

## Blockade of $\text{Na}^+$ and $\text{K}^+$ Currents by Local Anesthetics in the Dorsal Horn Neurons of the Spinal Cord

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**Background:** The dorsal horn of the spinal cord is a pivotal point for transmission of neuronal pain. During spinal and epidural anesthesia, the neurons of the dorsal horn are exposed to local anesthetics. Unfortunately, little is known about the action of local anesthetics on the major ionic conductances in dorsal horn neurons. In this article, the authors describe the effects of bupivacaine, lidocaine, and mepivacaine on voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  currents in the membranes of these neurons.

**Methods:** The patch-clamp technique was applied to intact dorsal horn neurons from laminae I–III identified in 200- $\mu\text{m}$  slices of spinal cord from newborn rats. Under voltage-clamp conditions, the whole-cell  $\text{Na}^+$  and  $\text{K}^+$  currents activated by depolarization were recorded in the presence of different concentrations of local anesthetics.

**Results:** Externally applied bupivacaine, lidocaine, and mepivacaine produced tonic block of  $\text{Na}^+$  currents with different potencies. Half-maximum inhibiting concentrations ( $\text{IC}_{50}$ ) were 26, 112, and 324  $\mu\text{M}$ , respectively. All local anesthetics investigated also showed a phasic, that is, a use-dependent, block of  $\text{Na}^+$  channels. Rapidly inactivating  $\text{K}^+$  currents ( $\text{K}_A$  currents) also were sensitive to the blockers with  $\text{IC}_{50}$  values for tonic blocks of 109, 163, and 236  $\mu\text{M}$ , respectively. The block of  $\text{K}_A$  currents was not use dependent. In contrast to  $\text{Na}^+$  and  $\text{K}_A$  currents, delayed-rectifier  $\text{K}^+$  currents were almost insensitive to the local anesthetics applied.

**Conclusions:** In clinically relevant concentrations, local anesthetics block  $\text{Na}^+$  and  $\text{K}_A$  currents but not delayed-rectifier  $\text{K}^+$  currents in spinal dorsal horn neurons. The molecular mechanisms of  $\text{Na}^+$  and  $\text{K}^+$  channel block by local anesthetics seem to be different. Characterization of these mechanisms

could be an important step in understanding the complexity of local anesthetic action during spinal and epidural anesthesia. (Key words: Bupivacaine; lidocaine; mepivacaine; patch-clamp; slice.)

SPINAL dorsal horn neurons receive sensory information from primary afferent terminals. Myelinated A $\delta$ -fibers and unmyelinated C-fibers responsible for pain transduction form synaptic contacts with the dorsal horn neurons located in laminae I–III of the spinal cord,<sup>1,2</sup> indicating an involvement of these neurons in processing of pain. During spinal and epidural anesthesia, the dorsal horn neurons are exposed to high concentrations of local anesthetic because of its diffusion directly into the spinal cord,<sup>3</sup> and neuronal excitability could be influenced by suppression of transmitter- and voltage-activated conductances.

It is generally accepted that local anesthetics suppress transmission of pain by blocking voltage-gated  $\text{Na}^+$  channels of peripheral nerve; however, an increasing number of studies have been performed that describe the action of local anesthetics on different types of voltage-gated<sup>4,5</sup> and background<sup>6,7</sup>  $\text{K}^+$  channels. Electrophysiologic experiments studying the action of local anesthetics were performed mostly on peripheral axons,<sup>8</sup> but our knowledge about the effects of local anesthetics on ionic currents in somata of different neurons is limited. The data reported for axonal channels cannot be applied directly to somatic channels of the neurons, as a high diversity of electrophysiologic and pharmacologic properties of voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels in axonal<sup>9–11</sup> and somatic<sup>12,13</sup> membranes has been reported.

Here we report the effects of bupivacaine, lidocaine, and mepivacaine—widely used local anesthetics with different lipophilic properties—on voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  currents in visually identified dorsal horn neurons. The experiments were performed on slice preparations<sup>14</sup> of spinal cord from newborn rats to record

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Na<sup>+</sup> and K<sup>+</sup> currents from intact cells in which channel properties and densities had not been modified by enzymatic treatment.

## Materials and Methods

### Preparation

Experiments were performed using the whole-cell patch-clamp technique<sup>15</sup> on 200- $\mu$ m thin slices cut from the lumbar enlargement (L3-L6) of the spinal cord of 2- to 5-day-old rats.<sup>16</sup> Animals were rapidly decapitated, and the spinal cords were cut out carefully in ice-cold preparation solution bubbled with O<sub>2</sub>-CO<sub>2</sub> (95%:5%). After removal of the pial membrane with fine forceps, the spinal cord was embedded in preparation solution containing 2% agar cooled to 39°C. To accelerate solidification of the agar, the beaker was placed in ice-cold water. The agar block containing the lumbar enlargement of the spinal cord was cut out and glued to the glass stage fixed in the chamber of the tissue slicer. The spinal cord was sliced in ice-cold preparation solution under continuous bubbling. The slices were incubated subsequently for 1 h at 37°C.<sup>16,17</sup> The standard procedure of cell cleaning by repetitive blowing and suction of Ringer's solution through a broken patch pipette was not used, as each slice contained numerous dorsal horn neurons with clean surfaces. The procedures used for animal decapitation were reported to the local veterinarian authority and are in accordance with the German guidelines.

