Lipophilic and Stereospecific Interactions of Amino-amide Local Anesthetics with Human Kv1.1 Channels

Mark A. Punke, M.D.,* Patrick Friederich, M.D.†

Background: This study’s aim was to investigate the interaction of amino-amide local anesthetics with human Kv1.1 potassium channels. These channels were chosen because of their proven physiologic role. By using a homolog series of local anesthetics with different lengths of the N-substituent, it was intended to elucidate the role of lipophilic interactions with Kv1.1. The use of stereoisomers allowed testing of the role of polar drug actions.

Methods: Human Kv1.1 channels were measured with the patch clamp technique. Concentration–response data were described by Hill functions. Gating changes were described by Boltzmann functions.

Results: Inhibition of Kv1.1 channels by dextrobupivacaine, bupivacaine, levobupivacaine, dextroropivacaine, levoropivacaine, and mepivacaine was concentration dependent, reversible, stereoselective, voltage dependent, and frequency independent. The IC₅₀ values were (mean ± SEM) 41 ± 3 µM (n = 20), 56 ± 3 µM (n = 26), 76 ± 8 µM (n = 24), 135 ± 29 µM (n = 23), 313 ± 32 µM (n = 25), and 1,451 ± 351 µM (n = 23), respectively. The midpoint of current activation was shifted into the hyperpolarizing direction. The inhibitory potency as well as the potency to induce gating changes correlated with the number of CH₂ groups in the side chain of the drugs (r > 0.9, P < 0.05).

Conclusions: Kv1.1 channels constitute an important biophysical model for elucidating molecular mechanisms underlying local anesthetic drug effects. Inhibition likely results from an open state–dependent blocking mechanism. Interaction of local anesthetics with the ion channel protein is determined by lipophilic drug properties.

LOCAL anesthetics inhibit the conductance of painful stimuli by blocking action potentials through the inhibition of voltage-gated ion channels.1 They exhibit their effects not only by binding to sodium channels1–5 but also by interacting with other ion channels, such as voltage-dependent potassium channels.6–11 In contrast to the effects on sodium channels, the molecular interaction of local anesthetics with voltage-dependent potassium channels is less well defined.9,11–16

Voltage-dependent potassium channels constitute a heterologous group of membrane-bound proteins with a significant physiologic and pathophysiologic role.17 Kv1.1 potassium channels are abundantly expressed in the central nervous system, including myelinated axons, synaptic terminals, and dorsal root ganglia.9,18,19 Kv1.1 channels are important for various cellular functions, such as action potential generation, repolarization of the membrane potential, spike frequency adaptation, and neurotransmitter release.20 Inhibition of Kv1.1 channels in dorsal root ganglia neurons offers a possible approach for the treatment of chronic pain.9 Experimental evidence shows that inhibition of Kv1.1 together with endogenous sodium channels may contribute to conduction block.9 On the other hand, mutated and functionally impaired Kv1.1 channels are associated with epilepsy,21 and Kv1.1 knockout mice display an epileptic phenotype.22 Interaction of amino-amide local anesthetics with Kv1.1 channels may, therefore, contribute not only to analgesic action9 but also to these drugs’ unwanted neurotoxic side effects, such as seizures.8,16

Local anesthetics block potassium channels preferentially from the open state by interacting with the internal ion channel pore and the S6 transmembrane segment.6,12,23 Differences regarding the pharmacologic effects of local anesthetics on potassium channels exist9,16,23 and likely result from subtype and even species differences in channel structure.23 Information on the effects of local anesthetics on Kv1.1 is limited to the interaction of bupivacaine23 and lidocaine with rat Kv1.1 channels.24 It is currently unknown whether the effects of local anesthetics on Kv1.1 channels result from lipophilic interactions, and it is unknown to what extent these effects may be influenced by stereospecific properties of the drugs.

