

Suppression of Potassium Conductance by Droperidol Has Influence on Excitability of Spinal Sensory Neurons

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Background: **During spinal and epidural anesthesia with opioids, droperidol is added to prevent nausea and vomiting. The mechanisms of its action on spinal sensory neurons are not well understood. It was previously shown that droperidol selectively blocks a fast component of the Na^+ current. The authors studied the action of droperidol on voltage-gated K^+ channels and its effect on membrane excitability in spinal dorsal horn neurons of the rat.**

Methods: **Using a combination of the patch-clamp technique and the "entire soma isolation" method, the action of droperidol on fast-inactivating A-type and delayed-rectifier K^+ channels was investigated. Current-clamp recordings from intact sensory neurons in spinal cord slices were performed to study the functional meaning of K^+ channel block for neuronal excitability.**

Results: **Droperidol blocked delayed-rectifier K^+ currents in isolated somata of dorsal horn neurons with a half-maximum inhibiting concentration of 20.6 μM. The A-type K^+ current was insensitive to up to 100 μM droperidol. At droperidol concentrations insufficient for suppression of an action potential, the block of delayed-rectifier K^+ channels led to an increase in action potential duration and, as a consequence, to lowering of the discharge frequency in the neuron.**

Conclusions: **Droperidol blocks delayed-rectifier K^+ channels in a concentration range close to that for suppression of Na^+ channels. The block of delayed-rectifier K^+ channels by droperidol enhances the suppression of activity in spinal sensory neurons at drug concentrations insufficient for complete conduction block.**

DORSAL horn neurons located in laminae I–II of the spinal cord receive most of their primary sensory input from myelinated Aδ-and nonmyelinated C-fibers and are involved in nociception.[1,2] During epidural and spinal anesthesia, these neurons are exposed to high concentrations of local anesthetics or opioids.[3] Application of opioids is usually accompanied by side effects such as nausea, emesis, and pruritus. To reduce nausea and emesis and to prolong analgesia, the neuroleptic droperidol is applied together with opioids.[4]

At the cellular level, droperidol was shown to act on dopaminergic receptors (D2) as well as voltage-gated Na^+ conductance.[5–8] The sensitivity of different components of Na^+ current to droperidol has further been studied in spinal dorsal horn neurons[9] by means of the "entire soma isolation" (ESI) method.[10,11] The ESI method allowed a visual identification of the sensory neurons within the spinal cord slice and further pharmacologic study of ionic channels in their isolated somata under conditions in which diffusion of the drug molecules is not impeded by the connective tissue surrounding the neuron.[9] It has been found that droperidol selectively suppresses the fast inactivating component of Na^+ current, while the slowly inactivating one remained resistant to the drug.[9] The effect of droperidol on spinal neurons may not only be restricted to the action on the fast inactivating Na^+ current, because some blockers of Na^+ channels were also shown to potently suppress different types of voltage-gated[12–14] as well as background K^+ channels.[15–17]

In the present study, the action of the neuroleptic droperidol on voltage-gated K^+ channels in spinal dorsal horn neurons as well as its functional meaning were studied.

Materials and Methods

Preparation

Experiments were performed by means of the patch-clamp technique[18] on 200-μm thin slices cut from the lumbar enlargement (L3–L6) of the spinal cord of 8–17-day-old rats.[14,19,20] Animals were rapidly decapitated, and the spinal cords were carefully removed 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 down to 39°C. To accelerate solidification of the agar, the beaker with 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 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 were reported

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to the local veterinarian authority and are in accordance with German guidelines.

Solutions

Preparation and experimental 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). Low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin used to prevent activation of Ca^{2+} and Ca^{2+}-dependent K^+ currents was obtained from the preparation solution by setting the concentration of Ca^{2+} to 0.1 mM, increasing Mg^{2+} to 5 mM, and addition of 100 nM charybdotoxin and 1 μM apamin. The study of K^+ channels was conducted in Na^+-free choline-Cl solution containing 141 mM choline-Cl, 0.6 mM KCl, 0.1 mM $CaCl_2$, 5 mM $MgCl_2$, 11 mM glucose, and 5 mM HEPES (pH 7.4 adjusted with 5 mM KOH). Tetrodotoxin 0.2 μM was added to this solution to block voltage-gated Na^+ channels.

Stock solution of droperidol (20 mM) was obtained by dissolving the drug in dimethyl-sulfoxide. It was added to bath solution to achieve the desired drug concentration. Tetrodotoxin and tetraethylammonium were directly added to the bath solution. Bovine serum albumin (0.05%) was added to charybdotoxin- and apamin-containing solution to prevent an unspecific adhesion of blocker molecules to the walls of the experimental chamber and the tubings. 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 apamin were purchased from Sigma (Deisenhofen, Germany), tetrodotoxin and charybdotoxin from Latoxan (Rosans, France), and tetraethylammonium from Merck (Darmstadt, Germany).

The pipette solution used in ESI experiments contained 5 mM NaCl, 144.4 mM KCl, 1 mM $MgCl_2$, 3 mM EGTA, and 10 mM HEPES (pH 7.3 by 10 mM NaOH). The pipette solution in all other experiments contained 5 mM NaCl, 144.4 mM KCl, 1 mM $MgCl_2$, 3 mM EGTA, and 10 mM HEPES (pH 7.3 adjusted with 10.6 mM KOH).

