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VANILLOID-INDUCED CONDUCTION ANALGESIA: SELECTIVE, DOSE-DEPENDENT, LONG-LASTING, WITH A LOW LEVEL OF POTENTIAL NEUROTOXICITY

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Abstract

Vanilloid agonists (capsaicin, resiniferatoxin, [RTX]) applied to the peripheral nerves provide conduction blockade. In contrast to the analgesic component of conduction anesthesia produced by local anesthetics, vanilloid agonists provide conduction analgesia not associated with suppression of motor or sensory functions not related to pain. Vanilloid agonists provide conduction analgesia selectively because their effect on the nerve trunks is limited to C- and A δ -fibers. RTX is much more potent than capsaicin and has a wider therapeutic window. In the rat experiments perineural RTX produced a long-lasting thermal and mechanical hypoalgesia with a very wide separation between effective concentrations [from 0.00003% to 0.001%] providing the effect lasting from several hours to several weeks. A nerve block with RTX prevented the development of thermal and mechanical hyperalgesia as well as pain behavior in a model of incisional pain. RTX-induced conduction blockade has an inherent drawback of TRPV1 agonists – the initial excitation (pain); therefore, a local anesthetic should be injected to prevent it. When RTX was applied to the rat's sciatic nerve in doses necessary to provide conduction analgesia, the frequency of unmyelinated fiber degeneration was more than an order of magnitude lower than that with the therapeutic concentration of lidocaine. These promising results should be confirmed by experiments in species other than rodents (pigs, sheep). Taken together, the data indicate possible clinical applicability of vanilloid-induced conduction analgesia.

1. Introduction

Although capsaicin-related research started more than half a century ago, the dramatic increase of the interest to this topic was instigated by the cloning of the first vanilloid (capsaicin) receptor in 1997 (1). The growth of publications on vanilloid receptors is such that the PubMed database for terms “capsaicin” and “TRPV1” has more than 5 thousand articles published for the last 10 years. The number of reviews exceeds one hundred. This growth of publication is due to the finding many new related areas of investigations: new receptors (TRPV2, TRPV3, TRPV4, TRPM8, TRPA1), endogenous TRPV1 activators (anandamide), new TRPV1 receptor antagonists, potential clinical applications other than pain (urinary urge incontinence, chronic cough, irritable bowel syndrome), etc. Multiple approaches to TRP-based therapies are

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Implication Statement

Vanilloid agonists (capsaicin, RTX) applied to the peripheral nerves provide conduction blockade. In contrast to the analgesic component of conduction anesthesia produced by local anesthetics, vanilloid agonists provide conduction analgesia not associated with suppression of motor or sensory functions not related to pain. The article summarizes data suggesting clinical applicability of vanilloid-induced conduction analgesia.

presented in Figure 1. This article will focus on conduction analgesia. Related topics, especially those based on the injections of vanilloid agonists (instillation into surgical field, infiltrations, neuraxial administrations) are also presented although briefly. Information on the pharmacology of TRP channels and prospects for TRP-based therapies other than conduction analgesia are covered in the following excellent reviews: 2 – 5.

2. General overview of vanilloid agonists

Vanilloid Receptors

TRPV1 (transient receptor potential vanilloid type 1 receptor) was initially identified as the receptor for capsaicin, the pungent ingredient in peppers. It is a non-selective cation channel, predominantly expressed by sensory neurons. TRPV1 is activated by a diverse range of chemical ligands (capsaicin, resiniferatoxin [RTX], endogenous lipid anandamide, and other endogenous capsaicin-like substances), as well as protons, heat, and it can behave as an integrator of the effects of many pain-producing agents (1,2,5). Ten years after the cloning of TRPV1 (1), several other TRPs have been described in dorsal root ganglia: TRPV2, TRPV3, TRPV4, TRPA1, and TRPM8 (4,5). These channels are emerging as sensory transducers that may participate in the generation of pain evoked by chemical, thermal and mechanical stimuli. Interaction (“teamwork”) between TRP channels was suggested for their role in many pain mechanisms. TRPV1 is the first described and most studied TRP receptor that may play both pronociceptive and protective roles in the various models of inflammatory and neuropathic pain syndromes. There are indications that TRPV1 may be sensitized and upregulated during inflammation and in other disease states. TRPV1 is present not only in primary sensory neurons but also in various brain nuclei, throughout the whole neuraxis, and is also expressed in non-neuronal tissues such as the epidermis (keratinocytes), urothelium, bronchial epithelium, alveolar cells, mast cells, fibroblasts and smooth muscle (5). Therefore, agents acting via TRPV1 may have therapeutic values in addition to pain relief.

