Research report

Block of sodium currents in rat dorsal root ganglion neurons by diphenhydramine

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Accepted 15 August 2000

Abstract

To elucidate the local anesthetic mechanism of diphenhydramine, its effects on tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium currents in rat dorsal root ganglion (DRG) neurons were examined by the whole-cell voltage clamp method. Diphenhydramine blocked TTX-S and TTX-R sodium currents with \( K_v \) values of 48 and 86 \( \mu \text{M} \), respectively, at a holding potential of \(-80 \text{ mV}\). It shifted the conductance–voltage curve for TTX-S sodium currents in the depolarizing direction but had little effect on that for TTX-R sodium currents. Diphenhydramine caused a shift of the steady-state inactivation curve for both types of sodium currents in the hyperpolarizing direction. The time-dependent inactivation became faster and the recovery from the inactivation was slowed by diphenhydramine in both types of sodium currents. Diphenhydramine produced a profound use-dependent block when the cells were repeatedly stimulated with high-frequency depolarizing pulses. The use-dependent block was more pronounced in TTX-R sodium currents. The results show that diphenhydramine blocks sodium channels of sensory neurons similarly to local anesthetics. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

A variety of adverse reactions are associated with local anesthetics, some of which are thought to be allergic. Local anesthetics are classified as ester or amide types according to their chemical structures. Esters are associated with a higher incidence of allergic reactions. Patients who are allergic to one type of local anesthetics can be treated with the other. In the instance that hypersensitivity to both ester and amide local anesthetics occurs, then alternative therapies are required [8].

Diphenhydramine is one of the first-generation histamine \( H_1 \) receptor antagonists. It is widely used in various allergic conditions such as rhinitis, urticaria and conjunctivitis, and in motion sickness. In addition diphenhydramine has been recommended as an alternative local anesthetic among others for patients claiming allergy to lidocaine and its chemical analogues. In clinical studies diphenhydramine has been proven to be as effective as lidocaine for achieving local anesthesia without clinically noticeable complications, although injection is more painful [10,12,19].

Local anesthetics exert their effect by preventing the generation and the conduction of the sensory nerve impulse. The nerve conduction block by local anesthetics is mainly caused by their inhibition of voltage-gated sodium channels in the nerve membrane [6]. Likewise the local anesthetic activity of diphenhydramine may arise from sodium channel inhibition. Indeed the interaction of diphenhydramine with sodium channels has been reported. Batrachotoxin (BTX) is a depolarizing agent that causes persistent activation of sodium channels at the resting membrane potential by altering the voltage sensitivity of both activation and inactivation of the sodium channels [5]. Diphenhydramine inhibited BTX-elicited sodium in-
Electrophysiological recording

2.2. Electrophysiological recording

Cells attached to cover slips were transferred into a recording chamber on the stage of an inverted microscope. Ionic currents were recorded under voltage-clamp conditions by the whole-cell patch clamp technique [13]. Suction pipettes (0.8–1.0 MΩ) were made of borosilicate glass capillary tubes (TW150F-4, World Precision Instrument, Sarasota, FL) using a two step vertical puller (PP-83, Narishige, Tokyo, Japan) and heat-polished with a microforge (MF-83, Narishige). The pipette solution contained (in mM): NaCl 10, CsCl 65, CsF 70, HEPES 10. The pH was adjusted to 7.2 with CsOH. The external solution contained (in mM): NaCl 50, choline chloride 90, tetraethylammonium chloride 20, d-glucose 5, HEPES 5, MgCl₂ 1, CaCl₂ 1. Lanthanum (LaCl₃, 10 μM) was used to block calcium channel currents. The solution was adjusted to pH 7.4 with tetraethylammonium hydroxide. An Ag–AgCl pellet/3 M KCl–agar bridge was used for the reference electrode. Membrane currents were recorded using an Axopatch-1D amplifier (Axon instruments, Foster City, CA). Signals were digitized by a 12-bit analog-to-digital interface (Digidata 1200A, Axon Instruments), filtered with a 8-pole lowpass Bessel filter at 5 kHz and sampled at 50 kHz using pCLAMP6 software (Axon Instruments) on an IBM-compatible PC. Series resistance was compensated 60–70%. Capacitative and leakage currents were subtracted by using a P+/P4 procedure [3]. For the study of use-dependent block of diphenhydramine this procedure was not used. The liquid junction potential between internal and external solution was an averaged −4 mV. The data shown in this paper were corrected for the liquid junction potential. All experiments were performed at 22–24°C. Stock solution of diphenhydramine was made in distilled water at a concentration of 100 mM and aliquots were stored at −20°C until used. They were diluted in the external solution to the desired concentrations just before experiment. The recording chamber was continuously perfused with the external solution or one containing diphenhydramine by gravity at a rate of 2 ml per min. The volume of the chamber was 0.5 ml, which facilitated the solution exchange.

