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Hyperpolarization-activated Current (I_h) Contributes to Excitability of Primary Sensory Neurons in Rats

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Abstract

In various excitable tissues, the hyperpolarization-activated, cyclic nucleotide-gated current (I_h) contributes to burst firing by depolarizing the membrane after a period of hyperpolarization. Alternatively, conductance through open channels I_h channels of the resting membrane may impede excitability. Since primary sensory neurons of the dorsal root ganglion show both loss of I_h and elevated excitability after peripheral axonal injury, we examined the contribution of I_h to excitability of these neurons. We used a sharp electrode intracellular technique to record from neurons in nondissociated ganglia to avoid potential artefacts due to tissue dissociation and cytosolic dialysis. Neurons were categorized by conduction velocity. I_h induced by hyperpolarizing voltage steps was completely blocked by ZD7288 (approximately 10 μ M), which concurrently eliminated the depolarizing sag of transmembrane potential during hyperpolarizing current injection. I_h was most prominent in rapidly conducting A α / β neurons, in which ZD7288 produced resting membrane hyperpolarization, slowed conduction velocity, prolonged action potential (AP) duration, and elevated input resistance. The rheobase current necessary to trigger an AP was elevated and repetitive firing was inhibited by ZD7288, indicating an excitatory influence of I_h . Less I_h was evident in more slowly conducting A δ neurons, resulting in diminished effects of ZD7288 on AP parameters. Repetitive firing in these neurons was also inhibited by ZD7288, and the peak frequency of AP transmission during tetanic bursts was diminished by ZD7288. Slowly conducting C-type neurons showed minimal I_h , and no effect of ZD7288 on excitability was seen. After spinal nerve ligation, axotomized neurons had less I_h compared to control neurons and showed minimal effects of ZD7288 application. We conclude that I_h supports sensory neuron excitability, and loss of I_h is not a factor contributing to increased neuronal excitability after peripheral axonal injury.

1. Introduction

Damage to the axons of peripheral sensory neurons may result in chronic neuropathic pain in human subjects. Numerous sequelae ensue at locations along the sensory pathway, including the brain and spinal cord. Altered properties of the sensory neuron itself may be noted at both the injury site and in the neuronal somata in the dorsal root ganglion proximal to the injury, where shifts in expression and function of voltage gated channels in the contribute to an altered biophysical phenotype. Specifically, diminished tetrodotoxin-insensitive Na⁺ current and increased tetrodotoxin-sensitive current (Cummins et al., 2000), diminished K⁺ currents (Everill and Kocsis, 1999; Sarantopoulos et al., 2007), and diminished high voltage-activated

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(Abdulla and Smith, 2001; Baccei and Kocsis, 2000; Hogan et al., 2000; McCallum et al., 2006) and low-voltage-activated (McCallum et al., 2003) currents may each contribute to hyperexcitability noted in DRG neurons after axotomy (Sapunar et al., 2005).

The hyperpolarization-activated, cyclic nucleotide-gated current, also termed the anomalous rectifier current or H-current (I_h), is expressed in sensory neurons, particularly large neurons with myelinated axons (Scroggs et al., 1994; Tu et al., 2004; Vasilyev et al., 2007). This current is activated by membrane potentials negative to -60mV (Yao et al., 2003) and does not inactivate, and therefore is in a conducting state at resting membrane potentials (RMPs) typical of sensory neurons. Neuronal I_h channels conduct Na^+ and K^+ and exhibit a reversal potential of approximately -35mV (Maccaferri and McBain, 1996; Mayer and Westbrook, 1983; Mercuri et al., 1995; Yagi and Sumino, 1998), so their activation usually results in depolarization of the neuronal membrane potential.