Actions and Mechanisms

Vanilloid agonists binding to TRPV1 open the channel pore and lead to cation, predominantly calcium, influx that causes membrane depolarization. When membrane depolarization reaches the threshold level, action potentials are generated and may be perceived as itch or pain (2). Vanilloid agonists release a variety of proinflammatory neuropeptides (calcitonin gene-related peptide [CGRP], cholecystokinin [CCK], substance P [SP], galanin, and somatostatin) from sensory nerve endings and initiate neurogenic inflammation. Excitation of sensory neurons by vanilloid agonists is followed by a refractory state in which neurons do not respond to various stimuli, including a subsequent vanilloid challenge. This process starts with receptor desensitization (rapid loss of activity of the receptor occupied by an agonist) and leads to inactivation (defunctionalization [2]) of vanilloid-sensitive neurons. The vanilloid-induced inactivation may be reversible or irreversible depending on the dose of agonist and its administration site. These different states induced by vanilloid agonists have led to their use for opposite aims: to induce pain (in experiments) and to relieve it. Inactivation of vanilloid-sensitive neurons is most likely not a single, well-defined biochemical process but rather a cascade of events that starts with TRPV1 activation, with calcium entry from outside the cell as well as calcium release from endoplasmic stores playing a leading role (2,6,7). This process includes a long-lasting desensitization of TRPV1. Depletion of neuropeptides, blockade of the axonal transport of macromolecules, sustained depolarization block that prevents action potential generation, inhibition of mitochondrial bioenergetics, and many other effects mediated by TRPV1 can be responsible for vanilloid-induced inactivation of sensory neurons. Vanilloid agonists have a spectrum of various effects that are not mediated by TRPV1. RTX differs from capsaicin in its spectrum of non-TRPV1-mediated actions (2).

Capsaicin vs. RTX

Capsaicin and RTX are the most commonly studied vanilloid agonists. The ability of capsaicin, used in high doses topically or systemically, to produce a novel type of analgesia was discovered by Nicholas Jancso (8). RTX is an irritant component of a cactus-like plant, *Euphorbia resinifera*. Because capsaicin and RTX share a vanillyl group essential for their activity (although they differ in the rest of the molecule), they are collectively termed vanilloids. RTX are much more potent than capsaicin. Their potency differences are variable depending on the assays. For Ca^{2+} uptake in DRG neurons, RTX is approximately 300-fold more potent; with desensitization of the urinary bladder to subsequent challenge, it is approximately 1000-fold more potent (2). At the same time, with activation of the cloned TRPV1, RTX is only 20-fold more potent than capsaicin (1). According to Szallasi and Blumberg (2), compared to capsaicin, RTX has a much wider therapeutic margin of safety (prevention of neurogenic inflammation vs. respiratory paralysis). Neuronal excitation caused by capsaicin is much more pronounced than that caused by RTX. Current-clamp experiments reveal that both capsaicin and RTX produce sustained membrane depolarization, but capsaicin generated a significantly greater number of action potentials (9). The generation of action potentials probably depends on the rate of the channel opening. Cation influx with RTX is probably less rapid than with capsaicin. That may be the reason why with intravesicular instillation of these vanilloids (for the treatment of urinary bladder hyperreflexia) RTX caused much less initial irritation in treated patients than capsaicin (10).

TRPV1 Antagonists

The other approach to find new therapeutic strategies involving TRPV1 is with the use of TRPV1 antagonists. The TRPV1-agonist-based analgesia is very different from that provided with TRPV1 antagonists. TRPV1 agonists silence the whole nerve terminal, whereas antagonists selectively block TRPV1 receptor. Interest in TRPV1 antagonists was significantly increased by recent advances suggesting that there are endogenous TRPV1 activators or “endovanilloids” and that anandamide may be one such compound (for review see references 3 and 11). The recognition of endovanilloid signaling via TRPV1 in pain and inflammatory hyperalgesia has intensified efforts to discover novel TRPV1 antagonists (for review see references 4 and 12).