Current Recording

Pipettes for whole-cell recording pulled from borosilicate glass tube (GC 150, Clark Electromedical Instruments, Pangbourne, United Kingdom) were fire-polished to give a final resistance of 3–4 MΩ. The patch-clamp amplifier was an EPC-7 (List, Darmstadt, Germany) in both voltage- and current-clamp experiments. The effective corner frequency of the low-pass filter was 3 kHz. The frequency of digitization was 10 kHz in all experiments. 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 voltage-clamp experiments using records with hyperpolarizing pulses that activated no currents. Offset potentials were corrected 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, voltage errors caused by resistance in series did not exceed 4 mV in most cases. All experiments were conducted at room temperature (21–23°C).

To make the action potentials or trains of action potentials comparable, we kept the membrane potential at approximately −70 mV in current-clamp experiments by injecting sustained depolarizing or hyperpolarizing currents through the recording electrode.

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, were able to generate action potentials, and showed spontaneous synaptic activity. The resting potentials measured in intact neurons were between −78 and −55 mV.

The Entire Soma Isolation Method

A detailed description of the ESI method has been given elsewhere.[9–11] 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 ("soma") if it had lost all of its processes during isolation and preserved only 10–20% of original Na^+ current recorded from the neuron in the slice before its 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 physiological state of the isolated structures was confirmed by a considerable increase in their input resistances (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] In the present study, no differentiation between the "somata" and "soma+axon" complexes were made; therefore, all isolated structures will be referred to as "somata".

Separation of A-type and Delayed-rectifier K^+ Currents

Potassium currents were recorded in isolated somata in choline-Cl bath solution. Inactivating A-type and delayed-rectifier K^+ currents were separated on the basis of a procedure described previously.[14] In brief, total K^+ currents activated by a depolarizing step to +20 mV after a 150-ms prepulse to −120 mV consisted of both rapidly inactivating A-type and delayed-rectifier components. A similar depolarization to +20 mV applied after a 150-ms prepulse to −60 mV (at which A-type channels

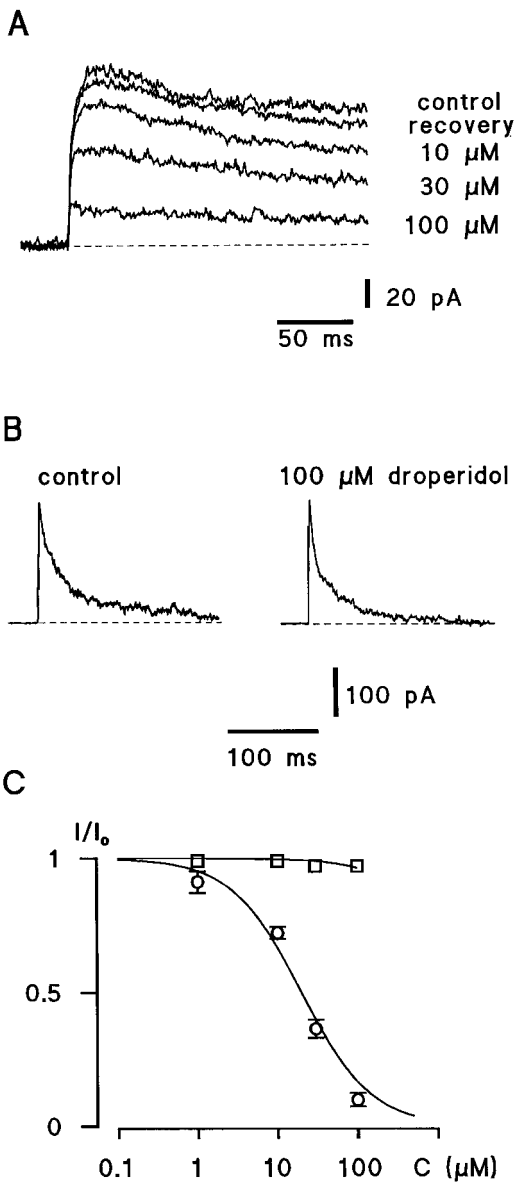

Fig. 1. Droperidol blocks delayed-rectifier but not A-type K^+ currents in isolated "somata" (*A*) Delayed-rectifier K^+ currents activated by a voltage pulse to +20 mV after a 150-ms prepulse to −60 mV in the presence of different droperidol concentrations (indicated near the corresponding traces). Holding potential, −80 mV. (*B*) Fast inactivating A-type K^+ channels obtained according to a procedure given in Materials and Methods in control choline-Cl solution and in the presence of 100 μM droperidol. (*C*) Concentration–effect relations of delayed-rectifier (circles) and A-type K^+ current (squares) suppression by droperidol (each seven "somata"). The data points for delayed-rectifier K^+ channels were fitted using a standard isotherm (equation 1) with IC_{50} of 20.6 ± 1.1 μM. The data for the A-type K^+ currents were fitted by eye.

in spinal neurons are almost completely inactivated[21]) elicited only a non-inactivating component of K^+ current that was considered as a delayed-rectifier current (fig. 1A). After digital subtraction of the delayed-rectifier component from the total K^+ current, the fast inactivating A-type component of K^+ current could be obtained (fig. 1B). The delayed-rectifier currents were measured at the end of a 200-ms depolarizing pulse, whereas the A-type currents were measured as the peak amplitudes of the subtracted component. The magnitude of A-type and delayed-rectifier K^+ currents were in the range of 110–340 and 100–370 pA, respectively.