### Solutions

Preparation solution contained (in mM) NaCl (115), KCl (5.6), CaCl<sub>2</sub> (2), MgCl<sub>2</sub> (1), glucose (11), NaH<sub>2</sub>PO<sub>4</sub> (1), and NaHCO<sub>3</sub> (25) (pH = 7.4 when bubbled with 95% O<sub>2</sub>:5% CO<sub>2</sub>). During all experiments, the slices were perfused with low-Ca<sup>2+</sup>, high-Mg<sup>2+</sup> solution (Ringer's solution) to reduce spontaneous synaptic activity in neurons. Ringer's solution was obtained from the preparation solution by setting the concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> to 0.1 mM and 5 mM, respectively. Tetraethylammonium-chloride-containing solution used for investigation of Na<sup>+</sup> channels (Ringer's tetraethylammonium solution) contained (in mM) NaCl (95), KCl (5.6), CaCl<sub>2</sub> (0.1), MgCl<sub>2</sub> (5), glucose (11), NaH<sub>2</sub>PO<sub>4</sub> (1), NaHCO<sub>3</sub> (25), and tetraethylammonium-chloride (20) (pH = 7.4 when bubbled with 95% O<sub>2</sub>:5% CO<sub>2</sub>). For recording K<sup>+</sup> currents, an external solution in which Na<sup>+</sup> ions were substituted with choline<sup>+</sup> ions (choline-

Ringer's solution) was used. Choline-Ringer's solution contained (in mM) choline-chloride (141), KCl (0.6), CaCl<sub>2</sub> (2), MgCl<sub>2</sub> (1), glucose (11), and HEPES (10) (pH adjusted to 7.4 with 5-mM KOH). Local anesthetics were added directly to the corresponding external solutions. The experimental chamber with a volume of 0.6 ml was perfused continuously by external solution at a rate of 2-3 ml/min.

The pipette solution used for Na<sup>+</sup> current recordings (high-Cs<sub>i</sub><sup>+</sup>) contained (in mM) NaCl (5.8), CsCl (134), MgCl<sub>2</sub> (1), EGTA (3), and HEPES (10) (pH of 7.3 adjusted with 9.2-mM NaOH). Standard pipette solution for recording K<sup>+</sup> currents (high-K<sub>i</sub><sup>+</sup>) contained (in mM) NaCl (5), KCl (144.4), MgCl<sub>2</sub> (1), EGTA (3), and HEPES (10) (pH adjusted to 7.3 by 10.6-mM KOH).

Bupivacaine-HCl and lidocaine-HCl were purchased from Sigma Chemical Company (St. Louis, MO). Mepivacaine was taken as Scandicaine (4%) from Astra Chemicals (Wedel, Germany). The hydrophilic quaternary derivative of lidocaine QX-314 was ordered from Alomone Labs (Jerusalem, Israel).

### Current Recordings

The whole-cell pipettes were pulled in two stages from a borosilicate glass tube (GC 150, Clark Electromedical Instruments, Pangbourne, United Kingdom) and were fire polished to give a final resistance of 5-8 M $\Omega$ . The patch-clamp amplifier was a List EPC-7 (Darmstadt, Germany). The effective corner frequency of the low-pass filter was 2 kHz in the experiments with Na<sup>+</sup> currents and 1 kHz in the experiments with K<sup>+</sup> currents. The frequency of digitization was at least twice that of the filter. The data were stored and analyzed by commercially available software (pCLAMP, Axon Instruments, Foster City, CA). Transients and leakage currents were digitally subtracted in all recordings using records with hyperpolarizing pulses. Offset potentials were nulled directly before formation of the seal. Errors in the clamped potential evoked by the series resistance of the electrode were not corrected. All experiments were performed at a room temperature of 21-23°C.

### Identification of Dorsal Horn Neurons

The dorsal horn neurons were identified in spinal cord slices as multipolar 8- to 12- $\mu$ m cells located in laminae I-III of the dorsal horn (fig. 1). Several criteria were used to distinguish between neuronal and glial cells. In spinal cord slices, some types of glial cells were shown to harbor some voltage-gated Na<sup>+</sup> channels. Full action potentials, however, could not be evoked by an injection