Clinical Applications

Clinical use of vanilloid agonists started with topical capsaicin (0.025 and 0.075% preparations for application on the skin). Topical capsaicin has been used for almost 20 years for the treatment of post-herpetic neuralgia, osteoarthritis, diabetic neuropathy, and other chronic musculoskeletal or neuropathic pain syndromes. The most recent systematic review of topical capsaicin for the treatment of chronic pain concluded that it has moderate to poor efficacy but may be useful as an adjunct or sole therapy for a small number of patients who are unresponsive to, or intolerant of, other treatments (13). The next step in the clinical application of capsaicin was in the form of intravesicular instillation in the treatment of urinary bladder overactivity. The multiple studies of patients with this pathology have demonstrated an improvement in urinary tract symptoms (14). However, the initial acute pain and irritation associated with capsaicin are major deterrents to its widespread use. The use of RTX instillation for the same purpose indicated that it is possible to have similar efficacy but with much less initial irritation (14). The most recent clinical application of capsaicin (in new formulation) was an intraoperative instillation into the wound during total knee arthroplasty (15). In this study, 50 patients received a single instillation of capsaicin (5 mg in 60 mL) or a placebo, both after the application of bupivacaine (0.25%). The capsaicin group demonstrated better postoperative analgesia, and there was no difference between the groups in reporting adverse events.

Neurotoxicity

Neurotoxicity of vanilloid agonists is a controversial subject. The main basis for this controversy is in inadequate assessment of vanilloids' margins of safety with different application sites. It is well established that capsaicin is able to kill adult sensory neurons in culture. This action is most likely mediated by calcium influx (16). Capsaicin given to newborn rats destroys the majority of small- to medium-sized dorsal root ganglion (DRG) neurons (17). In adult rats, a significant loss of DRG neurons induced by systemic capsaicin was also reported (18); however, the dose was extremely high. Jancso initially used capsaicin in doses up to 80 mg/kg s.c. to render adult rats fully insensitive to chemically evoked pain for 1 to 3 months. A more aggressive treatment protocol included 950 mg/kg capsaicin given s.c. over a period of 5 days (19). Systemic RTX used in a single dose of up to 150 µg/kg caused desensitization against chemogenic pain, noxious heat, and neurogenic inflammation lasting 4 to 6 weeks (20–22). Comparisons of the dose ranges for desensitization (limited by respiratory paralysis) of systemic capsaicin and RTX in rats demonstrated that both agents embrace doses that differ by two orders of magnitude with capsaicin and three orders of magnitude with RTX (2). The spectrum of vanilloid biological activities that can cause neurotoxicity can be specific (via TRPV1) or nonspecific (2). At least three of the vanilloid actions are able to destroy sensory neurons under certain circumstances. One of them is their inhibitory effect on axoplasmic transport (23–24). For example, it was postulated that capsaicin in neonatal rats kills developing neurons by blocking axonal transport of nerve growth factor (NGF) from the periphery, where it is produced (26). The other likely mechanism of toxicity is involvement of calcium-activated proteases (16). Another effect of vanilloid agonists-related neurotoxicity is apoptosis (26,27). Capsaicin and RTX cause mitochondrial damage (28) and the resulting increase in intracellular reactive oxygen species can initiate apoptosis. The central role in the initiation of the above mechanisms is played by the vanilloid-induced calcium influx. At the same time, although calcium seems to be an important orchestrator of vanilloid-induced neuronal degeneration in vitro, the question remains open whether the rise in intracellular calcium by vanilloid agonist can achieve sufficiently high levels in adult sensory neurons in vivo to cause irreversible neuronal damage (2).

Vanilloid agonists display various mechanisms of action that can possibly underlie both therapeutic potential and neurotoxicity. At different target sites for the effects of vanilloids (the brain areas related to sensory neurons, dorsal horns of the spinal cord, dorsal root ganglia, nerve trunks, and distal nerve terminals), the contribution of different mechanisms of actions may vary substantially. As a result, the degree of separation between the doses producing a desirable effect and those producing a toxic effect may depend on their application site. The main question is whether the separation between the desired effect and possible toxic effect is large enough with the particular target site. Local anesthetics are good for comparison in this regard: Depending on the concentration they can provide both reversible anesthesia and neurolytic block (29).

3. Conduction analgesia with vanilloid agonists

When local anesthetics are applied to a peripheral nerve the analgesic effect can be observed at a time when motor function and touch are less affected (30) which is termed differential block. In general, local anesthetics cannot produce analgesia without at least some effect on motor and sensory functions not related to pain. Therefore, the outcome resulting from a nerve blockade was termed conduction anesthesia (31). In contrast to the analgesic component of conduction anesthesia produced by local anesthetics, true conduction analgesia is also possible: It is observed with an application of vanilloid agonists to a peripheral nerve.

