is also present in the human heart, is believed to be an important contributor to repolarization of atrial myocytes. Thus, bupivacaine plasma concentrations of 4–12 μg/ml (~12–36 μM) can result from intravascular injection, which can significantly inhibit K+ currents in cardiac tissue. However, these plasma concentrations are associated with much lower free-drug concentrations, because lipid-soluble local anesthetics, such as bupivacaine, are highly protein bound.

K+ channel blockade by local anesthetics also may contribute to conduction block. Drachman and Strichartz showed that K+ channel blockers potentiate impulse inhibition by local anesthetics. In addition, local anesthetic inhibition of baseline K+ channels expressed by thin myelinated peripheral nerves may be a component of differential nerve block. These findings suggest that the effects of local anesthetics in neural tissue may extend beyond the known direct actions on voltage-gated Na+ channels.

Recently, we cloned a new mammalian tandem pore domain K+ channel (TASK) that is expressed in heart and brain tissue and inhibited by local anesthetics. Tandem pore domain K+ channels represent a new family of ion channels that contribute to baseline membrane conductances. In addition, they resemble physiologic channels known to contribute to the resting...
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Table 1. Q Values and IC₅₀s of Local Anesthetics on TASK Currents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Q (pH 7.6)</th>
<th>IC₅₀ (μM)</th>
<th>Q × IC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mepivacaine</td>
<td>29</td>
<td>709 ± 190</td>
<td>21</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>62</td>
<td>222 ± 46</td>
<td>14</td>
</tr>
<tr>
<td>R(+)-Ropivacaine</td>
<td>168</td>
<td>51 ± 13</td>
<td>9</td>
</tr>
<tr>
<td>S(−)-Ropivacaine</td>
<td>168</td>
<td>53 ± 15</td>
<td>9</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>336</td>
<td>668 ± 214</td>
<td>225</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>507</td>
<td>41 ± 10</td>
<td>21</td>
</tr>
<tr>
<td>Etidocaine</td>
<td>1157</td>
<td>39 ± 9</td>
<td>45</td>
</tr>
</tbody>
</table>

Octanol-buffer distribution coefficients (Q) at pH 7.6, approximate half-maximal inhibition concentrations (IC₅₀), including standard errors of the estimates of various local anesthetics for blocking TASK currents, and the product of Q and IC₅₀ are summarized.

Approximate IC₅₀ values were obtained by fitting the relative responses to the Hill equation. Q values were calculated according to the formula (1) in Methods. TASK = TWIK-related acid-sensitive K⁺ channel.

membrane potential of excitable cells, such as the Aplysia S channel or the flicker channel. These new ion channels have four or eight transmembrane segments and two pore domains in tandem within the primary amino acid sequence. Whereas the ion conduction pathway of voltage-gated K⁺ channels is thought to be composed of four pore domains of four independent channel subunits, the tandem pore domain K⁺ channels presumably constitute dimers of these tandem pore domain subunits, thereby yielding a tetrafoi structure, as seen with other K⁺ channels.

Although we have yet to identify specific inhibitors of tandem pore domain K⁺ channels, bupivacaine is among the most potent pharmacologic inhibitors of the TASK channel. Therefore, we evaluated the mechanisms of local anesthetic inhibition of cloned tandem pore domain K⁺ channels expressed by excitable tissues. First, we evaluated bupivacaine inhibition of the outwardly rectifying K⁺ channel TOK1, the open-rectifier K⁺ channel ORK1, the tandem pore domains weakly inwardly rectifying K⁺ channel TWIK-1, the outwardly rectifying TWIK-related K⁺ channel TREK-1, and the TWIK-related acid-sensitive K⁺ channel TASK. Second, based on the reported pH dependence of bupivacaine block of a physiologic baseline K⁺ channel, we studied bupivacaine inhibition of tandem pore domain K⁺ channels at different pH values. Third, also based on reports of agent specificity of local anesthetic inhibition of the flicker channel, we evaluated possible agent-specific effects of local anesthetics in different concentrations on these channels. Fourth, we studied the effects of stereoisomers of local anesthetics on tandem pore domain K⁺ channels based on reports of the stereoselectivity of local anesthetic inhibition of several ion channels.

