Inhibition of a Hyperpolarization-Activated Current by Clonidine in Rat Dorsal Root Ganglion Neurons

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Yagi, Junichi and Rhyuji Sumino. Inhibition of a hyperpolarization-activated current by clonidine in rat dorsal root ganglion neurons. J. Neurophysiol. 80: 1094–1104, 1998. Whole cell voltage- and current-clamp recordings were carried out to investigate the effects of clonidine, an α2-adrenoceptor agonist, in L2 and L5 dorsal root ganglion (DRG) neurons of the rat. In voltage-clamp mode, application of 20 μM clonidine reversibly reduced the inward current evoked by hyperpolarizing voltage steps. The “clonidine-sensitive current” was obtained by subtracting the current during clonidine application from the control current, and its properties were as follows. 1) It was a slowly activating inward current evoked by hyperpolarization. 2) The reversal potential in the standard extracellular solution ([K⁺]o = 5 mM, [Na⁺]o = 151 mM) was −38.3 mV, and reduction of [Na⁺]o shifted it to a more negative potential, whereas an increase of [K⁺]o shifted it to a more positive potential, indicating that the current was carried by Na⁺ and K⁺ (PNa/PK = 0.22). 3) The relationship between the chord conductance underlying the clonidine-sensitive current and voltage could be fitted by a Boltzmann equation. These results indicate that the clonidine-sensitive current corresponds to a hyperpolarization-activated current (Ih), i.e., clonidine inhibits Ih in rat DRG neurons. DRG neurons were classified as small (15.9–32.9 μm diam), medium-sized (33–42.9 μm), and large (43–63.6 μm), and 7 of 19, 24 of 25, and 22 of 22 of these types exhibited Ih with a mean ± SE clonidine-induced inhibition values of 36.1 ± 3.5% (n = 7), 43.1 ± 3.7% (n = 24), and 35.1 ± 2.7% (n = 22), respectively. Clonidine application to L4 and L5 DRG neurons excised from rats the sciatic nerves of which had been transected 14–35 days previously (transected DRG neurons) also reduced Ih. In current-clamp mode, 9 of 13 intact and 4 of 6 transected medium-sized DRG neurons that exhibited Ih responded to clonidine with hyperpolarization (>2 mV). Some medium-sized DRG neurons exhibited repetitive action potentials in response to a depolarizing current pulse, and clonidine reduced the firing discharge frequencies in 8 of 11 intact and 3 of 4 transected neurons tested. Injection of a hyperpolarizing current pulse produced time-dependent rectification in DRG neurons that exhibited Ih, and clonidine blocked this rectification in all intact and transected neurons tested. These results suggest that inhibition of Ih due to α2-adrenoceptor activation contributes to modulation of DRG neuronal activity in rats. On the basis of our findings, we discuss the possible mechanisms whereby sympathetically released norepinephrine modulates the abnormal activity of DRG neuronal cell bodies after nerve injury.

INTRODUCTION

It has been demonstrated that slowly activating inward currents are evoked by hyperpolarizing voltage steps in a variety of neurons, including dorsal root ganglion (DRG) neurons, and in cardiac cells. Such hyperpolarization-activated currents, which have been termed Ih (H current), Ii (funny current), and IAR (anomalous rectification current) by various investigators (DiFrancesco et al. 1986; Larkman and Kelly 1992; Mayer and Westbrook 1983; McCormick and Pape 1990; Spain et al. 1987; Yanagihara and Irisawa 1980), will be referred to as Ih here. The characteristics of Ih channels are: slow opening at negative potentials, permeability to both Na⁺ and K⁺ ions, and sensitivity to blockade with Cs⁺ ions. In addition, Ih is also called the “pacemaker current,” because it is thought to play a significant role in cell excitability, especially the firing frequency. Activation of Ih at negative potentials can result in a slow depolarization, which is identified as time-dependent rectification during injection of a hyperpolarizing current (Mayer and Westbrook 1983; Scroggs et al. 1994; Villiére and McLachlan 1996). Such a depolarizing influence could accelerate neuronal firing discharges. Therefore, modulation of Ih could affect cell excitability. Modulation of Ih by neurotransmitters and intracellular second messengers in various cells has been reported (Akopian and Witkovsky 1996; DiFrancesco et al. 1986; DiFrancesco and Tortora 1991; Pape and McCormick 1989; Tokimasa and Akasu 1990; Watts et al. 1996) and the mechanisms responsible for such modulation vary. However, little is known about Ih modulation in DRG neurons. In this communication, we report that clonidine, a selective α2-adrenergic receptor agonist, inhibits Ih in DRG neurons of the rat.

DRG neurons are heterogeneous, and the diameters of their somata vary: the smallest cells correspond to Aβ- and C-type DRG neurons that give rise to slow-conducting axons, whereas the larger cells are Aα- and Aβ-type DRG neurons that give rise to fast-conducting axons (Harper and Lawson 1985). The DRG neurons of these different size classes have distinct sensory modalities and electrophysiological properties. Ih is observed in most medium and large DRG neurons, but infrequently in the smallest cells (Scroggs et al. 1994). We have found that clonidine leads to inhibition of Ih in most DRG neurons that exhibit Ih.