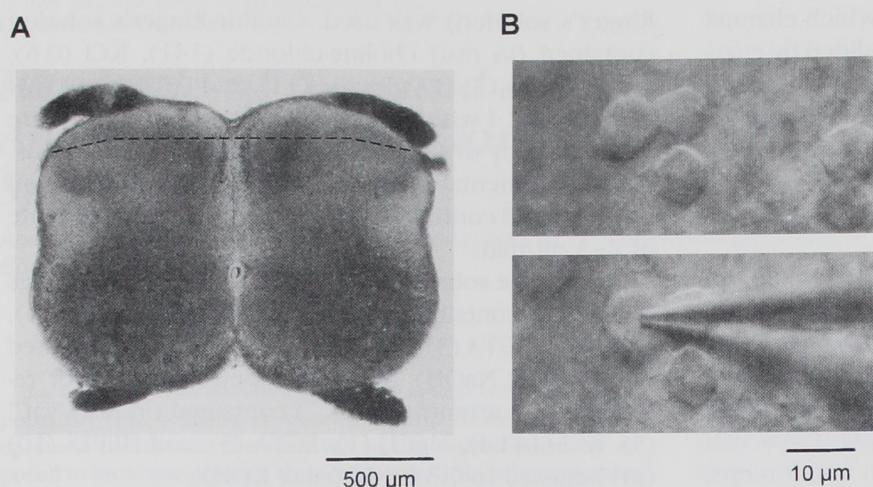


Fig. 1. Dorsal horn neurons in the spinal cord slice. (A) Slice from the lumbar enlargement (L3–L6) of 5-day-old rat spinal cord. Dorsal horn neurons were identified in laminae I–III of the spinal cord. The lower border of this region is indicated by a dashed line. (B) A dorsal horn neuron in the spinal cord slice. The same neuron is shown below during the whole-cell recording.

tion of the current pulses into the cell because of either small amplitude of  $\text{Na}^+$  currents or low ratios of  $\text{Na}^+$  to  $\text{K}^+$  or  $\text{Na}^+$  to leakage conductances.<sup>18</sup> Further, neurons but not glial cells demonstrated spontaneous synaptic activity.<sup>18</sup> In our experiments with  $\text{K}^+$  channels, the neurons were perfused with high- $\text{K}_i^+$  solution, and it was possible to record action potentials in external Ringer's solution. Only cells generating full action potentials were investigated. The resting potentials in the neurons studied were between  $-80$  and  $-50$  mV. In experiments with  $\text{Na}^+$  currents, the neurons were perfused with high- $\text{Cs}_i^+$  solution, and action potentials could not be recorded. A cell was considered a neuron if the amplitude of  $\text{Na}^+$  current exceeded 1 pA and if spontaneous synaptic currents were observed. The current study is based on recordings from 56 dorsal horn neurons.

### Statistical Analysis

The data points in concentration-effect curves were fitted using the nonlinear least-squares method as indicated in the legends to figures 3C and 5B. Fitted values are given as means  $\pm$  SE, and numerical values are presented as means  $\pm$  SEM.

### Sodium and Potassium Current Recording

Tetrodotoxin-sensitive  $\text{Na}^+$  currents were recorded in whole-cell patch-clamp mode from the somata of dorsal horn neurons in Ringer's-tetraethylammonium solution. The pipettes were filled with high- $\text{Cs}_i^+$  solution. To reduce the amplitude of  $\text{Na}^+$  currents and thus the voltage error due to the resistance in series, holding potential

was set to  $-80$  mV at which about 50% of  $\text{Na}^+$  channels were inactivated.  $\text{Na}^+$  currents were activated by 50-ms voltage steps to  $-30$  mV.

Whole-cell  $\text{K}^+$  currents were recorded in choline-Ringer's solution. The pipettes were filled with high- $\text{K}_i^+$  solution. The currents activated by depolarizing voltage steps consisted of rapidly inactivating  $\text{K}^+$  ( $\text{K}_A$ ) current and noninactivating delayed-rectifier ( $\text{K}_{DR}$ ) current components.<sup>17</sup> Figure 2 demonstrates the method used for separation of  $\text{K}_A$  and  $\text{K}_{DR}$  current components.  $\text{K}^+$  currents were activated by depolarization to  $+20$  mV, either after 150-ms prehyperpolarization to  $-120$  mV (total current =  $\text{K}_A + \text{K}_{DR}$  current) or after 150-ms predepolar-

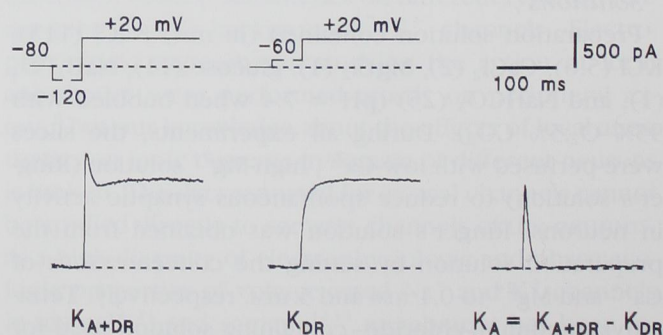


Fig. 2. Separation of a total voltage-gated  $\text{K}^+$  current on inactivating A ( $\text{K}_A$ ) and noninactivating delayed-rectifier ( $\text{K}_{DR}$ ) components in dorsal horn neurons. Total  $\text{K}^+$  current ( $\text{K}_A + \text{K}_{DR}$ ) was activated by a potential step to  $+20$  mV after a 150-ms prepulse to  $-120$  mV (left). The delayed-rectifier component of the  $\text{K}^+$  current ( $\text{K}_{DR}$ ) was activated by a voltage step to  $+20$  mV after a 150-ms prepulse to  $-60$  mV (middle). Inactivating A current was obtained by digital subtraction of delayed-rectifier current from total current:  $\text{K}_A = \text{K}_{A+DR} - \text{K}_{DR}$ .