Finally, we evaluated the effects of local anesthetic inhibition on the resting membrane potential of cells heterologously expressing these tandem pore domain K⁺ channels.

Materials and Methods

The following studies were approved by the University of California, San Francisco, Committee on Animal Research. Mature female Xenopus laevis were obtained from Nasco (Fort Atkinson, WI). The frogs were anesthetized by submersion in 0.3% 3-aminobenzoic acid ethyl ester for 30 min. Oocytes were removed surgically and defolliculated by treatment, for 1 h at room temperature, with 2 mg/ml collagenase A (Boehringer-Mannheim, Indianapolis, IN) in oocyte Ringer’s solution containing a high magnesium concentration (OR-Mg; 82 mM NaCl, 2 mM KCl, 5 mM HEPES, 20 mM MgCl₂, pH 7.4). After washing with OR-Mg, followed by washing with modified Barth’s solution (88 mM NaCl, 1 mM KCl, 10 mM HEPES, 7 mM NaHCO₃, 1 mM CaCl₂, 1 mM Ca(NO₃)₂, pH 7.0), stage V and VI oocytes were selected for injection. On the same day as removal, the oocytes were injected with 5–10 ng of TASK, TREK-1, TOK1, ORK1, or TWIK-1 cRNA in oocyte saline (100 mM KCl and 20 mM NaCl in diethylypyrocarbonate-treated water) or with oocyte saline alone as controls. Injected oocytes were incubated at 18°C in modified Barth’s solution with 50 mg/ml gentamycin, 2.5 mM sodium pyruvate, 5% heat-inactivated horse serum, and 5 mM theophylline, with gentle rotation for 2–4 days.

We studied three local anesthetics without a piperidine group (lidocaine, etidocaine, and tetracaine) and four with a piperidine group (mepivacaine, R(+) ropivacaine, S(−)-ropivacaine, and bupivacaine). We also studied the permanently neutral local anesthetic ethyl-p-aminobenzoate (benzocaine). Lidocaine, bupivacaine, tetracaine, and benzocaine were purchased from Sigma Chemical Company (St. Louis, MO). Mepivacaine, R(+) ropivacaine, and S(−)-ropivacaine, and etidocaine were provided by ASTRA Pharmaceuticals (Södertälje, Sweden). Stock solutions of local anesthetics (10 mM lidocaine, 10 mM mepivacaine, 10 mM tetracaine, 3 mM bupivacaine, 1 mM R(+) and 1 mM S(−)-ropivacaine, and 1 mM etidocaine) were prepared in frog Ringer’s solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, pH 7.6) and
maintained at 4°C for no more than 4 weeks. A stock solution of 1 M benzocaine in ethanol was prepared immediately before the experiments.

TASK, TREK-1, TOK1, ORK1, and TWIK-1 plasmids were linearized by restriction digestion with Xho I for TASK, HindIII for TREK-1, Not I for TOK1 and ORK1, and BamHI I for TWIK-1. After linearization, plasmids were purified with phenol and chloroform and used as templates. Capped transcript was prepared using T3 (TASK) and T7 (TREK-1, TOK1, ORK1, TWIK-1) mMessage mMachines (Ambion, Austin, TX). cRNA was precipitated with lithium chloride and resuspended in oocyte saline to a final concentration of approximately 0.5 mg/ml.

