LABORATORY INVESTIGATIONS

Anesthesiology
2000; 92:1667-76

Differential Block of Fast and Slow Inactivating Tetrodotoxin-sensitive Sodium Channels by Droperidol in Spinal Dorsal Horn Neurons

Andrea Olschewski, Dr.med., Michael E. Bräu, P.D., Dr.med.,* Gunter Hempelmann, Prof.Dr.med.Dr.h.c.,† Werner Vogel, Prof.Dr.rer.nat.,‡ Boris V. Safronov, Ph.D.§*

Background: **Dorsal horn neurons of the spinal cord participate in neuronal pain transmission. During spinal and epidural anesthesia, dorsal horn neurons are exposed to local anesthetics and opioids. Droperidol is usually given with opioids to avoid nausea and vomiting. A recently developed method of "entire soma isolation" has made it possible to study directly the action of droperidol on different components of Na^+ current in dorsal horn neurons.**

Methods: **Using a combination of the whole-cell patch-clamp recording from spinal cord slices and the entire soma isolation method, we studied the direct action of droperidol on two types of Na^+ currents in dorsal horn neurons of young rats.**

Results: **The tetrodotoxin-sensitive Na^+ current in isolated somata consisted of a fast inactivating (τ_F, 0.5–2 ms; 80–90% of the total amplitude) and a slow inactivating (τ_S, 6–20 ms; 10–20% of the total amplitude) component. Droperidol, at concentrations relevant for spinal and epidural anesthesia, selectively and reversibly suppressed the fast component with a half-maximum inhibiting concentration (IC_{50}) of 8.3 μM. The slow inactivating component was much less sensitive to droperidol; the estimated IC_{50} value was 809 μM.**

Conclusions: **Droperidol selectively blocks fast Na^+ channels, the fast and slow components of the Na^+ current in dorsal horn neurons are carried through pharmacologically distinct types of Na^+ channels, and the effects of droperidol differ from those of local anesthetics and tetrodotoxin, which equipotently suppress both components. Droperidol may be suggested as a pharmacologic tool for separation of different types of inactivating tetrodotoxin-sensitive Na^+ channel. (Key words: Electrophysiology; ion channels; neuroleptics.)**

* Anesthesiologist.

† Professor and Chair of Anesthesia.

‡ Professor of Physiology.

§ Physiologist.

Received from the Departments of Anesthesiology and Intensive Care Medicine and Physiology, Justus-Liebig-University, Giessen, Germany. Submitted for publication September 30, 1999. Accepted for publication January 28, 2000. Supported in part by the Deutsche Forschungsgemeinschaft Grant Vo 188/16, Bonn, Germany, and by the Förderverein für Anästhesie e.V., Giessen, Germany.

Address reprint requests to Dr. Safronov: Physiologisches Institut, Justus-Liebig-Universität, Aulweg 129, D-35392 Giessen, Germany. Address electronic mail to: Boris.Safronov@physiologie.med.uni-giessen.de

DORSAL horn neurons located in laminae I-III of the spinal cord receive most of their primary sensory input from myelinated Aδ fibers and nonmyelinated C fibers, and therefore participate in processing and transduction of nociceptive information.[1,2] During epidural and spinal anesthesia, the dorsal horn neurons are exposed to high concentrations of local anesthetics and/or opioids that directly diffuse into the spinal cord.[3] Application of opioids is usually accompanied by side effects such as nausea, emesis, and pruritus. To reduce these side effects and to prolong analgesia, the neuroleptic droperidol is often applied with opioids during epidural and spinal anesthesia.[4] However, the mechanisms of droperidol action on the central nervous system are not completely understood. Although it is generally accepted that droperidol acts on dopaminergic receptors (D2), there are several indications that it also suppresses voltage-gated Na^+ conductance. For example, a subcutaneous application of droperidol was shown to result in a reversible local anesthesia.[5] Droperidol blocks voltage-gated Na^+ channels in cardiomyocytes[6-8] and mammalian nonmyelinated nerve.[9] However, little information is available about the action of droperidol on diverse types of Na^+ channels expressed in spinal cord neurons.

The recently developed method of "entire soma isolation" (ESI), *i.e.*, isolation of the soma from its neuron during whole-cell recording, has allowed a description of the properties and spatial distributions of two distinct types of inactivating Na^+ channels in dorsal horn neurons of the spinal cord.[10] In addition, the ESI method provides a possibility to study pharmacologic properties of the channels in identified neurons under conditions where diffusion of the blocker molecules is not impeded by connective tissue surrounding the neuron. In the current investigation we applied the ESI method to dorsal horn neurons from laminae I-III to study the effect of droperidol on fast and slow types of inactivating tetrodotoxin (TTX)-sensitive Na^+ channel.[10] It has been

found that the fast Na^+ channels are two orders of magnitude more sensitive to droperidol than the slow channels and the effect of droperidol differs from that of local anesthetics and TTX, which equipotently suppress both components of the Na^+ current.

Materials and Methods

Preparation

Experiments were performed by means of the patch-clamp technique[11] on 200-μm-thin slices, cut from the lumbar enlargement (L3–L6) of the spinal cord of 6–12-day-old rats.[12,13] The animals were rapidly decapitated, and the spinal cords were carefully cut out in ice-cold preparation solution bubbled with 95% O_2–5% CO_2. After removal of the pial membrane with fine forceps, the spinal cord was embedded in a preparation solution containing 2% agar cooled to 39°C. 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 that was continuously bubbled with 95% O_2–5% CO_2. The slices were thereafter incubated for 1 h at 32°C. The standard procedure of cell cleaning by repetitive blowing and suction of the bath solution through a broken patch pipette was not used because each slice contained numerous dorsal horn neurons with clean surfaces.