In various neuronal cell types, I_h has been demonstrated to influence neuronal excitability through two opposing properties. The activation of I_h upon membrane repolarization following an action potential (AP) results in a depolarization that may initiate subsequent APs and a rhythmic burst, especially in conjunction with T-type calcium current (Pape, 1996). Accordingly, I_h has been shown to support spontaneous burst firing in most brain sites (Chan et al., 2004; Ghamari-Langroudi and Bourque, 2000; Lupica et al., 2001; Maccaferri and McBain, 1996; Tanaka et al., 2003), although not all (Mercuri et al., 1995). In contrast to this excitatory influence, ionic conductance through I_h channels reduces the input resistance of neurons and thereby limits AP initiation in response to a depolarizing current (Doan et al., 2004; Gasparini and DiFrancesco, 1997; Maccaferri and McBain, 1996). Since I_h is expressed in axons (Birch et al., 1991; Takigawa et al., 1998), reduced input resistance may increase conductance failure through points of impedance mismatch, such as the T-branch where the central and peripheral limbs of the sensory neuron axon meet with the axonal stem that leads to the neuronal soma (Luscher et al., 1994; Stoney, 1985).

In a prior investigation (Sapunar et al., 2005), we have noted a significant loss of the hyperpolarization-induced transmembrane potential relaxation ("sag") in sensory neurons after axotomy. Since the sag phenomenon is attributable to I_h (Maccaferri and McBain, 1996; Tanaka et al., 2003; Yao et al., 2003), we hypothesized that loss of I_h may contribute to the hyperexcitability also seen in sensory neurons following axotomy (Sapunar et al., 2005). Few observations are available to discern the role of I_h in sensory neurons. Block of I_h with the nonspecific agent clonidine resulted in reduced repetitive firing in partially digested DRG neurons (Yagi and Sumino, 1998), as did the specific agent ZD7288 in a small number of dissociated DRG neurons (Tu et al., 2004). However, measurement of I_h is influenced by both temperature and cytoplasmic dialysis (Cuevas et al., 1997), and dissociation produces an injury-like effect on neuronal excitability (Zheng et al., 2007). We have therefore examined the regulation of sensory neuronal excitability by I_h , using ZD7288 to selectively block I_h (BoSmith et al., 1993; Larkman and Kelly, 2001; Satoh and Yamada, 2000) in neurons of nondissociated DRGs at 35°C , using a sharp electrode technique that limits cellular alterations associated with patch dialysis (Blatt and Slayman, 1983).

2. Results

2.1 I_h in neurons of non-dissociated DRGs

Discontinuous single-electrode voltage clamp (DSEVC) recording was employed to confirm blockade of I_h by ZD7288. I_h was identified during as a slowly developing inward current during hyperpolarizing voltage commands (Fig. 1A). ZD7288 (approximately $10\mu\text{M}$, see methods) was delivered *via* a pressurized system. Control experiments with comparable application of vehicle without ZD7288 produced no changes in measured parameters (data not

shown). Three minute application of ZD7288 resulted in complete inhibition of the time-dependent component of inward current (Fig. 1A, Table 1), which confirms the identity of this component as I_h and allows its measurement as the difference current obtained by digitally subtracting the baseline trace from the post-ZD7288 trace (Fig. 1B). The functional effect of I_h may be discerned in its production of the transmembrane potential sag, seen in discontinuous current-clamp (DCC) mode as a slowly developing depolarization during sustained hyperpolarizing current pulses recorded (Fig. 1C,D). In the same neurons in which I_h was blocked by ZD7288, sag was also entirely eliminated (Fig 1C, Table 1), which confirms that I_h generates the sag. This causal relationship is further supported by the strong cell-by-cell correlation ($R=0.80$, $P<0.001$) between I_h and sag, which was measured as the percentage return of the potential back to RMP (Villiere and McLachlan, 1996).

Reliable membrane capacitance determination is not possible using our recording technique due to the influence of space-clamp limitations in non-dissociated tissue, so I_h was not normalized for capacitance. The larger size of A α / β neurons ($47\pm6\mu\text{m}$) compared to A δ ($37\pm3\mu\text{m}$) and C-type neurons ($34\pm3\mu\text{m}$) may in part contribute to the larger I_h . However, as a reflection of current density, the functional impact of I_h may be estimated by the degree of sag in the different categories of neurons (Table 1). The prominent sag in A α / β -type neurons, compared to a smaller average sag in A δ and near absence in C-type neurons, indicates that the large I_h recorded in A α / β neurons and intermediate I_h recorded in A δ do not represent merely the larger size of these neurons in comparison to the slight I_h in C-type neurons.