Two-electrode Voltage-clamp and Current-clamp Recording

Oocytes were studied by two microelectrode voltage-clamp and current-clamp techniques using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Microelectrodes (0.3–1.5 MΩ) were backfilled with 3 M KCl. For the voltage-clamp experiments, the holding potential was −80 mV. Voltage pulse protocols used 1-s steps ranging in most experiments from −140 to +40 mV in 20-mV increments, with 1.5-s interpulse intervals. Electrophysiologic studies were performed at room temperature (20–23°C) in a 25-μl recording chamber superfused with frog Ringer’s solution at a rate of approximately 4 to 5 ml/min. Local anesthetic solutions were applied for 2–4 min before voltage pulse protocols, and washout experiments were performed after 2–4 min of superfusion with local anesthetic-free frog Ringer’s solution. Similar experiments with anesthetic solution at a different pH were performed with a washout period between changes of the various treatment solutions. Saline-injected oocytes used as controls underwent the same treatments as cRNA-injected oocytes.

For most experiments, current signals were low-pass filtered (eight-pole Bessel filter; Frequency Devices, Haverhill, MA) at 40 Hz, sampled at 100 Hz, digitized, and stored on a Power Macintosh 7100 computer (Apple Computers, Cupertino, CA) using data acquisition software (ADInstruments, Milford, MA). To quantify responses, leakage currents of saline-injected oocytes were averaged and subtracted from currents of cRNA-injected oocytes. Ion currents recorded during superfusion with local anesthetics were normalized to averaged currents measured with drug-free frog Ringer’s solution before and after the application of local anesthetics.

TWIK-1 cRNA-injected oocytes were studied when their resting membrane potential was more negative than −55 mV and when reversible barium inhibition (1 mM) was observed. TWIK-1 cRNA-injected oocytes that did not fulfill these criteria were excluded.

Experiments measuring changes in the membrane potentials of oocytes were performed with the same equipment using the current-clamp mode.

Data Analysis and Statistics

Except when stated otherwise, results are expressed as the mean ± SD and n values indicate the number of oocytes studied. Octanol:buffer distribution coefficients (Q) for local anesthetics were calculated according to the formula

\[ Q = (\beta P^+ + P^0) / (1 + \beta) \] (1)

where \( P^+ \) and \( P^0 \) are the octanol:buffer partition coefficients for the charged and neutral species of the local anesthetic, respectively, and \( \beta = 10^{9k_{bs-pH}} \) as described by Strichartz et al.\(^{21}\)

The approximate IC\textsubscript{50} values for the inhibition of TASK by various local anesthetics were estimated by fitting the logistic transformed response data to the Hill equation using a linear least-squares fitting procedure (JMP; SAS Institute Inc., Cary, NC).

\[ R = \frac{IC_{50}^n}{[L]a^n + IC_{50}^n} \] (2)

where \( R \) = response (\( I_{LA}/I_{Control} \)), \( IC_{50} = \) the concentration at which 50% inhibition is observed, \([L]a = \) the concentration of local anesthetic, and \( n = \) the Hill coefficient.

The voltage dependence of local anesthetic inhibition of TASK currents was analyzed by normalization of leakage-subtracted currents in the presence of local anesthetic to matching controls to yield a fractional block \( f \) at each voltage. Data at potentials greater than −40 mV were fit to the Woodhull equation by regression to estimate the Woodhull coefficient \( \delta \) (the fraction of the electric field that the blocker experiences).\(^{22}\) For calculations of the Woodhull coefficients, the effective charge \( z \) was assumed to be +1.

We used paired or unpaired Student \( t \) tests to identify differences in data in two groups when appropriate. To identify differences in more than two groups, we used one-way analysis of variance followed by the Student-Newman-Keuls test for multiple comparisons. Significance was defined as \( P < 0.05 \).
Results

Bupivacaine Inhibition of Tandem Pore Domain K⁺ Channels

We evaluated the sensitivity of cloned tandem pore domain K⁺ channels to 1 mM bupivacaine. Figure 1 shows the current-voltage relations of TASK, TREK-1, TOK1, ORK1, and TWIK-1 cRNA-injected oocytes in the presence and absence of 1 mM bupivacaine. All tandem pore domain K⁺ channels were inhibited by bupivacaine, with TASK, TREK-1, and TOK1 showing the greatest inhibition. TWIK-1 showed inhibition of outward and inward currents by 1 mM bupivacaine.