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 $CaCl_2$, 1 mM $MgCl_2$, 11 mM glucose, 1 mM NaH_2PO_4, and 25 mM $NaHCO_3$ (pH 7.4 when bubbled with 95% O_2–5% CO_2). In the experimental chamber, the slices were perfused with low-Ca^{2+}/high-Mg^{2+} solution to reduce spontaneous synaptic activity in neurons. This solution was obtained from the preparation solution by setting the concentration of Ca^{2+} and Mg^{2+} to 0.1 mM and 5 mM, respectively. Tetraethylammonium (TEA^+) containing solution used for the investigation of Na^+ channels (TEA solution) contained 95 mM NaCl, 5.6 mM KCl, 0.1 mM $CaCl_2$, 5 mM $MgCl_2$, 11 mM glucose, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 20 mM TEA-Cl (pH 7.4 when bubbled with 95% O_2–5% CO_2). Stock solution of droperidol (20 mM) was obtained by dissolving this drug in dimethyl sulfoxide. The stock solution was added to TEA solution to achieve the desired drug concentration. The addition of dimethyl sulfoxide did not change the pH value in our experimental solution and had no effect on Na^+ currents studied here. The local anesthetic lidocaine was directly added to the external TEA solution. The experimental chamber with a volume of 0.4 ml was continuously perfused by external solution at a rate of 2–3 ml/min. Droperidol and lidocaine-HCl were purchased from Sigma Chemical Co. (Deisenhofen, Germany), TTX was purchased from Latoxan (Rosans, France), and TEA was purchased from Merck (Darmstadt, Germany).

The pipette solution used for Na^+ current recordings (high-Cs_i^+) contained 5.8 mM NaCl, 134 mM CsCl, 1 mM $MgCl_2$, 3 mM EGTA, and 10 mM HEPES (pH 7.3 adjusted with 9.2 mM NaOH).

Current Recording

Pipettes for whole-cell recording were pulled in two stages from borosilicate glass tube (GC 150; Clark Electromedical Instruments, Pangbourne, United Kingdom) and were fire-polished to give a final resistance of 3–5 MΩ. The patch-clamp amplifier was an EPC-7 (List, Darmstadt, Germany). The effective corner frequency of the low-pass filter was 3 kHz. The frequency of digitization was at least twice that of the filter. The data were stored and analyzed by using 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. In experiments performed on the isolated *somata*, these errors did not exceed 4 mV in most cases. All experiments were conducted 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 cells with a soma (8–12-μm diameter) located in laminae I–III.[10] Neurons were distinguished from glial cells in voltage-clamp mode on the basis of a procedure described previously.[10] All neurons studied possessed a large Na^+ current exceeding 1 nA and showed spontaneous synaptic activity.[14] The restingpotentials in intact neurons measured just after breaking the membrane and establishing the whole-cell recording configuration (before diffusion of Cs_i^+-containing pipette solution into the cell) were between −80 and −50 mV.

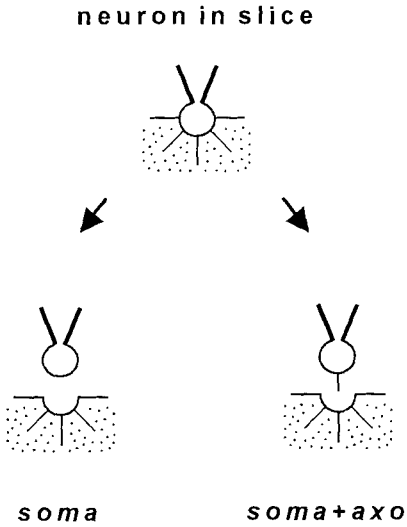

Fig. 1. Scheme of the entire soma isolation (ESI) method. In whole-cell recording mode, the entire soma of the neuron was isolated from the slice by slow withdrawal of the recording pipette. The isolated structure without an attached process was classified as *soma*. The isolated structure with one process was identified as *soma+axon* complex if the remaining Na^+ current exceeded 90% of the current recorded from the intact neuron in the slice just before its isolation.

The Method of Entire Soma Isolation

A detailed description of the ESI method has been published elsewhere.[10] Briefly, in whole-cell recording mode, the entire soma of the neuron was isolated from the slice by slow withdrawal of the recording pipette (see fig. 1). The isolated structure was classified as soma (*soma*) if it had lost all of its processes during isolation and preserved only 10–20% of original Na^+ current recorded in the slice before isolation. The isolated structure was classified as *soma+axon* complex if it contained one process and preserved more than 90% of the original Na^+ current. The good physiologic state of the isolated structures was confirmed by a considerable increase in their input resistance (reflecting a decrease in membrane leakage conductance), by stable or even improved membrane resting potentials, and by the ability of *soma+axon* complexes to generate action potentials.[10] Because the Na^+ currents in the *somata* and *soma+axon* complexes had very similar kinetics,[10,15] we assumed that they were carried through the same types of Na^+ channel.

Statistical Analysis and Fitting

The normalized amplitudes of peak Na^+ currents in concentration-effect curves were fitted by means of a nonlinear least squares procedure using a standard isotherm:

$$I/I_0 = (1 - Res)/(1 + (c/IC_{50})) + Res \quad (1)$$

where I was the current measured in the presence of a given drug concentration, I_0 was the control current measured in the absence of drug, c was the drug concentration, IC_{50} was the concentration giving a half-maximum effect, and Res was the residual or non-blocked component. The binding of one drug molecule to the receptor was assumed to be sufficient for channel suppression.

Fitting of the inactivation kinetics of the total Na^+ currents was performed with two exponentials using the following equation:

$$I(t,c) = I_F(c) \times \exp(-(t - t_0)/\tau_F) + (I_0(c) - I_F(c)) \times \exp(-(t - t_0)/\tau_S) \quad (2)$$

where I(t,c) was the current magnitude as a function of time and blocker concentration (c), t_0 was the time at which the inward current had reached its maximum, $I_0(c)$ was the maximum inward current at a given blocker concentration, $I_F(c)$ was the amplitude of the fast current component at t_0, τ_F (0.5–2 ms) was the inactivation time constant of the fast component, and τ_S (6–20 ms) was the inactivation time constant of the slow component. For each cell, the values of τ_F and τ_S had been determined by fitting the recording in control (c = 0) and were kept fixed in all further fittings of currents recorded in the presence of the blocker. By fitting the inactivation kinetics of suppressed currents, the values of $I_0(c)$ and $I_F(c)$ for each blocker concentration were determined. The data points plotted on the concentration-effect relations in figure 4B were obtained by normalizing the component amplitudes in the presence of the blocker by the amplitude in control: $I_F(c)/I_F(0)$ for the fast component and $[I_0(c) - I_F(c)]/(I_0(0) - I_F(0))$ for the slow component, assuming that the amplitude of the slow component $I_S(c) = I_0(c) - I_F(c)$.