Analysis of Changes in Repetitive Firing Behavior

For each neuron, several (at least five) corresponding pairs of recordings were subjected to a detailed analysis of changes in repetitive firing behavior. Each pair contained one train of action potentials recorded in control and one in the blocker-containing solution, both activated by current pulses of the same strength. For each individual recording, the mean firing frequency was determined as $f = (N - 1)/\Delta T$, where N was the number of complete spikes observed during current injection and ΔT was the time interval between the first and the last spikes in the train. For each pair, a ratio between the mean firing frequencies in blocker-containing (f_B) and control (f_C) solutions (k) was chosen as a measure of changes in firing behavior, $k = f_B/f_C$. The k values determined for at least five pairs of recordings were then averaged to give the k value for one neuron. The k values given in the text represent the mean values from five different neurons.

Statistical Analysis and Fitting

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

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

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 the drug, c was the drug concentration, and IC_{50} was the concentration giving a half-maximum effect. The binding of one drug molecule to the receptor was assumed to be sufficient for channel suppression.

The changes in the action potential overshoot and duration induced by the drug application were analyzed using analysis of variance followed by *post hoc* Tukey honest significant difference test. The changes in firing rates expressed by k were analyzed with nonparametric Mann–Whitney U test. The differences were considered as significant at *P* less than 0.05. *P* values given in table 1 correspond to significant differences between control *versus* drug and drug *versus* recovery groups. No significant differences between control *versus* recovery groups were found.

The present study is based on recordings from 71 intact neurons in the spinal cord slice and 39 isolated "somata." All numerical values are given as mean ± SEM.

Table 1. Effects of 10 nM TTX, 10 mM TEA, and 100 μM Droperidol on Single Spikes and Frequencies of Repetitive Firing in Dorsal Horn Neurons

	Parameter	Units	n	Control	Drug	Recovery	*P*
TTX	AP overshoot	mV	7	+49.8 ± 1.9	+29.5 ± 1.4	+49.7 ± 2.0	< 0.001
	AP duration	ms	7	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	NS
	Firing rate (*k*)		5	1.00	1.03 ± 0.01	1.03 ± 0.01	NS
TEA	AP overshoot	mV	12	+54.0 ± 3.1	+53.5 ± 3.1	+53.9 ± 3.1	NS
	AP duration	ms	12	1.9 ± 0.1	5.8 ± 0.3	1.9 ± 0.1	< 0.001
	Firing rate (*k*)		5	1.00	0.72 ± 0.06	1.00 ± 0.01	< 0.01
Droperidol	AP overshoot	mV	28	+50.8 ± 1.2	+36.3 ± 1.1	+50.7 ± 1.2	< 0.001
	AP duration	ms	28	1.9 ± 0.1	5.0 ± 0.2	1.95 ± 0.1	< 0.001
	Firing rate (*k*)		5	1.00	0.68 ± 0.08	0.99 ± 0.02	< 0.01
Droperidol*	AP overshoot	mV	14	+48.4 ± 1.6	+31.1 ± 1.3	+48.5 ± 1.6	< 0.001
	AP duration	ms	14	1.8 ± 0.1	4.1 ± 0.2	1.8 ± 0.1	< 0.001
	Firing rate (*k*)		5	1.00	0.71 ± 0.03	0.99 ± 0.01	< 0.01

Action potential (AP) duration was measured at the level of 0 mV for all neurons. The values are given as mean ± SEM. Values of *P* correspond to significant differences between control *versus* drug and drug *versus* recovery groups. No significant differences between control *versus* recovery groups were found for all experiments.

TTX = tetrodotoxin; TEA = tetraethylammonium; NS = nonsignificant difference; droperidol* = the data for droperidol obtained in a low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin.

The parameters obtained by fitting the data points using a nonlinear least-squares procedure are given as mean ± SE.

Results

Droperidol Blocks Delayed-rectifier K^+ Channels

Droperidol at concentrations between 10 and 100 μM reduced the delayed-rectifier current in isolated "somata" (fig. 1A). In contrast, the inactivating A-type currents were not affected by 100 μM droperidol (fig. 1B). The drug did not produce any detectable change in the kinetics of both currents. The concentration–effect relation for the block of delayed-rectifier K^+ channels was fitted with a standard isotherm with IC_{50} = 20.6 ± 1.1 μM (fig. 1C).

Functional Relevance of the Delayed-rectifier Current Block by Droperidol

These results indicate that the delayed-rectifier K^+ channels show a similar sensitivity to droperidol as voltage-gated Na^+ channels (IC_{50} = 8.3 μM).[9] The following experiments were designed to address the question of the functional significance of K^+ channel block by the substances that suppress both Na^+ and K^+ channels. According to a standard point of view, full suppression of Na^+ currents by high concentrations of anesthetics leads to complete conduction block in the neuronal membrane regardless of the drug action on K^+ currents. On the other hand, most neurons possess Na^+ currents with amplitudes several times larger than those needed for the action potential generation (see Discussion), and therefore incomplete block of the Na^+ conductance at blocker concentrations even considerably exceeding the IC_{50} would not result in conduction block. Such a phenomenon may be relevant during the onset of spinal or local anesthesia or recovery from it. Under such conditions, block of K^+ channels may have an influence on the cell excitability.

This assumption was tested in the experiments in which the actions of tetrodotoxin, tetraethylammonium, and droperidol on the single action potential were compared. The first two substances are classical blockers of Na^+ and delayed-rectifier K^+ channels, respectively, whereas droperidol suppresses both channels with a similar potency. The action potentials were recorded from intact neurons in the spinal cord slice. The concentrations of the blockers were chosen to suppress approximately half of the current, *i.e.*, 10 nM tetrodotoxin, 10 mM tetraethylammonium, and 100 μM droperidol. Because the currents show lower sensitivity to blockers when studied in the slice preparation,[9] these concentrations exceeded those sufficient for half-maximal current suppression in isolated "somata."