TTX-R sodium currents were isolated by blocking TTX-S sodium currents with TTX (100 nM). For the study of TTX-S sodium currents, cells that expressed only TTX-S sodium currents were used. TTX-S sodium currents were first identified by their fast kinetics and then a complete inhibition of the currents by TTX (100 nM) at the end of experiments. A period of 5–10 min was allowed after the establishment of the whole-cell recording configuration to ensure adequate equilibration between the internal pipette solution and the cell interior and to obtain a stable membrane current.

2.3. Data analysis

Data were analyzed by a combination of pCLAMP6 programs and SigmaPlot (Jandel Scientific, San Rafael, CA). All results are presented as mean±S.E.M. and n represents the number of the cells examined. Statistical
comparisons were made by paired Student’s t-test. The level of significance was considered at $P<0.05$.

3. Results

3.1. Block of sodium currents by diphenhydramine

Typical TTX-S and TTX-R sodium currents are shown in Fig. 1. The currents were evoked by step depolarizations to 0 mV from a holding potential of $-80$ mV. TTX-S sodium currents exhibited much faster time courses of activation and inactivation than TTX-R sodium currents. Both types of sodium currents were blocked after bath application of diphenhydramine. Fig. 1A and B show the time course of diphenhydramine block of TTX-S and TTX-R sodium currents, respectively.

The current amplitude decreased rapidly within 2 min after the drug application and then the rate of decrement was slowed reaching a steady state within 5 min. The currents were restored after 3 to 5 min of washout with drug-free solution. At a concentration of 100 $\mu$M, diphenhydramine for 5 min application reduced TTX-S and TTX-R sodium currents to $33\pm3\%$ ($n=7$) and $45\pm2\%$ ($n=7$) of control, respectively. Upon washout with drug-free solution for 5 min they were restored to $89\pm4\%$ and $88\pm1\%$ of control, respectively.

The dose–response relationships for diphenhydramine block of sodium currents are shown in Fig. 2. The fraction of control current remaining after diphenhydramine treatment is plotted as a function of the diphenhydramine concentration. The curves are fits to data points of the Hill equation, $I/I_{\text{CONTROL}}=1/(1+[DH]/K_d)^h$, where $I_{\text{CONTROL}}$ is control current amplitude, $I_{DH}$ is current amplitude remaining after diphenhydramine treatment, [DH] is the concentration of diphenhydramine, $K_d$ is the apparent dissociation constant for diphenhydramine block.

![Fig. 1. Time course of diphenhydramine (DH) block of TTX-S (A, $n=7$) and TTX-R (B, $n=7$) sodium currents. Currents were evoked by step depolarizations to 0 mV from a holding potential of $-80$ mV at an interval of 15 s. Diphenhydramine 100 $\mu$M was applied for 5 min and then washed out with diphenhydramine-free external solution as indicated in the box. Representative current traces recorded at times a and b are shown in the right panel.](image)

![Fig. 2. Dose–response relationship for diphenhydramine block of TTX-S (●) and TTX-R (○) sodium currents. Currents were evoked by step depolarizations to 0 mV from a holding potential of $-80$ mV. Current amplitude was measured before and after diphenhydramine treatment for 5 min, and the fraction of control current amplitude was plotted as a function of diphenhydramine concentration ($n=7$ for each data point). The curves are drawn according to the Hill equation.](image)
of sodium currents, and \( h \) is the Hill coefficient. The curves were best fitted when \( K_a \) values were 48 and 86 \( \mu M \), and Hill coefficients were 1.2 and 1.4, for the TTX-S and TTX-R sodium currents, respectively.