Because external pH in the range of 4.3 to 8 has no effect on TOK1 activity, the TOK1 clone was used to investigate the effect of pH alteration on local anesthetic blockade of a tandem pore domain K⁺ channel. TOK1 currents at the end of the depolarizing steps from −80 to +40 mV were measured first without a local anesthetic at pH 7.6 and pH 6.4. In the presence of 1 mM bupivacaine, the inhibition of TOK1 was significantly greater at pH 7.6 (61 ± 15%, n = 9) than at pH 6.4 (17 ± 2%, n = 4; P < 0.05). Similar experiments were performed with TASK at three different extracellular pH levels and the relative responses were normalized to the corresponding pH. The inhibition of TASK currents by 10 μM bupivacaine also was significantly greater at higher pH values (pH 8.4: 28 ± 5%, n = 3; pH 7.6: 25 ± 3%, n = 5; and pH 7.0: 10 ± 3%, n = 4, respectively; P < 0.05).

Dose-dependent Block of Tandem Pore Domain K⁺ Channels by Local Anesthetics

Figure 2 shows current tracings from a TASK cRNA-injected oocyte obtained in the absence and presence of 1 mM lidocaine during application of 1-s voltage steps from −120 to +40 mV in 20-mV increments (holding potential, −80 mV). TASK currents activated instantaneously and did not inactivate throughout the voltage pulse, with or without lidocaine superfusing the oocyte. Inhibition of the currents was fully reversible after washout.

TASK currents were inhibited in a dose-dependent manner by all the local anesthetics studied (fig. 3). Table 1 summarizes the approximate IC₅₀ values for the inhibition of TASK by various local anesthetics. The mean Hill coefficient (equation 2 in Materials and Methods) for all local anesthetics studied was close to unity (0.8 ± 0.2). Woodhull coefficients δ for local anesthetic inhibition of TASK currents were small and ranged from 0.04 for 10 μM etidocaine to 0.14 for 10 μM S(-)-ropivacaine, implying that the voltage-dependence of local anesthetic inhibition is not strong. The Woodhull coefficient δ for 10 μM bupivacaine was 0.12, which is similar to that described for the human cardiac K⁺ channel Kv1.5.²⁰ The percentage of inhibition of TASK currents by 10 μM bupivacaine increased from 23 ± 3% at −20 mV to 29 ± 5% at +40 mV (n = 8; P < 0.05). Such an increase in inhibition in a voltage-dependent manner is expected if bupivacaine reaches the TASK channel from inside cells. With the exception of tetracaine, the potency of a local anesthetic to inhibit TASK currents was correlated directly with the octanol-buffer distribution coefficient (R² = 0.78, P < 0.05).

The permanently neutral local anesthetic benzocaine at doses of 1 mM and 100 μM also inhibited TASK currents by 51 ± 6% (n = 5) and 19 ± 6% (n = 4), respectively. Because of the poor solubility of benzocaine in aqueous solutions, we dissolved the higher concentration with ethanol, 0.1%. Ethanol, 0.1%, used alone as control inhibited TASK currents by 12 ± 5% (n = 6), which is significantly less than the inhibition observed with 1 mM benzocaine dissolved with ethanol, 0.1%.

Steroselectivity

Inhibition of TASK by the stereoisomers of ropivacaine, R(+) and S(-), was studied at 10 μM, 100 μM, and 1 mM. No significant difference in the inhibition produced by the isomers was observed. The inhibition of TASK currents by R(+) ropivacaine was 18 ± 5, 55 ± 7, and 94 ± 5%, whereas S(-)-ropivacaine inhibited the currents by 19 ± 6 (n = 11), 50 ± 7 (n = 7), and 93 ± 5% (n = 3) at the different concentrations.

