

# Meperidine Suppresses the Excitability of Spinal Dorsal Horn Neurons

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**Background:** In addition to local anesthetics, meperidine has been successfully used for local anesthesia. When applied intrathecally, the dorsal horn neurons of the superficial laminae are exposed to high concentrations of meperidine. These cells represent an important point for the transmission of pain information. This study investigated the blocking effects of meperidine on different ionic currents of spinal dorsal horn neurons and, in particular, its impact on the generation of action potentials.

**Methods:** Using a combination of the patch clamp technique and the entire soma isolation method, the action of meperidine on voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  currents in spinal dorsal horn neurons of rats was described. Current clamp recordings from intact neurons showed the functional relevance of the ion current blockade for the generation of action potentials.

**Results:** Externally applied meperidine reversibly blocked voltage-gated  $\text{Na}^+$  currents with a half-maximum inhibiting concentration ( $\text{IC}_{50}$ ) of 112  $\mu\text{M}$ . During repetitive stimulation, a slight phasic block occurred. In addition, A-type  $\text{K}^+$  currents and delayed-rectifier  $\text{K}^+$  currents were affected in a dose-dependent manner, with  $\text{IC}_{50}$  values of 102 and 52  $\mu\text{M}$ , respectively. In the current clamp mode, single action potentials were suppressed by meperidine. The firing frequency was lowered to 54% at concentrations (100  $\mu\text{M}$ ) insufficient for the suppression of a single action potential.

**Conclusions:** Meperidine inhibits the complex mechanism of generating action potentials in spinal dorsal horn neurons by the blockade of voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels. This can contribute to the local anesthetic effect of meperidine during spinal anesthesia.

SYSTEMICALLY applied meperidine is widely used for analgesia and anesthesia. In addition, meperidine produces conduction block when applied locally to peripheral nerve, suggesting that it is a local anesthetic.<sup>1,2</sup> Subarachnoid application of meperidine results in spinal anesthesia<sup>3,4</sup> including motor blockade<sup>4-6</sup> sufficient for surgery of the lower limbs,<sup>3</sup> the perineum,<sup>4</sup> urologic surgery,<sup>6,7</sup> and cesarean delivery.<sup>8</sup> Meperidine is also useful for intravenous anesthesia either as a sole agent<sup>9,10</sup> or in combination with local anesthetics.<sup>11,12</sup>

In *in vitro* experiments, meperidine blocks action po-

tential conduction in peripheral nerves<sup>13</sup> and muscle<sup>14</sup> by inhibiting voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels.

Spinal dorsal horn neurons receive their inputs from primary afferent terminals and therefore participate in processing the sensory information from noxious receptors. In contrast to the peripheral nerve, which only conducts the action potential in the region where local anesthetics are applied during spinal anesthesia, dorsal horn neurons must generate an action potential before transmitting it to supraspinal targets. During spinal and epidural anesthesia, the applied drugs diffuse directly into the spinal cord,<sup>15</sup> especially to the superficial neurons in laminae I and II, which are important for the transmission of primary sensory input such as pain and thermoception.<sup>16-18</sup> Although most clinical studies regarding the local anesthetic effects of meperidine deal with its effects on the spinal cord,<sup>1,3,7,8,19-21</sup> the majority of electrophysiologic studies were performed on peripheral nerves or on artificially expressed ion channels.<sup>2,13,22-25</sup> These experiments demonstrated the effects of meperidine on voltage-gated ion channels and on action potential conduction, which is explained by blockade of voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels. However, it remains unclear how meperidine influences the generation of action potentials in superficial spinal dorsal horn neurons.

In this study, we investigated the effects of meperidine on the generation of single action potentials and on series of action potentials in spinal dorsal horn neurons of the rat. Furthermore, the effect on voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels was investigated.

## Materials and Methods

### Preparation

Experiments were performed by means of the patch clamp technique<sup>26</sup> on 200- $\mu\text{m}$  slices cut from lumbar enlargements (L3-L6) of the spinal cord of 3- to 11-day-old rats.<sup>27-29</sup> The animals were rapidly decapitated, and the spinal cords were carefully cut out in ice-cold preparation solution bubbled with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . After removal of the pial membrane with fine forceps, the spinal cord was embedded in a preparation solution containing 2% agar cooled down to 39°C. To accelerate solidification of the agar, the beaker with the preparation was placed in ice-cold water. The agar block containing the lumbar enlargement of the spinal cord was cut out and glued to a glass stage fixed in the chamber of the tissue slicer. The spinal cord was sliced in ice-cold preparation solution under continuous bubbling. The

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slices were thereafter incubated for 45 min at 32°C. The procedures of animal decapitation have been reported to the local veterinarian authority and are in accordance with the German guidelines.

### Solutions

Preparation solution contained 115 mM NaCl, 5.6 mM KCl, 2 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 11 mM glucose, 1 mM  $\text{NaH}_2\text{PO}_4$ , and 25 mM  $\text{NaHCO}_3$  (pH 7.4 when bubbled with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ ). In the experimental chamber, the slices were superfused by low- $\text{Ca}^{2+}$  solution to reduce spontaneous synaptic activity and to prevent activation of  $\text{Ca}^{2+}$  currents and  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  currents. Low- $\text{Ca}^{2+}$  solution was obtained from the preparation solution by setting the concentration of  $\text{Ca}^{2+}$  to 0.1 mM and increasing the concentration of  $\text{Mg}^{2+}$  to 5 mM (referred to as Ringer's solution). Tetraethylammonium-containing solution (TEA solution) used for  $\text{Na}^+$  current recordings contained 95 mM NaCl, 5.6 mM KCl, 0.1 mM  $\text{CaCl}_2$ , 5 mM  $\text{MgCl}_2$ , 11 mM glucose, 1 mM  $\text{NaH}_2\text{PO}_4$ , 25 mM  $\text{NaHCO}_3$ , and 20 mM TEA-Cl (pH 7.4 when bubbled with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ ). The study of  $\text{K}^+$  currents was performed in  $\text{Na}^+$ -free choline-Cl solution containing 141 mM choline-Cl, 0.6 mM KCl, 0.1 mM  $\text{CaCl}_2$ , 5 mM  $\text{MgCl}_2$ , 11 mM glucose, and 10 mM HEPES; pH 7.4 was adjusted with 1 M KOH. KCl was then added to give a final  $\text{K}^+$  concentration of 5.6 mM.