### 3.2. Effects of diphenhydramine on sodium current activation

Effects of diphenhydramine on the current–voltage curve and the conductance–voltage curve are illustrated in Fig. 3A for TTX-S sodium currents and in Fig. 3B for TTX-R sodium currents. TTX-S sodium current started to activate around \(-50 \text{ mV} \) reaching a peak amplitude around \(-20 \text{ mV} \), and its direction reversed near \(+40 \text{ mV} \). Diphenhydramine at 30 \( \mu M \) for 5 min decreased TTX-S sodium current amplitude over the entire test potentials without a change in the reversal potential. Voltage-dependence of TTX-S sodium current activation was changed by diphenhydramine. In control experiments, the half-maximum conductance (\( V_{g_{0.5}} \)) was calculated to be \(-34.6 \pm 1.5 \text{ mV} \) and the slope factor (potential required for an \( e \)-fold change) was \( 5.4 \pm 0.4 \text{ mV} \) (\( n=8 \)). In the presence of diphenhydramine 30 \( \mu M \), the values were changed by \(+4.1 \pm 1.0 \text{ mV} \) (\( P<0.01 \)) and \(+1.3 \pm 0.3 \text{ mV} \) (\( P<0.01 \)), respectively.

TTX-R sodium current started to activate around \(-30 \text{ mV} \) reaching a peak amplitude around \(-5 \text{ mV} \). Diphenhydramine at 100 \( \mu M \) blocked TTX-R sodium current at all test potentials. In the absence of diphenhydramine \( V_{g_{0.5}} \) was \(-12.0 \pm 1.1 \text{ mV} \) and the slope factor was \( 5.2 \pm 0.3 \text{ mV} \) (\( n=7 \)). Diphenhydramine changed the values by \(+1.2 \pm 0.7 \text{ mV} \) and \(+0.4 \pm 0.5 \text{ mV} \), respectively, but the differences were not statistically significant.

### 3.3. Effects of diphenhydramine on the time constant of inactivation

The effect of diphenhydramine on the decaying phase of sodium current was investigated (Fig. 4). Currents were evoked by step depolarizations to 0 \text{ mV} from a holding potential of \(-80 \text{ mV} \). The decay of sodium current was fitted by a single exponential function and the time constant was calculated. The time constants for TTX-S sodium currents were 0.29\pm 0.02 and 0.30\pm 0.03 ms (\( n=7 \)) in the absence and in the presence of diphenhydramine 100 \( \mu M \), respectively (Fig. 4A). When the peak amplitude of sodium current in the presence of diphenhydramine was multiplied to match that of control current, they overlapped almost completely, indicating an absence of the effect on the time course of TTX-S sodium current.

On the other hand, the decay of TTX-R sodium current was accelerated by diphenhydramine. The time constants in the absence and in the presence of diphenhydramine 100 \( \mu M \) were calculated to be 5.92\pm 0.39 and 4.81\pm 0.40 ms (\( P<0.001 \), \( n=7 \)), respectively (Fig. 4B). The acceleration of TTX-R sodium current decay by diphenhydramine is evident when the peak amplitudes are matched. The effect was reversible upon washout of the drug and the time constant was 5.49\pm 0.51 ms (\( P<0.01 \)).

### 3.4. Effects of diphenhydramine on the steady-state inactivation

TTX-S sodium channels were inactivated completely at holding potential above \(-50 \text{ mV} \) and were relieved from inactivation at holding potential below \(-100 \text{ mV} \) (Fig. 5A). Diphenhydramine at 100 \( \mu M \) for 5 min treatment shifted the steady-state inactivation curve in the hyperpolarizing direction. The maximum current amplitude at the holding potential of \(-140 \text{ mV} \) was also reduced by 13\pm 1\% (\( n=7 \)). In control experiments, the half-maximum inactivation potential (\( V_{h_{0.5}} \)) was estimated to be \(-79.8 \pm 1.4 \text{ mV} \) and the slope factor was \( 6.3 \pm 0.2 \text{ mV} \) (\( n=7 \)). Diphenhydramine at 100 \( \mu M \) shifted \( V_{h_{0.5}} \) by \(-12.8 \pm 1.1 \text{ mV} \) (\( P<0.001 \)) and increased the slope factor by \( 0.8 \pm 0.2 \text{ mV} \) (\( P<0.01 \)).