2.2 Functional role of I_h in DRG neurons

The influences of I_h blockade with ZD7288 upon action potential (AP) parameters (Fig. 2) and neuronal excitability (Table 1) were determined in neurons grouped by conduction velocity (CV).

The majority of neurons in the A α / β category conduct non-nociceptive sensory information (Lawson, 2002). Block of I_h in this group resulted in small but significant hyperpolarization of RMP, attributable to removal of a resting conductance through the noninactivating I_h channels. This is also demonstrated by an increased input resistance during I_h blockade. Loss of I_h also resulted in slight slowing of conduction velocity, consistent with the expression of these channels in the axons of DRG neurons (Birch et al., 1991), where they support conduction (Grafe et al., 1997). Despite an increase in input resistance, the rheobasic current necessary to initiate an AP was elevated by ZD7288, indicating an excitatory influence of I_h . This was also evident in the firing patterns elicited by further depolarization, during which 7 of 8 repetitively firing A α / β neurons showed decreased AP generation after ZD7288 (4.7 ± 2.7 APs at baseline, 2.0 ± 1.8 APs during ZD7288, $P=0.023$, $n=8$; Fig. 3).

In A δ neurons, which generally conduct nociceptive afferent traffic, a diminished effect of ZD7288 was seen compared to A α / β neurons. A smaller fractional increase in input resistance was seen (10% in A δ vs. 28% in A α / β neurons), no significantly RMP hyperpolarization developed, and rheobase was not significantly affected. However, 5 of 6 repetitively firing neurons in this category also demonstrated decreased AP generation after ZD7288 (4.5 ± 3.0 APs at baseline, 2.1 ± 2.4 APs during ZD7288, $P=0.026$, $n=6$). The ability of the neurons to successfully conduct bursts of APs during tetanic axonal stimulation (following frequency) was also diminished in A δ neurons (Fig. 4, Table 1). Following frequency is limited by currents that underlie the afterhyperpolarization (AHP) (Luscher et al., 1994), which increased in amplitude during ZD7288 administration in A δ neurons. This may result from preferential activation of I_h during the AHP (Maccaferri and McBain, 1996). The absence of this effect in A α / β neurons may be a consequence of the different I_h kinetics in these DRG neuronal populations (Tu et al., 2004).

In slowly conducting, mostly nociceptive C-type neurons, the only change noted during ZD7288 administration was a diminished AHP duration (Table 1). Since only a very slight ZD7288-sensitive I_h was observed during DSEVC, this finding is unlikely to be attributable to loss of I_h . No change in firing pattern was noted in 7 repetitively firing C-type neurons (1.4 ± 1.1 APs at baseline, 2.3 ± 1.7 APs during ZD7288, $P=0.18$).

2.3 I_h in axotomized DRG neurons

The main goal of this study was to identify whether increased excitability seen after sensory neuron injury can be attributed to the loss of I_h . Data presented so far indicates a contrary finding that selective loss of I_h does not increase neuronal excitability. It is possible, however, that residual I_h in injured neurons could have a different functional role in the context of diverse axotomy-induced cellular alterations compared to the context of an uninjured neuron. We therefore examined the effect of ZD7288 on sensory neurons from rats subjected to spinal nerve ligation (SNL), which produced hyperalgesia ($34 \pm 14\%$ hyperalgesic responses to plantar application of a pin, $n=4$ rats vs. $0 \pm 0\%$ in control animals, $n=18$ rats). Compared to neurons from control animals, neurons in DRGs removed 19 ± 3 days after SNL showed a diminished I_h (Table 2) in both A α/β neuron ($P<0.01$) and A δ neurons ($P<0.01$), with proportionately smaller voltage sag. Application of ZD7288 to axotomized sensory neurons produced minimal functional changes (Table 2). Unlike control neurons, firing rate in repetitively firing axotomized neurons had no consistent response to ZD7288 (2.3 ± 0.8 APs at baseline, 2.2 ± 1.2 APs during ZD7288, $P=0.5$, $n=6$).