Meperidine (Dolantin®; Aventis Pharma, Bad Soden, Germany) was directly added to control solutions. The experimental chamber with a volume of 0.4 ml was continuously perfused by external solution at a rate of 2-3 ml/min; the pH of solutions containing drug was tested and corrected to eliminate potential pH-induced effects.

The pipette solution used for  $\text{K}^+$  current recordings from intact neurons contained 5 mM NaCl, 144.4 mM KCl, 1 mM  $\text{MgCl}_2$ , 3 mM EGTA, and 10 mM HEPES; pH 7.3 was adjusted with 1 M KOH. KCl was then added to give a final  $\text{K}^+$  concentration of 155 mM. The pipette solution used for  $\text{K}^+$  current recordings in experiments with isolated somata contained 5 mM NaCl, 144.4 mM KCl, 1 mM  $\text{MgCl}_2$ , 3 mM EGTA, and 10 mM HEPES; pH 7.3 was adjusted with 1 M NaOH. NaCl was then added to give a final  $\text{Na}^+$  concentration of 15 mM. In experiments with  $\text{Na}^+$  currents, the pipette solution contained 5.8 mM NaCl, 134 mM CsCl, 1 mM  $\text{MgCl}_2$ , 3 mM EGTA, and 10 mM HEPES; pH 7.3 was adjusted with 1 M NaOH. NaCl was then added to give a final  $\text{Na}^+$  concentration of 15 mM.

### Recording Conditions

Pipettes pulled from borosilicate glass tubes (GC 150; Clark Electromedical Instruments, Pangbourne, United Kingdom) were fire polished to give a final resistance of 3-7 M $\Omega$ . The patch clamp amplifier was an Axopatch 200B (Axon Instruments, Foster City, CA). In experiments with  $\text{Na}^+$  currents, the effective corner frequency

of the low-pass filter was 5 kHz;  $\text{K}^+$  currents were filtered with 1 kHz. The frequency of digitization was twice that of the filter frequency. In current clamp mode, action potentials were filtered with 5 kHz and sampled at 10 kHz. The data were stored and analyzed using commercially available software (pCLAMP; Axon Instruments). Transients and leakage currents were digitally subtracted in all experiments using records with hyperpolarizing pulses that activated no currents. 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. For currents recorded in the isolated somata, voltage errors due to resistance in series in most cases did not exceed 4 mV. All experiments were performed at room temperature of 21-23°C.

Ion currents were investigated in voltage clamp mode.  $\text{Na}^+$  currents were recorded in external TEA solution using pipettes filled with CsCl solution. They were activated by a voltage step to -20 mV after a 50-ms prepulse to -120 mV. Holding potential was -80 mV. For investigation of use-dependent blockade (30 pulses at 2, 5, and 10 Hz), no hyperpolarizing prepulse was applied.  $\text{K}^+$  currents were recorded in external choline-Cl solution.  $\text{K}^+$  currents were separated on the basis of a procedure described previously.<sup>30</sup> Total  $\text{K}^+$  currents activated by depolarizing steps to +40 mV after a 150-ms prepulse to -120 mV consisted of both rapidly inactivating A-type and delayed-rectifier components. A similar depolarization applied after a 150-ms prepulse to -60 mV (which almost completely inactivates  $\text{K}_A$  current) elicited only a noninactivating component of  $\text{K}^+$  current, considered to be delayed-rectifier current. The amplitudes of the  $\text{K}_{DR}$  currents were measured at the end of a 250-ms depolarizing pulse.

Action potentials were recorded in current clamp mode. To make the action potentials or trains of action potentials comparable, we kept the membrane potential at approximately -80 mV in current clamp experiments by injecting sustained depolarizing or hyperpolarizing currents through the recording electrode. The duration of the current pulse was 10 ms for single action potentials and 500 ms for series of action potentials.

To reduce the resistance in series, experiments in voltage clamp mode were performed using the method of entire soma isolation (see The Method of Entire Soma Isolation). The identification of a neuron in the spinal cord slice was followed by the isolation procedure. In isolated somata, the voltage errors due to the resistance in series were smaller than 4 mV.

### Identification of Dorsal Horn Neurons

The dorsal horn neurons were identified in spinal cord slices as multipolar cells with a soma (8- to 12- $\mu\text{m}$  diameter) located in laminae I-III. Neurons were distinguished from glial cells in voltage clamp mode on the

basis of a procedure described previously.<sup>29</sup> In all neurons studied, a large  $\text{Na}^+$  current exceeding 1 nA could be elicited; they were able to generate action potentials and sometimes showed spontaneous synaptic activity. The resting potentials measured in intact neurons lay between  $-84$  and  $-50$  mV.

#### Method of Entire Soma Isolation

A detailed description of the entire soma isolation method has been given elsewhere.<sup>29</sup> Briefly, in whole cell recording mode, the entire soma of the neuron was isolated from the slice by slow withdrawal of the recording pipette. The isolated structure was classified as *soma* if it had lost all of its processes during isolation and preserved only 10–20% of original  $\text{Na}^+$  current recorded from the neuron in the slice before its isolation. The isolated structure was classified as *soma-plus-axon complex* if it contained one process and preserved more than 90% of the original  $\text{Na}^+$  current. The sound physiologic condition of an isolated structure was confirmed by a considerable increase in its input resistance (reflecting a decrease in membrane leakage conductance), by stable or even improved membrane resting potentials, and by the ability of soma-plus-axon complex to be excitable *i.e.*, to generate action potentials.

#### Statistical Analysis and Fitting

The current study is based on recordings from 20 intact neurons in the spinal cord slice and 61 isolated somata. Numerical values are given as mean  $\pm$  SEM. The parameters obtained by fitting the data points are given as mean  $\pm$  SE.