The nonlinear fitting of the steady state inactivation curves (see fig. 5) were performed using a standard Boltzmann equation:

$$I/I_0 = 1/[1 + \exp(-(E - E_{H50})/k)] \quad (3)$$

where I was a current measured at a given potential (E), I_0 was the maximum current, E_{H50} was the potential at which half of Na^+ channels was inactivated, and k was a steepness factor.

The present study is based on recordings from 22 intact neurons in the slice, 49 isolated *somata*, and 10 isolated *soma+axon* complexes. All numerical values are given as mean ± SEM. The parameters obtained by fitting the data points using a linear least-squares procedure are given as mean ± SE.

Results

Na^+ currents were recorded from the isolated *somata* of dorsal horn neurons in external TEA solution; the pipettes were filled with high-Cs_i^+ solution. Holding potential was set to −80 mV and Na^+ currents were activated by 50-ms voltage steps to −20 mV after a 50-ms prepulse to −120 mV. The peak Na^+ currents remaining in the isolated *somata* after separation from the axon initial segment had a small amplitude of 150–300 pA (10–20% of original Na^+ current measured in most intact dorsal horn neurons). Therefore, the voltage error caused by the resistance in series was small, and problems of a poor space clamp of the neuronal membrane were avoided. As a consequence of the improved space clamp condition, the kinetics of the Na^+ current in an isolated *soma* became considerably faster than in an intact neuron (fig. 2A).

Pharmacologic Studies with the ESI Method

The suitability of the ESI method for pharmacologic investigations of ionic channels in spinal neurons was tested in the experiments shown in figure 2. Sensitivities of Na^+ currents recorded from intact dorsal horn neurons in the spinal cord slice and from their isolated *somata* to the local anesthetic lidocaine were compared.

To reach the steady state block in experiments with intact neurons, the slices had to be perfused at least 2–5 min with lidocaine-containing solution before the measurements were performed. The hyperpolarizing prepulse to −120 mV was omitted to reduce the amplitude of the Na^+ current. Lidocaine suppressed the Na^+ current with an IC_{50} of 112 ± 8 μM (eight neurons) in intact neurons in the spinal cord slice (fig. 2B).

The IC_{50} value for lidocaine obtained in experiments with isolated *somata* was 12.6 ± 1.9 μM (five *somata* and four *soma+axon* complexes). It should be noted that a prepulse to −120 mV preceding the depolarization in isolated *somata* is known to reduce the channel sensitivity to the blocker. Nevertheless, approximately nine times lower concentrations of lidocaine were needed to evoke the same effect as in intact neurons. It is therefore concluded that the connective tissue surrounding the intact neuron in the spinal cord slice remarkably impedes the drug diffusion toward the cell membrane and that the apparent IC_{50} values measured in pharmacologic experiments performed using the slice preparation represents an underestimation of the real channel sensitivity to the blocker. Therefore, we per-

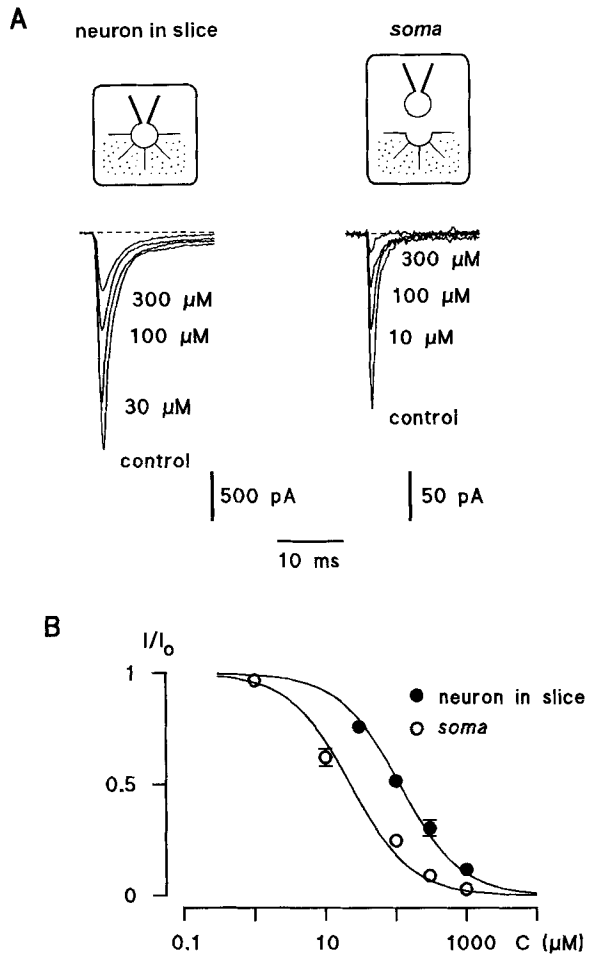

Fig. 2. Testing the suitability of the ESI method for pharmacologic investigation. Action of the local anesthetic lidocaine on Na^+ currents recorded from intact neurons in the spinal cord slice and from isolated *somata*. (*A*) Na^+ currents recorded in the presence of different concentrations of lidocaine. Holding potential was −80 mV. The currents in isolated *somata* were activated by a 50-ms voltage pulse to −20 mV after a 50-ms prepulse to −120 mV. For intact neurons, the hyperpolarizing prepulse was not applied to reduce the current amplitude. (*B*) Concentration-dependent inhibition of Na^+ current by lidocaine in intact neurons (solid symbols, eight cells) and isolated *somata* (open symbols, nine *somata*). The data points were fitted using equation 1 with Res = 0, giving IC_{50} values of 112 ± 8 μM for intact neurons and 12.6 ± 1.9 μM for isolated *somata*. Here and in the following figures, error bars indicate ± SEM if exceeding symbol size.