Figure 2 shows effect of 10 nM tetrodotoxin on single spike. In control solution, recording of the spike was followed by switching the amplifier to voltage-clamp mode and measurement of Na^+ and K^+ currents. After addition of tetrodotoxin, Na^+ current was reduced to 39%, whereas K^+ current at the end of the pulse remained unchanged. In the presence of tetrodotoxin, the amplifier was again switched to the current-clamp mode and the spike was recorded. After a suppression of more than half of the Na^+ current, the neuron was still able to fire a spike. Comparison of the spikes recorded in the presence and absence of tetrodotoxin (fig. 2, right) indicated that a suppression of Na^+ current resulted only in a small and reversible reduction in the overshoot potential (table 1).

It is well known that the velocity of membrane depolarization (dE/dt) is proportional to the magnitude of the flowing current (I) according to the equation

$$dE/dt = I/C \quad (2)$$

where C is the membrane capacity. Therefore, it might be reasonably assumed that suppression of Na^+ current could reduce the velocity of membrane depolarization, leading to a delayed activation of K^+ conductance that

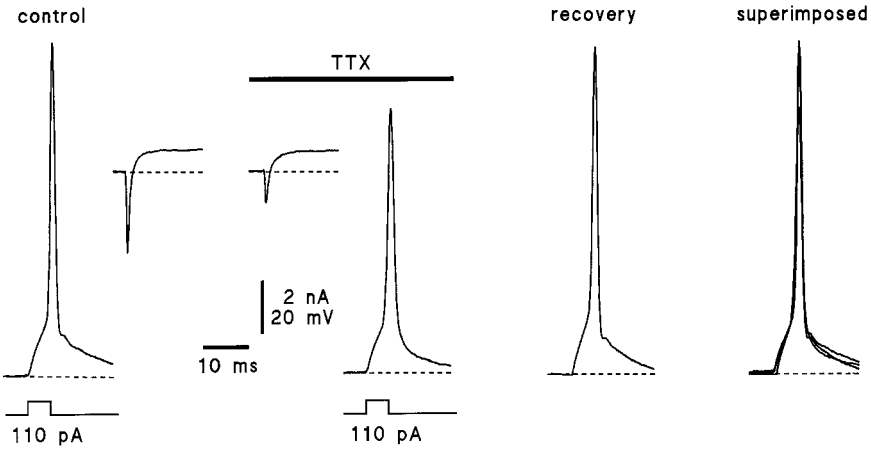

Fig. 2. Effect of 10 nM tetrodotoxin on the shape of a single spike in intact neurons. The action potentials and inward Na^+ and outward K^+ currents were recorded in absence and presence of 10 nM tetrodotoxin (TTX). Under the current-clamp conditions, the action potentials were elicited by 5-ms depolarizing current pulses of 110 pA. In voltage-clamp experiments, ionic currents were activated by a voltage step to 0 mV after a 50-ms prepulse to −120 mV. Holding potential, −80 mV. (*Right*) All three action potentials are shown superimposed.

could change the shape of the whole spike. In contrast, the apparent duration of the spike in figure 2 was not changed by tetrodotoxin. To clarify this point, we plotted both spikes from figure 2 at higher time resolution and digitally differentiated the traces (fig. 3). Indeed, the maximum velocity of depolarization decreased in tetrodotoxin from 183 to 100 V/s, in good agreement with the predictions of equation 2. Nevertheless, the apparent duration of the spike was not considerably changed. It could be assumed that the slowing of the rising phase did not change the width of the spike in dorsal horn neurons because the duration of the rising phase (0.36 ± 0.03 ms when potential changed from −20 to +20 mV; control, five neurons) was considerably shorter than the spike duration at −20 mV (1.51 ± 0.11, five neurons).

Addition of 10 mM tetraethylammonium led to a reversible block of approximately a half of delayed-rectifier K^+ current (measured at the end of the pulse), whereas the

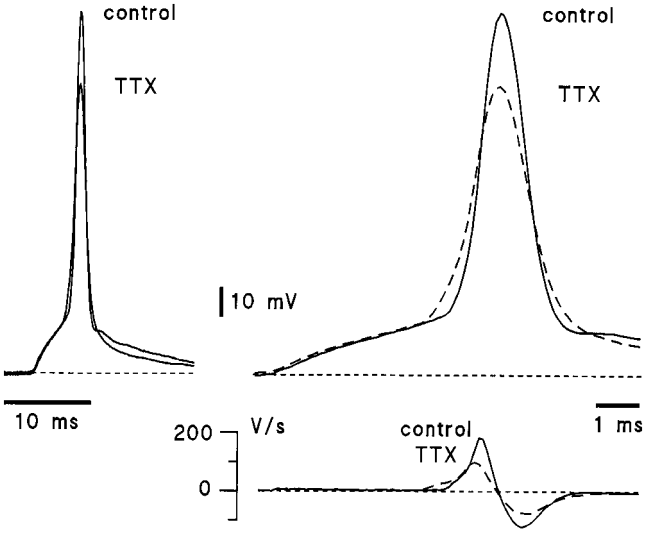

Fig. 3. Analysis of the tetrodotoxin-induced changes in the shape of action potential. Superimposed action potentials recorded in absence and presence of 10 nM tetrodotoxin (TTX) at normal (*left*) and higher (*right*) time resolutions. (*Right, bottom*) The first derivatives of upper traces. The neuron is the same as in figure 2.

Na^+ current was not changed (fig. 4). As expected, suppression of half of the delayed-rectifier K^+ conductance resulted in a considerable slowing of the repolarizing phase of the action potential (table 1), whereas the overshoot potential remained unchanged.

