Transient opening of the perineurial barrier for analgesic drug delivery

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AUTHOR SUMMARY

Peripheral nerves are separated from the environment by the perineurial barrier, which protects against nerve damage by molecules like cytokines and protons that impair nerve conduction. However, this barrier also impedes drug delivery to the nerves. The perineurial barrier is formed by a basal membrane and a layer of perineurial cells, which express tight-junction proteins that limit the permeability of this barrier. For example, the tight-junction proteins claudin-1, claudin-5, and occludin are expressed at lower levels during peripheral nerve injury when the perineurial barrier is transiently damaged (1). How this occurs at the molecular level is unknown. Here, we demonstrate that claudin-1 is the major sealing component of the perineurial barrier. This finding could potentially help researchers develop more effective methods for delivering therapeutic agents to the peripheral nerve.

In peripheral nerves, selective targeting of sensory or nociceptive neurons still remains a clinically unachieved goal. Several potentially interesting drugs, including sodium channel (NaV 1.7)-selective blockers (2) or - and -opioid receptor-selective agonists (3), are not effective in vivo, presumably because the delivery of peripherally injected drugs is impeded by the perineurial barrier. Hypertonic solutions have been used to open barriers to enhance drug delivery to the brain. Hypertonic saline also increases perineural permeability and facilitates peripheral opioid analgesia at nerve terminals (4). Here, we used the perineural injection of hypertonic saline together with the sodium channel blocker TTX or Proctoxin-II, or the opioid receptor agonist [D-Ala2,N-Me-Phe4, Gly5-ol]-enkephalin (DAMGO) (agonist for -opioid receptors) or [D-Pen2, d-Pen5]-enkephalin (DPDPE) (agonist for -opioid receptors) to unravel the opening of the perineurial barrier in full mechanistic detail (Fig. 1). Perineural injection of hypertonic solution, as a tool to open the barrier, facilitated an analgesic effect of sodium channel blockers and of opioids in normal rats and rats with hind paw inflammation. Hypertonic pre-treatment also enabled certain sodium channel blockers to block the conduction of A- or C-fibers in isolated sciatic nerves from normal rats. The effect in vivo was restricted to nociception, because the drugs did not impair motor function. The perineurial barrier remained functionally open for several hours after treatment with hypertonic saline. In parallel, the content of claudin-1 in the membranes of perineurial cells decreased. That claudin-1 is a ubiquitous sealing protein of the tight junction is known from studies of claudin-1 KO mice that are not viable because of loss of water through the skin.

How does hypertonic saline reduce the content of claudin-1? Metalloproteinases (MMPs) have been implicated in the property of hypertonic solutions of opening the blood–brain barrier. MMPs also contain a hemopexin domain (MMP9 PEX) that controls the activity of the enzyme. In our system, MMP9 protein expression was found in the perineurium. Blockade of MMP9 PEX inhibited the facilitating effect of hypertonic saline on analgesia induced by opioids and inhibited the down-regulation of claudin-1. In contrast, perisciatric injection of

Editor’s Summary: The perineurium is an important barrier that protects peripheral nerves from the environment. It is comprised of the basal membrane and a layer of perineurial cells that express tight junction proteins for permeability control. The authors present a novel approach for delivering drugs to peripheral nerves by transiently opening the perineurial barrier through the use of hypertonic saline. This method facilitates peripheral opioid analgesia and reduces claudin-1 content in perineural membranes, potentially opening a new avenue for therapeutic delivery.


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purified MMP9 PEX protein instead of hypertonic saline had the same effect on nociceptive thresholds or on claudin-1 expression. MMP9 PEX can bind to low-density lipoprotein receptor-related protein-1 (LRP-1) and triggers phosphorylation of intracellular p42/44 ERK (5). This pathway, including MMP9 PEX binding to LRP-1 and subsequent ERK phosphorylation, is active in the perineurium as well. LPR-1 is expressed abundantly in the perineurium in close proximity to claudin-1. Inhibitors of LRP-1 abolish the effects of hypertonic saline and of MMP9 PEX. In the perineurium, ERK is rapidly phosphorylated after treatment with hypertonic solution or MMP9. When ERK phosphorylation is blocked using an ERK inhibitor, claudin-1 content remains unchanged and no analgesic effects of opioids together with hypertonic solution are detected. The pathway is active in the perineurium as well as in a colonic epithelial cell line: MMP9 PEX treatment increases paracellular transport of ions and small molecules and decreases expression of claudin-1.

Our data presented here establish the function and mechanism of regulation of claudin-1 in the perineurial barrier of normal rats as well as in rats with hind paw inflammation. Thus, claudin-1 acts as an important sealing component of the perineurial barrier. Modulation of its regulatory pathway may allow the delivery of drugs or gene therapy vectors to the peripheral nerve, which is of clinical importance because it could facilitate the peripheral application of therapeutics for transport to the CNS. Further, our findings provide a rational basis for resealing the barrier after breakdown of the perineurium under pathophysiological conditions. It is likely that this knowledge can be applied to other physiological barriers, such as the blood–brain or intestinal mucosal barrier.