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ization to  $-60$  mV ( $K_{DR}$  current). The amplitude of  $K_{DR}$  current was measured at the end of depolarizing pulses.  $K_A$  current was obtained as the difference between  $K_{A+DR}$  and  $K_{DR}$  current. The time constants of  $K_A$  current inactivation measured at  $+20$  mV were 8–20 ms. The contribution of  $K_A$  current to the total  $K^+$  current appeared to be fairly minimal compared with that of  $K_{DR}$  current (fig. 2, left). The current subtraction, however, revealed that the amplitudes of  $K_A$  current were approximately equal to or exceeded those of  $K_{DR}$  currents in typical dorsal horn neurons (fig. 2, middle and right).

In experiments studying the use-dependent block,  $Na^+$  and  $K^+$  currents were activated at a frequency of 1 Hz, first in control solution and then in the presence of local anesthetics.

Each slice was perfused for  $\geq 2$ –5 min with external solution containing different local anesthetic concentrations before the measurements were made. In all cases, the steady-state block was reached.

## Results

### Sodium Currents

The tonic block (binding of the blocker to closed channel) of  $Na^+$  currents by local anesthetics is shown in figure 3A. To minimize the contribution of the use-dependent block, we considered only the currents activated by the first depolarizing pulse in the presence of each new local anesthetic concentration. Externally applied bupivacaine at concentrations of 1–100  $\mu M$  blocked  $Na^+$  currents in a concentration-dependent manner. Less hydrophobic local anesthetics, such as lidocaine and mepivacaine, were not as potent in a tonic suppression of whole-cell  $Na^+$  currents (fig. 3B). The concentration–effect curves revealed half-maximum inhibiting concentrations ( $IC_{50}$ ; presented as mean  $\pm$  SE) of  $26 \pm 3$   $\mu M$  ( $n = 7$ ) for bupivacaine,  $112 \pm 8$   $\mu M$  ( $n = 5$ ) for lidocaine, and  $324 \pm 4$   $\mu M$  ( $n = 8$ ) for mepivacaine (fig. 3C). At a holding potential of  $-80$  mV, wash-out of  $Na^+$  channel blockade by external mepivacaine or lidocaine required  $\approx 10$  min, whereas that of bupivacaine took  $\approx 30$  min.

In the following experiments, the use dependence of  $Na^+$  current block by local anesthetics (*i.e.*, phasic block) was studied.  $Na^+$  currents were activated every second by voltage steps from  $-80$  to  $-30$  mV, first in control solution and then in the presence of local anesthetics. Control current and several consecutive currents recorded with 100- $\mu M$  bupivacaine are shown

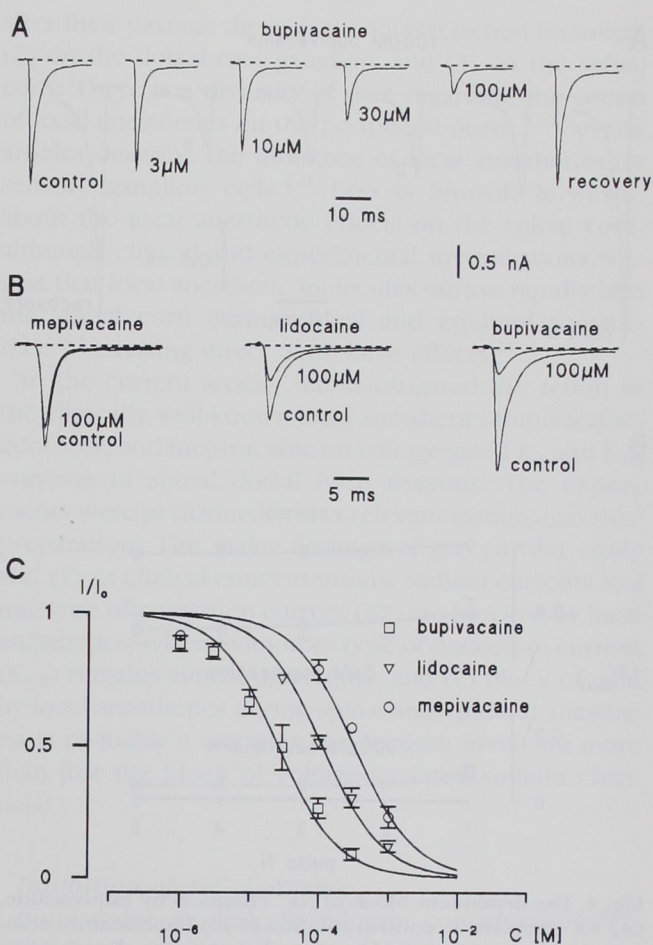
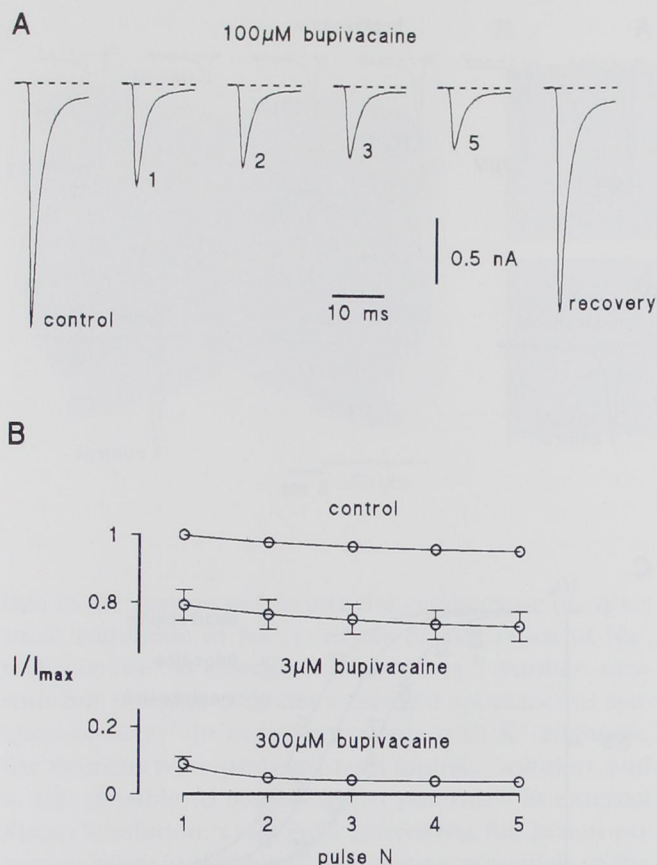