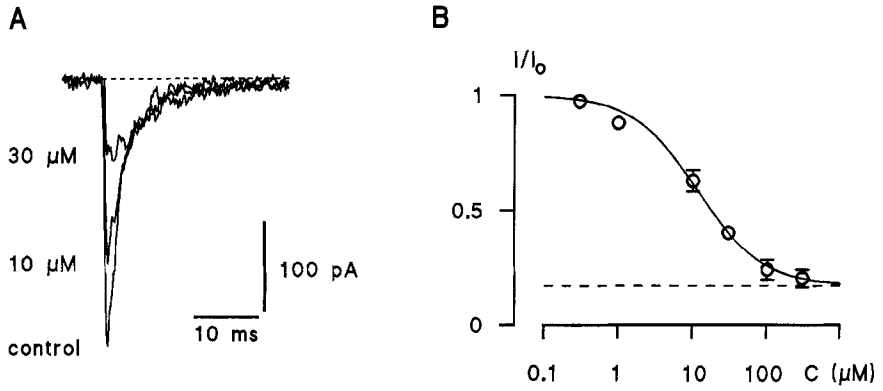

Fig. 3. Effect of droperidol on the inactivating Na^+ current in *somata*. (*A*) Recordings of Na^+ current in the control solution and in the presence of 10 and 30 μM droperidol. (*B*) Concentration dependence of the total Na^+ current suppression by droperidol (12 *somata*). The data points were fitted using equation 1, giving IC_{50} = 11.6 ± 2.0 μM and Res = 0.17.

formed the following experiments on isolated *somata* or *soma+axon* structures.

Suppression of the Total Na^+ Current in Isolated Somata

Inactivating Na^+ current in isolated *somata* of dorsal horn neurons consisted of fast inactivating (τ_F, 0.5–2 ms; 80–90% of total current) and slow inactivating (τ_S, 6–20 ms; 10–20% of the total current) components.[10] Both fast and slow components of the transient inward current studied here were carried through Na^+ rather than Ca^{2+} channels, because they were observed under conditions in which Ca^{2+} currents were suppressed either by lowering Ca^{2+} to 0.1 mM and increasing Mg^{2+} to 5 mM in external solution or by addition of 50 mM F^- to the pipette solution. In isolated *somata*, both components of the Na^+ current disappeared after substitution of external NaCl with choline-Cl or were completely blocked by 100 nM TTX.[10] Investigation of the steady state Na^+ channels located in the processes of dorsal horn neurons[10] was beyond the scope of the present study.

Addition of 0.3–300 μM droperidol to the bath solution produced a concentration-dependent reduction of the peak Na^+ current (fig. 3). It can be seen that 10 and 30 μM droperidol suppressed the fast component of the current, whereas the slow component remained relatively unchanged (fig. 3A). The highest concentration of the drug tested (300 μM) blocked only 83% of the total peak current (fig. 3B). Fitting the data points of the concentration–effect relation with equation 1 gave an IC_{50} of 11.6 ± 2.0 μM and a Res of 0.17 ± 0.03 (12 *somata*). It is assumed that the droperidol-resistant component of the current was mostly carried through slow inactivating channels.

To test the sensitivity of each component of Na^+ current to droperidol, we performed a two-exponential fitting of inactivation kinetics of the total Na^+ current (equation 2) in the presence of different droperidol concentrations (fig. 4A). Suppression of the fast component was complete and could be adequately described using one isotherm (equation 1) with IC_{50} = 8.3 ± 1.1 μM and Res = 0 (eight *somata*; fig. 4B). The slow component was much less sensitive to droperidol, being reduced to only 0.83 at 300 μM. Fitting of the data points for the slow component with an isotherm assuming Res = 0 gave an IC_{50} of 809 ± 247 μM (eight *somata*; fig. 4B). Thus, the slow component was approximately two orders of magnitude less sensitive to the drug than the fast one.

The two-exponential fitting of inactivation kinetics was further performed to study whether the fast and slow components of Na^+ current also have different ranges of steady state inactivation. The Na^+ currents were activated in isolated *somata* by a voltage pulse to −30 mV after a prepulse (50 or 160 ms long) to different potentials ranging between −130 and −40 mV. For a 50-ms prepulse, the fitting of the data points with equation 3 gave the E_{H50} = −73.8 ± 0.8 mV and k = −8.8 mV for the fast component and E_{H50} = −62.9 ± 0.9 mV and k = −8.6 mV for the slow one (fig. 5, five *somata*). At a 160-ms prepulse, both components inactivated at more negative potentials: E_{H50} = −84.6 ± 0.5 mV, k = −9.0 mV for the fast component and E_{H50} = −74.7 ± 0.8 mV, k = −8.8 mV for the slow component (five *somata*). Thus, the fast Na^+ currents inactivated at approximately 10 mV more negative potentials than the slow ones.