The clonidine-induced inhibition of Ih in rat DRG neurons observed in this study will modulate neuronal excitability and appears to be associated with noradrenergic modulation of the ectopic spontaneous discharges of DRG neurons. After injury of a peripheral nerve, a proportion of neuronal cell bodies in the DRG develop ectopic spontaneous discharges (Burchiel 1984; Kajander et al. 1992; Petersen et al. 1996; Wall and Devor 1983). Furthermore, morphological and electrophysiological studies have revealed that nerve injury triggers sympathetic-sensory coupling within DRGs and that spontaneously active DRG neurons respond to sympathetic...
activation with increased or decreased discharge frequencies (Devor et al. 1994; McLachlan et al. 1993; Michaelis et al. 1996; Xie et al. 1995), suggesting that sympathetically released norepinephrine probably regulates different types of ion channel that play important roles in DRG neuronal activity. On the other hand, pharmacological studies of pain have well documented that α2-adrenoceptor activation by clonidine produces antinociceptive effects (Davis et al. 1991; Kayser et al. 1995). Here, we analyze the effect of clonidine on \( I_h \) and the influence of the modulated \( I_h \) on DRG neuronal activity and discuss the possible mechanisms whereby α2-adrenoceptor activation modulates the abnormal activity of DRG neuronal cell bodies after nerve injury.

METHODS

Surgical and cell preparation

Adult female rats (Sprague Dawley, 6- to 12-wk old) were anesthetized deeply with pentobarbital sodium (80 mg/kg) injected intraperitoneally. Bilateral L4 and L5 DRGs were excised and placed immediately in oxygenated artificial cerebrospinal fluid (ACSF) comprising (in mM) 125 NaCl, 3.8 KCl, 2.0 CaCl\(_2\), 1.0 MgCl\(_2\), 1.2 KH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 10 d-glucose (pH 7.4). For simplicity, this solution is referred to henceforth as standard ACSF. The DRGs were digested with collagenase (1 mg/ml, Wako) for 30 min at room temperature (24 ± 2°C). After the treatment the epineurium around each was removed as carefully as possible. Each ganglion was placed on the bottom of a recording chamber (volume, ~0.8 ml) on the stage of an upright microscope (Nikon, X2-UD) with Nomarski optics, fixed with a grid of nylon threads glued to a platinum frame (Edwards et al. 1989) and superfused with a pipette with a tip diameter of 40–50 μm for 10–30 min at room temperature (Yawo and Chuhma 1994).

To study the effect of axotomy on DRG neuronal sensitivity to clonidine, the sciatic nerves of another group of rats were transected before experimentation. Under anesthesia with pentobarbital (50 mg/kg ip), the left sciatic nerve was exposed at the midthigh, ligated (4-0 silk suture) near the sciatic notch, then transected distal to the ligature site, and a silicone cap was sutured to the end of the proximal stump to promote formation of a neuroma (Oyelese et al. 1995). After this procedure, 60–70% of the neurons in the L4, L5 DRGs would have been axotomized (Devor et al. 1985; Himes and Tessler 1989). Fourteen to 35 days after axotomy, the left L4 and L5 DRGs were excited, and cell preparation was carried out as described above for intact rats.

After electrophysiological recording, a microphotograph of each DRG neuron was taken (H-III, Nikon) and the soma diameter (the average of the widths of its longest and shortest axes) was determined. The DRG neurons were classified, according to their diameters, as small (15.9–32.9 μm), medium (33–42.9 μm), and large (43–63.6 μm).

Electrophysiological techniques

The whole cell patch-clamp technique (Hamill et al. 1981) was applied to the DRG neurons superfused with ACSF. Patch pipettes were made by pulling borosilicate thin-walled glass capillaries (GC150T-10, Clark Electromedical) in two stages on a vertical puller (PC-10, Narishige) and then lightly fire-polished using a microforge (MF-90, Narishige). According to the procedure of Yawo and Chuhma (1994), a continuous satellite cell around the target DRG neuron was ruptured by the patch pipette with application of positive pressure (Fig. 1), immediately after which the pipette pressure was switched to negative to form a gigahm seal (>5 GΩ). The internal solution comprised (in mM) 155 KCl, 5 NaCl, 0.25 CaCl\(_2\), 2 MgCl\(_2\), 0.5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 5 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 2 Na\(_2\)ATP, and 0.2 Na\(_2\)GTP, pH adjusted to 7.2 with KOH, and the total [K\(^+\)] after pH adjustment was 162 mM. The pipette filled with this internal solution had a resistance of 1.8–2.4 MΩ for the small neurons and 1.4–2.0 MΩ for the medium-sized and large ones. The liquid junctional potential (<3 mV) was measured using the current-clamp procedure and was not corrected.

Voltage- and current-clamp experiments were carried out using an Axopatch 200A patch-clamp amplifier (Axon Instruments) at room temperature (24–27°C). The electrode and membrane capacitative transient were minimized by the front panel of the patch-clamp amplifier before and after seal rupture, respectively, and the series resistance was measured. The series resistance was usually <6 MΩ and compensated (50–70%) by a standard circuit. The input resistance measured during a 10-mV hyperpolarization from a holding potential of −60 mV by low-pass Bessel filter and digitized at 10 kHz. Data acquisition and analysis were carried out by an on-line IBM PC/AT clone computer programmed with the pClamp 6.0 system (Axon Instruments) via the digitizer (Digitdata 1200, Axon Instruments). In RESULTS, all the values are expressed as means ± SE.

In current-clamp mode, the effect of clonidine on the resting membrane potential was investigated as follows. When the resting membrane potential was stable for 3 min after whole cell recording...
was established, the standard ACSF was changed to that containing 20 μM clonidine. After 3 min, the membrane potential was measured four times every 5 s, and the mean value, which was considered to be the resting membrane potential in the presence of clonidine, was compared with that before drug application.

**Drug application**

DRG neurons were bathed with the required solution perfused at a constant rate (3 ml/min) through a manifold (MP-6, Waner Instrument) connected to the chamber in a near-zero dead space configuration for the purpose of fast solution exchange. Clonidine and other drugs were applied by changing the solution superfusing the DRG to one containing the required drug. A concentration-response curve was generated for clonidine (Fig. 7A), and 20 μM clonidine was found to evoke almost saturated responses. Therefore this concentration was used in all the experiments except those for investigation of pharmacological properties.

