C-fiber-Selective Peripheral Nerve Blockade

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Abstract: Despite the clinical demand, current uses of local anesthetics do not allow selective blockade of nociceptive fibers. Regional anesthesia produces an analgesic effect accompanied with undesired side effects due to block of motor, non-nociceptive sensory and autonomic fibers. These side effects limit the clinical use of local anesthetics and affect the recovery and rehabilitation period after surgical procedures. Therefore one main goal of research in the field of regional anesthesia is selectively targeting nociceptive fibers. Recent studies describing the role of nociceptive specific sodium channels in generation and propagation of nociceptive signals make these channels ideal targets for pain selective blockade. In addition, novel methods of targeted delivery of charged local anesthetics selectively into nociceptors provide another potentially successful approach for c-fiber specific nerve block. This review summarizes currently on-going studies on several promising targets and methods to achieve pain selective anesthesia.

INTRODUCTION

Local anesthetics (LAs) are frequently used in clinical practice when anesthesia of limited body area is desired. LAs block voltage-gated sodium channels, thereby preventing generation of action potentials and their propagation along the nerve [1]. However, LAs block sodium channels not only in sensory fibers but also in motor and sympathetic fibers. To date, no agent or method translatable into current clinical practice has been shown to elicit usable pain-selective nerve blocks. In general, clinicians agree that there is a slight but detectable difference among local anesthetics in motor vs. sensory blockade; e.g., bupivacaine in general has somewhat more sensory/nociceptive block than motor block, while etidocaine confers more motor than sensory block. The following pages review treatments that are far more selective or exclusively pain-fiber selective than currently used clinical and experimental local anesthetics. Nociceptive-selective nerve block has been attempted with concentrations of LAs that are high enough for only certain nerve fibers (smaller-diameter, thinly myelinated A-delta or unmyelinated C-fibers), but not for others (larger-diameter, myelinated nerves such as A-beta). Nevertheless, studies have demonstrated that nerve block does not always follow this size principle, and motor fibers are blocked before nociceptive fibers [2]. Therefore, complete pain relief is generally accomplished only with simultaneous low-threshold sensory sympathetic and motor blockade, leading to numerous adverse effects. Improving the sensory-selectivity of LAs will clearly extend their clinical utility. (Of note, especially in the clinical

anesthesia literature, the terms "sensory-selective" and "differential block" are commonly used and are roughly interchangeable with "pain-selective" and "nociceptor-selective.") We review novel methods to achieve nociceptor-selective peripheral nerve blockade.

Considering the properties of pain-specific peripheral nerve fibers is fundamental in exploring differential nerve blockade. Noxious stimuli are received and interpreted by selective-type peripheral sensory neurons, i.e. nociceptors. Nociceptors are unique among other peripheral neurons for their expression of high-threshold transducer receptors that transform noxious chemical, thermal, and/or mechanical stimuli into electrical signals [3]. Those receptors include channels from the transient receptor potential family; TRPV1, V2, M8, A1, and the purinoreceptor P2X [4-6]. TRPV1, V2, and M8 are involved in perception of heat [4, 5, 7, 8], and A1 is involved in sensing various chemical irritants [9]. Purinoreceptor P2X is activated by the presence of ATP [10]. The activation of these receptors results in ion influx and depolarization of the membrane of the nerve terminal [11]. If the depolarization is strong enough to activate sodium channels, it will result in action potentials that propagate along A delta and C fibers. These nociceptors express a unique repertoire of sodium channels including both TTX-sensitive and -resistant subtypes. A-delta fibers are associated with transmission of superficial, sharp pain. The C fibers are associated with transmission of dull, throbbing pain [12]. It is now agreed that within the peripheral nervous system C-fibers express TRPV1 and $Na_{(v)}1.7$ almost exclusively [13-17]. Therefore this review focuses mainly on TRPV1 and $Na_{(v)}1.7$.