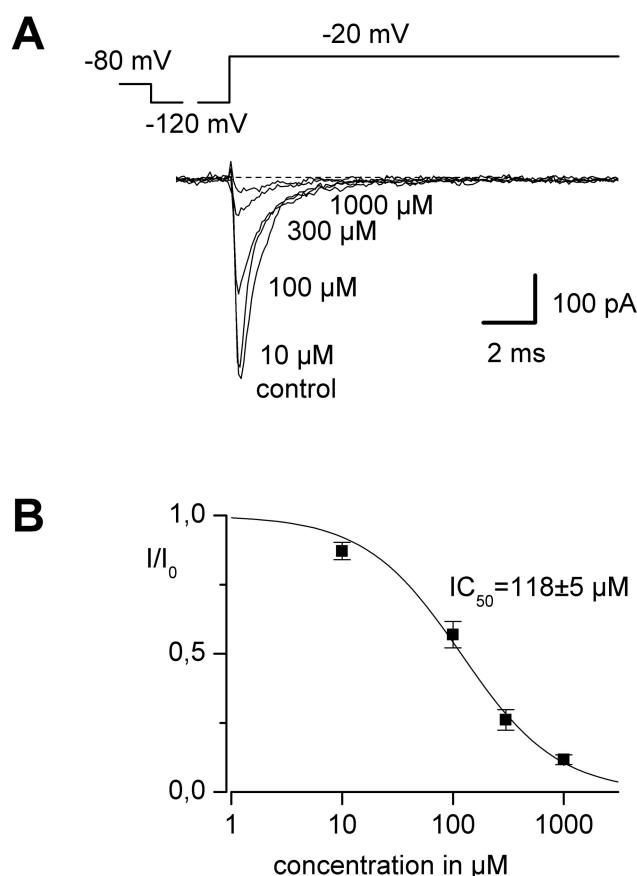
Data points were fitted using a nonlinear least-squares method with the equation:  $f(C) = 1 - \text{RES} \times C/(C + \text{IC}_{50})$ , where  $C$  is the blocker concentration,  $\text{IC}_{50}$  is the half-maximum inhibiting concentration, and RES is the fraction of current that cannot be blocked by meperidine (only needed for the  $\text{K}_{\text{DR}}$  currents). Because the Hill coefficient was less than 1 (0.93 for  $\text{I}_{\text{Na}}$  and  $\text{K}_{\text{A}}$  and 0.75 for  $\text{K}_{\text{DR}}$ ), in all curves, it was set to 1 to account for a 1:1 binding.

Intergroup differences were assessed by a factorial analysis of variance with *post hoc* analysis with the Fisher least significant difference test. The Student paired *t* test was used to compare the relative amplitudes of  $\text{Na}^+$  currents at the 30th pulse before and after 100  $\mu\text{M}$  meperidine (phasic blockade) and threshold potentials in control and after application of meperidine. *P* values less than 0.05 were considered significant.

## Results

#### $\text{Na}^+$ Currents

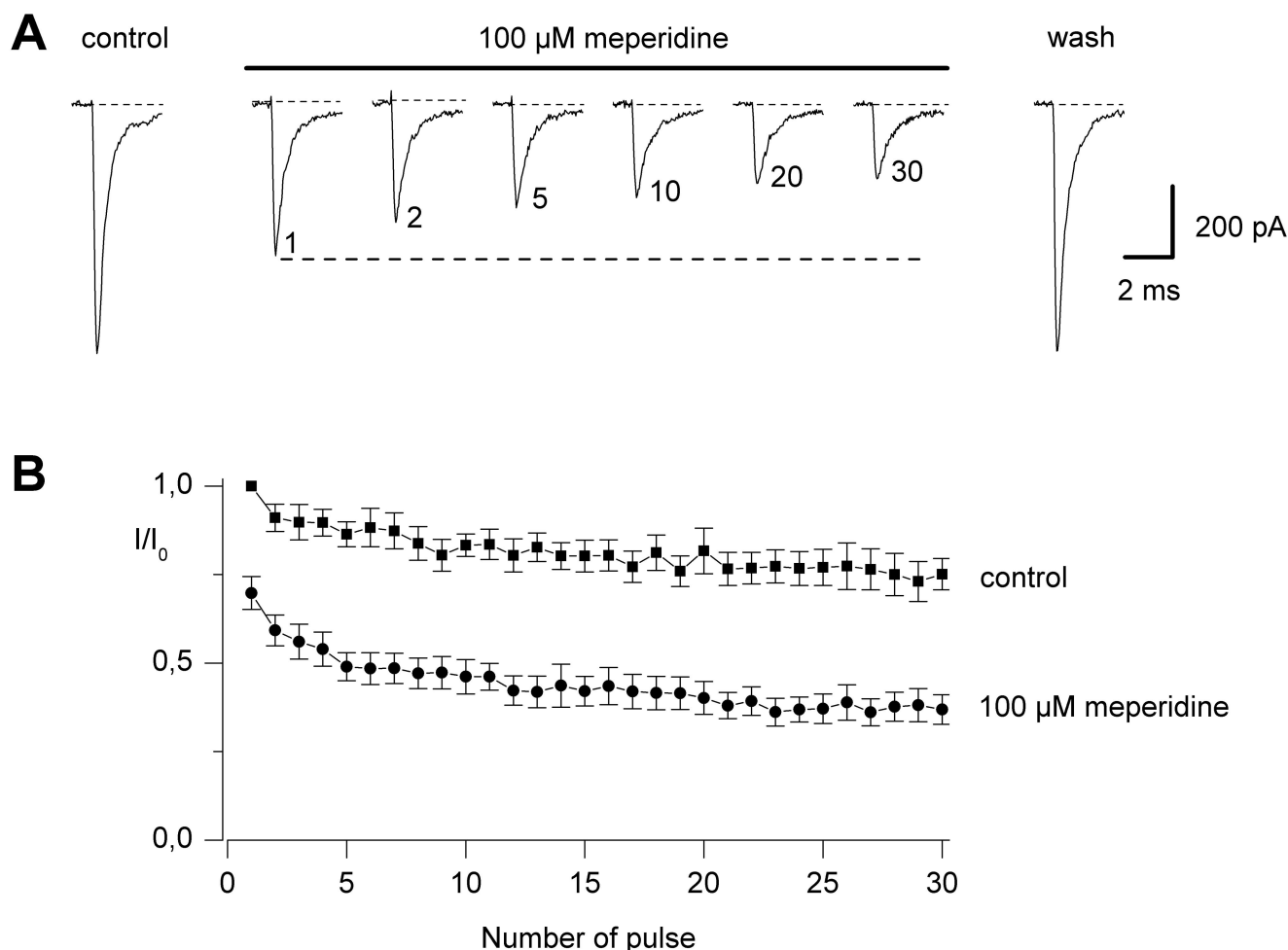
$\text{Na}^+$  currents were recorded from isolated somata of dorsal horn neurons in external TEA solution using pi-