TRPV1 in Peripheral Nerves

TRPV1 was found in different parts of the primary afferent neuron: the cell body (dorsal root ganglia), the central and peripheral terminals, and the axons (32–33). Immunolocalization of TRPV1 in the axons of the rodent sciatic nerve revealed that they are present in unmyelinated axons along the axonal plasma membrane where spots of immunopositivity formed an almost gap-free lining (34). It was also reported that capsaicin-induced stimulation of TRPV1 in the peripheral nerves leads to CGRP exocytosis along unmyelinated axons (34). TRPV1 receptors were found to be located on the inner face of the plasma membrane (35); they are also located on all internal membranes (36). The study of subcellular location of TRPV1 within DRG indicated that it was associated with plasma membrane and the Golgi complex of small- to medium-sized neurons (37). When DRG neurons were immunohistochemically stained with antibodies against TRPV1 in addition to the use of A-fiber markers it was found that double-labeled neurons were predominantly small and medium sized (38). These results suggest that TRPV1 is likely to be expressed by A δ -fiber neurons as well as C-fiber neurons.

Conduction Block

Perineural application of vanilloid agonists results in the selective conduction blockade of C- and, to a lesser extent, A δ -fibers. Studies demonstrating this effect of vanilloids are almost exclusively conducted with capsaicin. In *in vivo* experiments with application of capsaicin (1%) to a peripheral nerve, conduction of C-fiber and, to a lesser extent, A δ -fiber (but not A α , β -fiber) components of the compound action potential across the application site were demonstrated to be profoundly decreased (39–42). Recordings from single fibers (stimulated proximal and distal to the application of capsaicin on the rat's nerve) revealed that nociceptive C-fibers responding to strong mechanical and heat stimulation were blocked without any changes in the conduction in A-fibers (39,43). When the effects of capsaicin (1% administered topically to the sural nerve) on the responses of neurons belonging to the spinothalamic tract were studied in monkeys, the responses to innocuous mechanical stimuli applied to the cutaneous receptive field were increased, the responses to noxious mechanical stimuli decreased, and the responses to heat stimuli almost completely eliminated (40). Almost all the studies on the effect of topical vanilloid agonists on peripheral nerve conduction were performed with the use of 1% solution of capsaicin. One of the exceptions was the *in vivo* study on the effects of capsaicin on conduction in cutaneous nerves in four mammalian species (rat, ferret, guinea pig and rabbit) (41). This study demonstrated that in the rat and ferret the decrease of the compound action potential with 0.11 mM of capsaicin was almost as profound (by 70% in rats) as with 33 mM (1% solution). In the guinea pig and rabbit, the effects of capsaicin were much smaller than those found in the rat and ferret. The effect of capsaicin was also explored *in vitro* with the use of isolated fascicles from human sural nerve (44). Capsaicin produced a substantial reduction of the C-fiber component of compound action potential with a concentration as low as 10 μ M.

One of the first studies on the mechanism of the capsaicin-induced conduction blockade put forward the concept of depolarization blockade (45). Both capsaicin and RTX induce sustained membrane depolarization (9) by opening non-selective cation channels. The other possibility for the blockade mechanism is a direct effect of vanilloid agonists on voltage-gated sodium channels. Voltage-clamp experiments showed that capsaicin inhibits Na⁺ currents in specific types of ganglion neurons (46,47). This effect might be related to TRPV1 or be independent of it, via changes in membrane elasticity (48) or via a non-TRPV1 receptor (49). Conduction blockade could be also associated with many other mechanisms of action demonstrated by vanilloid agonists (for example, insufficient energy supply due to mitochondrial dysfunction or inhibition of axonal transport).

Analgesic Effects

Jancso G et al. (50) reported that in rats, capsaicin (1%, 0.1 mL) applied to the sciatic or saphenous nerve for 15 min produced a long-lasting (more than 4 weeks) increase in nociceptive threshold to heat and prevented neurogenic inflammation in the affected paw. Since the motor functions of the rat's paw remained intact the authors concluded that perineural capsaicin produced a selective analgesic effect. It was later demonstrated that perineurally applied capsaicin also inhibits the responses to noxious mechanical stimuli (40). Experiments in rats also demonstrated that, similar to capsaicin, perineural RTX (0.001%, 0.1 mL) produced a long-lasting thermal and mechanical hypoalgesia (51). In a study with the percutaneous administration of RTX to the sciatic nerve, it was found that the depth of RTX-induced analgesia and its duration is dose-dependent with a very wide separation between concentrations producing relatively short hypoalgesia and long-lasting nociceptive block: from 0.00003% to 0.001%. The highest concentration (0.001%) produced blockade of responses to the noxious heat with recovery in 2 weeks. Comparison of the responses to noxious pressure and heat revealed the predominance of the RTX effect on thermal responses (Figure 2). The blockade of the responses to noxious pressure occurred without changes in the response to the touch [von Frey filaments] (52).