TRPV1 CHANNELS

The vanilloid receptor subtype 1 (VR1) (TRPV1) is a member of the superfamily of transient receptor potential ion

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channels [4]. TRPV1 is expressed peripherally in primary afferent nociceptors [13] and is stimulated and sensitized by noxious heat, protons, and various inflammatory mediators that comprise the 'inflammatory soup' including bradykinin, adenosine triphosphate and arachidonic acid derivatives such as prodtagrandings and leukotriene B4 [3]. TRPV1 is a nonselective cation channel that permits calcium and sodium ions to pass through the membrane of the primary sensory/nociceptive neurons, causing membrane depolarization and leading to nociceptive responses. However, initial excitation of the nociceptive neuron by capsaicin is followed by a long refractory state, including desensitization of the receptor or channel,changes in axon terminals, mitochondrial swelling, release of calcitonin gene-related peptide, displacement of adenosine triphosphate by the calcium sensor calmodulin, depletion of substance P, and, at higher concentrations, the possibility of axonal atrophy and terminal degeneration [18-20]. This desensitization and the longer-lasting atrophic/degenerative changes led to clinical use of capsaicin in topical ointments to relieve neuropathic pain such as post-herpetic neuralgia and minor aches and pains associated with arthritis, strains, and sprains [19]. A single high-dose local injection of capsaicin is also currently being investigated for controlling post-surgical and osteoarthritis pain[19].

The ultrapotent TRPV1 agonist RTX has also been well-studied and is currently clinically used to treat certain urological conditions such as bladder hyperactivity [21-25]. As a TRPV1 activator, RTX is known to be 20-fold more potent than capsaicin [4] and, similar to capsaicin, produces prolonged membrane depolarization of TRPV1-laden nerve fibers. The peripheral injection of RTX has been shown to prevent development of thermal and mechanical allodynia in inflammatory rat pain models [26-29].

Recently Binshtok *et al*. postulated that activation of the TRPV1 channel allows otherwise impermeant molecules such as QX-314, a bulky positively charged lidocaine derivative, to enter the nerve cells (Fig. **1**). Indeed, in a rat sciatic nerve block model, the injection of QX-314 followed by capsaicin demonstrated an expected nociceptor-selective,

long-lasting blockade while leaving motor impulse conduction intact. [30] Moreover, such an activation of TRPV1 channels will lead to a predominantly nociceptor-selective blockade with experimental (amitriptyline or N-methyl amitriptyline) and clinically used (lidocaine and bupivacaine) LA agents [31].

In addition, the narrow dosage range within which LAs such as lidocaine and bupivacaine can be safely administered without significant toxicity is one of the most severely limiting aspects of these drugs in clinical practice. For instance, the dose of lidocaine that produces toxic effects is only several folds greater than the dose necessary to produce a therapeutic effect. The therapeutic ranges of capsaicin and RTX are known to be two and three orders of magnitude greater, respectively [32]. The combination of LAs with capsaicin allows lower concentration of LAs to achieve the desired effect and hence increases their safety margin. Nevertheless, whether the facilitation of LAs by TRPV1 agonists is accompanied by proportionally increased cellular toxic effects has yet to be determined.

One clinically limiting aspect of these agents is the initial burning sensation when they are applied topically. The combined use of capsaicin with LA has the additional benefit of 'anesthetizing' the nerve first, preventing the burning sensation. Furthermore, at least in rats, the subcutaneous or sciatic perineural injection of high concentrations of capsaicin (0.1%) or RTX (0.001%), administered with clinically used LAs like lidocaine or bupivacaine to awake animals, does not elicit any immediate behavioral changes suggestive of pain expected of the initial activation of TRPV1 by these agents (Gerner, P. unpublished results).

Similarly, other high-conductance nociceptive-selective nonspecific cation transducer channels could be used to produce nociceptive-selective local anesthesia.