As blocker of Na^+ and delayed-rectifier K^+ channels, droperidol (100 μM) reduced both currents recorded in voltage-clamp mode to approximately a half (fig. 5). It reversibly changed the shape of the spike by lowering the overshoot potential and increasing the spike duration (table 1).

The changes in repetitive firing behavior of dorsal horn neurons induced by 10 nM tetrodotoxin, 10 mM tetraethylammonium, and 100 μM droperidol were analyzed using recordings of trains of action potentials (fig. 6 and table 1). In tetrodotoxin, the amplitudes of the spikes were slightly reduced in comparison with those of the corresponding control spikes, indicating a partial block of Na^+ currents. Nevertheless, the frequency of repetitive firing during the depolarization was not influenced by tetrodotoxin (k = 1.03). Addition of tetraethylammonium to a control solution did not change the amplitude of the first spike in the train but resulted in a considerable reduction of the frequency of repetitive firing (k = 0.72). Application of droperidol led to a slight reduction in the amplitude of the first spike but resulted in a pronounced reduction in the firing frequency (k = 0.68). The observed effects on the firing rates were reversible for all three substances.

The current-clamp experiments previously described were performed in external solution with physiologic level of calcium ions (2 mM). Under these conditions, in addition to the voltage-gated Na^+ and K^+ currents, Ca^{2+}-dependent K^+ (K_{Ca}) conductances can be also involved in repolarization of single action potential and modulation of the repetitive firing, as was shown for several types of central neurons.[20,22–25] A possible participation of K_{Ca} conductances makes the interpretation of the present results more complicated, because the data about the expression and function of the different types of K_{Ca} channels in spinal

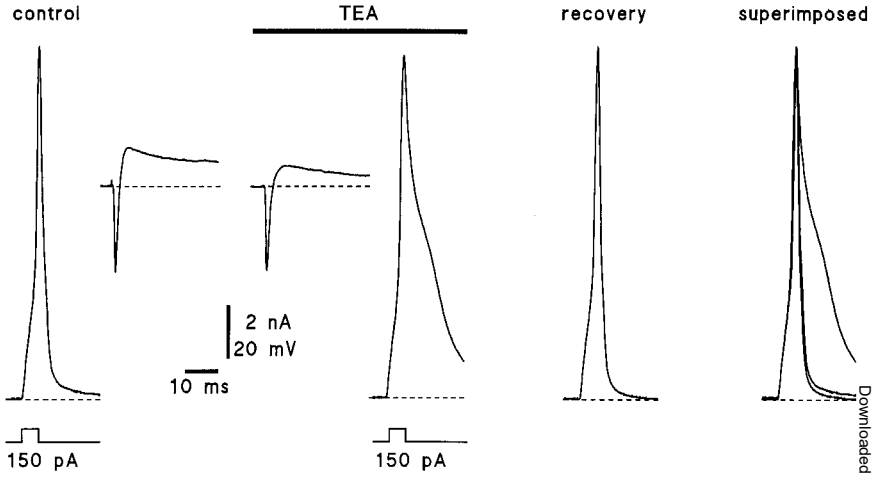

Fig. 4. Prolongation of the action potential by 10 mM tetraethylammonium (TEA). The action potentials and ionic currents were recorded in the absence and presence of 10 mM tetraethylammonium. The action potentials were elicited by 5-ms depolarizing current pulses of 150 pA. Na^+ and K^+ currents were activated by a voltage step to 0 mV after a 50-ms prepulse to −120 mV. Holding potential, −80 mV. (*Right*) The superimposed action potentials.

dorsal horn neurons as well as about their sensitivity to droperidol are not available at the moment. Therefore, we performed experiments in which the activation of K_{Ca} conductances was prevented by perfusing the slice with low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin. Charybdotoxin is a specific blocker of the large conductance K_{Ca} channels contributing to the spike repolarization, whereas apamin is known as a blocker of the small conductance K_{Ca} channels responsible for the slow after-hyperpolarization controlling firing rate in many types of neurons.[20,24–27]

To test whether charybdotoxin and apamin have some effect on voltage-gated K^+ channels, we first recorded total (A-type and delayed-rectifier) K^+ currents in isolated "somata" in the presence and absence of the blockers (fig. 7A). Addition of 100 nM charybdotoxin and 1 μM apamin did not change the amplitude and the time course of the total K^+ current (five "somata"). Thus, it could be concluded that both blockers do not affect voltage-gated A-type and delayed-rectifier K^+ channels.

When single spikes were recorded from the intact neurons in the spinal cord slice in external low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin, application of 100 μM droperidol resulted in a reversible prolongation of the action potential (fig. 7B and table 1). In experiments with injection of long-lasting depolarizing current pulses, a reversible reduction of the firing rate was observed (k = 0.71; table 1). Thus, in low-Ca^{2+}–high-Mg^{2+} solution containing charybdotoxin and apamin, under conditions in which the active membrane conductance consisted of voltage-gated Na^+ and K^+ (A-type and delayed-rectifier) components only, droperidol increased the duration of single spike and slowed the firing rate in a manner similar to that observed at physiologic Ca^{2+} concentrations.