The aim of the study was to systematically investigate the pharmacologic effects on human Kv1.1 channels of a homolog series of amino-amide local anesthetics with different lengths of the N-substituent. Kv1.1 channels were chosen because of their physiologic significance. The homolog series of amino-amide local anesthetics was chosen because the length of the N-substituent determines the lipophilicity of these agents and, therefore, allows elucidation of the role and the structural requirements for lipophilic interactions of local anesthetics with Kv1.1 channels. The use of stereoisomers allowed determination of the role of polar interactions of amino-amide local anesthetics and Kv1.1 channels. Taken together, this information is an important prerequisite for understanding the molecular mechanisms contributing to local anesthetic drug effects.

Materials and Methods

Cell Culture

Human Kv1.1 channels (accession No. L02750) were cloned by HindIII and KpnI into the eucaryotic expression vector pcDNA3 (Invitrogen, Karlsruhe, Germany)
and stably expressed in human embryonic kidney 293
cells. Human embryonic kidney 293 cells were cultured in 50-ml flasks (NUNC, Roskilde, Denmark) at 37°C in
Dulbecco medium (GIBCO, Invitrogen, Carlsbad, CA)
with 10% fetal calf serum (Biochrom, Berlin, Germany),
100 U/l penicillin, and 100 mg/ml streptomycin (GIBCO)
in a humidified atmosphere (5% CO₂). The cells were
grown as nonconfluent monolayers and subcultured in
monodishes (35-mm diameter; NUNC) at densities be-
tween 2 and 3 × 10⁴ cells per dish before the electro-
physiologic experiments.

Patch Clamp Recordings

Whole cell currents were measured with the voltage
clamp method of the patch clamp technique using an
EPC-9 amplifier (HEKA Elektronik, Lambrecht, Germany)
and Pulse software version 8.53 (HEKA Elektronik). The
currents were filtered at 1 kHz, and series resistance was
actively compensated by at least 80%. A p/4 leak sub-
traction protocol was used in the study, with the excep-
tion of the experiments for frequency dependence. The
patch electrodes were fabricated from borosilicate glass
capillary tubes with filament (World Precision Instru-
mements, Saratoga, FL) using a Sutter P-97 puller (Sutter
Instrument Company, Novato, CA). The pipettes had a
resistance of 2–4 MΩ and were filled with a solution con-
taining the following electrolyte concentrations: 160
mM KCl, 0.5 mM MgCl₂, 10 mM HEPES, and 2 mM Na₂ATP,
with a pH of 7.25 adjusted with KOH. The extracellular
solution consisted of 135 mM NaCl, 5 mM KCl, 2 mM
CaCl₂, 2 mM MgCl₂, 5 mM HEPES, 10 mM sucrose, and
0.01 mg/ml phenol red, with a pH of 7.4 adjusted with
NaOH. All experiments were performed at room tem-
perature. The local anesthetics were dissolved in the wetary
extracellular solution, yielding stock solutions of 1 mM
dextrobupivacaine, bupivacaine, levobupivacaine,
and dextroropivacaine. For levoropivacaine, a stock so-
lution of 3 mM was prepared, and for mepivacaine, we
used a stock solution of 10 mM (all chemicals were
purchased from Sigma, Deissenhofen, Germany, except
for levobupivacaine, dextrobupivacaine, and dextroropi-
vacaine, which were gifts from AstraZeneca, Södertälje,
Sweden). The drugs were applied on the cells by a
perfusion system driven by hydrostatic pressure with
Teflon tubing (N Research Europe, Guemlingen, Switzer-
land). The inflow of the perfusion system did not have
significant influence of the currents.