Similar results were observed with TTX-R sodium channels (Fig. 5B). Almost all TTX-R sodium channels were inactivated at holding potential above \(-30 \text{ mV} \) and...
were free of inactivation at holding potential below −80 mV. In the absence of diphenhydramine the steady-state inactivation curve was best fitted when \( V_{h_{0.5}} \) was −50.7±1.3 mV and the slope factor was 4.8±0.2 mV (n=6). Diphenhydramine at 100 μM for 5 min shifted the curve by −13.5±1.1 mV (\( P<0.001 \)) and increased the slope factor by 1.5±0.2 mV (\( P<0.001 \)). The maximum current amplitude at the holding potential of −110 mV was reduced by about 41±7%.

### 3.5. Effects of diphenhydramine on the time-dependent inactivation

Effects of diphenhydramine on the time-dependent inactivation of sodium channels are shown in Fig. 6A,B. TTX-S sodium currents started to inactivate 0.4 ms after the depolarizing pulse to −40 mV. The current amplitude then decreased exponentially and disappeared almost completely after 3000-ms pre-pulse. In the absence of diphenhydramine the time course was best described by three exponential functions, 0.59exp(−\( t/2.0 \))+0.25exp(−\( t/43.4 \))+0.16exp(−\( t/852.4 \)), where \( t \) is the pre-pulse duration in ms. After treatment of diphenhydramine 100 μM for 5 min the slowest component observed in the control disappeared, and the time course was best described by two exponential functions, 0.65exp(−\( t/2.5 \))+0.35exp(−\( t/33.6 \)).

TTX-R sodium currents started to inactivate 2 ms after the depolarizing pulse to −20 mV and were completely inactivated after 2000-ms pre-pulse. In the absence of diphenhydramine the time course was best described by two exponential functions, 0.60exp(−\( t/66.6 \))+0.40exp(−\( t/504.8 \)). In the presence of diphenhydramine 100 μM for 5 min the time course was best described by two exponential functions, 0.51exp(−\( t/13.1 \))+0.49exp(−\( t/115.7 \)).

### 3.6. Effects of diphenhydramine on the recovery from inactivation

TTX-S sodium channels were inactivated by 5-s depolarizing step to −20 mV from a holding potential of −100 mV and then repolarized to −100 mV for various durations followed by a step depolarization to 0 mV. The resultant current amplitude was plotted as a function of repolarizing duration (Fig. 6C). The time course of the recovery was best described by three exponential functions, 0.19(1−exp(−\( t/6.8 \)))+0.46(1−exp(−\( t/1085.1 \)))+0.34(1−exp(−\( t/7648.6 \)), where \( t \) is the repolarizing duration (ms). In the presence of diphenhydramine 100 μM for 5 min the fastest component disappeared and the time course was best described by two exponential functions, 0.73(1−exp(−\( t/1210.6 \)))+0.27(1−exp(−\( t/11440.5 \)).