Discussion

The basic finding of the present study is that neuroleptic droperidol, in addition to a suppression of voltage-gated Na^+ current,[9] also blocks delayed-rectifier K^+ current in spinal dorsal horn neurons, and that this block of K^+ conductance changes the neuronal firing behavior under conditions of incomplete Na^+ conductance block. This observation is of a special interest because clinically used local anesthetics such as bupivacaine, lidocaine, or mepivacaine also affect both Na^+ and K^+ channel systems,[12–15] but the meaning of this complex block is not well understood. Here we tried do determine conditions under which suppression of K^+ channels by Na^+ chan

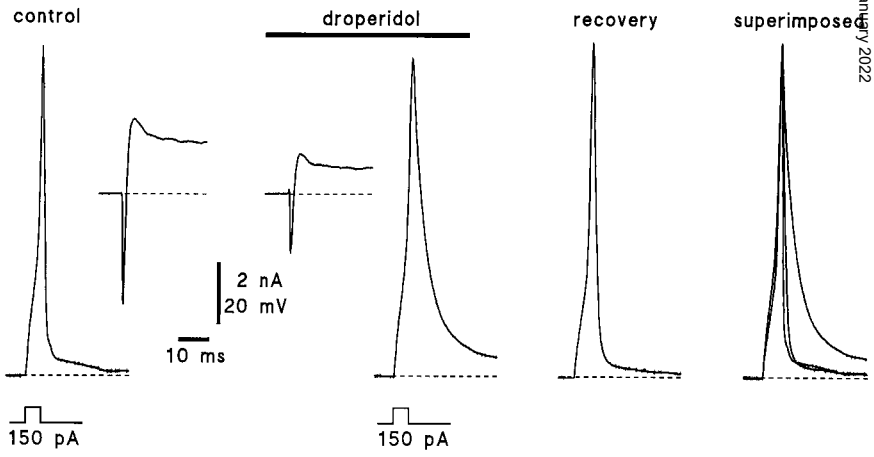

Fig. 5. Dual effect of 100 μM droperidol on the single action potential. The spikes were elicited by 5-ms current pulses of 150 pA in control solution and in the presence of 100 μM droperidol. The Na^+ and K^+ currents were activated by a voltage step from a holding potential of −80 mV to 0 mV after a 50-ms prepulse to −120 mV. The application of droperidol affected neither membrane resting potential nor the input resistance of the neuron (1.54 ± 0.21 GΩ in control *vs.* 1.57 ± 0.20 GΩ in droperidol, measured in voltage-clamp mode at voltage step from −80 to −120 mV, five neurons).

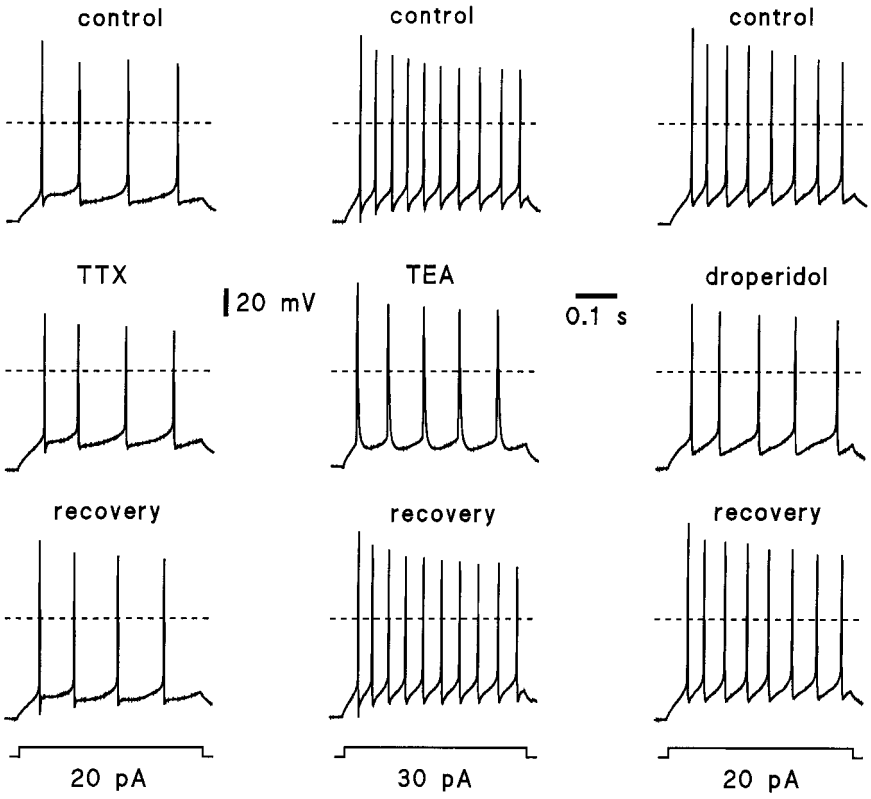

Fig. 6. Effects of tetrodotoxin (TTX), tetraethylammonium (TEA), and droperidol on repetitive firing in dorsal horn neurons. (*Left*) Trains of action potentials elicited in control (control and recovery) and 10 nM tetrodotoxin-containing solutions (*left*), control (control and recovery), and 10 mM tetraethylammonium-containing solutions (*middle*) as well as control (control and recovery) and 100 μM droperidol-containing solutions (*right*). Data are from three different neurons. The trains of action potentials were evoked by injecting depolarizing 0.5-s current pulses into the cells. For all recordings in one column, the strength of the injected current was equal and is given below the lowermost trace.

nel blockers can influence the excitability in spinal sensory neurons and addressed the question of functional consequences of applying the drugs with dual blocking effect.

Relation between the IC_{50} Value and the Block of the Physiologic Function

According to reported IC_{50} values, delayed-rectifier K^+ channels are less sensitive to droperidol than Na^+ channels (20.6 μM *vs.* 8.3 μM[9]). However, the IC_{50} values measured in voltage-clamp experiments do not give a direct information about the effectiveness of conduction block in a neuron or nerve fiber. For example, it is known that the axon possesses approximately five times more Na^+ channels than the amount necessary for the spike generation (the safety factor for the nerve conduction is approximately five).[28] In other words, conduction block can be achieved only if four fifths of Na^+ channels are blocked (80% block). Assuming the one-to-one binding reaction (equation 1) the 80% block is achieved at 4 times IC_{50}.