A single injection of RTX (0.001%) to the sciatic and saphenous nerves in rats was found to prevent completely the development of thermal and mechanical hyperalgesia induced by carrageenan inflammation (51). A similar antihyperalgesic effect was reported in a rat model of incisional pain (Figure 3) (52). RTX also prevented the incision-induced pain behavior associated with weight bearing. The selective blockade of nociceptive fibers with RTX not only prevented hyperalgesia itself but also formation of long-term hyperalgesia-related memory (53). Thus, pain can be preempted by selective and prolonged blockade of nociception with vanilloid agonists.

A single perineural administration of RTX prevented the development of hyperalgesia in at least one type of experimental neuropathy – produced by loose ligation of the sciatic nerve (Figure 4) [54]. In this model, neuroinflammation plays a significant role in the ensuing hyperalgesia (55). The positive effect can be explained on the following basis. Stimulation of the peripheral nerve leads to CGRP exocytosis along unmyelinated axons (34). The release of CGRP and other neuropeptides causes vasodilatation and plasma extravasation, both important elements of neuroinflammation. At the same time, various inflammatory mediators, released after nerve damage, can activate or sensitize the TRPV1 receptors (56). Taken together, these findings may explain the protective effect of RTX in pain caused by neuroinflammation.

The analgesic and antihyperalgesic effects of perineural vanilloid agonists are preceded by a short excitatory phase that is much more pronounced with capsaicin than with RTX. Electrophysiological studies with capsaicin indicated that the background activity of the neurons belonging to the affected sensory fibers was profoundly increased (5-fold) but lasted only for 5–10 min (40).

4. Toxicity of vanilloid agonists related to their perineural administration

As indicated above, vanilloid agonists display many mechanisms that can lead to neurotoxicity. Most of these mechanisms are activated by the vanilloid-induced rise in intracellular calcium. The approach to the neurotoxicity of vanilloid agonists is complicated by the following two factors: the site of vanilloid administration and the method of unmyelinated fiber visualization. The site of a vanilloid administration can be an important factor that determines its toxicity. It is well known that in newborn rats, sensitivity of small- to medium-sized DRG neurons to capsaicin is especially high. Various parts of the primary afferent neurons (central terminals, cell bodies, axons, and peripheral terminals) have their own spectrum of specific mechanisms

sensitive to vanilloid agonists. Therefore the toxic effects of vanilloid with intrathecal, perineural, or subcutaneous administration may be quite different. For example, peripheral sensory axons have different sensitivity to capsaicin with the subepidermal part of the axon being the most vulnerable (57).

Presently there is a controversy related to the use of immunohistochemical methods for the detection of vanilloid-induced damage to the nerve fibers. In studies on the neurotoxic effects of capsaicin applied to the skin (58,59), administered perineurally (60), or into the urinary bladder (61) in order to provide nerve fiber visualization, the authors used immunohistochemical methods based on determination of SP, CGRP, and/or the pan-neuronal marker protein gene product (PGP 9.5). These studies demonstrated a profound loss of nerve fiber staining in the epidermis (or the urinary bladder wall) that led many of the authors to the conclusion that capsaicin induced axonal degeneration. However, none of these studies used electron microscopy to confirm this conclusion. When Avelino and Cruz (62) re-examined the problem of capsaicin-induced axonal degeneration in the rat bladder with the parallel use of immunohistochemical and electron microscopic methods, they found that both capsaicin and RTX caused a profound reduction in SP and CGRP immunoreactivity without causing significant changes determined by electron microscopy. The authors concluded that vanilloids applied intravesically (in the urinary bladder) in full desensitizing concentrations exert an effect on the bladder (including depletion of SP and CGRP) that lasts for 8 to 12 weeks without nerve fiber degeneration. They suggested that PGP immunoreactivity, which was used in several capsaicin studies as an index of axon destruction, could be lost because vanilloids cause axonal transport blockade that slows the arrival of PGP to peripheral axons, which occurs without nerve degeneration. Thus, when the axonal traffic is affected, equating of the disappearance of TRPV1 staining with neuronal loss is questionable.