SODIUM CHANNELS

Each sodium channel consists of a large functional alpha-subunit and one or two much smaller auxiliary beta-subunits. Subtypes of sodium channels arise from variation in the
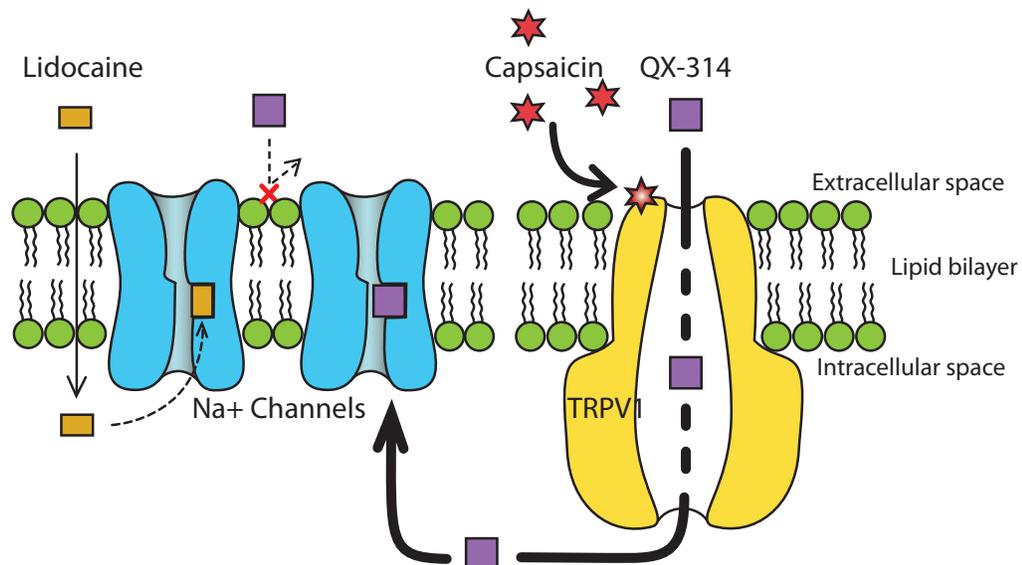

Fig. (1). Hypothesized mechanism of QX-314's entry into the nerve. The activation of the TRPV1 channels opens the pore, allowing molecules such as QX-314 enter the cell to block the sodium channel.

homologous alpha-subunit genes. The nine subtypes now known in mammals (Na$_{(v)}$1.1 through 1.9) are differentially expressed in various tissues, which suggests their functional correlation. For example, multiple subtypes of sodium channels in the dorsal root ganglion (DRG) are believed to be involved in multiple events along the pain pathway such as transmission, signal amplification, and action potential electrogenesis. Nociceptors express several types of sodium channels including both TTX-sensitive (Na(v) 1.1, 1.6, 1.7 and 1.3) and TTX-resistant (Na$_{(v)}$ 1.8 and 1.9) subtypes. Some of the sodium channels such as Na$_{(v)}$1.8, Na$_{(v)}$1.9, and Na$_{(v)}$ 1.7 are expressed exclusively on peripheral [33-35] but not vagal [36] nociceptors.

Currently the "hottest" target for nociceptive-specific blockade is the Na$_{(v)}$1.7 channel, since this subtype determines the ability of a nerve to transmit pain sensation [37].

The importance of Na$_{(v)}$ 1.7 has become increasingly evident through genetic correlation of this channel with congenital abnormality of pain perception [38]. Loss-of-function mutations of Na$_{(v)}$1.7 are reported in patients with channelopathy-associated insensitivity, in which patients have isolated lack of sensory function for pain and smell [37]. On the other hand, several gain-of-function mutations of genes related to the regulation or function of Na$_{(v)}$1.7, resulting in over-activity of this channel, are found in patients with two painful congenital disorders, erythermalgia (also termed erythermelalgia) and paroxysmal extreme pain disorder, congenital conditions whereby patients are afflicted by episodic severe pain attacks accompanied by cutaneous flushing [39-41]. Na$_{(v)}$1.7 also appears to be involved in the development of inflammatory pain, as demonstrated in an animal study that showed inflammation-induced upregulation of Na$_{(v)}$1.7 in the DRG [42]. Furthermore, a recent preclinical study has reported that ProTX-II, a Na$_{(v)}$1.7-selective antagonist from spider venom, prevented the propagation of action potentials in small-diameter nociceptive fibers, while larger fibers remained intact [43]. The further identification of such a selective Na$_{(v)}$1.7 blocker is an area of great interest.