Comparison with Effects of TTX and Lidocaine

To test whether the differential block of fast and slow components of the Na^+ current is a specific effect of droperidol, the action of 30 nM TTX and 100 μM lidocaine on the currents recorded in isolated *somata* were

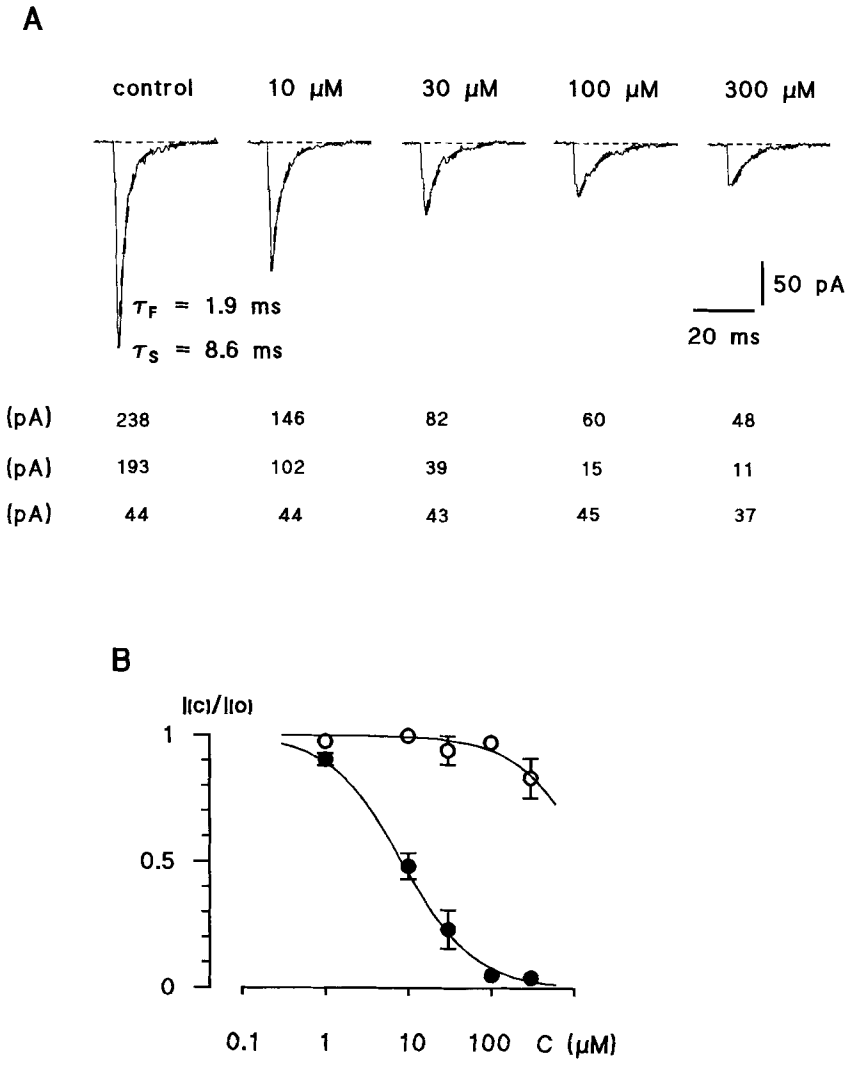

Fig. 4. Differential block of two components of the Na^+ current by droperidol. (*A*) Na^+ currents recorded in an isolated *soma* in control and in the presence of 10, 30, 100, and 300 μM droperidol. Inactivation kinetics of the currents were fitted with two exponentials using equation 2. The time constants of inactivation of fast and slow components are indicated near the control recording. The amplitudes of the total current (I_0) and of fast (I_F) and slow ($I_S = I_0 - I_F$) components are given below the corresponding traces. (*B*) Concentration dependence of suppression of fast (filled symbols) and slow (open symbols) components by droperidol (eight *somata*). The data points for the fast component were best fitted with equation 1: IC_{50} = 8.3 ± 1.1 μM, Res = 0. Fitting the data points for the slow component under the assumption that Res = 0 resulted in an IC_{50} value of 809 ± 247 μM.

compared (fig. 6). TTX and lidocaine proportionally reduced both fast and slow components, whereas droperidol predominantly suppressed the fast one. This phenomenon can be seen clearly if the peak of the blocked current was normalized to the peak of control current (traces on the right in fig. 6). The currents recorded in the presence of TTX (seven *somata* and six *soma+axon* complexes) and lidocaine (five *somata* and four *soma+axon* complexes) had nearly the same kinetics as control recordings. In contrast, the trace in 100 μM droperidol was considerably slower than the trace in control, indicating a dominance of the slow component (fig. 6). It can be concluded that neither TTX nor lidocaine are able to produce a differential block of the fast and slow Na^+ currents.

Use-dependent Block by Droperidol

In the following experiments with isolated *somata*, we studied the ability of droperidol to produce a use-dependent block of the total Na^+ current. A train of 10 Na^+ currents was activated at a frequency of 1 Hz by 50-ms depolarizing pulses from −80 to −20 mV in the absence and presence of 10 μM droperidol (fig. 7A). Droperidol, similar to local anesthetics, produced a use-dependent block of the current.[16] Figure 7B shows the amplitudes of the Na^+ currents normalized to the amplitude of the first current recorded in control solution (seven isolated *somata*). In the absence of the drug, the amplitude of the 10th current was reduced by 0.11, presumably because of insufficient recovery of Na^+ channels from slow inactivation. In the presence of 10

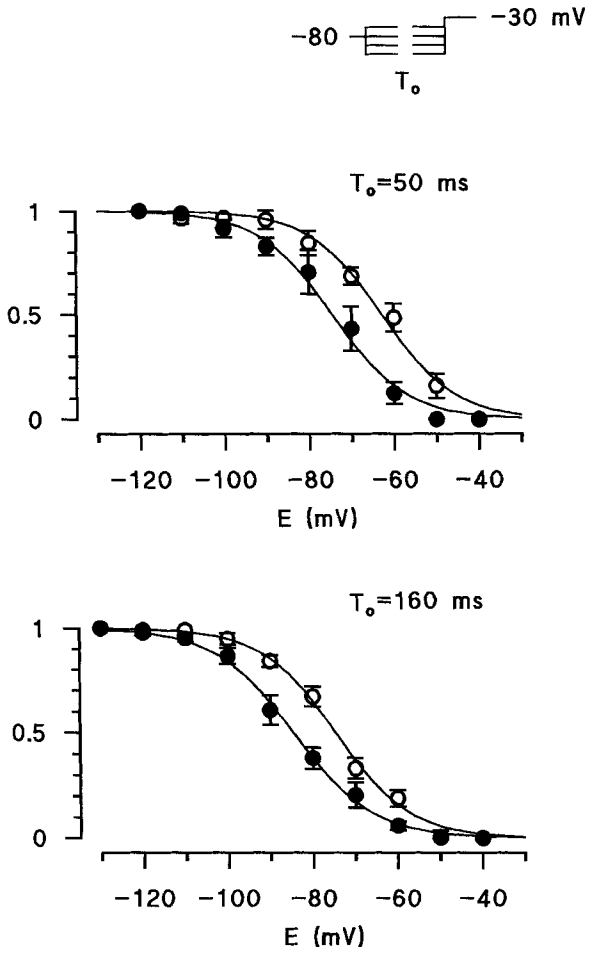

Fig. 5. Inactivation characteristics of fast and slow components of Na^+ current. The inactivation characteristics of the fast (filled symbols) and slow (open symbols) components on Na^+ currents activated by a voltage step to −30 mV after a 50-ms (*upper*) or a 160-ms (*lower*) prepulse to different potentials. The fitting parameters are given in Results.