For TTX-R sodium channels the inactivating potential of −20 mV for 5 s from a holding potential of −80 mV and the repolarizing potential of −80 mV were used (Fig. 6D). The time course of the recovery from inactivation was best described by two exponential functions, 0.25(1−exp(−\( t/633.6 \)))+0.75(1−exp(−\( t/6937.0 \)) in the absence of diphenhydramine. In the presence of diphenhydramine 100 μM
Fig. 5. Effects of diphenhydramine on the steady-state inactivation curves for TTX-S (A, n=7) and TTX-R (B, n=6) sodium channels. The membrane potential was held at various levels for 20 s, and then current was evoked by a step depolarization to 0 mV. The current amplitude is plotted as a function of the holding potential. The curves are drawn according to the equation, $I/I_{\text{max}} = 1/(1+\exp((Vh-V_{h50})/k))$, where $I$ is current amplitude, $I_{\text{max}}$ is maximum current amplitude, $Vh$ is holding potential, $V_{h50}$ is the potential at which $I$ is 0.5 $I_{\text{max}}$, and $k$ is the slope factor. Diphenhydramine 100 µM shifted $V_{h50}$ and $k$ of TTX-S channels by $-12.8 \pm 1.1$ mV and $+0.8 \pm 0.2$ mV, respectively, and those of TTX-R channels by $-13.5 \pm 1.1$ mV and $+1.5 \pm 0.2$ mV, respectively. (○) control; (●) diphenhydramine 100 µM, normalized to its own maximum; (▲) diphenhydramine 100 µM, normalized to the maximum of control.

µM for 5 min the time course was best described by two exponential functions, $0.02(1-\exp(-t/0.5)) + 0.98(1-\exp(-t/15288.9))$.

3.7. Use-dependent block

Diphenhydramine caused a use-dependent block of sodium currents during repetitive stimuli. TTX-S sodium currents were evoked by 50 consecutive 5-ms pulses to 0 mV from a holding potential of $-80$ mV at frequencies ranging from 1 to 20 Hz in the absence and in the presence of diphenhydramine 100 µM for 5 min (Fig. 7A). Even in the absence of diphenhydramine the current amplitude was reduced with increasing pulse number and frequency due to the accumulation of the channel inactivation. The use-dependent effect of diphenhydramine was not pronounced at pulse frequencies of 1 and 2 Hz. At frequencies of 10 and 20 Hz, however, a marked use-dependent block was observed.

TTX-R sodium channels showed a greater sensitivity to use-dependent block by diphenhydramine than TTX-S sodium channels (Fig. 7B). TTX-R sodium currents were evoked by 50 consecutive 25-ms pulses to 0 mV from a holding potential of $-80$ mV at frequencies ranging from 0.5 to 5 Hz in the absence and in the presence of diphenhydramine 100 µM for 5 min. There was a profound use-dependent block of the current by diphenhydramine even at 0.5 Hz stimuli, at which the control current was reduced minimally. The difference in current amplitude between before and after diphenhydramine treatment
became bigger at 1 and 2 Hz stimuli, but it was reduced at 5 Hz stimuli.

4. Discussion

Diphenhydramine caused a reversible blockade of TTX-S and TTX-R sodium currents in rat DRG neurons. The $K_d$ values for the current block were calculated to be 48 and 86 $\mu$M for TTX-S and TTX-R sodium currents, respectively, at a holding potential of −80 mV. Thus TTX-S sodium channels appear to be more sensitive to diphenhydramine than TTX-R sodium channels. However, when the holding potential was lowered to remove the effect of diphenhydramine on the steady-state inactivation of the channels, the drug at 100 $\mu$M reduced TTX-S and TTX-R sodium currents by 13% and 41%, respectively (Fig. 5). This result implies that at resting state TTX-R sodium channels would be more sensitive to the drug.

The $K_d$ value for TTX-S sodium currents is comparable to the results obtained with lidocaine ($K_d$ 42–50 $\mu$M) at the same condition. The sensitivity of TTX-R sodium currents to diphenhydramine, however, is greater than that to lidocaine by a factor of 2.3 [21,24]. This would be advantageous for diphenhydramine over lidocaine in that diphenhydramine can block the slow conducting sensory fibers more efficiently. Higher concentrations of lidocaine are required to suppress the slow conducting C-fibers involved in pain transmission than the fast conducting A-fibers [11,26]. TTX-R sodium currents are expressed mainly in the slow conducting fibers with a smaller diameter, and the higher concentrations of lidocaine re-
quired to block the conduction of these fibers is partly attributable to the lower sensitivity of TTX-R sodium currents to lidocaine [24,25].

Since the \( K_d \) values are a lot greater than the therapeutic plasma concentrations of diphenhydramine measured at usual dosages (0.7 \( \mu \)M) [14], the drug would barely bind to the sodium channels in the usual clinical situations such as in allergy treatment. However, a local administration of the drug would achieve concentrations sufficient to block the sensory sodium channels without systemic effects.