Fig. 3. Blockade of  $Na^+$  currents in dorsal horn neurons by bupivacaine, lidocaine, and mepivacaine. (A) Whole-cell  $Na^+$  currents activated by voltage steps from a holding potential of  $-80$  to  $-30$  mV in the presence of different concentrations of bupivacaine (indicated near the corresponding traces). (B) Comparison of potencies of channel block produced by 100- $\mu M$  mepivacaine, lidocaine, and bupivacaine.  $Na^+$  currents were activated by voltage steps from  $-80$  to  $-30$  mV. (C) Concentration dependence of  $Na^+$  current block by bupivacaine (squares), lidocaine (triangles), and mepivacaine (circles). Data points were fitted using a nonlinear least-squares method with the equation:  $f(C) = 1 - C/(C + IC_{50})$ , where  $C$  is the blocker concentration and  $IC_{50}$  is the constant of dissociation.  $IC_{50}$  values (mean  $\pm$  SEM) were  $26 \pm 3$   $\mu M$  for bupivacaine ( $n = 7$ ),  $112 \pm 8$   $\mu M$  for lidocaine ( $n = 8$ ), and  $324 \pm 4$   $\mu M$  for mepivacaine ( $n = 5$ ). Error bars indicate  $\pm$ SEM if exceeding symbol size.

in figure 4A. The amplitudes of peak  $Na^+$  currents as a function of pulse number for 0- (control solution), 3-, and 300- $\mu M$  bupivacaine ( $n = 8, 8$ , and 6, respectively) are given in figure 4B.  $Na^+$  currents recorded in the presence of local anesthetic were normalized to first currents recorded in control solution. The strongest



**Fig. 4.** Use-dependent block of  $\text{Na}^+$  channels by bupivacaine. (A)  $\text{Na}^+$  currents in control solution ( $0\text{-}\mu\text{M}$  bupivacaine) compared with those activated by several consecutive depolarizing pulses at a frequency of 1 Hz in the presence of  $100\text{-}\mu\text{M}$  bupivacaine (the number of the pulse is shown near the corresponding trace). The currents were activated by voltage pulses from  $-80$  to  $-30$  mV. (B) Normalized amplitudes of  $\text{Na}^+$  currents recorded in control solution and in the presence of 3- and  $300\text{-}\mu\text{M}$  bupivacaine as a function of pulse number. Each corresponding current was normalized to the first  $\text{Na}^+$  current recorded in control solution. Error bars indicate  $\pm\text{SEM}$  if exceeding symbol size.