TASK Modulation of the Resting Membrane Potential

We studied the effects of various local anesthetics on the resting membrane potential of TASK cRNA-injected oocytes. Local anesthetics caused an immediate and reversible depolarization, defined as the resting membrane potential during treatment with local anesthetic minus pretreatment values. Figure 4A shows tracings of membrane potentials before, during, and after treatment of TASK cRNA-injected oocytes and saline-injected oocytes (controls) with either 1 mM tetracaine or 1 mM bupivacaine. The bupivacaine-induced depolarization of TASK cRNA-injected oocytes (51 ± 7 mV; n = 9) and the tetracaine-induced depolarization of TASK cRNA-injected oocytes (22 ± 6 mV; n = 4) were significantly greater than the corresponding depolarizations of saline-
Fig. 1. Current-voltage relations of cloned tandem pore domain K⁺ channels. Raw signals without leakage subtraction are shown. Voltage pulses were from -140 to +40 mV in 20-mV increments from a holding potential of -80 mV. (A–F) Current-voltage relations of TASK, TREK-1, TOK1, ORK1, and TWIK-1 cRNA-injected oocytes in the absence and presence of 1 mM bupivacaine are shown. (F) Current-voltage curves from control saline-injected oocytes in the presence and absence of 1 mM bupivacaine are also shown. Data are mean ± SD.
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![Image of experiment setup with control, lidocaine 1 mM, and washout stages]

Fig. 2. The effects of lidocaine on TASK currents. Representative current responses from a TASK cRNA-injected oocyte (A) before, (B) during, and (C) after the application of 1 mM lidocaine. Voltage pulses ranged from −120 to +40 mV in 20-mV increments from a holding potential of −80 mV. Data were low-pass filtered (eight-pole Bessel filter; Frequency Devices, Haverhill, MA) at a 100-Hz cutoff before sampling at 1 kHz.

injected oocytes (tetracaine = 7 ± 1 mV and bupivacaine = 6 ± 4 mV, respectively; fig. 4B). Mepivacaine, 1 mM, also was tested and showed a depolarization of the resting membrane potential of TASK cRNA-injected oocytes of approximately 20 mV. Because the magnitude of the depolarization induced in oocytes by local anesthetics could depend on resting membrane potential, control oocytes were hyperpolarized by constant current injection to the more negative membrane potential of −70 mV observed with TASK-expressing oocytes. In these experiments, 1 mM bupivacaine still only depolarized the membrane potential of the control oocytes 7 ± 6 mV (n = 7). The small depolarizations by local anesthetics at the 1-mM concentration observed in saline-injected oocytes may have resulted from inhibition of endogenous baseline K⁺ channels, which have been described previously.²⁴ Extracellular alkalinity (pH 8.4), which is known to potentiate TASK currents,¹⁰ hyperpolarized TASK cRNA-injected oocytes (−4 ± 1 mV; n = 4) but did not affect saline-injected oocytes (−1 ± 2 mV; n = 8) (P < 0.05).

Discussion

Recently, a new family of K⁺ channels characterized by two pore domains in tandem within their primary amino acid sequence has been identified.¹³ Six members of this new family have now been cloned: TOK1¹⁵ (yeast), ORK1¹⁶ (Drosophila), TWIK-1¹²,²³ (mouse, human), TREK-1¹⁷ (mouse), TASK-1¹¹,²⁶ (rat, human, mouse), and TRAAK.²⁷ (mouse). The tandem pore domain K⁺ channels are expressed in most tissues and are particularly abundant in the brain, heart, and lung, but they are also found in the kidney, pancreas, and liver. These tandem pore domain K⁺ channels are not voltage gated and are believed to be important contributors, along with inward rectifiers, to baseline or leak potassium conductances.¹⁰⁻¹² Therefore, modulators of these channels will influence the resting membrane potential. A native K⁺ channel similar to TASK with respect to inhibition by acidity, local anesthetics, and Zn²⁺ has been described as the flicker channel of thin myelinated nerves.²⁸ Local anesthetics applied to isolated membrane patches from myelinated nerves that express the flicker channel indeed cause depolarization.²⁹ However, the affinity of bupivacaine for the flicker channel is extremely high (220 nM) compared with TASK (41 µM). Other known physiologic baseline K⁺ channels include the K⁺ channel in myelinated axons,²⁹ the muscarine-sensitive channel in sympathetic ganglia,²⁰ and K⁺ channels expressed by corneal endothelia³¹ and pancreatic acinar cells.²² Whether these physiologic baseline K⁺ channels also are inhibited by local anesthetics remains to be investigated.