Na$_{(v)}$1.8 is expressed predominantly in small nociceptive neurons [34] and has been demonstrated to be the main source of sodium influx during action potential electrogenesis [44, 45]. Knock-out (KO) studies demonstrated that this channel also underlies the ability of nociceptive neurons to fire repetitively [46]. Accumulating evidence has shown the intimate relationship of Na$_{(v)}$1.8 to generation of inflammatory and neuropathic pain [46-51]. The injection of complete Freund's adjuvant or carageenan increased expression of Na$_{(v)}$1.8 in the rat DRG. In mice, knocking down the Na$_{(v)}$1.8 gene by antisense oligonucleotides attenuated the development of inflammatory hyperalgesia [52,53]. Currently A-803467, a Na$_{(v)}$1.8-selective blocker, has been shown to attenuate mechanical allodynia in a dose-dependent fashion in animal pain models including sciatic nerve injury, spinal nerve ligation, and chemically induced thermal allodynia and secondary allodynia [51, 54]. Ambroxol, a relatively selective blocker of Na$_{(v)}$1.8. has also been shown to produce effective analgesia in inflammatory and neuropathic pain models in animals[55]. Another subtype,

Na$_{(v)}$1.9, is also found only in small DRG neurons [51, 56-58]. Na$_{(v)}$1.9 is thought to be responsible for a slow persistent sodium current with low threshold and activated over a wide range of voltage. This current is postulated to influence the subthreshold excitable properties of the membrane, which may explain the mechanism of sensitization by causing the membrane resting potential to fluctuate [46, 49, 57, 59]. In addition, Na$_{(v)}$1.9 KO mice failed to display inflammation-induced excitability or up-regulation of Na+ channels[60]. Subsequent introduction of cloned Na$_{(v)}$1.9 reinstituted this effect[60].

The differential effect on nociceptive-specific sodium channels may also underlie the analgesic effects of some anticonvulsants and antidepressants. For example, Lacosamide is an anticonvulsant that also has Na$_{(v)}$1.8-blocking properties and has been shown to be effective in treating neuropathic pain in preclinical studies[61].

The restricted expression of sodium channels Na$_v$1.7, Na$_v$1.8, and Na$_v$1.9 on peripheral nociceptors and the direct link of Nav1.7 to pain states in humans make them ideal targets for development of more effective drugs with fewer undesirable side effects. However, since specific blockers of sodium channels demonstrate low bioavailability, none of the existing compounds have proven suitable for clinical use. Recently demonstrated expression of TTX-resistant sodium channels on A-fibers of nodose vagal sensory neurons reduce the selectivity of these specific blockers to nociceptors [36]. Moreover, chronic pain disorders are multifactorial, and the efficacy of drugs is also greatly influenced by the up- and down-regulation of various sodium channel subtypes in the different stages of inflammatory and neuropathic pain [50, 62-66].

Therefore the approaches targeting sodium channel blockers specifically to nociceptive neurons are preferable.

FUTURE STUDIES

Many compounds targeting specific sodium channel subtypes have been identified and are currently awaiting detailed testing *in vivo* in various models. Among them, the above-mentioned A-803467 appears promising in treating neuropathic and inflammatory pain. However, no clinical trials are currently underway using this agent (clinical-trials.gov). Lacosamide is now under phase 3 clinical trials for mainly neuropathic pain such as painful diabetic neuropathy, migraine, and post-herpetic neuralgia. Ralfinamide is also in phase 2 clinical trials for various neuropathic pain conditions.

Besides capsaicin and RTX, further interest in nociceptive-selective blockade may be explored among the members of TRP ion channel family agonists or antagonists. Given the suggested critical role of TRP channels in the pain pathways in both the central and peripheral nervous systems, the surge of interest in the TRP family as a target for the next generation of analgesic agents has led to the discovery of numerous TRP agonists and antagonists. Some of the clinically used local and inhalation anesthetic agents are now known to be TRPV1 and TRPA1 agonists, including lidocaine, tramadol, and isoflurane [67-69]. These agents may generate synergistic differential analgesic effects with other local or general anesthetics through mechanisms similar to