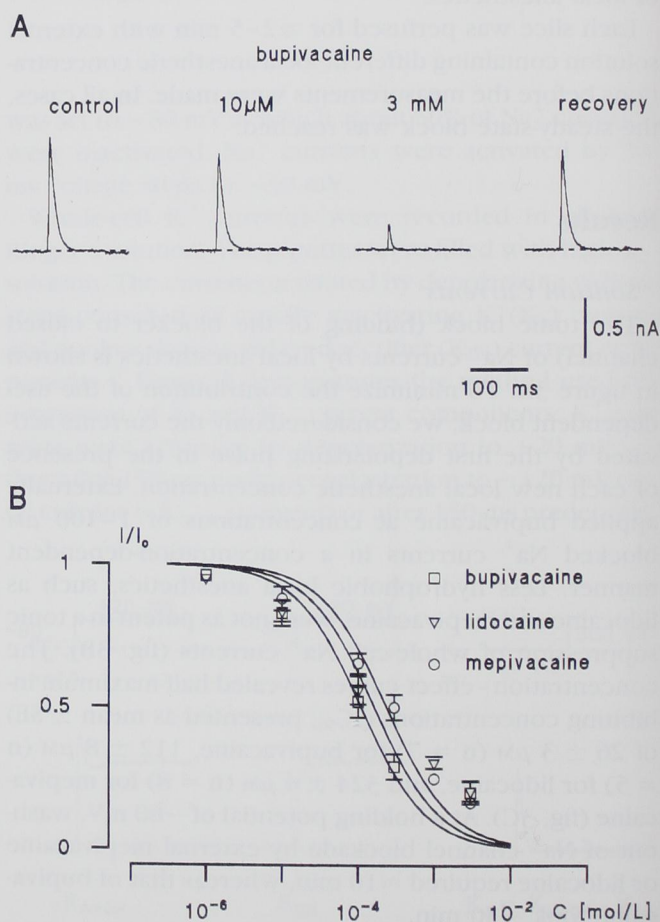
reduction in the amplitude was seen in the currents activated by the first two pulses (N1 and N2; fig. 4B). In the presence of  $300\text{-}\mu\text{M}$  bupivacaine, the  $\text{Na}^+$  current activated by the second pulse was reduced by  $40.0 \pm 4.9\%$  ( $n = 6$ ) compared with that activated by the first one. Use dependence of block was less pronounced at lower local anesthetic concentrations. A reduction in the amplitudes of only  $3.4 \pm 0.5\%$  ( $n = 8$ ) was seen between the first two pulses in  $3\text{-}\mu\text{M}$  bupivacaine. For comparison, the reduction of the second  $\text{Na}^+$  current in the absence of bupivacaine (control solution), which resulted from incomplete recovery of  $\text{Na}^+$  channels

from inactivation during 1-s intervals between depolarizing pulses, was  $2.1 \pm 0.4\%$  ( $n = 8$ ).

Similar use dependence of  $\text{Na}^+$  current blockade was observed also for lidocaine and mepivacaine (data not shown). In general, observed use-dependent block of  $\text{Na}^+$  channels by local anesthetics was very similar to that observed in several other preparations.<sup>8</sup>

#### Inactivating Potassium and Delayed-Rectifier Currents

Rapidly inactivating  $\text{K}^+$  currents were sensitive to externally applied local anesthetics at concentrations of  $1\text{-}\mu\text{M}$ – $3\text{-mM}$  (figs. 5A and 5B). The highest applied concen-



**Fig. 5.** Effect of local anesthetics on  $\text{K}_A$  currents. (A)  $\text{K}_A$  current in control solution and in the presence of  $10\text{-}\mu\text{M}$  and  $3\text{-mM}$  bupivacaine. (B) Concentration dependence of  $\text{K}_A$  current block by local anesthetics. The points were fitted by the equation:  $f(C) = 1 - C/(C + \text{IC}_{50})$ , where C is the blocker concentration and  $\text{IC}_{50}$  is the constant of dissociation.  $\text{IC}_{50}$  values (mean  $\pm$  SE) were  $109 \pm 16\text{-}\mu\text{M}$  for bupivacaine ( $n = 8$ ),  $163 \pm 31\text{-}\mu\text{M}$  for lidocaine ( $n = 7$ ), and  $236 \pm 28\text{-}\mu\text{M}$  for mepivacaine ( $n = 5$ ). Error bars indicate  $\pm\text{SEM}$  if exceeding symbol size.

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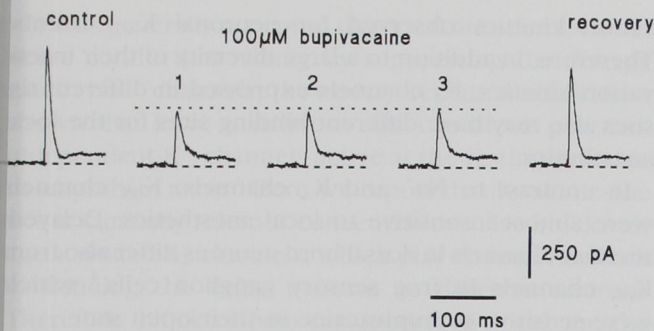


Fig. 6. Lack of use dependency of  $K_A$  current block by local anesthetics.  $K_A$  currents obtained in control solution and three consecutive  $K_A$  currents activated with 1-s interval in the presence of 100- $\mu$ M bupivacaine.

trations of local anesthetics (1–3 mM) suppressed up to 80% of the current. The  $IC_{50}$  values (mean  $\pm$  SE) for tonic block by bupivacaine, lidocaine, and mepivacaine were  $109 \pm 16 \mu\text{M}$  ( $n = 8$ ),  $163 \pm 31 \mu\text{M}$  ( $n = 7$ ), and  $236 \pm 28 \mu\text{M}$  ( $n = 5$ ), respectively (fig. 5B).

