Peripheral Block of the Hyperpolarization-Activated Cation Current (I_h) Reduces Mechanical Allodynia in Animal Models of Postoperative and Neuropathic Pain

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Background and Objectives: Block of the hyperpolarization-activated inward current (I_h) reduces excitability of peripheral axons during stimulation and decreases ectopic discharges in axotomized sensory neurons. Changes in I_h expression in DRG neurons have been suggested to partially underlie sensitization after nerve injury and inflammation. We hypothesized that peripheral block of I_h on axons would produce an antiallodynic effect in postoperative as well as neuropathic conditions, and we tested perineural administration of ZD 7288, a specific blocker of I_h, on pain-associated behavior in animal models of neuropathic and postoperative pain.

Methods: Under halothane anesthesia, partial sciatic nerve injury or hind-paw incision were performed on adult male rats as previously described. Mechanical allodynia was inferred by demonstration of a decrease in paw withdrawal threshold by application of calibrated von Frey filaments. After surgery, animals received either a saline or a ZD 7288 solution either by sciatic perineural injection or by intraplantar injection.

Results: Perineural administration of ZD 7288 (100 μM) significantly reduced mechanical allodynia induced by partial sciatic nerve injury and hind-paw incision. Saline and 10 μM of ZD 7288 had no significant effect on mechanical allodynia. Contralateral administration of ZD 7288, 100 μM, did not affect ipsilateral paw withdrawal threshold after nerve injury. Intraplantar injection of ZD 7288 failed to reduce mechanical allodynia after nerve injury. Sedation and motor effects were not observed.

Conclusions: The current study shows that peripheral block of I_h produces an antiallodynic effect, which suggests that I_h channels represent a novel target for nerve block treatment of postoperative and neuropathic pain. *Reg Anesth Pain Med 2005;30:243-248.*

Key Words: Postoperative pain, Neuropathic pain, Ion channels, Peripheral afferents, Peripheral nerve block.

After an action potential, a brief period occurs in which the cell membrane is hyperpolarized and rendered refractory to further stimulation. This effect reduces the maximum frequency at which peripheral nerves can fire. This period of hyperpolarization itself induces an opposite current that brings the resting membrane potential back to normal. This opposite current (inward rectification), induced by membrane hyperpolarization, occurs in both myelinated and unmyelinated axons of the mammalian peripheral nervous system,[1-4] including human peripheral nerves.[5] Axonal inward rectification is thought to reflect activation of both the hyperpolarization-activated cationic current (I_h) and the inwardly rectifying potassium current (I_{KIR}).[4] This inward rectification maintains membrane potential at an appropriate level during and after firing, when outwardly rectifying channels or electrogenic Na^+/K^+-pump activity otherwise might result in excessive hyperpolarization. In other terms, activation of inward rectification limits activity-dependent conduction block and, thus, provides a mechanism of maintenance of conduction of action potentials and allows sustained transmission of sensory information. According to this hypothesis, inhibition of axonal inward rectification should reduce excitability and conduction velocity of peripheral myelinated and unmyelinated axons during stimulation, as has been observed experimentally,[2,6] and may as a result produce an antinociceptive effect.

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Reduction of inward rectification in myelinated fibers has been reported in human diabetic neuropathy, as well as in streptozocin-induced experimental diabetes.[7] In contrast, increased inward rectification is exhibited in Charcot-Marie-Tooth disease type 1A.[8] No direct evidence indicates that these abnormal inward rectifications reflect alteration in axonal ion channels, either in their function or in their level of expression. The I_h current is decreased, however, in dorsal root ganglion (DRG) neurons after axotomy of the sciatic nerve[9] and is increased in large-diameter DRG neurons in several neuropathic pain models.[10-11] Because the I_h current controls rhythmic activity in spontaneously active cells,[12] these changes in I_h current density may contribute to generation of abnormal ectopic discharges developed in sensory neuronal cell bodies and axons after injury. The role of the I_h current in axons after injury to induce abnormal peripheral neural traffic and maintain central plasticity that leads to neuropathic pain is unexplored. However, its importance in allowing the transmission of pain in neuropathic conditions is suggested by the fact that block of the I_h current *in vitro* decreases firing frequency of ectopic discharges that originate from Aβ fibers and Aδ fibers.[10] Additionally, the effect of various inflammatory mediators on the I_h current suggests that axonal inward rectification might be increased during local inflammation of the nerve.[13] The activation of the I_h current may enhance excitability of the axon and its responsiveness to excitatory input, which further contributes to pain sensation.

Abnormal firing has been reported not only at the site of chronic injury[14] but also along normal nerve after skin injury.[15] These observations and the potential importance of the I_h current in the determination of excitability and conduction of impulses in pathophysiological conditions suggest that a peripheral block of the I_h current would be effective in treating postoperative as well as neuropathic pain. To assess this hypothesis, we investigated the effect of perineural administration of ZD 7288, a specific blocker of the I_h current, on pain-associated behavior in animal models of postoperative and neuropathic pain. Furthermore, because the presence of I_h channel on the afferent terminals in the skin have been reported,[16] we also examined the effect of block of the I_h current in terminals by intraplantar injection of ZD 7288.

Materials and Methods

Male Sprague-Dawley rats (250 to 350 g) were studied after approval by the Animal Care and Use Committee. Animals were housed at 22°C and under a 12-hour light–12-hour dark cycle, with food and water *ad libitum*.

Hind-Paw Incision

Hind-paw incision was performed as previously described by Brennan et al.[17] Under halothane anesthesia, a 1-cm longitudinal incision was made with a scalpel blade through skin and fascia of the plantar aspect of the left hind paw, starting 0.5 cm from the proximal edge of the heel and extending to a point just proximal to the first set of foot pads. The plantaris muscle was elevated and incised longitudinally. The skin was closed with 2 mattress sutures of 5-0 silk, and the wound site was covered with iodine solution. Animals were allowed to recover for 4 hours before behavioral testing.

Partial Sciatic-Nerve Injury

Partial sciatic-nerve injury was performed as previously described by Seltzer et al.[18] Under halothane anesthesia, the left sciatic nerve was exposed in the left hindlimb at midthigh level and carefully cleared of surrounding connective tissue. A 6.0 polypropylene suture was inserted into the nerve with a three-eighth–inch curved needle, and the dorsal third to half of the nerve was tightly ligated. In some experiments, the nerve was also partially sectioned. The wound was closed with 4 skin sutures and covered with iodine solution. Animals were allowed to recover for 4 to 6 weeks before behavioral testing.

Paw Withdrawal Testing

Mechanical allodynia was inferred by demonstration of a decrease in paw withdrawal threshold by application of calibrated von Frey filaments (Semmes-Weinstein Aesthesiometer Kits). For behavioral testing, animals were placed in a Plexiglas chamber on an elevated wire-mesh table and were allowed to acclimate for 30 minutes. Filaments were applied perpendicularly to the plantar surface of the left hind paw for 5 seconds, with a frequency of 1/s. Withdrawal threshold was determined by sequential increase and decrease of the stimulus strength and the lowest force; the force at which the animal withdrew the paw in at least 2 of the 3 trials was taken as the withdrawal threshold. For this study, 28 g was recorded as the paw withdrawal threshold if no withdrawal response to the next lowest filament (15 g) occurred. Before surgery, animals were tested for their baseline mechanical threshold. Only animals with a threshold less than 5.5 g after surgery were included for further behavioral studies. Animals were randomly assigned to