**Fig. 1.** Effect of meperidine on the inactivating  $\text{Na}^+$  current. (A) Recordings of the tetrodotoxin-sensitive  $\text{Na}^+$  current in control solution and in the presence of 10, 100, 300, and 1,000  $\mu\text{M}$  meperidine. The currents were activated by a voltage step to  $-20$  mV after a 50-ms prepulse to  $-120$  mV. Holding potential was  $-80$  mV. (B) Concentration dependence of the  $\text{Na}^+$  current suppression by meperidine (13 somata). The  $\text{IC}_{50}$  value for  $\text{Na}^+$  currents was  $118 \pm 5 \mu\text{M}$ . The currents ( $I$ ) were normalized by the amplitude of the corresponding current recorded in control solution ( $I_0$ ).

pettes filled with  $\text{Cs}^+$  containing solution. Externally applied meperidine at concentrations ranging from 10 to 1,000  $\mu\text{M}$  reversibly blocked the peak  $\text{Na}^+$  current in a concentration-dependent manner (fig. 1A). Figure 1B depicts currents of 13 experiments normalized to control recordings and plotted to the corresponding concentration of meperidine. These dose-response curve was fitted with the Hill equation, assuming that one blocker molecule interacts with one receptor: The corresponding half-maximum inhibiting concentration ( $\text{IC}_{50}$ ) was  $118 \pm 5 \mu\text{M}$ . The addition of naloxone (0.1 and 1  $\mu\text{M}$ ,  $n = 6$ ; not shown) did not influence meperidine (100  $\mu\text{M}$ ) block of  $\text{Na}^+$  current.

To investigate the use dependence of  $\text{Na}^+$  current block by meperidine,  $\text{Na}^+$  currents were activated at a frequency of 2 Hz first in control solution and then in the presence of 100  $\mu\text{M}$  meperidine. The current in control and several consecutive currents recorded in 100  $\mu\text{M}$  meperidine are shown in figure 2A. Figure 2B shows the



**Fig. 2.** Use-dependent block of Na<sup>+</sup> currents by meperidine. (A) Na<sup>+</sup> currents in control solution (left) and in the presence of 100 μM meperidine (middle) activated by 30 consecutive depolarizing pulses at a frequency of 2 Hz (the pulse number is indicated near the corresponding trace). The currents were activated by a 10-ms voltage step to −20 mV. Holding potential was −80 mV. The effects of meperidine on Na<sup>+</sup> currents are reversible (right). (B) Normalized amplitudes of Na<sup>+</sup> currents recorded in control solution and in the presence of 100 μM meperidine as a function of pulse number. Each current was normalized to the amplitude of the first Na<sup>+</sup> current recorded in control solution (8 somata). Stronger effects can be observed at higher frequencies (5 and 10 Hz).

peak amplitudes of the Na<sup>+</sup> currents in control and after addition of 100 μM meperidine normalized to the amplitude of the first current recorded in control solution ( $n = 8$ ). In the absence of meperidine, the amplitude of the 30th current was reduced by 25%, probably because of the insufficient recovery of Na<sup>+</sup> channels from slow inactivation. In the presence of 100 μM meperidine, the relative amplitude was reduced from 0.75 to 0.37 ( $P < 0.001$ ) at the 30th pulse. This corresponds to a current reduction of 49% caused by slow recovery from inactivation and by use dependent blockade. A similar effect could be observed at frequencies of 5 and 10 Hz (table 1).

#### K<sup>+</sup> Currents

K<sup>+</sup> currents were recorded from isolated somata of spinal dorsal horn neurons in external choline-Cl solution using pipettes filled with high-K<sup>+</sup> solution. Meperidine was applied externally at concentrations ranging from 10 to 3,000 μM. Fast inactivating K<sub>A</sub> currents were

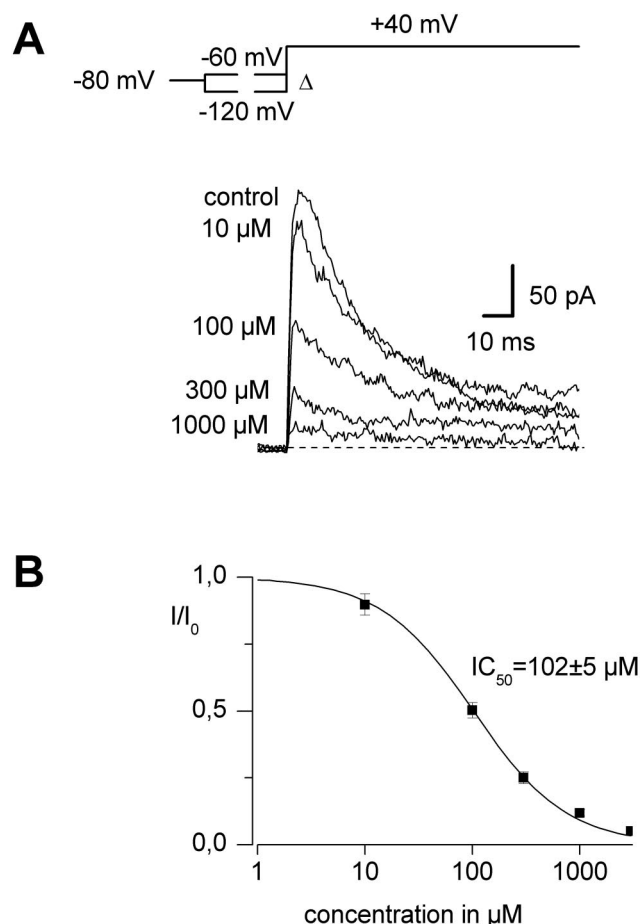
blocked by externally applied meperidine (fig. 3A). The corresponding half-maximum inhibiting concentration was  $102 \pm 5 \mu\text{M}$  ( $n = 34$ ; fig. 3B). A slightly higher sensitivity to meperidine could be observed in K<sub>DR</sub> currents (fig. 4A). The dose-response curve was fitted with the Hill equation, giving an IC<sub>50</sub> value of  $52 \pm 10 \mu\text{M}$  ( $n = 31$ ; fig. 4B). Even at high concentrations of meperidine (3,000 μM), the blockade of K<sub>DR</sub> currents was not