In contrast to a difference of one order of magnitude in  $IC_{50}$  values obtained in our experiments with  $Na^+$  currents (fig. 3C), those values for the  $K_A$  current block by local anesthetics were in a narrow range of concentrations and, therefore, were only weakly dependent on lipid solubility of the drugs. The block of  $K_A$  currents by the local anesthetics was not use dependent (data shown for bupivacaine in fig. 6).

Externally applied QX-314, a hydrophilic quaternary derivative of lidocaine, which permanently carries a positive charge and cannot penetrate the membrane, did not reduce the amplitude of  $K_A$  currents (3-mM concentration,  $n = 4$ ; data not shown).

In contrast to  $Na^+$  and  $K_A$  currents,  $K_{DR}$  currents were only slightly reduced by externally applied mepivacaine ( $n = 7$ ), lidocaine ( $n = 7$ ), and bupivacaine ( $n = 5$ ), as demonstrated in figure 7.

## Discussion

Numerous studies performed during the past 40 yr have shown that the mechanisms of the local anesthetic action during epidural and spinal anesthesia are complex<sup>19</sup> and cannot be interpreted as a simple suppression of an ion conductance in the axonal membrane. For a more comprehensive understanding of the principles of local anesthesia in this region, it is necessary to consider the action of local anesthetics on three major sites: (1) on mixed nerves in the paravertebral spaces

after their passage through the intervertebral foramina; (2) on the dorsal root ganglion; and (3) on the spinal cord. There is a diversity of data regarding the action of local anesthetics on the peripheral nerve.<sup>8,20</sup> Several articles describe the influence of local anesthetics on sensory ganglion cells.<sup>4,21</sup> Less is known, however, about the local anesthetic effects on the spinal cord, although clinical and experimental investigations suggest that local anesthetic molecules diffuse rapidly into the spinal cord during spinal and epidural anesthesia<sup>3,22,23</sup> exerting direct depressive effects.<sup>23</sup>

In the current article, we investigated the action of the clinically well-known local anesthetics bupivacaine, lidocaine, and mepivacaine on voltage-gated  $K_A$  and  $K_{DR}$  currents in spinal dorsal horn neurons. The experiments were performed with a relevant mammalian slice preparation. The major findings of the current study are: (1) At clinical concentrations, sodium currents and one type of potassium current ( $K_A$ ) are blocked by local anesthetics, whereas another type of potassium current ( $K_{DR}$ ) remains almost unaffected; and (2) Block of pain by local anesthetics during spinal and epidural anesthesia is probably a complex mechanism, involving more than just the block of voltage-activated sodium channels.

### Inhibition of $Na^+$ Currents

Bupivacaine at clinically relevant concentrations<sup>24,25</sup> and lidocaine and mepivacaine exhibited both tonic and phasic blocks of  $Na^+$  currents in the membranes of dorsal horn neurons.  $IC_{50}$  values for tonic block were 26, 112, and 324  $\mu\text{M}$  for bupivacaine, lidocaine, and mepivacaine, respectively. Similar values were reported for the tonic inhibition of  $Na^+$  currents by bupivacaine in amphibian node of Ranvier<sup>4</sup> (25  $\mu\text{M}$ ) and in frog gan-

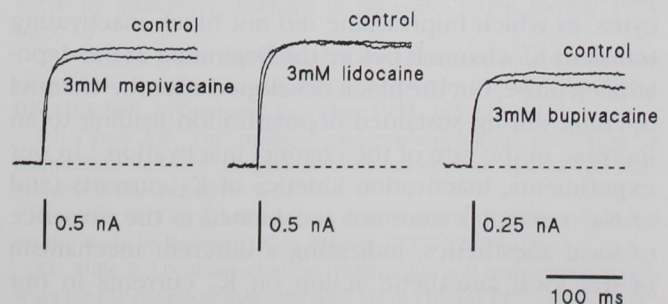


Fig. 7. Effect of local anesthetics on  $K_{DR}$  current.  $K_{DR}$  currents in the presence and absence of 3-mM mepivacaine, 3 mM lidocaine, and 3-mM bupivacaine.

gion cells<sup>4</sup> (95 and 160  $\mu\text{M}$ ). The potency of local anesthetics obtained in the current study increased with their hydrophobicity,<sup>26</sup> suggesting that (1) Externally applied local anesthetic molecules had to diffuse through the neuronal membrane before they could reach a specific binding site on the channel molecule, or (2) A hydrophobic interaction exists between local anesthetic molecules and the binding site itself.

In our experiments, the potency of local anesthetic action was increased during repetitive cell stimulation. Such a use-dependent block is usually explained by a higher sensitivity of open or inactivated  $\text{Na}^+$  channels to local anesthetics, compared with their sensitivity in the "resting" state (modulated-receptor model).<sup>20,27,28</sup> Therefore,  $\text{IC}_{50}$  values for the tonic block reported here may underestimate the real channel sensitivity to local anesthetics, and lower local anesthetic concentrations may be sufficient for block of  $\text{Na}^+$  channels during repetitive firing in neurons under normal physiologic conditions. Further, in this study, the neurons were held at a potential of  $-80$  mV, which appears to be more negative than the resting potential in neurons *in vivo*. More depolarized resting potentials in dorsal horn neurons *in vivo* would lead to stronger steady-state inactivation of  $\text{Na}^+$  channels and, as a consequence, to their additional blockade by local anesthetics.