The safety factor of five determined for the conduction in the axon has been assumed. This value is close to that in spinal dorsal horn neurons, which can be simply estimated from our experiments. The neurons were always able to generate stable action potentials if the maximum Na^+ currents activated after a prepulse to −120 mV were approximately 1 nA. The mean Na^+ current in neurons of older rats was larger than 5 nA.[29] Thus, the safety factor for the spike generation in spinal dorsal horn neurons could be also estimated as five, and effective conduction block could be expected at drug concentrations exceeding 4 times IC_{50}.

Delayed-rectifier K^+ channels are involved in spike repolarization and therefore determine its duration. The safety factor for this function of K^+ channels is not known. If it were lower than that for the Na^+ channel function (depolarization), one would expect to see measurable effects of the blocker on the K^+ channel function (repolarization) at lower concentrations. Indeed, these assumptions were supported in our experiments with single action potentials.

Block of approximately half of the Na^+ current by tetrodotoxin did not suppress membrane excitability. The neuron was still able to generate single spikes with a duration similar to that observed in control solution. In contrast, a block of approximately half of the voltage-gated K^+ current by tetraethylammonium led to a considerable prolongation of the spike, indicating that the safety factor for the K^+ channel function is lower than two. According to the expectation, an addition of droperidol at a concentration reducing both Na^+ and K^+ currents to approximately a half did not result in a spike suppression but significantly increased its duration.

Effect on Repetitive Firing

A functional consequence of the spike prolongation by tetraethylammonium and droperidol was a reduction in the frequency of repetitive firing in spinal sensory neurons. This reduction was not observed in the presence of

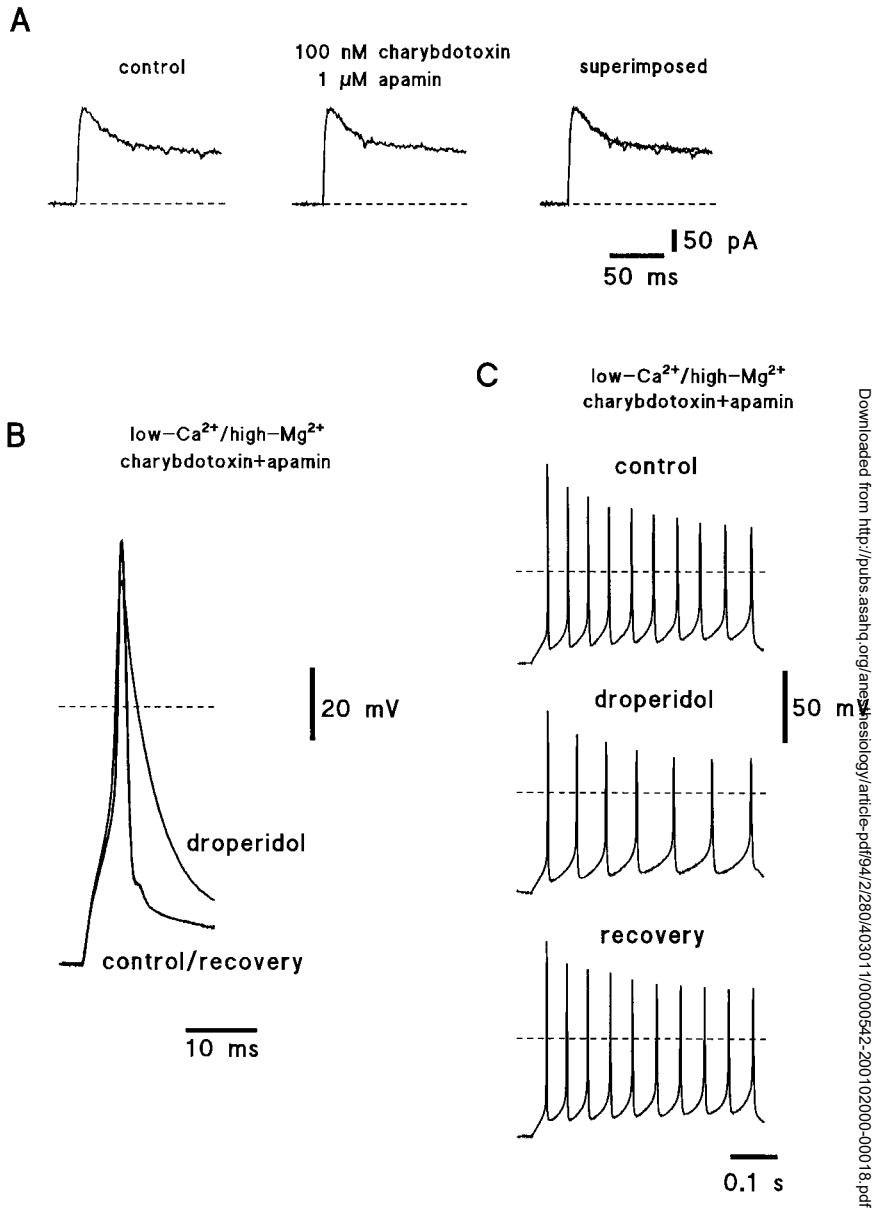

Fig. 7. Droperidol changes the shape of the action potential and the firing rate in low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin (*A*); 100 nM charybdotoxin and 1 μM apamin do not affect voltage-gated K^+ currents. Total (A-type and delayed-rectifier) K^+ currents were activated in isolated "somata" by a voltage pulse to +20 mV after a 150-ms prepulse to −120 mV. The holding potential was −80 mV. Control, external choline-Cl solution. (*B*) Action potentials recorded from intact neuron in spinal cord slice in low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin in absence (control–recovery) and presence of 100 μM droperidol. (*C*) Trains of action potentials activated by 500-ms current pulses of 20 pA in low-Ca^{2+}–high-Mg^{2+} solution containing 100 nM charybdotoxin and 1 μM apamin in absence (control–recovery) and presence of 100 μM droperidol.

tetrodotoxin, indicating that it resulted from the suppression of K^+ rather than Na^+ channels.