**Table 1.** Use-dependent Blockade of Sodium Currents at Frequencies of 2, 5, and 10 Hz

Frequency, Hz	Control	100 μM Meperidine	P Value
2	$0.75 \pm 0.04$	$0.37 \pm 0.04$	<0.001
5	$0.57 \pm 0.04$	$0.25 \pm 0.04$	<0.001
10	$0.46 \pm 0.04$	$0.16 \pm 0.02$	<0.001

Values represent the relative amplitudes of the 30th pulse in control solution and after application of 100 μM meperidine compared with the first pulse in control solution. Sodium currents are activated by a voltage pulse to −20 mV (10 ms); holding potential was −80 mV. Data are given as mean ± SEM.



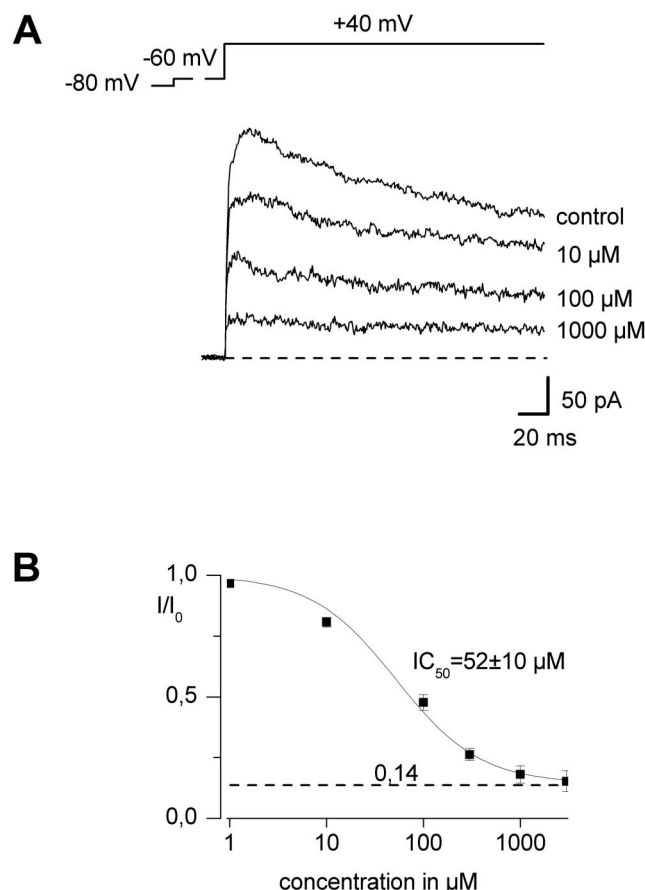


**Fig. 3.** Effect of meperidine on K<sub>A</sub> currents. (A) K<sub>A</sub> current in control solution and in the presence of 10, 100, 300, and 1,000 μM meperidine. The currents were activated by a 250-ms voltage step to +40 mV after a 150-ms prepulse to -120 mV. Holding potential was -80 mV. The procedure of separating K<sub>A</sub> currents from other K<sup>+</sup> currents is explained in Materials and Methods. (B) Concentration dependence of the K<sub>A</sub> current suppression by meperidine (34 somata). The data points were fitted using the equation:  $f(C) = 1 - C/(C + IC_{50})$ , where C is the blocker concentration and IC<sub>50</sub> is the half-maximum inhibiting concentration. The IC<sub>50</sub> value for K<sub>A</sub> current was  $102 \pm 5$  μM.

complete. The remaining current was  $14 \pm 3\%$  of the K<sub>DR</sub> current in control solution. Further investigations of K<sub>DR</sub> subtypes were not performed.

#### Action Potentials

Current clamp experiments were performed in intact neurons in the spinal cord slice using Ringer's solution as bath and pipettes filled with high-K<sup>+</sup> solution. Single action potentials were elicited using a 10-ms depolarizing current pulse of increasing amplitude (fig. 5 and table 2; n = 12). A concentration of 10 μM meperidine had little effect on the shape of a single action potential. Only a slight reduction of the repolarizing velocity was observed. The addition of 100 μM meperidine caused marginal changes in a single action potential. The overshoot potential was slightly reduced, and the width of the action potential increased. Most noticeably, the hy-



**Fig. 4.** Effect of meperidine on K<sub>DR</sub> currents. (A) K<sub>DR</sub> current in control solution and in the presence of 10, 100, and 1,000 μM meperidine. The currents were activated by a 250-ms voltage step to +40 mV after a 150-ms prepulse to -60 mV. Holding potential was -80 mV. (B) Concentration dependence of the K<sub>DR</sub> current suppression by meperidine (31 somata). The data points were fitted using the equation:  $f(C) = (1 - RES \times C)/(C + IC_{50})$ , where C is the blocker concentration, IC<sub>50</sub> is the half-maximum inhibiting concentration, and RES is the fraction of current that cannot be blocked by meperidine. The IC<sub>50</sub> value for K<sub>DR</sub> current was  $52 \pm 10$  μM. Approximately 14% of the K<sub>DR</sub> current remained unblocked even during application of 3,000 μM meperidine.

perpolarizing afterpotential disappeared. These effects were more pronounced with 300 μM meperidine. The application of 1,000 μM meperidine resulted in disappearance of the action potential; the membrane response was only passive to the stimulus current. A detailed analysis of different action potential parameters is given in table 2.