#### Blockade of $\text{K}^+$ Currents

$\text{K}_\text{A}$  channels play an important role in defining the firing patterns in somata of different neurons.<sup>29</sup> In our experiments, local anesthetics exhibited tonic but not phasic block of  $\text{K}_\text{A}$  currents in the membrane of spinal dorsal horn neurons, with  $\text{IC}_{50}$  values of 109, 163, and 236  $\mu\text{M}$  for bupivacaine, lidocaine, and mepivacaine, respectively.

Block of  $\text{K}_\text{A}$  currents by local anesthetics observed in the current study was not similar to that reported for transient outward  $\text{K}^+$  currents in rat ventricular myocytes, in which bupivacaine did not block inactivating transient  $\text{K}^+$  channels before the beginning of the depolarizing pulse, but the block developed after the channel opening during sustained depolarization leading to an increase in the rate of the channel inactivation.<sup>5</sup> In our experiments, inactivation kinetics of  $\text{K}_\text{A}$  currents (and of  $\text{Na}^+$  currents) were not accelerated in the presence of local anesthetics, indicating a different mechanism of the local anesthetic action on  $\text{K}_\text{A}$  currents in our preparation. Moreover,  $\text{K}_\text{A}$  currents in ventricular myocytes inactivated relatively slowly, with a time constant of about 75 ms, in contrast to the fast 8- to 20-ms inacti-

vation kinetics observed for neuronal  $\text{K}_\text{A}$  currents. Therefore, in addition to a large diversity of their inactivation kinetics,  $\text{K}_\text{A}$  channels expressed in different tissues also may have different binding sites for the local anesthetics.

In contrast to  $\text{Na}^+$  and  $\text{K}_\text{A}$  channels,  $\text{K}_\text{DR}$  channels were almost insensitive to local anesthetics. Delayed-rectifier channels in dorsal horn neurons differ also from  $\text{K}_\text{DR}$  channels in frog sensory ganglion cells,<sup>4</sup> which were sensitive to bupivacaine in their open state.

Thus, in addition to the well-known diversity of the electrophysiologic properties of  $\text{K}^+$  channels and their different levels of sensitivity to classical  $\text{K}^+$  channel blockers, such as tetraethylammonium and 4-aminopyridine,  $\text{K}^+$  channels in neuronal membranes also may differ regarding their levels of sensitivity to local anesthetics.

#### Binding Sites for Local Anesthetics

It has been suggested that local anesthetic molecules can reach the binding site at the  $\text{Na}^+$  channel on two different pathways.<sup>30-32</sup> On the hydrophilic pathway, local anesthetic molecules can diffuse through the cell membrane and block the open channel from inside. The blocker can be trapped in the channel if the gates are closed, and it can leave the channel only after gate reopening. In the hydrophobic pathway, external local anesthetic molecules can diffuse directly to the binding site within the membrane and can leave it without channel reopening. These two pathways could explain tonic and phasic block of  $\text{Na}^+$  currents observed in the current study. A diffusion of the local anesthetic molecule through the cell membrane also may be the reason for the higher potency of the more hydrophobic local anesthetics obtained for tonic  $\text{Na}^+$  current block.

In contrast to  $\text{Na}^+$  currents, the block of  $\text{K}_\text{A}$  currents by local anesthetics was not use dependent. Therefore, we suppose that local anesthetics can block open and closed  $\text{K}_\text{A}$  channels equally. More hydrophobic local anesthetics were only slightly more potent in blocking  $\text{K}_\text{A}$  currents, indicating that the interaction between the local anesthetic molecule and the binding site is less hydrophobic for the  $\text{K}_\text{A}$  channel than for the  $\text{Na}^+$  channel. Further, a quaternary derivative of lidocaine, QX-314, which does not penetrate the membrane, failed to block  $\text{K}_\text{A}$  currents when applied externally. This is consistent with the idea that the binding site for local anesthetics is not directly accessible from the external side of the membrane and therefore may be located either within the membrane or on its inner side.

## LOCAL ANESTHETICS BLOCK ION CHANNELS IN SPINAL NEURONS

It was generally accepted that local anesthetics block nerve conduction mainly by suppressing the voltage-gated  $\text{Na}^+$  channels; however, recent studies have shown that local anesthetics also can block potential-independent  $\text{K}^+$  channels active at the resting potential in amphibian axon.<sup>6</sup> Here we report that local anesthetics at clinically relevant concentrations also block voltage-activated  $\text{K}_A$  channels, which play an important role in defining the firing patterns in different neurons. Therefore, block of pain conduction by local anesthetics should be considered as a complex mechanism, which includes effects on the resting membrane potential and on neuronal firing due to suppression of different types of  $\text{K}^+$  channels.

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