3. Discussion

The observations reported here are the first to use a microelectrode recording technique in nondissociated DRGs to define the functional role of I_h in primary sensory neurons. These technical details may have critical importance. I_h in primary sensory neurons is highly sensitive to cytosolic cyclic nucleotides and to ATP, both producing a positive shift in the activation curve (Ingram and Williams, 1996; Komagiri and Kitamura, 2003). I_h is also sensitive to cytoplasmic Ca^{2+} levels (Pan, 2003; Schwandt et al., 1992). These mechanisms may explain neuronal I_h sensitivity to temperature and to cytoplasmic dialysis by patch electrodes (Cuevas et al., 1997). Using a microelectrode method that minimizes dialysis (Blatt and Slayman, 1983) and 35°C bath temperature may help avoid disruption of natural cytosolic conditions that regulate I_h . Preservation of the natural ganglionic environment and neuron/glia interactions by avoiding dissociation may add further validity to our observations, particularly regarding neuronal excitability. Although this preparation is not perfused, the bicarbonate-based bath solution is oxygenated and only superficial neurons were impaled. Also, the dorsal root must be sectioned, but at a much greater distance from the soma than occurs with dissociation, which thereby avoids the immediate phenotypic shifts that follow dissociation (Zheng et al., 2007). Our findings confirm that DRG neurons possess inward currents induced by hyperpolarization that are characteristic of I_h . Similar to previous observations (Scroggs et al., 1994), we found I_h is maximal in rapidly conducting sensory neurons.

We performed this study to test whether loss of I_h may contribute to the hyperexcitability seen in sensory neurons after peripheral nerve injury. Specifically, rheobase is diminished after spinal nerve ligation in both A α/β and A δ neurons, while repetitive firing during depolarization is elevated in A δ neurons (Sapunar et al., 2005). Axotomy has been shown to reduce sag (Sapunar et al., 2005) and I_h (Abdulla and Smith, 2001) in rat DRG neurons, so a possible stabilizing effect of I_h on DRG neurons might be considered. However, contrasting findings have also been reported. Elevated pain behavior that follows peripheral nerve injury is decreased after ZD7288 given systemically (Chaplan et al., 2003) or applied to the nerve injury site (Dalle and Eisenach, 2005). However, intrathecal ZD7288 has no effect on injury-induced

pain behavior (Chaplan et al., 2003), and the mechanistic insights from these *in vivo* experiments are limited since the tissue site of action cannot be discerned. Also, while Chaplan et al. (Chaplan et al., 2003) showed increased I_h in DRG neurons after axotomy, interpretation of that study is complicated by their concurrent finding that injury decreases expression of protein and message for HCN1 and HCN2, the principal genes encoding I_h channels in sensory neurons.

Sensory neuron axotomy is followed by numerous changes in cellular processes. In order to isolate the effects of I_h loss, we examined the effects of ZD7288 on uninjured neurons. Our present findings do not support our hypothesis that diminished I_h contributes to increased excitability of DRG neurons after axonal injury, but rather indicate the opposite. With I_h blockade by ZD7288, rheobase increased in A α / β neurons despite an elevated input resistance, and no effect was seen in slower conducting neurons. The generation of repetitive APs during membrane depolarization was diminished in A α / β and A δ neurons during ZD7288 application, as was the ability of A δ neurons to conduct rapid trains of APs through the T-branch and into the cell soma. The use of ZD7288 is a reliable means of identifying the role of I_h as prior reports have noted an absence of effects of ZD7288 on conductances other than I_h (BoSmith et al., 1993; Larkman and Kelly, 2001). Consistent with this, our findings confirm a complete elimination of I_h that accompanies these manifestations of depressed neuronal excitability. We note, however, a reduction in AHP duration in C-type neurons with application of ZD7288, possibly indicating an effect on currents other than I_h , as has been noted in prior reports (Chevalleyre and Castillo, 2002; Ghamari-Langroudi and Bourque, 2000).