Stimulation Protocols and Data Analysis

The holding potential during all experiments was −80
mV. The current-voltage relation was established by
depolarizing the cell membrane in steps of 10 mV for
100 ms between the membrane potentials −70 and +60
mV. To characterize the concentration-dependent ef-
fects of the local anesthetics, outward currents of Kv1.1
channels were measured at +40 mV at the end of the
test potential. The inhibition of the outward currents by
local anesthetic agents was determined as the inhibition
of the maximum current and calculated by the following
formula: inhibition = 1 − (Idrug/(Icontrol + Iwashout)/2). The
ratio of the maximum current (I) under influence of
the local anesthetic agents and the mean of maximum
current under control and washout conditions was sub-
tracted from 1. The data of the concentration-response
curves were mathematically described by Hill functions:
f = 1/(1 + (IC₅₀/c)ᵢ), where IC₅₀ is the concentration of
the half-maximal inhibition, c is the drug concentration,
and h is the Hill coefficient, using Kaleidagraph software
(Synergy-Software, Reading, PA). SIs of calculated Hill
parameters were used as defined by Kaleidagraph.
The whole cell conductance was calculated using the follow-
ing formula: Gmax = Imax/(V_m − E_k), where I_max is the
maximum current of each test potential, V_m is the mem-
brane potential, and E_k is the Nernst potential for potas-
sium. The conductance-voltage relation was mathemat-
ically described by a Boltzmann equation: 1 = I_max/(1 +
exp(V_m - V_m)/k)), where V_m is the voltage of half-
maximal activation, V_m is the membrane potential, and k
is the slope factor. The fitting procedure was performed
with Kaleidagraph software. Frequency dependence of
Kv1.1 channels was analyzed with repetitive 30-ms pulses
to +40 mV with three different interpulse durations (100,
300, and 1,000 ms). The remaining current under the
influence of the local anesthetic agents in the steady state
phase at the end of the test potential was normalized to
control values. The normalized current values were plotted
against a time axis according to the different interpulse
durations. Time constants of current inactivation were
determined by fitting current decay with a monoexponential
function using Pulsefit software version 8.53.

Statistical Analysis

Statistical significance was tested using analysis of vari-
ance and the Tukey-Kramer multiple comparisons test
(Graph Pad; Prism, San Diego, CA) or two-sided paired
and unpaired Student t test as appropriate (Excel; Mi-
crosoft, Redmond, WA). Data points are given as mean ±
SD unless stated otherwise; n values indicate the number
of experiments.

Results

Original current recordings of Kv1.1 channels exhib-
ited typical delayed rectifier currents devoid of fast inac-
tivation (figs. 1A–F). The currents activated at membrane
potentials more positive than −40 mV, with a midpoint of
current activation (Vₘₐₓ) of −26 ± 1 mV (n = 5) and
a slope factor of 7.1 ± 0.5 (n = 5).

The inhibitory effects of dextrobupivacaine, racemic bu-
pivacaine, levobupivacaine, dextroropivacaine, levoropi-
vacaine, and racemic mepivacaine were analyzed at a mem-
brane potential of +40 mV and quantified as the reduction of the steady state outward current at the end of each test potential. All local anesthetics inhibited currents through Kv1.1 channels (figs. 1A–F). Inhibition of Kv1.1 channels was concentration dependent, stereoselective, and reversible. The concentration–response data for inhibition of Kv1.1 channels were mathematically described by Hill equations (fig. 2). Dextrobupivacaine inhibited Kv1.1 channels with the highest potency. The IC50 value was 41 ± 3 µM, and the corresponding Hill coefficient was 1.0 ± 0.1 (mean ± SEM; n = 20). The sensitivity of Kv1.1 channels for the different local anesthetic agents decreased in the following order: dextrobupivacaine > bupivacaine > levobupivacaine > dextroropivacaine > levoropivacaine > mepivacaine (fig. 2; for overview, see table 1). Stereoselectivity of the effects was analyzed by comparing the current inhibition of Kv1.1 channels by dextroisomers and levoisomers. Both dextroisomers inhibited Kv1.1 channels more potently than the respective levoisomers did. For dextrobupivacaine (30 µM) and levobupivacaine (30 µM), Kv1.1 channel inhibition was 74 ± 9% (n = 5) versus 59 ± 14%.

Fig. 1. Effects of different amino-amide local anesthetics on human Kv1.1 stably expressed in human embryonic kidney (HEK) 293 cells. Shown are original current traces of Kv1.1 channels evoked by depolarizing pulses ranging from −80 to +60 mV in 10-mV steps, demonstrating inhibition of Kv1.1 channels by dextrobupivacaine (A), levobupivacaine (B), racemic bupivacaine (C), dextroropivacaine (D), levoropivacaine (E), and racemic mepivacaine (F). Shown are current traces under control conditions and under the influence of the different drugs.