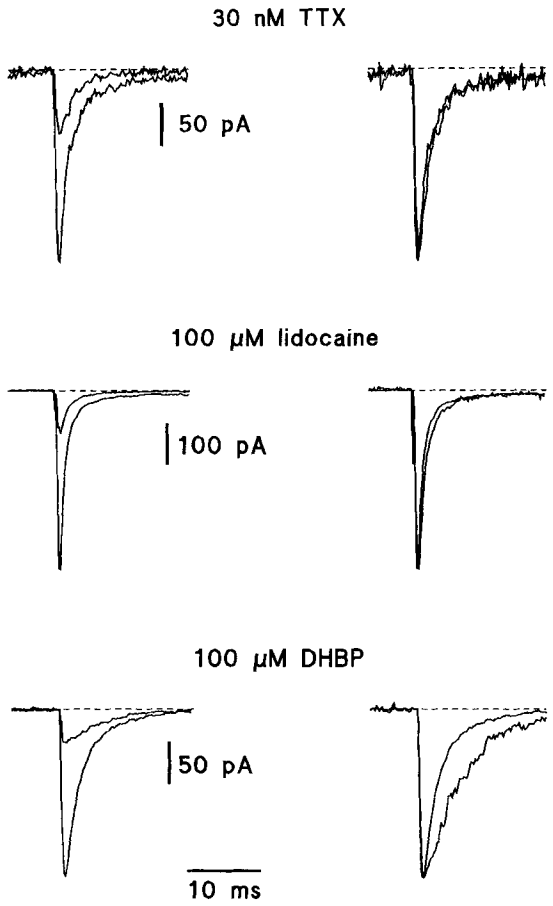

Fig. 6. Comparison of effects of tetrodotoxin (TTX), lidocaine, and droperidol on Na^+ currents in isolated *somata*. (*Left*) Na^+ currents recorded in control solution and in the presence of 30 nM TTX, 100 μM lidocaine, and 100 μM droperidol. (*Right*) To compare the inactivation kinetics, each current recorded in the presence of the blocker was normalized to the peak amplitude of the corresponding control current.

μM droperidol, the relative amplitude of the Na^+ current was reduced from 0.59 at the first pulse to 0.35 at the 10th pulse. Thus, slow inactivation and use-dependent block induced by 10 μM droperidol reduced the total Na^+ current by 41% (10th current *vs.* first current).

It can be seen in figure 7A that the total Na^+ current was reduced because of apparent reduction in the amplitude of the fast component, whereas the slow component remained unchanged. To describe the use-dependent block of each component, the two exponential fittings of the inactivation kinetics of the total Na^+ current were performed. Figure 7C shows the amplitudes of the fast (six *somata*) and slow (six *somata*) components in control solution and in the presence of 10 μM droperidol normalized to the amplitude of the corresponding component of the the first current activated in a control solution. The data points for the slow component in the presence of the blocker were almost indistinguishable from those in a control solution. The amplitude of the fast component in 10 μM droperidol was reduced from 0.45 to 0.20 (first *vs.* 10th current). Thus, the use-dependent block of the fast component was 56%.

Discussion

The current study has demonstrated that the ESI method can be adequately used in pharmacologic investigations of ionic channels and that the neuroleptic