A similar reduction of the firing frequency by tetraethylammonium was reported for facial motoneurons from guinea pig[23] and hypoglossal motoneurons of neonatal rat.[24] In both cases, a prolongation of the single spike in tetraethylammonium enhanced the later phase of after-hyperpolarization, leading the authors to the conclusion that an increased influx of Ca^{2+} ions during prolonged spikes was the major factor slowing the firing rate. The mechanism of reduction of the firing rate in spinal dorsal horn neurons by tetraethylammonium and droperidol reported here seems to be of another origin, because the application of tetraethylammonium did not result in an enhancement of the later phase of after-hyperpolarization (fig. 6), and the firing rate was also slowed by application of droperidol in low-Ca^{2+}–high-Mg^{2+} solution containing charybdotoxin and apamin, where Ca^{2+} influx into the cell as well as the K_{Ca} channels were blocked. It should also be noted that droperidol does not seem to produce a direct blocking effect on K_{Ca} conductances, as their suppression usually leads to an increase, rather than a decrease, in the rate of repetitive firing.[22–24]

A possible reason for the reduction of the firing frequency in dorsal horn neurons after the block of delayed-rectifier K^+ channels by tetraethylammonium and droperidol may be a stronger inactivation of voltage-gated Na^+ channels during prolonged action potentials, leading to an increased time needed for the channel recovery from inactivation. Prolonged action potentials may also increase a use-dependent block of Na^+ channels by droperidol described previously.[9] Although our experiments with tetraethylammonium showed that the firing rate is considerably slowed even without any use-

dependent block of Na^+ channels, the latter may contribute to the frequency reduction induced by droperidol. Precise estimation of the influence of the use-dependent block of Na^+ channels on the repetitive firing is not possible at the moment because local anesthetics such as bupivacaine, lidocaine, and mepivacaine showing this type of Na^+ channel block additionally suppress voltage-gated K^+ channels in spinal dorsal horn neurons[9] and therefore also produce a complex blocking effect. Thus, suppression of delayed-rectifier K^+ channels by the blocker of Na^+ channels may result in a reduction of firing abilities of spinal sensory neurons.

Relevance of K^+ Channel Block

The neuroleptic droperidol exerts a dual effect on spinal sensory neurons, suppressing both Na^+ and voltage-gated K^+ channels. In this respect, it shows some similarities with standard local anesthetics that block, in addition to Na^+ channels, also different types of K^+ channels in neuronal preparations.[14,15] The functional meaning of this complex block is still unclear. It was assumed that the block of background K^+ channels by local anesthetics in peripheral nerve may result in membrane depolarization, leading to partial inactivation of Na^+ channels and increased conduction block.[15] The block of inactivating A-type K^+ channels was also assumed to influence the firing behavior in spinal sensory neurons.[14] In the present study we combined the measurements of the drug potency in isolated "somata" with the current-clamp recordings from intact neurons to directly examine whether suppression of K^+ channels by the Na^+ channel blockers may influence the mechanisms of conduction in neuronal membrane.

The block of K^+ channels by droperidol reduces the ability of neurons to generate repetitive spikes. In these terms, the suppression of K^+ channels can facilitate conduction block in neurons and nerve fibers. It is interesting to note that clinically used local anesthetics such as bupivacaine, lidocaine, and mepivacaine were shown to exert a similar dual effect, suppressing both voltage-gated Na^+ and K^+ conductances in spinal sensory neurons[14]; therefore, they may also be able to influence membrane excitability *via* action on K^+ conductance. Such a phenomenon may appear during conditions in which the drug concentration is insufficient for complete conduction block, for example, during the onset of spinal or local anesthesia or recovery from it. It may also be the case during standard regional anesthesia in the neighboring spinal cord segments where the drug concentration is lower.

In conclusion, despite the broad clinical usage of neuroleptic droperidol, there is no univocal opinion about the mechanisms of its action, and the literature describing clinical and laboratory studies of its effects is controversial. Although it is generally believed that epidural droperidol applied with opioids reduces nausea and emesis,[4,30] there are some reports showing that only intravenous, but not epidural, droperidol alleviates these side effects.[31] There are also controversial reports about the antinociceptive effects of the drug. It was found in a clinical study that epidural droperidol reduces the duration of analgesia produced by epidural sufentanil.[30] Another laboratory study with mammalians suggests that intrathecal droperidol has no antinociceptive effect itself or in combination with morphine,[32] whereas some clinical investigations indicate that low doses of droperidol given epidurally might also potentiate the antinociceptive effect of epidural morphine.[4] Our present and previous[9] results also indicate that droperidol itself may exert an antinociceptive effect that can be of clinical relevance, for example, for the cerebral action of the drug during intravenous application. Despite difficulties with the interpretation of the data of clinical and laboratory investigations available at the moment, this study of droperidol effects performed at the cellular level may be helpful in understanding the complex mechanisms of the drug action on the central nervous system.

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