Fig. 2. Current inhibition was quantified as the reduction of the steady state current at a potential of +40 mV. The concentration–response data were described by Hill functions. For overview, see table 1.
The shift of the activation midpoint (V_{mid}) into the hyperpolarizing direction (figs. 3A–F).

Agents shifted the voltage dependence of Kv1.1 channel activation into the hyperpolarizing direction by all local anesthetic agents significantly with concentration (figs. 4A–F). The effects were dependent on the lipophilic properties of the drugs.

For the analysis of the voltage dependence of inhibition, the size of the outward current at the end of each test potential (I_{max}) under the influence of the different local anesthetic agents was related to the mean of control and washout current. The extent of inhibition was plotted against the membrane potential. All local anesthetic agents inhibited Kv1.1 channels in a voltage-dependent manner (figs. 5A–F; analysis of variance; P < 0.05). Inhibition of Kv1.1 channels was not frequency dependent, because the stimulation frequency of the depolarizing pulse did not significantly influence local anesthetic-induced inhibition (figs. 6A–F).

Apart from mepivacaine, all local anesthetics induced inactivation-like behavior of Kv1.1 currents characterized by an acceleration of macroscopic current decline (figs. 1A–F and 7A). Comparing inhibition of the peak current at the beginning of the depolarizing test pulse...
and inhibition of the outward current at the end of each test pulse (fig. 7B) revealed that apart from mepivacaine, inhibition of the peak currents by the local anesthetics was significantly smaller than inhibition of the steady state currents (fig. 7B). The time constants of inactivation were determined at concentrations close to the IC50.

Fig. 4. The shift of voltage of half-maximal activation (V_mid) into the hyperpolarizing direction by all local anesthetic agents significantly increased with concentration (tested with analysis of variance; P < 0.05). (A) Dextrobupivacaine, (B) levobupivacaine, (C) racemic bupivacaine, (D) dextroropivacaine, (E) levoropivacaine, and (F) racemic mepivacaine. * Significant difference. n.s. = no significant difference.

Fig. 5. All local anesthetic agents inhibited Kv1.1 channels in a voltage-dependent manner (tested with analysis of variance; P < 0.05). (A–C) At a concentration of 100 µM, dextrobupivacaine inhibited the maximum current (I_max) at −30 mV with 58 ± 11% versus 76 ± 8% at +60 mV (n = 5 paired experiments), whereas bupivacaine inhibited I_max at −30 mV with 45 ± 3% versus 71 ± 6% at +60 mV (n = 5 paired experiments), and at the same concentration, levobupivacaine inhibited I_max at −30 mV with 39 ± 8% versus 61 ± 4% at +60 mV (n = 6 paired experiments) (P < 0.05). (D) and (E) Dextroropivacaine and levoropivacaine (300 µM) inhibited I_max at −30 mV with 69 ± 6% versus 75 ± 4% at +60 mV (n = 5 paired experiments) and with 29 ± 11% at −30 mV versus 53 ± 7% at +60 mV (n = 7 paired experiments) (P < 0.05). (F) Finally, mepivacaine inhibited I_max at −30 mV with 24 ± 13% versus 66 ± 4% at +60 mV (n = 6 paired experiments) (P < 0.05).
value of the respective drug by fitting a monoeXponential function to the declining current at a membrane potential of $-60$ mV. Both dextroisomers induced a faster current decline than the respective levoisomers did. Dextrobupivacaine (300 μM) and levobupivacaine (300 μM) inactivated Kv1.1 channels with time constants of $3.3 \pm 1.1 \text{ ms}$ (n = 6) and $9.3 \pm 0.4 \text{ ms}$ (n = 5), respectively ($P < 0.05$). Racemic bupivacaine (30 μM) yielded a time constant of $7.1 \pm 2.2 \text{ ms}$ (n = 6) (fig. 7C). The time constants for dextroropivacaine (100 μM) and levoropivacaine (100 μM) were $4.5 \pm 1.1 \text{ ms}$ (n = 5) and $37.9 \pm 5.2 \text{ ms}$ (n = 5), respectively ($P < 0.05$).