Clonidine hydrochloride (Wako), yohimbine hydrochloride (Wako), phenylephrine hydrochloride (Wako), norepinephrine bitartrate (Funakoshi), isoproterenol hydrochloride (Funakoshi), tetrodotoxin (Wako), and N-methyl-D-glucamine (Sigma) were obtained from a commercial source.

**RESULTS**

**Responses to clonidine application and their time courses**

The modulation by clonidine (selective α₂-adrenoceptor agonist) of the inward and outward current evoked by voltage steps in adult rat DRG neurons were examined. Figure 2 shows the response of a medium-sized DRG neuron in voltage-clamp mode.
mode that was subjected to test voltage steps of hyperpolarization (−120 mV, 200 ms) and depolarization (−40 mV, 200 ms) every 5 s from a holding potential of −60 mV. About 1 min after changing the standard ACSF containing 1 μM tetrodotoxin to that also containing 20 μM clonidine, the magnitude of the inward current evoked by hyperpolarization declined slowly, reaching its lowest level in ~2 min. The holding current shifted slightly outward with a time course almost the same as that of the inward current reduction. The latency of the response (time between the onset of clonidine application and the initial reduction) was 50–80 s and the mean latency was 66 ± 3 s (n = 8). Recovery was slow and varied from cell to cell: 90% recovery usually occurred between 6 and 12 min after the clonidine-containing solution was replaced by standard ACSF. Desensitization to clonidine in the inward current reduction was not observed in the presence of clonidine.

**Clonidine-sensitive current**

The inward currents evoked by hyperpolarizing voltage steps from a holding potential of −60 mV in rat DRG neurons consisted of two current components: an instantaneous inward current (I_{inst}) and a slowly activating inward current (hyperpolarization-activated current; I_{h}) (Figs. 3A and 6A). The expression of I_{h} in DRG neurons varied depending on their diameters (Scroggs et al. 1994; Villiére and McLachlan 1996). All the large and most of the medium-sized DRG cells expressed I_{h}, but many of the small ones did not, as described later and shown in Fig. 6.

Figure 3A shows the currents of a medium-sized DRG neuron in voltage clamp mode elicited by 200-ms command pulses to between −40 and −120 mV from a holding potential of −60 mV in 10-mV increments before, during, and during application of 20 μM clonidine.

![Figure 4](http://jn.physiology.org/). Reversal potential of the clonidine-sensitive current. A: representative recordings for measuring the reversal potential. In voltage-clamp mode, whole cell currents (bottom) of a medium-sized (39.9 μm diam) DRG neuron in standard ACSF ([K⁺]₀ = 5 mM, [Na⁺]₀ = 151 mM) containing 1 μM tetrodotoxin were recorded in response to a twin-pulse protocol (top) comprising a 200-ms hyperpolarizing prepulse to −120 mV from a holding potential of −60 mV, which made the chord conductance underlying the inward current constant, followed by a test pulse to different voltage steps from −70 to −20 mV in 10-mV increments before and during application of 20 μM clonidine. B: tail current of the clonidine-sensitive current was obtained by subtracting the current recorded in the presence of clonidine from the control current shown in A. Rate of the clonidine-sensitive current deactivation was slow (time constant = 127 ms at −60 mV in this case). This sample data were plotted in C (○). −, in A and B, 0-current level. C: examples of the relationship between the tail current amplitude and test voltage steps in standard ACSF (○) and low Na⁺ ([Na⁺]₀ = 80 mM; □) and high K⁺ ([K⁺]₀ = 20 mM; Δ) extracellular solutions. For the low Na⁺ solution, NaCl was replaced by an equimolar amount of N-methyl-D-glucamine and for the high K⁺ solution, KCl was added to standard ACSF. Data were obtained from different neurons. Unbroken lines show the least-squares fit for the data.
after application of 20 μM clonidine. Hyperpolarizing voltage steps evoked both \( I_{inst} \) and \( I_h \) in this representative cell. Large reductions of the slowly activating inward currents were observed in the presence of clonidine, and recovery was partial after wash-out for 6 min. \( I_{inst} \) also was reduced slightly by clonidine and the holding current was shifted outward. “Clonidine-sensitive currents” were obtained by calculating the difference between the currents before and during clonidine application (Fig. 3B), and they reflected the currents through the ion channels that were modulated by clonidine. The voltage dependence of the clonidine-sensitive current was demonstrated by the current-voltage relationship (Fig. 3C). The clonidine-sensitive current was a slowly activating inward current evoked by hyperpolarizing voltage steps, and the current-voltage curve showed rectification in the inward direction over the voltage range −40 to −120 mV.

The reversal potential of the clonidine-sensitive current was determined by measuring the tail current in mediumsized cells (Fig. 4). As the instantaneous component of the tail current was assumed to be a product of the chord conductance underlying the clonidine-sensitive current at the end of the initial hyperpolarizing step and the driving force (the test pulse potential minus the reversal potential), a change in the tail current should be proportional to a change in the test pulse potential (Fig. 4C). The estimated mean reversal potential of the clonidine-sensitive current was −38.3 ± 0.6 mV (n = 8) in standard ACSF ([K⁺]o = 5 mM, [Na⁺]o = 151 mM). We measured the mean reversal potential of the clonidine-sensitive current in two other extracellular solutions: one with a low Na⁺ concentration ([K⁺]o = 5 mM, [Na⁺]o = 80 mM) and the other with a high K⁺ concentration ([K⁺]o = 20 mM, [Na⁺]o = 151 mM) using the same method (Fig. 4C). The reversal potential was shifted toward a more negative potential (−49.4 ± 1.0 mV, n = 4) in the low Na⁺ solution, whereas it was shifted to a more positive potential (−28.9 ± 0.8 mV, n = 5) in the high K⁺ solution. These results suggest that the channel modulated by clonidine is permeable to both Na⁺ and K⁺, i.e., the clonidine-sensitive current is a mixed Na⁺-K⁺ current.