Local Neurotoxicity

In initial studies with perineural administration of vanilloids, only capsaicin was used and almost exclusively in high concentrations – 1% to 1.5%. With these concentrations, the effect of capsaicin was still present at the end of the study period (4 to 6 weeks). This was the basis for the suggestion that the local treatment of peripheral nerves with capsaicin results in a permanent impairment of the function of capsaicin-sensitive afferent nerve fibers and possibly in their degeneration. However, the initial studies conducted with the use of electron microscopy found no axonal degeneration after topical (cornea) (63) or perineural (sciatic nerve) (64) application of capsaicin even in high concentrations of up to 1.5%. Two subsequent studies by the same group of authors on the effect of perineural capsaicin on the C-fibers with the use of electron microscopy resulted in conflicting outcomes. In rats, treatment of the saphenous nerve with 1% capsaicin caused the C:A fiber ratio to decrease by approximately 35%, although cross-sections of treated nerves had essentially normal appearances (43). At the same time, in rabbits (also with the saphenous nerve), 1% capsaicin did not change the C:A fiber ratio (65). The authors suggested that a concentration of capsaicin lower than 1% may suppress nociception in the rat without C-fiber degeneration. This suggestion was based on the finding that even 0.01% capsaicin applied to rat nerves cause long-lasting suppression of nociceptive responses.

Quantitative electron microscopic evaluation of the unmyelinated fibers following the RTX-induced nerve blockade was recently performed in rats (66). Two concentrations of RTX were used for percutaneous injection at the sciatic nerve, one providing blockade for approximately a day (0.0001%, 0.1 mL) and another for one week (0.001%, 0.1 mL); the sciatic nerves were removed for evaluation in 2 and 8 days after the injections. Cross-sections of the nerve had essentially normal appearances. One of the rarely observed findings was the irregularly compacted membranous deposits in the unmyelinated axons (Figure 5). The recovery of the

thermal nociception by the 8th day after RTX (0.001%) and the absence of any motor deficit or changes in the responses to the hindpaw stimulation with von Frey filaments during and after the nociceptive block was the other evidence that functions of the nerve were not irreversibly damaged.

Frequency of degeneration among unmyelinated axons was approximately one per thousand (Table 1). Compared with the effect of local anesthetics (including lidocaine up to 3%) on rat sciatic nerves (degeneration of approximately 5% of unmyelinated fibers) this frequency of degeneration is more than an order of magnitude lower. Taking into account that the duration of blockade with RTX lasts at least 10 times longer than the blockade with local anesthetics the difference in toxicity is impressive.

Acute Systemic Toxicity

When capsaicin was administered in rats in four increasing subcutaneous doses to a cumulative amount of 21–66 mg/rat, half of the animals died (67). LD₅₀ values determined in several animal species demonstrated a profound difference in sensitivity to capsaicin with a higher susceptibility in rats, mice, and guinea pigs and a lower susceptibility in hamsters and rabbits (68). For example, capsaicin LD₅₀ values with intraperitoneal administrations were 13 mg/kg (2mg/rat) in rats and more than 120 mg/kg in hamsters. The authors concluded that the possible cause of death from capsaicin was respiratory paralysis. There are no reported ED₅₀ values for RTX; however, the maximal doses of this agent, given subcutaneously in rats to study its pharmacodynamics, reached 100 µg/rat. The therapeutic dose range for RTX (s.c. injection in rats) was limited by the dose producing respiratory paralysis (2).

When comparing a dose of RTX used for blockade of the sciatic nerve in rats (100 ng for a blockade lasting for 24h) with the systemic doses indicated above, it becomes clear that the blocking dose is more than a thousand times less than the dose producing acute systemic toxicity. For comparison, it should be noted that the dose of bupivacaine producing sciatic nerve block in rats is 0.5 mg/rat (the block lasts only 1.5 h) and the bupivacaine LD₅₀ value -15 mg/rat (61 mg/kg) (69). Thus, the blocking dose of bupivacaine (with incomparably less lasting blockade than RTX) is only 30 times less than bupivacaine LD₅₀ value.

Inadvertent intravascular injection of a large dose of vanilloids can result in pulmonary chemoreflex, characterized by apnea, bradycardia, and hypotension lasting for 2 to 6 min. This reflex is due to the stimulation of pulmonary C-fiber afferents and can be blocked by TRPV1 receptor antagonists (capsazepine) (70). In rats, in contrast to capsaicin, RTX did not elicit the reflex triad; however, bradypnea was observed in some of the animals (71). In dogs, intrathecal RTX (1.2µg/kg) elicited transient hemodynamic effects (72).