RTX and capsaicin and are potential candidates for future studies to achieve differential nerve block. However, one drawback is that manipulation of TRPV1 activity also affects its thermoregulatory function. It has been demonstrated in both human and animal studies that TRPV1 blockade can lead to hyperthermia in susceptible individuals [70, 71]. These results obviously pose significant challenges to the clinical use of TRPV1 antagonists. Nonetheless, these results are informative for future study of the role of TRPV1 channel activation in thermoregulatory processes. Further research will hopefully identify other, more selective TRPV1 antagonists that they interfere only with the nociceptive transmission while sparing other TRPV1-mediated activities.
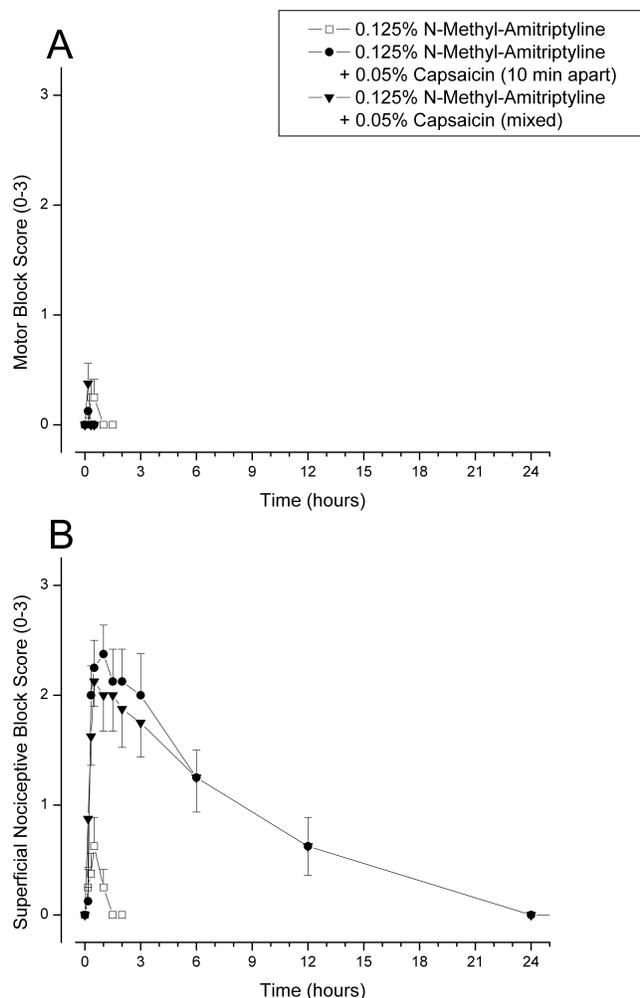

Fig. (2). Sciatic nerve block with 0.2 ml of N-methyl amitriptyline at 0.125 %, alone or in combination with 0.05 % capsaicin (simultaneously applied/mixed or 10 min later), n=8 rats per group. Data are presented as mean ± SE. **A.** Motor Block Score (0-3): Motor function was assayed by a digital platform balance. 0 = no block (or baseline); 1 = minimal block; 2 = moderate block; 3 = complete block (force of 20 g or less). **B.** Superficial Nociceptive Block Score (0-3): Nociception was evaluated by the nocifensive withdrawal reflex and vocalization to pinch of a skin fold. 3 = complete block, no nocifensive reaction or vocalization; 2 = moderate block, vocalization accompanied by slow withdrawal and flexion of the leg; 1 = minimal block, brisk flexion of the leg, with some sideways movement of the body or other escape response and loud vocalization; 0 = baseline with no block and all nocifensive responses listed above.

Besides identifying drugs for pain-specific sodium channel subtypes and/or TRPV1 agonist/antagonists, and combining TRPV1 channel agonists with permanently charged LAs (Fig. **2**), another future approach might be to combine TRPV1 channel agonists with specific positively charged sodium channel subtype antagonists to further maximize pain selectivity. The combination of a TRPV1 agonist and LA is currently awaiting regulatory approval for clinical trials.

In summary, several approaches are currently being pursued in the development of agents for C-fiber-selective peripheral nerve blockade. A considerable amount of effort has been put into identification of compounds specific for sodium channel subtypes and TRP agonists as well as antagonists. An exciting new approach is combining TRPV1 agonists with permanently charged large LA molecules, [31] selectively allowing these otherwise impermeable compounds only into C-fibers.

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