Next, we estimated the ratio of the permeability coefficients of Na⁺ and K⁺ in the channel that was modulated by clonidine using the following equation, which is a rearranged version of the Goldman-Hodgkin-Katz equation (Goldman 1943; Hodgkin and Katz 1949) and is solved for the ratio

\[
P_{Na} \left( \frac{P_{Na}}{P_K} \right) = \left[ \frac{[K^+]_o - [K^+]_i}{[Na^+]_o - [Na^+]_i} \right] \exp \left( \frac{EF}{RT} \right)
\]

where \( P_{Na} \) and \( P_K \) represent the permeability coefficients of Na⁺ and K⁺, respectively, \( E \) is the reversal potential, \( F \) the Faraday constant, \( R \) the universal gas constant, and \( T \) the temperature (K). Assuming that the intracellular ionic concentrations became equal to the ionic concentrations of the patch pipette solution, \([Na^+]_o \) and \([K^+]_o \) would be 9.6 and 162 mM, respectively. As the mean reversal potential was −38.3 mV in standard ACSF ([K⁺]o = 5 mM, [Na⁺]o = 151 mM) and the temperature was 24°C, \( P_{Na}/P_K \) calculated using the above equation was 0.21. The values of this ratio in low Na⁺ and high K⁺ solutions were 0.23 and 0.22, respectively. The mean value of \( P_{Na}/P_K \) was 0.22.

The voltage dependence of activation of the clonidine-sensitive current was demonstrated by the relationship between the chord conductance underlying the clonidine-sensitive current (\( G_{cs} \)) and voltage (Fig. 5). \( G_{cs} \) was obtained by dividing the clonidine-sensitive current amplitude by the driving force (\( V - V_{rev} \)), where \( V \) is the step potential and \( V_{rev} \) the reversal potential. In voltage-clamp mode, whole cell currents of medium DRG neurons that exhibited \( I_h \) were recorded in response to 200-ms hyperpolarizing steps to between −50 and −140 mV from a holding potential of −50 mV in 10-mV increments in standard ACSF, and the clonidine-sensitive current amplitudes at the end of the pulse were measured. The mean reversal potential of −38.3 mV in standard ACSF (see preceding text) was used to calculate the \( G_{cs} \) value at each potential, and it was normalized to the value at −140 mV, at which \( G_{cs} \) was assumed to be maximal (\( G_{cs \ max} \)). As shown in Fig. 5, the mean normalized data were plotted and fitted using the following Boltzmann equation

\[
G_{cs} = G_{cs \ max} / [1 + \exp \{ (V - V_{1/2}) / k \}]
\]

where \( G_{cs} \) is the chord conductance underlying the clonidine-sensitive current, \( G_{cs \ max} \) represents the maximal chord conductance, and \( V_{1/2} \) and \( k \) are parameters determining the half-activation voltage and the steepness factor, respectively. The equation was fitted to the data points by nonlinear least-squares curve fitting using an analysis program (Origin, MicroCal Software), and the parameter values were \( V_{1/2} = −85.9 \) mV, \( k = 12.9 \) mV (n = 5).

These biophysical properties of the clonidine-sensitive current are consistent with the following findings reported previously in relation to \( I_h \) in DRG neurons (Mayer and Westbrook 1983; Scroggs et al. 1994): \( I_h \) is a slowly activating inward current evoked by hyperpolarization, and the

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**FIG. 5.** Voltage dependence of activation of the clonidine-sensitive current. Mean normalized chord conductances underlying the clonidine-sensitive current (\( G_{cs} \)) were plotted against the membrane potential. Vertical bars indicate SE. Data were fitted using a Boltzmann equation: \( G_{cs} = G_{cs \ max} / [1 + \exp \{ (V - V_{1/2}) / k \} \] , where \( G_{cs} \) is the chord conductance underlying the clonidine-sensitive current, \( G_{cs \ max} \) represents the maximal chord conductance, and \( V_{1/2} \) and \( k \) are parameters determining the half-activation voltage and the steepness factor, respectively. Parameter values were \( V_{1/2} = −85.9 \) mV, \( k = 12.9 \) mV (n = 5). These were recordings of medium-sized DRG neurons.
current-voltage curve shows rectification in the inward direction; the reversal potential of $I_h$ is close to $-34 \text{ mV}$ in media of physiological composition and $I_h$ is a mixed $\text{Na}^+$ and $\text{K}^+$ current; and the relationship between the chord conductance of $I_h$ and voltage can be fitted by a Boltzmann equation. Therefore, we conclude that the clonidine-sensitive current corresponds to $I_h$, i.e., clonidine inhibits $I_h$ in rat DRG neurons.

**Inhibitory effects of clonidine on $I_h$ in DRG neurons of different sizes**

The effects of clonidine on $I_h$ in individual DRG neurons with different diameters were studied. As mentioned earlier, the DRG neurons tested could be divided into two groups on the basis of the presence or absence of $I_h$. An $I_h$ was obvious detected as a slowly activating inward current generated by a 200-ms hyperpolarizing pulse from a holding potential of $-60$ to $-120 \text{ mV}$ (Fig. 6A), but some DRG cells did not exhibit $I_h$ (Fig. 6B). $I_h$ was observed in 7 of 19 (36.8%) small, 24 of 25 (96.0%) medium-sized, and 22 of 22 (100%) large DRG cells tested, a result similar to that reported by Scroggs et al. (1994).