Lipophilicity of the different local anesthetic agents$^{26,27}$ correlated significantly with the number of CH$_2$ groups in the side chain ($r = 1.63 + 0.01x; r = 0.90, P < 0.05$). The IC$_{50}$ values for Kv1.1 channel inhibition and the shift of V$m_{50}$ were correlated with the length of the N-substituent of the homolog series of the local anesthetics (figs. 8A and B). Both the inhibitory potency and the potency to shift of the midpoint of current activation significantly correlated with the number of CH$_2$ groups ($r = 0.96, P < 0.05$; and $r = 0.94, P < 0.05$, respectively).

**Discussion**

This study established the effects of different aminoamide local anesthetics on human Kv1.1 channels. The biophysical properties of Kv1.1 wild-type channels used in our study were in accord with biophysical properties of these channels reported in previous studies.$^{23,28}$ Kv1.1 channels exhibited fast activating currents devoid of fast inactivation. The effects of racemic mixtures of bupivacaine and mepivacaine as well as the $R(+)$ and the $S(-)$ enantiomers of bupivacaine and ropivacaine on Kv1.1 channels were analyzed. All local anesthetic agents studied in this investigation inhibited Kv1.1 currents in a concentration-dependent, reversible, stereoselective, voltage-dependent, and frequency-independent manner. The normalized current values were plotted against a time axis according to the different interpulse durations. Inhibition of Kv1.1 channels was not frequency dependent, because the frequency of the depolarizing pulse did not significantly influence local anesthetic-induced inhibition. (A) Dextrobupivacaine, (B) levobupivacaine, (C) racemic bupivacaine, (D) dextroropivacaine, (E) levoropivacaine, and (F) racemic mepivacaine.

**Fig. 6.** Frequency dependence of local anesthetic induced block was analyzed at three different interpulse durations (100 ms [triangle], 300 ms [square], and 1,000 ms [circle]). The remaining current under the influence of the local anesthetic agents in the steady state phase at the end of the test potential was normalized to control values. The normalized current values were plotted against a time axis according to the different interpulse durations. Inhibition of Kv1.1 channels was not frequency dependent, because the frequency of the depolarizing pulse did not significantly influence local anesthetic-induced inhibition. (A) Dextrobupivacaine, (B) levobupivacaine, (C) racemic bupivacaine, (D) dextroropivacaine, (E) levoropivacaine, and (F) racemic mepivacaine.
manner. Voltage-dependent inhibition and the observed inactivation-like behavior are characteristic features of open channel block and are compatible with a direct interaction of the blocker with the pore region of Kv1.1 channels. Binding sites for local anesthetics on voltage-dependent potassium channels have been postulated to reside in the internal pore of the ion channel as well as in the region of the S6 helix and the S5–S6 linker of the channel protein. The structural homology of the pore region in Kv1.1, Kv1.5, and Kv4.3 allows interpreting the results of Kv1.1 channel block with reference to Kv1.5 as well as to Kv4.3 channels. Amino-amide local anesthetic binding to Kv1.5 channels involves lipophilic interaction at the position leucin 508 and valine 512 in the S6 segment of the channel as well as interaction with threonine 477 within the internal tetraethylammonium binding domain. Because of current decline, peak current inhibition was significantly smaller compared with the inhibition of the steady state current under the influence of the bupivacaine and the ropivacaine enantiomers as well as under racemic bupivacaine. The time constant of inactivation was analyzed close to the IC50 value of the respective drug, yielding time constants of 3.3 ± 1.1 ms (n = 6) for dextrobupivacaine (30 µM) and 9.3 ± 0.4 ms (n = 5) for levobupivacaine (30 µM) (P < 0.05). Dextroropivacaine (100 µM) and levoropivacaine (100 µM) inactivated Kv1.1 channels with time constants of 4.5 ± 1.1 ms (n = 5) and 37.9 ± 5.2 ms (n = 5), respectively (P < 0.05). The time constant for racemic bupivacaine (30 µM) was 7.1 ± 2.2 ms (n = 6). * Significant difference. B = racemic bupivacaine; M = racemic mepivacaine; n.s. = no significant difference; RB = dextrobupivacaine; RR = dextroropivacaine; SB = levobupivacaine; SR = levoropivacaine.