Local Anesthetic Inhibition of Tandem Pore Domain K⁺ Channels

We studied currently published cloned tandem pore domain K⁺ channels for sensitivity to local anesthetics.
Bupivacaine in a dose of 1 μM inhibited the currents of TASK, TREK-1, TOK1, ORK1, and TWIK-1, but had no effect on the current-voltage relation of control saline-injected oocytes (fig. 1). The open rectifier TASK, the currents of which satisfy the Goldman-Hodgkin-Katz current equation for an open channel, and the outward rectifiers TREK-1 and TOK1, which pass outward K⁺ current with an activation potential that is coupled to the equilibrium potential for potassium, showed the strongest inhibition by bupivacaine. TWIK-1 currents expressed in oocytes were small, but both inward and outward currents were diminished by bupivacaine.

We studied three local anesthetics without a piperidine group (lidocaine, etidocaine, and tetracaine) and four with a piperidine group (mepivacaine, R[+]-ropivacaine, S[−]-ropivacaine, and bupivacaine) to determine whether octanol-buffer distribution coefficients (Q) of specific local anesthetics correlate with their potency to inhibit TASK currents. Q is defined as the ratio at equilibrium of the concentration of local anesthetic in an organic phase at a certain pH to the concentration in an aqueous phase and is used to quantify the degree of hydrophobic partitioning at that pH. The Woodhull coefficients of local anesthetic inhibition of TASK currents were small, suggesting a relatively peripheral site of local anesthetic action. The approximate IC₅₀ of lidocaine on TASK (222 μM) is between the reported affinity for the resting state (1.4 μM) and the inactivated state (11 μM) of voltage-gated Na⁺ channels. With the exception of tetracaine, which showed a much weaker block than predicted, there was a good correlation be-

Fig. 3. Normalized responses of TASK cRNA-injected oocytes to local anesthetics. (A) Local anesthetic concentration of 10 μM. (B) The local anesthetic concentration of 100 μM. (C) The local anesthetic concentration of 1 mM. Studies were performed under a two-electrode voltage clamp in frog Ringer’s solution at pH 7.6. The normalized response is defined as current measured for the −80-mV to +40-mV pulse during the treatment condition compared with control. Data are expressed as the mean ± SD. The numbers in parentheses above the error bars refer to the number of oocytes studied. Local anesthetics are ordered by their octanol-buffer distribution coefficients (Q, increasing from left to right).
Fig. 4. Depolarization of TASK cRNA-injected and saline-injected oocytes during exposure to local anesthetics. (A) Representative tracings of the resting membrane potential before, during, and after treatment with 1 mM tetracaine or 1 mM bupivacaine. Local anesthetics were applied for 30 s (indicated by vertical bars). Resting membrane potentials of TASK cRNA-injected oocytes and saline-injected control oocytes were $-69 \pm 12$ mV ($n = 21$) and $-35 \pm 12$ mV ($n = 21$), respectively ($P < 0.05$). (B) Depolarization in millivolts of both TASK cRNA-injected and control oocytes. Data are expressed as the mean $\pm$ SD. The numbers in parentheses above the error bars refer to the number of oocytes studied. *$P < 0.05$ treatment with local anesthetic of TASK cRNA-injected oocytes versus control oocytes.