The $I_h$ and $I_{\text{inst}}$ components were discriminated visually. However, $I_{\text{inst}}$ would include the current component which is conducted through $I_h$ channels activated tonically at the holding level (see DISCUSSION). Here, the amplitude of $I_h$ was determined by subtracting the amplitude of $I_{\text{inst}}$ from that of the total current at the end of the pulse (Fig. 6A), indicating that it corresponds to the current conducted through $I_h$ channels evoked by a 200-ms hyperpolarizing pulse. Next, the amplitude of the clonidine-sensitive current was determined by calculating the difference between the $I_h$ amplitudes before and during application of clonidine and then the inhibitory effect of clonidine on $I_h$ was calculated as the proportion of the amplitude of the clonidine-sensitive current to that of $I_h$ before clonidine application. In Fig. 6C, the inhibitory effects of 20 $\mu\text{M}$ clonidine on $I_h$, as a

![Fig. 6. Inhibitory effects of clonidine on $I_h$ in DRG neurons of different sizes. A: representative example of the inhibitory effect of clonidine on $I_h$ in a DRG neuron expressing $I_h$. Whole cell currents in a medium-sized (41.5 $\mu\text{m}$ diam) DRG neuron were recorded under voltage clamp in response to 200-ms step to $-120 \text{ mV}$ from $-60 \text{ mV}$ before and during application of 20 $\mu\text{M}$ clonidine. $[\text{K}^+]_o = 5 \text{ mM}$. $[\text{Na}^+]_o = 151 \text{ mM}$. Instantaneous inward current and $I_h$ amplitudes before clonidine application at the end of the pulse are indicated by $I_{\text{inst}}$ and $I_{hb}$, respectively. Clonidine-sensitive current amplitude ($I_{cs}$) was determined by calculating the difference between the $I_h$ amplitudes before and during application of clonidine at the end of the pulse, and the magnitude of inhibition of $I_h$, as a percentage, was calculated using $I_{cs} / I_{hb} \times 100$. B: representative example of the current response of a DRG neuron lacking $I_h$. Current elicited by a 200-ms step to $-120 \text{ mV}$ from a holding potential of $-60 \text{ mV}$ was recorded in a small (20.4 $\mu\text{m}$ diam) DRG neuron, that did not exhibit a slowly activating inward current. $\rightarrow$, in A and B, 0-current level. C: percentage values for inhibition of $I_h$ by 20 $\mu\text{M}$ clonidine in individual DRG cells, which were divided into 2 groups: with (●) and without (○) $I_h$. For the neurons that did not exhibit $I_h$, the values were plotted as “0” on this graph. Vertical dotted lines divide the cells into small (S), medium-sized (M), and large (L) classes.](http://jn.physiology.org/doi/10.1152/jn.1999.181.11.1099)
percentage, on individual DRG cells of different sizes were plotted. Clonidine application led to inhibition of \( I_h \) in most of the DRG neurons that naturally exhibited \( I_h \). In small, medium-sized, and large DRG neurons that exhibited \( I_h \) were inhibited by 36.1 ± 3.5% \((n = 7)\), 43.1 ± 3.7% \((n = 24)\), and 35.1 ± 2.7% \((n = 22)\), respectively. There were no significant differences between the magnitudes of inhibition of \( I_h \) in cells of different sizes \([\text{1-way factorial analysis of variance (ANOVA) test}, P > 0.05]\).

**Pharmacological profile and identification of adrenergic receptors involved in modulation of \( I_h \)**

Pharmacological properties and characterization of the adrenoceptors involved in inhibition of \( I_h \) were explored in medium-sized DRG neurons. We recorded the drug-induced change in the amplitude of \( I_h \) evoked by hyperpolarizing voltage steps from \(-60\) to \(-120\) mV. Figure 7A shows concentration-response data for inhibition of \( I_h \) by clonidine. Clonidine was applied to individual medium-sized DRG neurons at several concentrations \((0.2–100 \mu M)\) starting with the lowest concentration and proceeding to higher concentrations in turn. The peak amplitude of the clonidine-sensitive current produced by each concentration of clonidine was normalized relative to the maximal inhibition produced by 100 \( \mu M \) clonidine in each cell. The data points were fitted with the function \( R = 1/[1 + (EC_{50}/[\text{clonidine}])^n] \), where \( R \) is the normalized response, \( EC_{50} \) is the half-maximal effective concentration, and \( n \) denotes the Hill coefficient. The fitting was carried out by nonlinear least-squares curve fitting program \((\text{Origin}, \text{MicroCal Software})\). \( EC_{50} \) was 2.2 \( \mu M \), and the Hill coefficient was 1.2.

Cesium ions \((\text{Cs}^+)\) have been shown to block \( I_h \) in many different cell types including DRG neurons \((\text{Mayer and Westbrook 1983; McCormick and Pape 1990; Scroggs et al. 1994})\). Application of 1 mM \text{Cs}^+ decreased the peak amplitude of \( I_h \) by 87.7 ± 3.0% \((n = 3)\), and the effects of clonidine on \( I_h \) were occluded completely by \text{Cs}^+ -induced blockade of \( I_h \) \((\text{Fig. 7B})\). Phenylephrine \((20 \mu M)\), an \( \alpha_1 \)-adrenergic receptor agonist, had little effect on \( I_h \) ampli-

![Fig. 7](http://jn.physiology.org/)