Series of action potentials were elicited using a depolarizing current pulse with a duration of 500 ms. The recording in control solution at different current pulses was followed by the application of 100 μM meperidine, a concentration that produced only slight effects on a single action potential. Figure 6 shows the effect of 100 μM meperidine on the firing frequency of series of action potentials. Compared with control recordings in eight neurons, the maximum firing frequency was re-

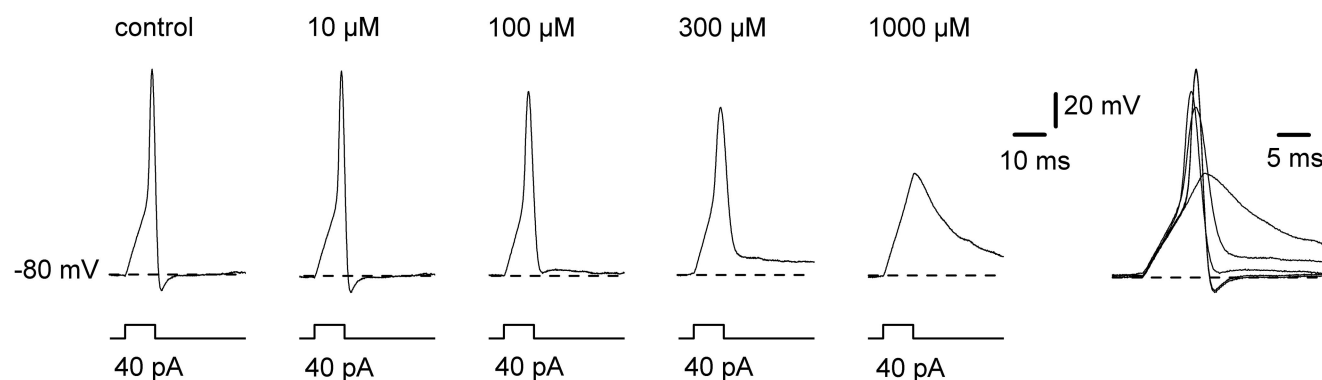


Fig. 5. Effects of meperidine on single action potentials. Recordings of action potentials in control solution (left) and after application of 10, 100, 300, and 1,000  $\mu\text{M}$  meperidine ( $n = 12$ ). The membrane potential was adjusted to  $-80$  mV by injecting a sustained current through the recording pipette. The impulse protocol is noted below the corresponding traces. (Right) All traces superimposed with a different time scale.

duced to  $54 \pm 8\%$  ( $n = 8$ ). At strong current pulses eliciting the maximum firing frequency in control solution, the neuron in the meperidine containing solution lost its ability to produce series of action potentials.

## Discussion

In addition to local anesthetics in clinical practice, numerous other drugs, such as opioids,<sup>5,8,31</sup> ketamine,<sup>32</sup> and clonidine,<sup>33</sup> can be used to achieve local anesthesia. Among opioids, meperidine takes an exceptional position and has already successfully been used in spinal anesthesia, where it produces a strong sensory and motor blockade comparable to that of lidocaine.<sup>20,34,35</sup> To understand the action of intrathecally applied meperidine, it is necessary to consider its effects on different sites: (1) mixed nerves after their passage through the intervertebral foramina, (2) dorsal root ganglion, and (3) spinal cord. Many investigators have studied the action of meperidine on peripheral nerve.<sup>13,24,36</sup> In contrast, little is known about the local anesthetic action of meperidine on the spinal cord or the dorsal root ganglia. However, in numerous clinical studies, meperidine has been administered intrathecally.<sup>1,4,7</sup>

In this article, we investigated the effects of meperidine on voltage-gated ion channels and on action potentials in spinal dorsal horn neurons. The major findings of

this study are as follows: (1) At clinical concentrations<sup>4–6,8,21</sup> of meperidine, voltage-gated  $\text{Na}^+$  and  $\text{K}^+$  channels are inhibited. (2) In the current clamp mode, the firing frequency of trains of action potentials is decreased at concentrations insufficient for the suppression of a single action potential (100  $\mu\text{M}$ ). (3) Single action potentials are suppressed by meperidine (1,000  $\mu\text{M}$ ).

Meperidine at clinically relevant concentrations during local anesthesia exhibits both tonic and a slight use-dependent block of  $\text{Na}^+$  currents in sensory neurons of laminae I and II of the spinal cord. The  $\text{IC}_{50}$  value for tonic block is  $118 \pm 5$   $\mu\text{M}$ . This value is in the same range as described for local anesthetics.<sup>30</sup> Similar values were reported for single  $\text{Na}^+$  channels in frog nerve fibers. Bräu *et al.*<sup>24</sup> found an  $\text{IC}_{50}$  value of 164  $\mu\text{M}$ . Significantly higher concentrations were reported for the  $\alpha$  subunit of  $\mu_1$   $\text{Na}^+$  channels expressed heterologously with rat brain  $\beta_1$  accessory subunit in *Xenopus* oocytes<sup>2</sup> ( $\text{IC}_{50} = 1.9$  mM). In our experiments, the fraction of blocked ion channels increased during repetitive stimulation from 30% in control to 63% in 100  $\mu\text{M}$  meperidine (2 Hz). This use-dependent blockade is usually explained by a higher affinity of the drug to open or inactivated  $\text{Na}^+$  channels compared with affinity to the resting state.<sup>37–39</sup> The blockade of  $\text{Na}^+$  currents and of single action potentials cannot be reversed by different concentrations of naloxone, indicating that the electrophysiologic effects de-

Table 2. Effect of Increasing Concentrations of Meperidine on Properties of Single Action Potentials