We found diminished I_h after axotomy by SNL, which confirms that our previous finding of diminished sag after injury (Sapunar et al., 2005) is due to loss of I_h . Application of ZD7288 to these injured sensory neurons had a diminished effect compared to control neurons, such that I_h blockade had no effect on RMP, AP duration, rheobase, or repetitive firing properties after axotomy. This suggests that the residual I_h in injured neurons plays no particular role in supporting hyperexcitability.

We conclude that axotomy-induced excitability in sensory neurons is attributable to events other than loss of I_h . Indeed, the loss of I_h might be considered a compensatory measure in the context of injury. While the present experiments do not indicate a mechanism by which I_h in decreased after axotomy, it is possible that axotomy-induced depression of both resting levels of cytosolic Ca^{2+} as well as the transient rises of Ca^{2+} that accompany neuronal activation (Fuchs et al., 2005; Fuchs et al., 2007) may result in lowered activity of Ca^{2+} /calmodulin-dependent protein kinase II, which is a regulator of I_h activity (Fan et al., 2005; Rigg et al., 2003).

4. Experimental Procedures

All procedures were approved by the Animal Care Committee of the Medical College of Wisconsin, Milwaukee, Wisconsin.

4.1 Animal subjects

Male Sprague-Dawley rats (Taconic Farms Inc., Hudson, NY) weighing 200–300 g were used. In a subset of animals, neuropathic pain behavior was induced by spinal nerve ligation (SNL). During isoflurane anesthesia (2% in oxygen), the spinal nerves at the fifth lumbar (L5) and L6 levels were exposed, ligated with 6-0 silk ligature, and transected. Accuracy was confirmed at the time of tissue harvest. Unlike the originally described method (Kim and Chung, 1992), paraspinal muscles and the adjacent articular process were not removed. The other animals received skin incisions alone.

4.2 Behavioral Testing

Sensory responsiveness after SNL was tested using a method that selectively identifies neuropathic hyperalgesia (Hogan et al., 2004). On 3 separate days in the second and third postoperative weeks after SNL, the plantar surface of each hind foot was touched 10 times with a 22g spinal anesthesia needle with pressure adequate to indent but not penetrate the skin. The frequency of a hyperalgesia-type response with sustained paw lifting, shaking or licking was determined. Control animals produce only a brief reflexive withdrawal.

4.3 Tissue preparation

DRGs were harvested during anesthesia with isoflurane (2–3% in oxygen). A laminectomy was performed up to the sixth thoracic level while the surgical field was perfused with artificial cerebrospinal fluid (aCSF, in mM: NaCl 128, KCl 3.5, MgCl₂ 1.2, CaCl₂ 2.3, NaH₂PO₄ 1.2, NaHCO₃ 24.0, glucose 11.0) bubbled by 5% CO₂ and 95% O₂ to maintain a pH of 7.35. The fourth and fifth lumbar DRGs and attached dorsal roots were removed and the connective tissue capsule dissected away from the ganglia under 20x magnification. Ganglia were transferred to a glass-bottomed recording chamber and perfused with 35°C aCSF. The proximal cut end of dorsal roots was placed on a pair of platinum wire stimulating electrodes. DRG neurons were viewed using an upright microscope equipped with differential interference contrast optics and infrared illumination. Neuronal soma diameter was determined with the focal plane adjusted to reveal the maximum somatic area, which was measured using a calibrated video image.