**FIG. 7.** Pharmacological profile involved in inhibition of \( I_h \). \( A \): dose-response data for clonidine. \( Aa \): mean normalized amplitudes of the clonidine-sensitive currents at various concentrations of clonidine plotted against \( \log[\text{clonidine}] \) \((n = 4\) for each point\). Error bars represent SE of mean. Concentration-response curve was fitted with the function \( R = 1/[1 + (EC_{50}/[\text{clonidine}])^n] \), where \( R \) is the normalized response, \( EC_{50} \) is the half-maximal effective concentration, and \( n \) denotes the Hill coefficient. \( EC_{50} \) was 2.2 \( \mu M \) and the Hill coefficient was 1.2. \( Ab \): representative examples of raw current traces in response to 200-ms steps to \(-120\) mV from \(-60\) mV. \( B \): occlusion of clonidine-induced inhibition of \( I_h \) by \( \text{Cs}^+ \). \( B \): antagonism of clonidine-induced inhibition of \( I_h \) by \( \text{yohimbine} \). Application of 2 \( \mu M \) clonidine resulted in a small decline of \( I_h \) in the presence of 2 \( \mu M \) \( \text{yohimbine} \), whereas after the yohimbine had been washed off the neuron for 8 min, 2 \( \mu M \) clonidine produced a larger reduction of \( I_h \) \((\bigcirc)\).
tude (percent change = \(-0.5 \pm 2.5\%\), \(n = 4\)) in the same medium-sized DRG neurons in which clonidine inhibited \(I_h\) (Fig. 7C). Figure 7D shows antagonism of the clonidine-induced inhibition of \(I_h\) by yohimbine, an \(\alpha_2\)-adrenergic receptor antagonist. When standard ACSF contained 2 \(\mu M\) yohimbine, application of 2 \(\mu M\) clonidine resulted in a small reduction of \(I_h\) amplitude (6.9 \(\pm 0.6\%\), \(n = 4\)), whereas after the yohimbine had been washed off the neurons, clonidine produced a significantly larger reduction of \(I_h\) amplitude (17.1 \(\pm 1.5\%\), \(n = 4\); paired \(t\)-test, \(P < 0.05\)). These results suggest that the clonidine-induced inhibition of \(I_h\) is mediated by \(\alpha_2\)-adrenoceptors.

Additionally, we observed that application of 10 \(\mu M\) noradrenaline produced an increase in \(I_h\) amplitude (12.1 \(\pm 1.9\%\), \(n = 4\)) in medium-sized DRG neurons and that in two of the three the increase was followed by suppression of \(I_h\). Also, 20 \(\mu M\) isoproterenol, a \(\beta\)-adrenoceptor agonist, increased \(I_h\) (7.6 \(\pm 1.1\%\), \(n = 3\)) (data not shown). It is suggested that \(\beta\)-adrenoceptor activation may be involved in the enhancement of \(I_h\) and that noradrenaline may produce two biphasic effects on \(I_h\) through activation of \(\alpha_2\)- and \(\beta\)-adrenergic receptors.

\(I_h\) inhibition by clonidine in DRG neurons after sciatic nerve transection

To investigate whether clonidine inhibited \(I_h\) in DRG neurons after experimental nerve injury (see METHODS), whole cell recordings were obtained from DRG neurons obtained from rats that underwent sciatic nerve transection 14–35 days previously (transected DRG neurons). The inhibitory effect of clonidine on \(I_h\) was measured with the procedure described in the legend to Fig. 6. Some transected DRG neurons did not exhibit \(I_h\), as observed with DRG neurons excised from rats with intact sciatic nerves (intact DRG neurons). \(I_h\) was observed in 7 of 15 small, 15 of 16 medium-sized, and 15 of 15 large transected DRG neurons. Application of 20 \(\mu M\) clonidine led to inhibition of \(I_h\) in most (35 of 37) of the transected DRG neurons that exhibited \(I_h\), and the mean values for clonidine-induced inhibition of \(I_h\) in small, medium-sized, and large DRG neurons were 46.3 \(\pm 7.5\%\) (\(n = 7\)), 35.6 \(\pm 4.2\%\) (\(n = 15\)), and 43.3 \(\pm 6.3\%\) (\(n = 15\)), respectively (Fig. 8). There were no significant differences between the magnitudes of the inhibition of \(I_h\) in cells of different sizes (1-way factorial ANOVA, \(P > 0.05\)). Comparison with the mean values for intact DRG neurons shown in Fig. 6 revealed no significant difference between the magnitudes of the inhibition of \(I_h\) for the intact and transected groups (2-way factorial ANOVA, \(P > 0.05\)).

These data demonstrated that clonidine inhibits \(I_h\) channels even in DRG neurons after nerve transection and that axotomy has no effect on the clonidine-induced inhibition of \(I_h\) in DRG neurons of rats 14–35 days after sciatic nerve transection.

Influence of clonidine on DRG neuronal excitability

In the last experiment of this study, we investigated the influence of clonidine on DRG neuronal excitability. Whole cell recordings of both intact and transected medium-sized DRG neurons that possessed \(I_h\) were carried out in current-clamp mode. Before application of clonidine, the mean resting membrane potential was \(-62.5 \pm 0.9\) mV (\(n = 13\)) and \(-61.0 \pm 1.1\) mV (\(n = 6\)) in intact and transected neurons, respectively (no significant difference; paired \(t\)-test, \(P > 0.05\)). Nine of 13 intact neurons and 4 of 6 transected neurons tested responded to clonidine with slight hyperpolarization (>2 mV) with a mean value of 3.0 \(\pm 0.5\) mV (\(n = 9\)) and 3.2 \(\pm 0.4\) mV (\(n = 4\)), respectively (no significant difference; paired \(t\)-test, \(P > 0.05\)), but application of clonidine had little effect on the resting membrane potentials of the other cells. Next, the effects of clonidine on the voltage responses of DRG neurons to injected current pulses were investigated (Fig. 9). Some medium-sized DRG neurons showed an action potential followed by an afterdepolarization or voltage humps in response to a given depolarizing current pulse, and a stronger current pulse evoked repetitive action potentials, probably due to a T-type Ca\(^{2+}\) current-mediated depolarization, as reported by others (Scroggs and Fox 1992; White et al. 1989). Application of 20 \(\mu M\) clonidine reduced the number and frequency of the firing discharges, and comparison of the action potentials elicited by a depolarizing current pulse in the absence of clonidine with those in its presence showed a reduction of the depolarizing influence after the initial phase of the afterhyperpolarization (Fig. 9B). The firing discharge frequencies were reduced by clonidine in 8 of 11 intact and 3 of 4 transected medium-sized DRG neurons that exhibited repetitive action potentials in response to a depolarizing current pulse. Clonidine increased the interspike interval between the initial and
dependent rectification in DRG neurons that exhibited activation by clonidine reversibly reduces current. The condition of the cells in this preparation was carried out. The condition of the cells in this preparation decreases. On the other hand, it has been shown that drug application was 0. Application of 20 μM clonidine in 3 min induced a 3.0-mV hyperpolarization of the resting membrane potential. Both the number and frequency of repetitive action potentials elicited by injection of a 50-ms depolarizing current pulse were reduced by clonidine. Application of clonidine also blocked time-dependent rectification produced by injection of a hyperpolarizing current pulse.