Fig. 7. (A) Original current traces under the influence of the different local anesthetic agents were overlaid to show inactivation-like behavior. (B) Local anesthetic inactivation-like behavior was quantified by measuring the maximum peak current at the beginning of the depolarizing test pulse and comparing it with the maximum outward current at the end of the respective test potential. Because of current decline, peak current inhibition was significantly smaller compared with the inhibition of the steady state current under the influence of the bupivacaine and the ropivacaine enantiomers as well as under racemic bupivacaine. (C) Time constants of inactivation were analyzed close to the IC50 value of the respective drug, yielding time constants of 3.3 ± 1.1 ms (n = 6) for dextrobupivacaine (30 µM) and 9.3 ± 0.4 ms (n = 5) for levobupivacaine (30 µM) (P < 0.05). Dextroropivacaine (100 µM) and levoropivacaine (100 µM) inactivated Kv1.1 channels with time constants of 4.5 ± 1.1 ms (n = 5) and 37.9 ± 5.2 ms (n = 5), respectively (P < 0.05). The time constant for racemic bupivacaine (30 µM) was 7.1 ± 2.2 ms (n = 6). * Significant difference. B = racemic bupivacaine; M = racemic mepivacaine; n.s. = no significant difference; RB = dextrobupivacaine; RR = dextroropivacaine; SB = levobupivacaine; SR = levoropivacaine.
lipophilic interaction of local anesthetics with this group of ion channels, thus, seems to constitute a common pharmacologic mechanism. In parallel to clinical knowledge, inhibition of Kv1.1 channels follows the same correlation of lipophilicity and analgetic potency. 30 To obtain equipotent analgesia in patients, higher concentrations of mepivacaine and ropivacaine have to be administered compared with bupivacaine. 30

All local anesthetics induced a concentration-dependent shift of the activation midpoint into the hyperpolarizing direction. This shift correlated with lipophilicity as well and may be explained by interference of local anesthetics with the coupling of voltage sensing and channel opening of Kv1.1 channels. 31 Interaction of local anesthetics with Kv1.1 channels is, therefore, unlikely to be restricted to single amino acids in the pore region of the channels. Rather, it seems to result from a more complex interaction with the channel protein. The interaction may likely involve regions of the S6 segment that are necessary for coupling the activation gate of these potassium channels to their voltage sensor residing in the S4 region. 23,31 Recently, a concerted contribution of the S4–S5 linker and the S6 segment to the modulation of voltage-dependent potassium channels by n-alkanols has been described. 31 A similar interaction of local anesthetics with the coupling of the activation gate to the voltage sensor of Kv1.1 channels rather than a direct interaction with the voltage sensor may, therefore, offer a possible explanation for the shift of the activation midpoint. Taken together, the effects on current inhibition and activation gating allow hypothesizing that local anesthetics, by binding to lipophilic amino acids L402 and V406 in the pore region, alter channel conductance and modulate gating by interference with the activation gate.