4.4 Electrophysiological Recording

Intracellular recordings were performed with microelectrodes fashioned from borosilicate glass (1mmOD, 0.5mmID, with Omega fiber – FHC Bowdoinham, USA) using a programmable micropipette puller (P-97, Sutter Instrument Company, Novato, CA). Microelectrode resistances were 80–120 MΩ when filled with 2M potassium acetate. Neurons were predominantly selected from the two outermost cell layers of the dorsal medial aspect of the DRG, and were impaled under direct vision by minimally indenting the membrane and then applying an oscillating current to the microelectrode. Data were acquired after stable recordings were achieved, typically within 5min of puncture. Membrane potential was recorded using an active bridge amplifier (Axoclamp 2B, Axon Instruments, Foster City, CA), except in protocols requiring simultaneous current injection through the electrode, for which we used discontinuous current clamp recording mode. This allows accurate recording of membrane voltage during the passing of current, although the noise level is increased compared to bridge mode. During discontinuous mode recording, the frequency of switching from current injection to recording was 2kHz, and full settling of the electrode charge was confirmed. Currents were filtered at 1 kHz (discontinuous mode) and 10kHz (bridge mode), and then digitized at 10kHz (discontinuous mode) or 40 kHz (bridge mode; Digidata 1322A and Axograph 4.9, Axon Instruments) for data acquisition and analysis. Somatic APs were elicited either by natural conduction from the site of dorsal root stimulation with square-wave pulses of up to 90mA lasting 0.06mS, or by direct membrane depolarization with current injection through the recording electrode. I_h was measured in voltage clamp using single-electrode discontinuous clamp mode, with a switching frequency of 1kHz.

Criteria for inclusion of data were a RMP negative to –50mV, and an AP amplitude greater than 40mV. Together, these excluded approximately 6% of recordings. AP measures (Fig. 2) were obtained from single traces after consistency of dimensions was confirmed by comparison to 10 sequential APs. Determinations of AP and AHP dimensions and the area under the curve for the AHP were performed digitally. Input resistance was calculated from the membrane potential shift generated by a 100ms injection of a hyperpolarizing current through the recording electrode, using a current amplitude (0.2–0.5nA) that failed to show any time-dependent rectification (“sag”) (Villiere and McLachlan, 1996). Sag in response to

hyperpolarization is predominantly attributable to I_h (Scroggs et al., 1994). This was quantified as the fractional return from the peak hyperpolarized membrane potential back towards RMP during injection of a 1.2nA hyperpolarization current for 100ms. Rheobase was determined as the minimum current able to elicit an AP during incremental depolarizing current injection of 0.5–10nA for 100ms. The pattern of impulse generation was determined during depolarizing current steps beyond rheobase, at which neurons either continued to produce single APs or fired repetitively. The influence of ZD7288 upon AP firing pattern was measured at a depolarizing voltage that first produced a drug-induced difference in the number of APs generated. This measure is a sensitive indicator of drug effect but cannot be used to compare inherent excitability between groups (such as uninjured neurons *versus* SNL neurons) at baseline conditions due to the highly variable number of APs that might be present at the depolarization level at which ZD7288 first has an effect.

The following frequency was determined by presenting trains of 20 axonal stimuli with 3s intervals between trains, each train having progressively shorter interstimulus intervals. The maximum frequency of stimulation (1/interstimulus interval) at which each stimulus in the train produced a full somatic AP was considered the somatic following frequency.

Neurons were classified by the method of Villiere and McLachlan (Villiere and McLachlan, 1996). Conduction velocity (CV) was measured by dividing the distance between stimulation and recording sites by the conduction latency. Neurons with dorsal root CV<1.5m/S were considered C-type, neurons with CV>15m/S were considered A α / β -type, and neurons with CV>1.5m/S but CV<10m/S were considered A δ -type. For neurons with CV between 10 and 15m/S, long AP duration was used to categorize the cells as A δ -types (Villiere and McLachlan, 1996). Stable recordings in C-type neurons are particularly challenging to acquire, so this group is necessarily smaller in number, and data were inadequate to evaluate following frequency.

4.5 Agents

ZD7288 (40 μ M, Tocris Bioscience, Bristol, UK) was dissolved in bath solution. This was delivered by a microperfusion technique from a pipette with a 4 μ diameter tip that was positioned 100 μ m from the impaled neuron, and ejected continuously by pressure applied to the back end of the pipette (Picospritzer II, General Valve Corp, Fairfield, NJ). Preliminary experiments in which cell depolarization was measured during microperfusion application of solutions of various K⁺ concentrations indicated an effective 4-fold dilution of pipette solution into the bath at the cell membrane, so the ZD7288 concentration was approximately 10 μ M at the cell surface. Preliminary experiments showed a full onset of action within 3min.