Short-lasting (up to 90 min) changes in rat's behavior following injection of RTX are described as somnolent and "pain-like behavior" (73). In experiments with preliminary injections of bupivacaine, such changes were not obvious (52).

5. Other approaches to analgesia with vanilloid agonists

In addition to conduction analgesia, vanilloid agonists can be used to provide other types of peripheral analgesia such as topical, intraarticular, intravesicular, and infiltration analgesia. These approaches are associated with the effects of vanilloids on the peripheral terminals of the primary afferent neurons. Targets of the vanilloid actions in the peripheral terminals are different from those in the nerve fibers. For example, mechanisms of peptide release from the nerve endings represent the targets different from those in the nerve trunks. There are a number of indications that peripheral nerve terminals are more sensitive to capsaicin than the nerve trunks (57,74). Topical capsaicin has been used clinically as an adjuvant analgesic in a variety

of pain conditions including postherpetic neuralgia, painful diabetic neuropathy, and postmastectomy pain syndrome (2). Capsaicin and RTX intravesicular (urinary bladder) administrations have been found effective in the treatment of detrusor hyperreflexia (75). An intraoperative (total knee arthroplasty) instillation of capsaicin solution (5 mg in 50 mL) into the wound (with bupivacaine) has been recently tried to provide a long-term postoperative analgesia with the positive outcome (15).

The application of vanilloid agonists to peripheral nerve terminals resulted in the loss of nerve fiber visualization determined with immunohistochemical methods (58,61,76,77). However, in a study of the effects of topical capsaicin (1%) applied to the eye of rats electron microscopy could not detect signs of nerve degeneration in the cornea (63). As indicated above, the parallel use of immunohistochemical and electron microscopic methods for the investigation of the changes of the terminal axons in the rat urinary bladder following intravesical capsaicin or RTX also indicated the absence of axon degeneration despite disappearance of immunohistochemical staining (62). These results question the equation of TRPV1 staining disappearance with neuronal loss when axonal traffic is blocked. The clinical effects of topical capsaicin or RTX could be explained by degeneration of terminal axons if these vanilloids are used in high concentrations. Nerve terminals probably have much higher sensitivity to vanilloid agonists than the nerve fibers. Therefore, degeneration of the nerve terminals following local application of vanilloid agonists is more likely than degeneration of nerve fibers following their application to the nerve trunks. However, the possibility of long-lasting defunctionalization without degeneration cannot be excluded even for topical application of vanilloid agonists. Their concentration probably determines the difference between inactivation without degeneration and inactivation with irreversible damage to the nerve terminals when the nerve functions can recover only due to regeneration.

The Iadarola group suggested the use of selective ablation of the nociceptive primary afferents as a way to delete neurons involved in pathological pain process (72,73,78,79). They suggested three strategies for pain modulation based on different applications of RTX: topical, intraganglionic, and intrathecal. Intrathecal RTX was used in a canine bone cancer model with positive results (79). The authors suggested that the therapeutic effect of RTX is due to its action on the neuronal cell bodies in the dorsal root ganglia. However, the dorsal horn terminals of primary afferents can also be the target of intrathecal RTX. Central terminals of primary afferents can be as highly sensitive to vanilloid agonists as peripheral terminals. The conclusion on the in vivo neuron degeneration was based on immunohistochemistry. Nevertheless, selective neurolytic block with high concentrations of RTX is an exciting possibility.

Conclusion

Vanilloid agonists (capsaicin, RTX) applied to the peripheral nerves provide conduction blockade. In contrast to the analgesic component of conduction anesthesia produced by local anesthetics, vanilloid agonists provide conduction analgesia not associated with suppression of motor or sensory functions not related to pain. Vanilloid agonists provide conduction analgesia selectively because their effect is limited to C- and A δ -fibers. The analgesic effect of vanilloid agonists is dose-dependent and long-lasting, from several hours to several weeks. The difference between minimal and maximal analgesic concentrations of RTX can reach two orders of magnitude. With very high concentrations of these agents the duration of the analgesia following sciatic nerve block in rats can exceed 3 weeks; however, after that the reversibility of the effect without nerve fibers regeneration is questionable. A nerve block with RTX prevented the development of thermal and mechanical hyperalgesia as well as pain behavior in a rat model of incisional pain. Vanilloid agonists have a potential for neurotoxicity that depends on many conditions of their administration (in vivo vs. in vitro models, newborn vs. adult animals, peripheral nerve terminals vs. nerve trunks, etc.). When RTX was applied to the