Between local anesthetic $Q$ values and their inhibition of TASK currents ($R^2 = 0.78$, $P < 0.05$). The product of $Q$ and the $IC_{50}$ of tetracaine was markedly elevated compared with other local anesthetics, implying resistance to this local anesthetic (table 1). Although hydrophobic sites are important determinants of local anesthetic inhibition of TASK currents, resistance to tetracaine implies that structural elements of local anesthetics also can contribute to the potency of inhibition.

The relative insensitivity of the ester-linked tetracaine to the TASK channel may result from unknown agent-specific characteristics. Interestingly, the flicker channel also has been reported to be resistant to inhibition by tetracaine and other ester-linked local anesthetics. However, the flicker channel also showed a marked difference in the potency of local anesthetics with and without a piperidine group, which we did not observe for the TASK currents.

In contrast to baseline $K^+$ channels, other ion channels have not been reported to be resistant to ester-linked anesthetics. Ester-linked local anesthetics, for a given distribution coefficient or partition coefficient of the neutral species, are more potent inhibitors of resting $Na^+$ channels and compound action potentials. $^{21,33,36}$

$pH$ Dependence of Local Anesthetic Inhibition of Tandem Pore Domain $K^+$ Channels

$pH$ affects the distribution of local anesthetics between the aqueous phase and the lipid membrane, and it also influences the charge of membrane-associated local anesthetics. Lower cytoplasmic $pH$ favors the protonated form of tertiary amine local anesthetics, thereby increasing their potency of inhibition of voltage-gated $Na^+$ channels. $^{37}$ In contrast, decreasing the external $pH$ will lead to a slower penetration because the neutral species penetrates the membrane more effectively. $^{37}$ The inhibition of TOK1 currents by bupivacaine was significantly greater at $pH$ 7.6 than at $pH$ 6.4. The inhibition of TASK currents also was considerably greater at higher $pH$. Interestingly, a similar $pH$ dependence has been shown for the inhibition of the flicker channel by bupivacaine. $^{9}$ Our findings suggest that the uncharged form of bupivacaine may be important for the inhibition of TOK1 and TASK currents. This suggestion is also supported by the observation that QX314, a permanently charged quaternary lidocaine derivative, which is therefore relatively lipid insoluble, has no effect on TASK currents. $^{10}$ In addition, benzoica, a permanently neutral local anesthetic, can inhibit TASK currents. Inhibition of TOK1...
and TASK currents at different pH levels correlated with the $Q$ values (calculated according to equation 1 in Materials and Methods) at the corresponding pH ($Q_{pH 8.4} = 1.559$, $Q_{pH 7.0} = 507$, $Q_{pH 7.0} = 150$, $Q_{pH 6.4} = 41$).

**Stereoselectivity**

There is increasing interest in the stereospecific effects and toxicity of local anesthetics, particularly of bupivacaine and ropivacaine. This interest derives from the relatively high clinical potency and low cardiac toxicity profile of the $S(-)$-stereoisomers of bupivacaine and ropivacaine compared with their $R(+)$-stereoisomers. In isolated frog peripheral nerve, stereoselective effects of bupivacaine on neuronal Na$^+$ channels for the tonic and phasic inhibition have been reported. For both these effects, the $R(+)$-enantiomer was found to be more potent, with stereopotency ratios ($R/S$) of 1.2 and 2.5:3, respectively.

Some known K$^+$ channels also display a stereoselective local anesthetic block. However, an outwardly rectifying K$^+$ channel, expressed in the rat heart, has been reported to be equally sensitive to the $R(+)$- and $S(-)$-stereoisomers of local anesthetics. We also did not find a statistically significant difference between the $R(+)$- and $S(-)$-stereoisomers of ropivacaine at three different concentrations with respect to TASK inhibition. The lack of a stereoselective block clearly distinguishes the TASK channel expressed in oocytes from the flicker channel in myelinated nerve, which shows a stereopotency ratio ($R/S$) of 20 for ropivacaine and 67 for bupivacaine.