4.6 Statistical analysis

Data is grouped by neuron type according to CV categories. The “n” for each group indicates the number of recorded neurons, which were obtained from between 5 and 11 different animals for each group of control neurons and from 4 animals for each group of SNL neurons. Data are expressed as means $\pm$ SD. The effect of ZD7288 was evaluated using paired Student’s *t*-test to identify significant drug effects in the context of natural variability between neurons under baseline conditions. Significance was accepted at $P<0.05$.

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Figure 1.

H-current (I_h) in sensory neurons. A. In discontinuous single electrode voltage-clamp mode, hyperpolarizing voltage commands (top panel, recorded actual voltage) produce an immediate inward current and an additional slowly activating component that is sensitive to ZD7288 (middle panel), shown as the digitally subtracted difference current (bottom panel), in an A α / β neuron. B. The current-voltage (I - V) relationship for I_h in this neuron. C. In the same neuron, discontinuous current clamp mode, hyperpolarizing current injections through the recording electrode (top panel) elicit time-dependent and voltage-dependent inward rectification ("sag", middle panel), that is eliminated by ZD7288 (approximately 10 μ M, bottom panel). D. The I - V relationship shows the loss of sag during ZD7288 administration (open squares) compared to baseline conditions (filled circles).

Figure 2. Measured action potential (AP) parameters. *RMP*, resting membrane potential; *APamp*, amplitude of AP; *AP95%*, duration of AP after 95% repolarization; *t*, latency following axonal stimulation; *AHPamp*, amplitude of afterhyperpolarization; *AHP80%*, duration of afterhyperpolarization until 80% recovery to baseline; *AHParea*, area of the AHP.

Figure 3.

Effect of ZD7288 (approximately 10 μ M) on firing pattern of an A α / β neuron during injection of depolarizing current through the recording electrode using discontinuous current clamp, during baseline conditions (A.) and during administration of ZD7288 to the same neuron (B.). In both cases, the upper panel shows voltage traces in response to the current commands shown in the lower panel. Traces in the two conditions are selected to show events at comparable degrees of membrane depolarization. As demonstrated by this neuron, ZD7288 hyperpolarized the resting membrane potential, increased the input resistance of the neuron from 22.5M Ω to 35.7M Ω , increased the voltage necessary to initiate an action potential, and eliminated this neuron's ability to fire multiple action potentials in response to sustained depolarization.

Figure 4.

Effect of ZD7288 (approximately 10 μ M) on the ability of an A δ neuron to conduct action potentials into the soma during tetanic stimulation. Under baseline conditions (A.), the neuronal soma can follow at 10Hz (left panel) and maintain successful firing at 50Hz (right panel), but the same neuron during ZD7288 (B.) drops action potentials at 50Hz (right panel). Note that the time scale is not the same in the right and left panels.

Table 1

Effects of H-current blocker ZD7288 on function of control dorsal root ganglion neurons.

		I_h (pA)	Sag ratio (%)	RMP (mV)	CV (m/s)	Apamp (mV)	AP95% (ms)	AHPamp (mV)	AHP80% (ms)	AHParea (mV·ms)	R_{in} (M Ω)	Rheobase (nA)	Following Freq. (Hz)
α/β =23	BL	297 ±94	24.5±12.1	-70.5±8.1	24.3±7.0	72.7±9.1	0.72±0.22	10.8±4.2	21.5±20.4	287±342	46±19	3.3±1.6	387±220
	ZD	0.0±0.0***	0.0±0.0***	-71.6±9.4*	23.8±6.8***	73.1±10.9	0.76±0.17*	10.9±4.9	41.6±69.6	347±451	61±25***	3.6±1.6*	387±212
$\Delta\delta$ =19	BL	163±129	14.2±13.9	-71.4±10.4	8.1±2.1	71.2±10.3	1.57±0.86	8.1±5.0	53±53	1165±1454	75±42	2.6±1.8	155±116
	ZD	0.0±0.0**	0.0±0.0***	-71.8±10.2	8.3±1.6	70.6±12.6	1.82±1.94	9.0±5.2**	156±468	1238±2195	85±38*	3.1±1.9	105±88*
C =8	BL	12±21	1.4±3.3	-63.4±4.1	0.5±0.1	76.2±12.5	3.31±0.55	14.3±5.4	41.5±14.6	2470±3023	153±28	1.2±0.3	
	ZD	0.0±0.0	0.0±0.0	-60.9±8.6	0.5±0.1	75.0±9.2	3.78±1.65	16.1±2.8	34.1±11.2*	3456±3569	136±57	1.0±0.3	