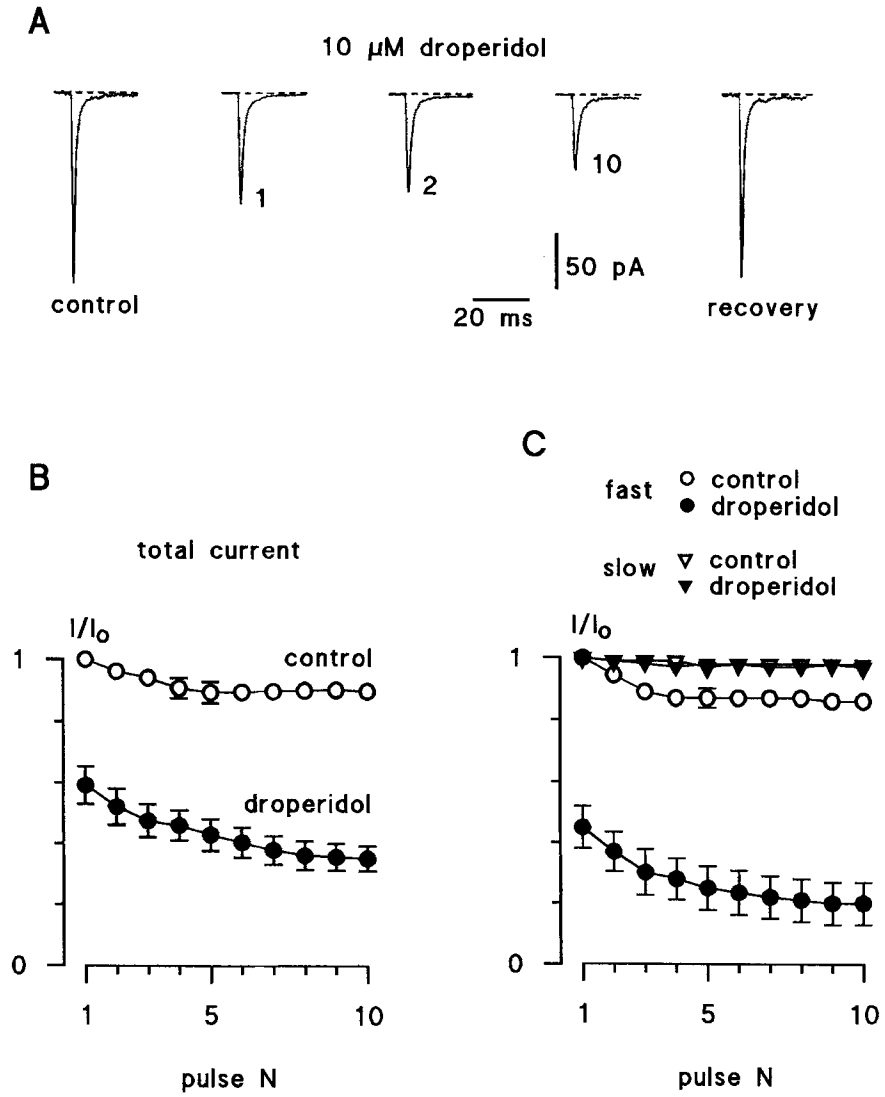

Fig. 7. Use-dependent block of Na^+ currents by droperidol. (*A*) Na^+ currents in control solution and in the presence of 10 μM droperidol activated by 10 consecutive depolarizing pulses at a frequency of 1 Hz (the number of the pulse is indicated near the corresponding trace). The currents were activated by 50-ms voltage pulses from −80 to −20 mV. (*B*) Normalized amplitudes of Na^+ currents recorded in control solution and in the presence of 10 μM droperidol as a function of pulse number. Each current was normalized to the amplitude of the first Na^+ current recorded in control solution (seven *somata*). (*C*) Normalized amplitudes of the fast (circles) and slow (triangles) components of Na^+ current in control (open symbol) and in 10 μM droperidol-containing solution (filled symbols). Each current was normalized to the amplitude of the corresponding component of the first current recorded in control solution. Data from six *somata*.

droperidol produces a differential block of fast and slow inactivating types of TTX-sensitive Na^+ channel in spinal dorsal horn neurons.

The ESI Method

Our experiments show that pharmacologic studies of ionic currents in isolated *somata* had several advantages in comparison with those performed on intact neurons in the spinal cord slice, excised membrane patches, or enzymatically dissociated neurons. First, relatively small amplitudes of the remaining Na^+ current (approximately 150–300 pA in the *somata* compared with 1–20 nA in intact neurons in the slice or in enzymatically dissociated neurons, which usually preserve at least a part of the axon initial segment) allow considerable reduction of the voltage error due to series resistance. On the other hand, remaining currents are sufficiently large to be studied directly, without averaging several consecutive recordings, as typically performed in pharmacologic studies with excised outside-out patches containing only a few channels. Furthermore, ionic currents in isolated *somata* show much less "run-down" effect than in outside-out patches.

Second, simple spherical geometry of the isolated *soma* with a diameter of approximately 10 μm provides adequate space clamp conditions. As a consequence, the

kinetics of Na^+ channels in isolated *somata* were considerably faster than in intact neurons. The isolated *somata* could also be used for measurements of changes in gating kinetics induced by different drugs.

Third, improved conditions for drug perfusion in isolated *somata* allows precise measurements of the binding constants for the drug investigated; the values obtained for intact neurons in the slice represent a considerable underestimation of the channel sensitivity, since connective tissue impedes diffusion of the blocker.

Fourth, in comparison with nucleated patches,[17] which also provide relatively large ionic currents, the whole isolated *somata* preserve their good physiologic state, being able to keep the resting potential (when the pipette contains physiologic high K^+ solution). The ESI methods gives the possibility to study effects of different substances on the somatic excitability under current-clamp conditions.

Fifth, the ESI method enables pharmacologic studies of the channels in the *soma* of neurons that have been identified before isolation on the basis of their location in the slice or patterns of electrical activity seen during whole-cell recording.

Differential Block of Na^+ Channels by Droperidol

Droperidol at concentrations between 0.1 and 300 μM suppressed the fast inactivating component of the TTX-sensitive Na^+ current, whereas the slow inactivating component remained almost unchanged. The IC_{50} value of 8.3 μM obtained for the fast component was approximately two orders of magnitude lower than that for the slow one (809 μM), indicating that droperidol is a selective blocker of fast Na^+ channels.

Two-component inactivation kinetics of TTX-sensitive Na^+ current were observed in a number of neuronal preparations.[10,18–21] In the frog node of Ranvier, the fast component of the Na^+ current was approximately eight times more sensitive to niflumic acid and approximately three times more sensitive to TTX than the slower one.[20]

In experiments with intact dorsal horn neurons in the spinal cord slice, a differential block of the fast and slow components by 1 μM TTX was only observed during perfusion of the slice with drug-containing solution.[10] The fast component had been completely suppressed within 1 min, whereas blockade of the slow component began several minutes later. In the isolated *somata*, however, the fast component was only slightly more sensitive to TTX, leading to the conclusion that the differential block of two components seen in experiments with intact neurons in the slice is unlikely to result from different TTX sensitivities of underlying channels but can be better explained by their different spatial distributions over the neuronal membrane. In this case, the connective tissue in the spinal cord slice impedes the diffusion of the blocker, and thus different regions of the neuronal membrane experience different TTX gradients. The idea of the existence of two independent types of inactivating Na^+ channels in dorsal horn neurons was also supported by single-channel measurements that revealed an approximately 25% larger unitary conductance for the slow channel.[10] Selective suppression of the fast component by droperidol reported here provides further evidence that fast and slow Na^+ currents in spinal dorsal horn neurons are carried through distinct types of ion channels. Droperidol, with its IC_{50} value two orders of magnitude lower for the fast channel compared with the slow one, seems to be the only substance described thus far that enables separation of the fast and slow components of TTX-sensitive Na^+ currents.