	Peak, mV	Ahp, mV	Threshold, mV	Duration, ms	Maximum Positive Slope, V/s	Maximum Negative Slope, V/s
Control	$46.5 \pm 3.9$	$-87.8 \pm 2.8$	$-39.2 \pm 1.3$	$1.9 \pm 0.1$	$114.1 \pm 9.2$	$-91.7 \pm 6.2$
10 $\mu\text{M}$	$41.0 \pm 4.3$	$-82.7 \pm 2.7$	$-40.7 \pm 1.1^*$	$2.2 \pm 0.1$	$102.9 \pm 10.6$	$-75.1 \pm 6.9^*$
100 $\mu\text{M}$	$27.1 \pm 5.0^\dagger$	$-77.2 \pm 1.3^\dagger$	$-41.9 \pm 1.1^\dagger$	$3.2 \pm 0.4$	$68.5 \pm 10.4^\dagger$	$-45.0 \pm 5.2^\dagger$
300 $\mu\text{M}$	$9.9 \pm 6.7^\ddagger$	$-74.8 \pm 1.2^\ddagger$	$-43.5 \pm 1.4^\ddagger$	$5.3 \pm 0.6^\ddagger$	$40.6 \pm 9.3^\ddagger$	$-23.9 \pm 4.0^\ddagger$
1,000 $\mu\text{M}$	$-14.6 \pm 4.8^\ddagger$	$-73.4 \pm 1.7^\ddagger$	$-42.7 \pm 0.8^*$	$10.3 \pm 1.4^\ddagger$	$16.4 \pm 4.1^\ddagger$	$-10.1 \pm 1.9^\ddagger$

Ahp is the maximum hyperpolarization after the action potential, durations of action potentials were measured at the half-maximum amplitude.<sup>43,50</sup> Positive and negative slopes are the corresponding maximum values after differentiation of the action potentials. Values are given as mean  $\pm$  SEM.

Significance levels are given as \*  $P < 0.05$ ,  $^\dagger P < 0.01$ , and  $^\ddagger P < 0.001$ .

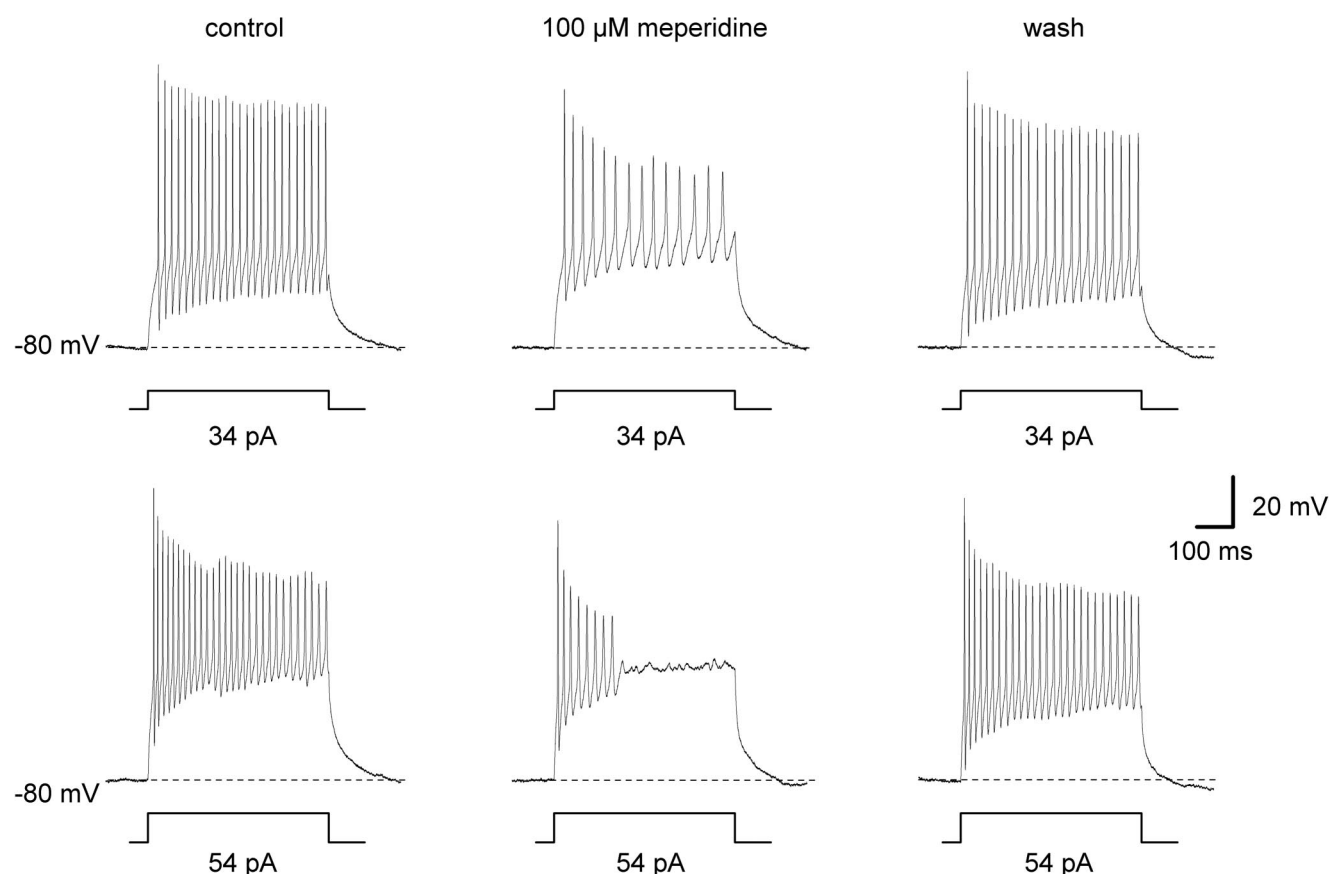


Fig. 6. Effects of meperidine on series of action potentials. Series of action potentials were evoked by 500-ms current pulses. The maximum firing frequency in control solution (left) was compared with that after application of 100  $\mu\text{M}$  meperidine (middle;  $n = 8$ ). The current pulse applied in the upper traces evoked the maximum firing frequency in meperidine containing solution. The current pulse applied in the lower traces evoked the maximum firing frequency in the control solution. The effects of meperidine on series of action potentials are completely reversible (right).

scribed in this article are not mediated *via* opioid receptors. This is in accord with observations in peripheral nerve.<sup>24</sup>