rat's sciatic nerve in doses necessary to provide conduction analgesia, the frequency of unmyelinated fiber degeneration was more than an order of magnitude lower than that with the therapeutic concentration of lidocaine. Presently, there are clinical data indicating that vanilloid agonists can produce positive effects with intravesicular instillation in urinary bladder overactivity (RTX, capsaicin) and provide postoperative analgesia with intraoperative instillation into the wound (capsaicin). Taken together, the data indicate possible clinical applicability of vanilloid-induced conduction analgesia. RTX-induced conduction blockade has an inherent drawback of TRPV1 agonists – the initial excitation (pain); therefore, a local anesthetic should be injected to prevent it. The major question related to the clinical applicability of RTX-induced conduction analgesia is the potential for local neurotoxicity, therefore the low level of neurotoxicity should be confirmed by experiments in species other than rodents (pigs, sheep).

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Figure 1. Approaches to TRP-based therapies. The box indicates a position of the article on conduction analgesia.

Figure 2.

Resiniferatoxin (RTX) time course curves for blockade of responses to noxious pressure and heat. Absence of the struggle response to pressure with a cutoff of 500g was determined as the blockade to pressure. Absence of the withdrawal response to heat (56°C) with a cutoff of 12 s was determined as the blockade to heat. RTX was applied percutaneously to the sciatic nerve (0.1mL; concentration range 0.00003% to 0.01%; $n = 5$ per group). The RTX time course curves were modeled and compared by using logistic regression analysis for a multivariate model. ^a $P < 0.0001$ vs. RTX 0.00003% and 0.0001%; ^b $P < 0.005$ vs. RTX 0.001%; ^c $P < 0.05$ vs. RTX 0.001%; ^d $P < 0.05$ vs. RTX 0.001% and 0.0003%; ^e $P < 0.001$ vs. RTX 0.00003% and 0.0001%; ^f $P < 0.05$ vs. RTX 0.00003% and 0.0001%.

[From Kissin et al. (52)]

Figure 3.

Preventative effect of resiniferatoxin (RTX) in incisional hyperalgesia as determined by measuring withdrawal threshold to application of von Frey filaments. RTX (0.0003%) was applied percutaneously to both the sciatic (0.1 mL) and saphenous (0.05 mL) nerves. To prevent an initial excitatory effect of RTX percutaneous bupivacaine (Bup 0.25%) nerve blockade preceded (5 min) injections of RTX or RTX vehicle (Veh; dimethyl sulfoxide in saline [Sal] with Tween 80). The plantar incision (Inc) was made 4 h after RTX injection. Four groups of rats ($n = 10$ per group) were used. The saline-vehicle (sal-veh) control did not have an incision. Withdrawal thresholds are in millinewtons. The results are expressed as medians with 95% confidence limits. ^a $P < 0.0001$ vs. Bup-Veh+Inc at baseline and 3.5 h; ^b $P < 0.05$ vs. Bup-Veh +Inc at baseline and 3.5 h; ^c $P < 0.0005$ vs. Bup-RTX+Inc at 6 h; ^d $P < 0.01$ vs. Sal-Veh at 6h; ^e $P < 0.05$ vs. Bup-RTX+Inc at 24 h; B = baseline

[From Kissin et al. (52)]

Figure 4.

Perineural resiniferatoxin (RTX) prevents the development of hyperalgesia and deficit in paw posture caused by the temporary placement of loose ligatures around the sciatic nerve. B = baseline, d = days after the drug administration and placement of ligatures. A single injection of RTX (0.0005%, 0.1mL) or its vehicle (0.1mL) was made at the sciatic nerve 3 h before the placement of ligatures. 10 d and 14 d values represent measurements taken 3 and 7 days after removal of the ligatures. Statistical significance: * = $P < 0.001$, + = $P < 0.01$, † = $P < 0.05$, all vs. vehicle group.

[From Kissin et al. (54)]

Figure 5. Examples of rarely observed (one per thousand fibers) deposits in the unmyelinated axons 48 hours after the perineural administration of resiniferatoxin (0.001%, 0.1 mL). Deposits are marked with **double arrows**, horizontal bars – **500 nm**. The two right-hand panels represent the same fiber with different degrees of magnification.
[From Kissin et al. (66)]

Table 1

Frequency of degeneration among unmyelinated axons.

Group	Number of rats	Approximate number of observed axons per rat	Number of degenerated axons per group	Percent of degenerated axons
Control (vehicle)	5	700	0	0
RTX 0.0001%	5	700	3	0.09
RTX 0.001%	5	700	5	0.14

[From Kissin et al. (66)]