The potency of $R(+)$-bupivacaine on a K$^+$ channel cloned from human ventricle (hKV1.5) is approximately seven times greater than that exhibited by $S(-)$-bupivacaine. A recent report of the molecular determinants of this stereoselective block shows that stereoselectivity required interaction with amino acid residues contained in the sixth transmembrane domain (threonine 505, leucine 508, and valine 512) of hKV1.5. Major structural differences between tandem pore domain and voltage-gated K$^+$ channels prevent accurate alignments of the protein sequences of TASK and hKV1.5. However, the hKV1.5 results suggest that site-directed mutagenesis of TASK within the postpore transmembrane domains might increase the stereoselectivity of TASK local anesthetic inhibition.

**Depolarization of the Resting Membrane Potential**

Historically, in a wide variety of experimental paradigms, local anesthetics have been reported to slow the rate of rise of compound action potentials rather than to affect the resting membrane potential. However, in frog sciatic nerves, the addition of the K$^+$ channel blockers tetraethylammonium ion or 3,4-diaminopyridine increased lidocaine-induced tonic and phasic inhibition by 15 and 28%, respectively. Similar results were achieved for the phasic inhibition by bupivacaine in the presence of the tetraethylammonium ion. The K$^+$ channel blockers alone depolarized the resting membrane potential by 4–6 mV. Local anesthetics preferentially bind to open and inactivated states of Na$^+$ channels that predominate at depolarized membrane potentials. Thus, K$^+$ channel blockers, by causing partial depolarization and Na$^+$ channel opening and inactivation, can increase the binding of local anesthetic to neuronal Na$^+$ channels. In addition, voltage-clamp studies of cells expressing Na$^+$ channels also have documented the potentiating effect of membrane depolarization on local anesthetic inhibition of Na$^+$ currents.

The membrane potentials of TASK cRNA-injected oocytes were depolarized by 20–30 mV by the application of 1 mM local anesthetic. When saline-injected control oocytes were hyperpolarized by constant current injection, bupivacaine still depolarized the membrane potential only by approximately 7 mV. This finding suggests that the local anesthetic-induced depolarization of TASK cRNA-injected oocytes does not depend on the relatively negative resting membrane potential of cRNA-injected oocytes.

Studies have shown that the resting membrane potential of several cell types is controlled by potassium channels that are not voltage gated. The inhibition of baseline tandem pore domain K$^+$ channels by local anesthetics could depolarize the resting membrane potential and augment the conduction block of peripheral nerves by promoting the formation of open and inactivated states of voltage-gated Na$^+$ channels, which are highly sensitive to local anesthetics.

**Conclusions**

The results of our study indicate that (1) the tandem pore domain K$^+$ channels TASK, TREK-1, TOK1, ORK1, and TWIK-1 are inhibited by bupivacaine; (2) bupivacaine inhibition of TOK1 and TASK is pH dependent, and the presence of the uncharged form of the local anesthetic is important for inhibition; (3) the local anesthetic inhibition of TASK currents is dose dependent, and the potency of the block by different local anesthet-
ics is predicted by their octanol-buffer distribution coefficients, with the exception of tetracaine, to which TASK is relatively insensitive; (i) the R(+)- and S(−)-stereoisomers of ropivacaine were equipotent with respect to the inhibition of TASK currents and, therefore, were not stereoselective; and (5) the local anesthetic inhibition of TASK currents depolarizes the resting membrane potential of cells expressing this channel, thereby possibly contributing to conduction block.

Future studies, such as mutagenesis experiments of existing local anesthetic-sensitive baseline K⁺ channels, the identification of new channels such as these, and the determination of their expression by sensory nerves will improve our understanding of local anesthetic action.

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