In contrast to droperidol, TTX and the local anesthetic lidocaine proportionally reduced both components and were not able to separate the current components in the isolated *somata*. It can be assumed that droperidol and local anesthetics show different mechanisms of action on Na^+ channels.

The neuroleptic droperidol and the local anesthetic lidocaine have some structural similarities. They comprise a lipophilic ring system on one end of the molecule that is connected by an aliphatic chain with a tertiary amine on the other end. The intermediate aliphatic chain contains an ester binding in droperidol and an amide binding in lidocaine. Because of the tertiary amine, the droperidol and lidocaine have pKa values of 7.6 and 8.2, respectively, and thus are partly protonated at physiologic pH. Their unprotonated forms are highly lipophilic with a partition coefficient (logP) of 3.5 for droperidol and 2.3 for lidocaine. In contrast, TTX is a permanently charged alkaloid with low lipid solubility. It binds with its charged guanidinium group to the outer mouth of the channel pore.

Numerous studies performed during the last 40 yr have shown that the mechanisms of local anesthetic action during epidural and spinal anesthesia are complex[22] and cannot be interpreted as a simple suppression of an ion conductance in the axonal membrane. The dorsal horn neurons of the spinal cord play a key role in neuronal pain transmission. They are exposed to local anesthetics, opioids, and neuroleptics during spinal and epidural anesthesia, and, therefore, suppression of the Na^+ conductance in dorsal horn neurons by these substances

contributes to analgesia. Droperidol blocks the fast Na^+ channels, providing a major portion of Na^+ current needed for fast membrane depolarization during the action potential. The IC_{50} value of 8.3 μM is three orders of magnitude lower than the concentration of droperidol injected into the epidural space during analgesia (6 mM syringe concentration, droperidol 2.5 mg/ml),[23] indicating that the clinically applied droperidol may affect the Na^+ conductance in spinal neurons. In addition, droperidol may be suggested as a pharmacologic tool in studying the properties and physiologic functions of fast and slow inactivating Na^+ channels.

The authors thank M. Wolff for critically discussing the manuscript and B. Agari for excellent technical assistance.

References

1. Light AR, Perl ER: Reexamination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibres. J Comp Neurol 1979; 186:117-32

2. Light AR, Trevino DL, Perl ER: Morphological features of functionally defined neurones in the marginal zone and substantia gelatinosa of the spinal dorsal horn. J Comp Neurol 1979; 186:151-72

3. Bromage PR, Joyal AC, Binney JC: Local anesthetic drugs: Penetration from the spinal extradural space into the neuroaxis. Science 1963; 140:392-4

4. Bach V, Carl P, Ravlo O, Crawford ME, Werner M: Potentiation of epidural opioids with epidural droperidol. Anaesthesia 1986; 41:1116-9

5. Greene MJ: Some aspects of the pharmacology of droperidol. Br J Anaesth 1972; 44:1972-6

6. Kern R, Einwachter HM, Haas HG, Lack EG: Cardiac membrane currents as affected by an neuroleptic agent: Droperidol. Pflügers Arch 1971; 325:262-78

7. Carmeliet E, Xhonneux R, Van-Glabbeek A, Reneman R: Electrophysiological effects of droperidol in different cardiac tissues. Naunyn-Schmiedeberg Arch Pharmacol 1976; 293:57-66

8. Dorticos FR, Garcia-Barreto D: Electrophysiological effects of droperidol on sinoatrial nodal fibers. Arch Int Pharmacodyn 1979; 240:137-42

9. Den Hertog A, Kleine JW: The effect of droperidol on mammalian non-myelinated nerve fibres. Eur J Pharmacol 1973; 21:1-5

10. Safronov BV, Wolff M, Vogel W: Functional distribution of three types of Na^+ channel on soma and processes of dorsal horn neurones of spinal cord. J Physiol 1997; 503:371-85

11. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 1981; 391:85-100

12. Edwards FA, Konnerth A, Sakmann B, Takahashi T: A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. Pflügers Arch 1989; 414:600-12

13. Takahashi T: Membrane currents in visually identified motoneurones of neonatal rat spinal cord. J Physiol 1990; 423:27-46

14. Chvátal A, Pastor A, Mauch M, Sykova E, Kettenmann H: Distinct populations of identified glial cells in the developing rat spinal cord slice: Ion channel properties and cell morphology. Eur J Neurosci 1995; 7:129-42

15. Safronov BV: Spatial distribution of Na^+ and K^+ channels in spinal dorsal horn neurones: Role of the soma, axon and dendrites in spike generation. Prog Neurobiol 1999; 59:217-41

16. Olschewski A, Hempelmann H, Vogel W, Safronov BV: Blockade of Na^+ and K^+ currents by local anesthetics in the dorsal horn neurons of the spinal cord. ANESTHESIOLOGY 1998; 88:172-9

17. Sather W, Dieudonné S, MacDonald JF, Ascher P: Activation and desensitisation of N-methyl-D-aspartate receptors in nucleated outside-out patches from mouse neurones. J Physiol 1992; 450:643-72

18. Chiu SY: Inactivation of sodium channels: Second order kinetics in myelinated nerve. J Physiol 1977; 273:573-96

19. Sigworth FJ: Covariance of nonstationary sodium current fluctuations at the node of Ranvier. Biophys J 1981; 34:111-33

20. Benoit E, Corbier A, Dubois JM: Evidence for two transient sodium currents in the frog node of Ranvier. J Physiol 1985; 361: 339-60

21. Huguenard JR, Hamill OP, Prince DA: Developmental changes in Na conductances in rat neocortical neurons: Appereance of a slowly inactivating component. J Neurophysiol 1988; 59:778-95

22. Butterworth JF IV, Strichartz GR: Molecular mechanisms of local anesthesia: A review. ANESTHESIOLOGY 1990; 72:711-34

23. Sanansilp V, Areewatana S, Tonsukchai N: Droperidol and the side effects of epidural morphine after cesarean section. Anaest Analg 1998; 86:532-7