Inhibition of \( I_h \) by clonidine

The clonidine-sensitive current in this study was an inward influence on \( I_h \)-K+ currents, as some other groups already have demonstrated, are partially activated at a holding potential of -60 mV in DRG neurons and the reversal potential of \( I_h \) is more positive than the holding level (Mayer and Westbrook 1983; Scroggs et al. 1994), \( I_h \) channels activated tonically at the holding level should produce a cation influx component. Thus \( I_{inst} \) evoked by hyperpolarizing voltage steps would include the current component that is conducted through tonically activated \( I_h \) channels, and a reduction in the number of tonically activated \( I_h \) channels by clonidine would lead to a reduction of \( I_{inst} \). Furthermore, inhibition of \( I_h \) by clonidine would shift the holding current outward because the cation influx component at the holding level decreases. On the other hand, it has been shown that \( I_{inst} \) includes a leak current carried largely by potassium ions \( (I_{leak}) \) and/or an inward rectifier potassium current \( (I_k) \) in DRG neurons (Scroggs et al. 1994). It is possible that the reduction of \( I_{inst} \) was associated with blockade of either of these currents by clonidine and that the reversal potential of the clonidine-sensitive current in this study was estimated more negative than that of net \( I_h \) due to the negative reversal of \( I_{leak} \) and \( I_k \). However, clonidine seemed to have little influence on \( I_{leak} \) and \( I_k \). If clonidine inhibits either of these currents, this would result in a larger reduction of \( I_{inst} \) and the holding current would show an inward shift or not change by offsetting of the inward and outward shift, since the equilibrium potential for K+ is much more negative than the holding potential. In fact, although 1 mM Cs+ produced a large reduction in the amplitudes of both the slowly activating inward current and \( I_{inst} \), it did not consistently produce a shift in the holding current, probably due to its simultaneous blockade of \( I_h \) and the other K+ current (Fig. 7B). Clonidine in fact produced an outward shift in the holding current. From these points, it is thought that the reduction of \( I_{inst} \) might result from the inhibition of tonically activated \( I_h \) rather than that of \( I_{leak} \) and \( I_k \), suggesting that clonidine inhibits \( I_h \) with little influence on \( I_{leak} \) and \( I_k \).

**Discussion**

In this study, whole cell patch-clamp recording of DRG neurons in the isolated ganglion preparation of the adult rat was carried out. The condition of the cells in this preparation would be expected to be fairly physiological in comparison with acutely dissociated or cultured cell preparations because enzymatic and mechanical damage to the cells could be minimized.

Inhibition of \( I_h \) by clonidine

The clonidine-sensitive current in this study was an inward current evoked by hyperpolarization and a mixed Na+-K+ current \((P_{Na}/P_K = 0.22)\), and its reversal potential was -38 mV in standard ACSF, similar to the characteristics of \( I_h \) described in other reports (Mayer and Westbrook 1983; McCormick and Pape 1990; Tokimasa and Akasu 1990; Watts et al. 1996). From this we conclude that \( \alpha_2 \)-adrenoceptor activation by clonidine reversibly reduces \( I_h \) in rat DRG neurons. In voltage-clamp mode, clonidine inhibited a slowly activating inward current evoked by hyperpolarizing voltage steps, also reduced \( I_{inst} \) slightly, and shifted the holding current outward (Figs. 2, 3, and 6A). The reduction of \( I_{inst} \) and the outward shift in the holding current appear to be due to inhibition of \( I_h \), which is based on the following consideration. Because \( I_h \) channels, as some other groups

![Image](http://jn.physiology.org/ by 10.220.32.246 on October 8, 2016)
In current-clamp mode, clonidine caused slight hyperpolarization of the membrane from the resting level (Fig. 9), and this was thought to result from the inhibition of $I_h$. $I_h$ is activated partially at a membrane potential of around $-60$ mV, which is close to the resting membrane potential of DRG neurons under our experimental conditions, as mentioned above. Therefore, it has been assumed that $I_h$ contributes to the resting membrane potential (Mayer and Westbrook 1983; Scroggs et al. 1994). The resting membrane potential is determined by the counterbalancing actions of some ionic conductances (Goldman 1943; Hodgkin and Katz 1949). The partial activation of $I_h$ at the resting level will play a role in positively shifting the membrane potential toward the reversal potential of $I_h$ (approximately $-38$ mV), and inhibition of $I_h$ by clonidine at the resting level will lead to a small hyperpolarization. Additionally, the fact that clonidine induces a hyperpolarization is compatible with the supposition that the agent scarcely inhibits $K^+$ current components other than $I_h$.