$\text{K}^+$  channels play an important role in regulating the firing patterns of different neurons. In our experiments, meperidine exhibits a tonic blocking action on  $\text{K}_A$  and on  $\text{K}_{DR}$  currents with  $\text{IC}_{50}$  values of 102 and 52  $\mu\text{M}$ , respectively. In contrast, local anesthetics such as lidocaine, bupivacaine, and mepivacaine affected  $\text{K}_{DR}$  currents in an ambivalent manner; low concentrations (1–10  $\mu\text{M}$ ) enhance and higher concentrations (1 mM) block  $\text{K}_{DR}$  current in spinal dorsal horn neurons.<sup>40</sup> The incomplete block of  $\text{K}_{DR}$  currents seems to be a common feature of meperidine and local anesthetics.<sup>41</sup> The same effect of meperidine with a similar residuum was observed in amphibian peripheral nerves.<sup>24</sup> It might result from a diversity of  $\text{K}^+$  channels contributing to this current; some subtypes may not be sensitive to meperidine. Another explanation is that meperidine or local anesthetics are not completely effective in blocking these currents. Local anesthetics such as lidocaine, bupivacaine, and mepivacaine block  $\text{K}_A$  currents with similar  $\text{IC}_{50}$  values ranging between 109 and 236  $\mu\text{M}$ .<sup>30</sup>

Single action potentials are suppressed by meperidine in a concentration-dependent manner. Low concentrations (10–300  $\mu\text{M}$ ) of meperidine reduce the overshoot potential, and the width of the action potential is increased. The slight reduction in the repolarization velocity and the decrease in the threshold potential is probably due the higher sensitivity of  $\text{K}^+$  currents to meperidine. At high concentrations (1,000  $\mu\text{M}$ ) of meperidine, total  $\text{Na}^+$  current blockade causes a complete suppression of the action potential. Lower concentrations of meperidine (100  $\mu\text{M}$ ;  $\text{IC}_{50}$  for  $\text{Na}^+$  currents) insufficient for suppression of a single action potential reduce the maximum firing frequency to 54%. A selective blockade of approximately 50% of the  $\text{K}_{DR}$  current by 10 mM tetraethylammonium reduces the firing frequency in spinal dorsal horn neurons to 72%. A similar reduction in firing frequency (to 68%) was caused by 100  $\mu\text{M}$  droperidol, blocking voltage-gated sodium channels and  $\text{K}_{DR}$  to approximately 50%. An exclusive blockade of voltage-gated  $\text{Na}^+$  currents by tetrodotoxin was insufficient to produce a reduction in firing frequency. Olschewski *et al.*<sup>42</sup> observed no reduction in firing frequency when 10 nM tetrodotoxin was applied to block

approximately 50% of Na<sup>+</sup> channels in spinal dorsal horn neurons. We conclude that the unexpected strong reduction in firing frequency by meperidine could originate from an additional block of K<sub>A</sub> currents.

It is difficult to correlate the blockade of Na<sup>+</sup> and K<sup>+</sup> currents with the effects on action potentials. There are different safety factors for the different channel types, meaning that only a fraction of the available ion channels are required to produce a single action potential. Madeja *et al.*<sup>43</sup> have demonstrated different IC<sub>50</sub> values for the blockade of sodium currents by tetrodotoxin (IC<sub>50</sub> = 6.4 nmol) and the reduction of action potential amplitude by tetrodotoxin (IC<sub>50</sub> = 104 nmol). At 100 μM meperidine, the action potential duration was not significantly increased, even though K<sup>+</sup> currents were significantly inhibited in isolated somata. This might be due to a preferred dendritic localization of K<sub>DR</sub> currents,<sup>44</sup> which are unlikely involved in the repolarization of the axon hillock where the action potentials are generated.<sup>29</sup> Unfortunately, little is known about the safety factors for K<sup>+</sup> channels in spinal dorsal horn neurons.

In comparison with other opioids, meperidine exhibits the strongest local anesthetic action at clinical concentrations.<sup>45,46</sup> This could be because of its low affinity for opioid receptors allowing the application of high concentrations of meperidine.<sup>24</sup> Most opioids in clinical practice have much higher affinities to opioid receptors. Therefore, they have to be applied at relatively low doses that do not exert a local anesthetic effect.<sup>13,23</sup> However, they will act as local anesthetics at concentrations exceeding those used in clinical settings. Fentanyl<sup>22,47</sup> and sufentanil<sup>47</sup> applied at unusually high concentrations (in the micromolar range) were also able to block nerve conduction in rabbit vagus nerve. When applied intrathecally, the usual dose of meperidine is 0.5–1 mg/kg.<sup>4–6,8</sup> Assuming that the volume of distribution after application of meperidine is 13 ml/kg,<sup>21</sup> the maximum cerebrospinal fluid concentration can be calculated at 310 μM (1 mg/kg meperidine). This concentration is in the range needed to block voltage-gated Na<sup>+</sup> and K<sup>+</sup> currents or single action potentials; a strong inhibiting effect on the generation of series of action potential can be expected. Because the plasma concentrations of intravenously applied meperidine are in the range of 1.3–2 μM,<sup>48,49</sup> it is unlikely that its systemic effects are mediated by an interaction with ion channels. For this reason, the local anesthetic effect of a single dose of subarachnoid meperidine can be explained by the local anesthetic effect of meperidine. However, an additional analgesic action of meperidine by its interaction with different transmitter systems, opioid receptors, or other ion channels could also contribute to the local anesthetic effect of meperidine.

In conclusion, we have shown that meperidine at concentrations reached after intrathecal application in the cerebrospinal fluid suppresses both voltage-gated

Na<sup>+</sup> and K<sup>+</sup> (K<sub>A</sub> and K<sub>DR</sub>) currents. In current clamp mode, single action potentials are suppressed in a dose-dependent manner. Concentrations insufficient for blockade of a single action potential produce a strong reduction in firing frequency of spinal dorsal horn neurons. Neuronal firing was also suppressed by blockade of voltage-gated K<sup>+</sup> channels. Therefore, block of pain transmission by meperidine should be considered a complex interaction that includes effects on different ion channels.

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