Stereoselective drug binding implies that the drug receptor is able to discriminate between the stereoisomers of a drug molecule. 32 Franqueza et al. 15 reported that bupivacaine block of Kv1.5 channels is stereoselective, with the R(+) enantiomer being sevenfold more potent than the S(−) enantiomer. In Kv1.5 channels, the stereoselective effects of bupivacaine enantiomers were reduced by substituting the amino acid threonine at position 505 by valine. 15 Threonine at the corresponding position 399 of Kv1.1 channels may suggest stereospecific interaction with Kv1.1 channels as well. Although this was the case, inhibition of Kv1.1 channels by aminoamide local anesthetics displayed a four- to five-times-weaker stereoselectivity than Kv1.5 channels. This quantitative difference in stereoselectivity between Kv1.1 and Kv1.5 channels supports the hypothesis that differences regarding the pharmacologic effects of local anesthetic action on potassium channels exist. 8,16,23 These difference likely result from subtype and even species differences in channel structure. 23 The difference in stereoselectivity between Kv1.1 and Kv1.5 channels may, furthermore, imply that despite structural similarities, the degree of lipophilic and polar interactions differ between different voltage-dependent potassium channel subtypes. Because the pore region is well conserved among different voltage-dependent potassium channels, such as Kv1.1 and Kv1.5, other regions of the protein or the lipid–protein interface may be involved in drug-channel interaction. In this context, it is interesting to note that the three-dimensional orientation of R(+) as compared with S(−) ropivacaine reduces the difference in potency between S(−) ropivacaine and S(−) bupivacaine introduced by an additional CH2 group in the latter by a factor of two. Furthermore, Kv1.1 is the only potassium channel that has been systematically studied with the same pattern of stereosepecificity as sodium channels. 33

In view of the physiologic significance of Kv1.1 channels, interaction with aminoamide local anesthetics may cause increased neuronal excitability. 9 Because Kv1.1 channels are predominately expressed in myelinated nerve fibers and large dorsal root ganglia neurons, 19 binding of local anesthetics to Kv1.1 channels may impair and delay the repolarization of the resting membrane potential of neurons. 9 This would slow the recovery of inactivated sodium channels and therefore decrease the availability of these channels, which would consequently increase the refractory period for action.
potential firing. Inhibition of Kv1.1 channels may therefore potentiate conduction block induced by sodium channel inhibition. A similar mechanism has been postulated for n-butyl-p-aminobenzoate, a new local anesthetic that blocks the delayed rectifier current of dorsal root ganglia neurons. On the other hand, central nervous system toxicity may also result from interaction of local anesthetics with potassium channels. Because of limitations of a heterologous expression system that was intentionally used to establish concentration-response curves in relation to clinical concentrations of the different local anesthetic agents, the obtained results of this study do not allow any valid conclusion to be drawn about clinical implications. However, it is striking that Kv1.1 channels would be inhibited at neurotoxic plasma concentrations reached after accidental intravascular injection of a dose of the local anesthetic used for regional anesthesia. Furthermore, the total plasma concentration of the different local anesthetics measured during convulsing correlates with systemic concentrations measured during neurotoxic side effects of these pharmaco-logic agents. Resolving whether human Kv1.1 channels play a significant role in local anesthetic drug effects, therefore, warrants further study. Regardless of future results, our study suggests that human Kv1.1 channels constitute an important biophysical model for elucidating molecular mechanisms underlying clinical effects or side effects of amino-amide local anesthetics.

The authors thank Andrea Zaiser (Technician, Institute of Neuronal Signal Transduction, Center for Molecular Neurobiology, University Medical Center Hamburg, Hamburg, Germany) for cell culture and Cornelia Siebrands, Ph.D. (Postdoctoral Fellow, Department of Biochemistry and Molecular Biology I, University Medical Center Hamburg), for critically reading the manuscript. The authors are grateful for the support of Olaf Pongs, Ph.D. (Director, Institute of Neuronal Signal Transduction, Center for Molecular Neurobiology, University Medical Center Hamburg).

References

2. Strichartz GR: Molecular mechanisms of nerve block by local anesthetics. ANESTHESIOLOGY 1976; 45:421–41
7. van den Berg RJ, Longobardo M, Delpont E, Caballero R, Tamargo J, Valenzuela C: Structural determinants of potency and stereoselective block of bkv1.5 channels induced by local anesthetics. Mol Pharmacol 1998; 54:162–9
37. Santos AC, DeArmas PI: Systemic toxicity of levobupivacaine, bupivacaine, and ropivacaine during continuous intravenous infusion to nonpregnant and pregnant ewes. ANESTHESIOLOGY 2001; 95:1256–64