Diphenhydramine accelerated the decay of TTX-R sodium current but not that of TTX-S sodium current. Another difference between two types of sodium currents was found with respect to the drug effect on the activation process. Diphenhydramine shifted the conductance–voltage curve for TTX-S sodium currents in the depolarizing direction accompanied by an increase in the slope factor, while it did not affect those for TTX-R sodium currents.

The diphenhydramine block was voltage-dependent as evidenced by the shift of the steady-state sodium channel inactivation curve to more negative potentials. The shift of the inactivation curve indicates that diphenhydramine molecules have a higher affinity for the inactivated channels than for the resting channels. Therefore, the action of diphenhydramine would be enhanced when the membrane is depolarized, which will cause a substantial reduction of membrane excitability.

Diphenhydramine facilitated the time-dependent inactivation of sodium channels. The inactivation of TTX-S sodium channels was best described by three exponential functions. Characteristically the slowest component disappeared after diphenhydramine treatment, while the first and the second components were hardly affected. The inactivation of TTX-R sodium channels was best described by two exponential functions and the time constants for both components became smaller by diphenhydramine. The recovery of sodium channels from inactivation was slowed by diphenhydramine. The recovery was best described by three exponential functions for TTX-S sodium channels and two exponential functions for TTX-R sodium channels. The first component of the recovery for TTX-S sodium channels was removed by diphenhydramine without appreciable changes in the second or the third components. Even though the recovery for TTX-R sodium channels was able to be described with two exponential functions after diphenhydramine, the first component was almost negligible and the time constant for the second component became larger resulting in right shift of the curve. It is predicted that the faster time-dependent inactivation and the slower recovery of the channels from inactivation caused by diphenhydramine would cause a use-dependent block during a rapid stimulation, which is a characteristic property of local anesthetics [20].

The use-dependent block of sodium currents by local anesthetics during high frequency depolarizing pulses is attributed to their binding to open and inactivated channels during stimulus and to their slow dissociation from the channels between stimulus [20]. This blocking action is enhanced by shortening the interpulse interval so that the number of the drug-bound channels increases cumulatively with successive pulses. The use-dependent block by diphenhydramine was more pronounced in TTX-R than TTX-S sodium currents. This difference might come from the longer pulses (25 ms) used for TTX-R sodium currents instead of 5-ms pulses used for TTX-S sodium currents. The different pulse durations were employed since the time courses are different between two types of currents. However, as evidenced by the acceleration of the decaying phase of TTX-R sodium currents by diphenhydramine, the drug may bind to the open state of TTX-R sodium channels. As a consequence diphenhydramine would bind to TTX-R sodium channels more avidly than to TTX-S sodium channels. The ability of diphenhydramine to cause the use-dependent block surpassed that of lidocaine. For TTX-S currents, lidocaine showed no appreciable use-dependent block even at pulse frequency of 33.3 Hz.

Lidocaine blocked TTX-R sodium currents in a use-dependent manner but less efficiently than diphenhydramine [21,24]. Local anesthetics blocked A-fibers more effectively than C-fibers at low frequency stimulation while the reverse was true at high frequency stimulation [26]. This phenomenon was explained by the highly use-dependent block of TTX-R sodium channels which are predominantly expressed in C-fibers [24]. A similar mechanism is expected to operate for diphenhydramine and a further study is required.

The present study demonstrated that diphenhydramine modulated sodium channels in a similar fashion with local anesthetics. Local anesthetics bind to a receptor site located in transmembrane S6 segment of domain IV of the sodium channel \( \alpha \) subunit [20]. The receptor site is the determinant of both the use-dependence and the voltage-dependence of the local anesthetic action. It was suggested that two phenyl groups separated by a certain distance and angle are important determinants for diphenhydramine to bind to the inactivated sodium channels, and diphenhydramine and local anesthetics would share the same binding site [16].

**Acknowledgements**

This work was supported by the Research Grant of Chung-Ang University College of Medicine Alumni Association in 1999 to J.-H.S.

**References**