H-current measured at during 150ms step to -120mV; ZD, ZD7288 (10 μ M); BL, Baseline condition; RMP, resting membrane potential; CV, conduction velocity; AHPamp, action potential amplitude; 95% action potential duration at 95% of amplitude; AHPamp, afterhyperpolarization amplitude; AHP80% afterhyperpolarization duration at 80% of amplitude; AHParea, afterhyperpolarization area under the curve; R_{in} , input resistance. Values are given as mean $\pm$ SD.

<0.05,

$P < 0.01$,

* $P < 0.001$ vs. baseline.

Table 2
Effects of H-current blocker ZD7288 on function of axotomized dorsal root ganglion neurons.

		I_h (pA)	Sag ratio (%)	RMP (mV)	CV (m/s)	Apamp (mV)	AP95% (ms)	AHPamp (mV)	AHP80% (ms)	AHParea (mV·ms)	R_{in} (M Ω)	Rheobase (nA)	Following Freq. (Hz)
Aα/β n=7	BL	91 $\pm$ 167	5.3 $\pm$ 7.0	-66.4 $\pm$ 6.6	19.5 $\pm$ 7.3	81.3 $\pm$ 7.7	0.78 $\pm$ 0.17	13.2 $\pm$ 3.8	51.9 $\pm$ 33.6	696 $\pm$ 360	29 $\pm$ 16	3.4 $\pm$ 2.1	328 $\pm$ 255
	ZD	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0*	-66.7 $\pm$ 7.0	19.2 $\pm$ 8.2	83.3 $\pm$ 5.0*	0.77 $\pm$ 0.19	14.4 $\pm$ 2.8	43.4 $\pm$ 35.0	696 $\pm$ 452	31 $\pm$ 16	3.4 $\pm$ 2.0	253 $\pm$ 215
Aδ n=8	BL	7 $\pm$ 15	0.8 $\pm$ 1.8	-65.6 $\pm$ 6.7	7.9 $\pm$ 2.8	80.0 $\pm$ 9.4	1.20 $\pm$ 0.26	13.6 $\pm$ 3.7	98.8 $\pm$ 68.6	1587 $\pm$ 1484	44 $\pm$ 12	3.2 $\pm$ 1.8	103 $\pm$ 113
	ZD	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	-65.0 $\pm$ 6.7	7.9 $\pm$ 2.7	77.9 $\pm$ 9.7*	1.29 $\pm$ 0.39	14.0 $\pm$ 3.0	88.7 $\pm$ 69.2	1484 $\pm$ 1351	40 $\pm$ 14	2.1 $\pm$ 1.3	113 $\pm$ 131

I_h , H-current measured at during 150ms step to -120mV; ZD, ZD7288 (10 μ M); BL, Baseline condition; RMP, resting membrane potential; CV, conduction velocity; AHPamp, action potential amplitude; AP95%, action potential duration at 95% of amplitude; AHP80%, afterhyperpolarization amplitude; AHP80%, afterhyperpolarization duration at 80% of amplitude; AHParea, afterhyperpolarization area under the curve; R_{in} , input resistance. Values are given as mean $\pm$ SD.

* $P < 0.05$,
 ** $P < 0.01$,
 *** $P < 0.001$ vs. baseline.