Reduction of firing discharge frequencies by clonidine

$I_h$ and $I_{2-}$ have been called the pacemaker currents because they are thought to be concerned with modulation of spike frequency (DiFrancesco and Tortora 1991; DiFrancesco et al. 1986; Mayer and Westbrook 1983; Yanagihara and Irisawa 1980). As shown in Fig. 9, application of clonidine reduced the number and frequency of the repetitive action potentials evoked by a depolarizing current, and it is likely that this reduction was attributable to the inhibition of $I_h$. Two findings throw light on the mechanism involved. First, clonidine caused slight hyperpolarization of the membrane from the resting level, as mentioned above. Therefore, the membrane potential would be expected to shift away from the level at which action potentials are triggered. Second, application of clonidine led to a reduction of the depolarizing influence after the initial phase of afterhyperpolarization (Fig. 9B), and such reduction also was identified as a clonidine-induced block of time-dependent rectification during injection of a hyperpolarizing current (Fig. 9A). $I_h$ activated by membrane hyperpolarization during an afterhyperpolarization could exert a depolarizing influence and could facilitate repetitive action potentials. Thus it is possible that the inhibition of $I_h$ by clonidine reduces the depolarizing influence and delays initiation of the next action potential, resulting in a reduction of the repetitive action potential frequency.

Possible functional role of clonidine-induced inhibition of $I_h$ in DRG neurons

In this study, we have shown that $\alpha_2$-adrenoceptor activation can inhibit $I_h$ and reduce the firing frequency in both intact and transected DRG neurons. Here we discuss the possible mechanisms whereby sympathetic activation modulates the abnormal activity of DRG neurons after nerve injury.

After injury of a peripheral nerve, a proportion of neuronal cell bodies in the DRG develop ectopic spontaneous discharges (Buchtel 1984; Devor et al. 1994; Kajander et al. 1992; Michaelis et al. 1996; Petersen et al. 1996; Wall and Devor 1983). In addition, nerve injury triggers sympathetic-sensory coupling within DRGs, and the spontaneously active DRG neurons respond to sympathetically released norepinephrine with increased or decreased discharge frequencies (Devor et al. 1994; McLachlan et al. 1993; Michaelis et al. 1996; Xie et al. 1995). On the other hand, on the basis of in situ hybridization and immunohistochemistry experiments on intact DRG neurons, Levine and colleagues have suggested that individual DRG neurons possess multiple $\alpha$-adrenergic receptor subtypes ($\alpha_{2B}$ and $\alpha_{2C}$ at least) and concluded that coexpression of multiple $\alpha_2$-adrenergic receptor subtypes enables a single transmitter to produce a number of effects on the same neuron (Gold et al. 1997). Thus we suggest that inhibition of $I_h$ by $\alpha_2$-adrenoceptor activation is one such effect of norepinephrine on DRG neurons, although we do not know which subtype of $\alpha_2$-adrenergic receptors are involved in this modulation. In DRG neurons showing spontaneous action potential activity due to nerve injury, if the afterhyperpolarization of the spontaneous action potentials is negative and lasts long enough to activate $I_h$, then $I_h$ would contribute to facilitation of the firing discharges. Therefore inhibition of $I_h$ by $\alpha_2$-adrenoceptor activation may reduce the discharge frequency of spontaneously active DRG neurons.

Electrophysiological observations have suggested that norepinephrine has not only an inhibitory but also an excitatory effect on spontaneously active DRG neurons (Devor et al. 1994; Michaelis et al. 1996; Petersen et al. 1996). These findings suggest that norepinephrine has some effects on ion channels via certain types of adrenergic receptors (presumably $\alpha_2$ and $\beta_2$-adrenergic receptor subtypes linked to excitatory pathways), other than the inhibitory effect on $I_h$. In our preliminary experiments norepinephrine produced an increase in $I_h$, followed by suppression in some medium-sized DRG neurons. Also, isoproterenol, a $\beta$-adrenoceptor agonist, increased $I_h$ (see the pharmacological profiles section in RESULTS). These observations suggest that $\beta$-adrenoceptor activation may be involved in the enhancement of $I_h$ and that norepinephrine may produce two opposing effects on $I_h$ through activation of $\alpha_2$ and $\beta_2$-adrenergic receptors. Presumably through a mechanism dependent on cell-to-cell differences in the relative proportion of adrenergic receptor subtypes, norepinephrine appears to participate in the modulation of DRG neuronal excitability (Devor et al. 1994; Michaelis et al. 1996). Furthermore, some types of adrenergic receptors seem to be upregulated after nerve injury, indicating that DRG neurons acquire de novo sensitivity to norepinephrine (Gold et al. 1997; Perl 1994). Further experiments on norepinephrine-induced modulation of ion channels are required to clarify the mechanisms whereby it modulates DRG neuronal activity.

Possibility of antinociceptive effects by clonidine-induced inhibition of $I_h$

It has been proposed that antinociceptive effects are produced by clonidine (Davis et al. 1991; Kayser et al. 1995). Davis and colleagues (1991) reported that application of a clonidine patch to the skin reduced hyperalgesia in patients with neuropathic pain, suggesting that clonidine inhibits peripheral neuronal activities. We interpret this result as being due to clonidine-induced reduction of the firing discharges of $A$-type DRG neurons.

Many small DRG neurons do not exhibit $I_h$ (Scroggs et al. 1994; Villiére and McLachlan 1996; this study), suggesting that many of the nociceptive $C$-type neurons, which
give rise to nonmyelinated, slow-conducting axons, do not possess \( I_h \) channels, because the smallest cells correspond to C-type DRG neurons (Harper and Lawson 1985). Therefore, many of the C-type DRG neurons may not be subject to influences associated with modulation of \( I_h \), as they do not seem to possess \( I_h \) channels. However, clonidine-induced inhibition of \( I_h \) should be produced in A-type neurons, which give rise to myelinated axons and correspond to medium-sized and large DRG neurons. It has been reported that even activities of low-threshold Aβ fibers increase sensitivity to inflammatory pain (Neumann et al. 1996). Thus it is possible that clonidine could produce antinociceptive effects via inhibition of \( I_h \) in A-type DRG neurons.

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