QX-314 Produces Long-lasting Local Anesthesia Modulated by Transient Receptor Potential Vanilloid Receptors in Mice

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Background: The quaternary lidocaine derivative QX-314 is now known to produce long-lasting local anesthesia despite its positive charge. However, recent research suggests that the transient receptor potential vanilloid receptor agonist, capsaicin, should reduce the onset and offset times, whereas the transient receptor potential vanilloid receptor antagonist, capsazepine, should delay the onset time of sensory blockade by QX-314.

Methods: Sensory blockade in the tail of the conscious mouse was investigated using QX-314 2.5% in combination with capsaicin 0.1% and/or capsazepine (50 μg/ml). After tail injection, onset and offset times of local anesthesia were measured using the hot water tail-flick latency test.

Results: Capsaicin reduced the onset time of local anesthesia by QX-314 by more than 75% (Mann-Whitney test, P = 0.007; n = 10 per group) with no effect on the offset time of QX-314. For QX-314 without capsaicin, the onset and offset times were 23 min (interquartile range 15–30 min) and 360 min (interquartile range 285–375 min), respectively. For QX-314 with capsaicin, the onset and offset times were 3 min (interquartile range 3–8 min) and 360 min (interquartile range 285–435 min), respectively. In the antagonist study, capsazepine without added capsaicin decreased QX-314's efficacy, as 6 out of 9 mice did not develop sensory blockade after 90 min (Fisher exact test, P = 0.009).

Conclusion: We have confirmed in a sensory blockade model that QX-314 is a local anesthetic with a slow onset and a long duration of reversible blockade. Capsaicin, a transient receptor potential vanilloid receptor agonist, accelerated QX-314's onset kinetics, whereas capsazepine, a transient receptor potential vanilloid receptor antagonist, decreased QX-314's efficacy. These observations raise the possibility that endovanilloids may modulate cell entry of QX-314.

QX-314, a quaternary derivative of lidocaine, has a permanent positive charge that theoretically impairs its ability to cross neuronal membranes. As a few older in vitro investigations have demonstrated at least some cell entry,1 we recently revisited this assumption and find that extracellular QX-314 is in fact a local anesthetic producing nociceptive, sensory, and motor blockade.2 The onset speed and efficacy are concentration-dependent, with a 10-fold prolongation in duration compared to the equal percentage concentration of lidocaine. Subsequently, Binshok et al.3 concluded from their in vivo finding, at a single concentration, that QX-314 is a nociceptive blocker, but only in the presence of capsaicin. Capsaicin is a prototypical agonist at the vanilloid subtype-1 receptor of transient receptor potential (TRPV1) channels. Inflammatory mediators, protons, and heat activate this ligand-gated non-selective cation channel during nociceptive transduction andafferent neurotransmission.4

To determine if TRPV1 activity modulates QX-314's local anesthetic action, we examined the ability of a TRPV1 agonist and antagonist to affect sensory blockade by QX-314. Using the mouse tail-flick test, we hypothesized that the agonist capsaicin would shorten QX-314's onset plus offset times and that the antagonist capsazepine would delay QX-314's onset time. In addressing solubility issues for capsaicin and capsazepine, we used Intralipid® (Baxter Corporation, Toronto, Ontario, Canada) as our vehicle instead of the more typical solvent ethanol,3 which has been shown to activate TRPV1 receptors.5

Materials and Methods

The Animal Care Committee of The University of British Columbia (Vancouver, British Columbia, Canada) approved our protocol for conscious mice involving restraint, bilateral tail injections, and repeated hot water (54°C) tail-flick testing. Using a randomized and controlled experimental design, the experimenter (Mr. Pillai) was blinded to group allocation during drug injection and hot water testing. Computer randomization§ was performed in lines of four or five (see Blinded TRPV1 agonist and antagonist experiment sections in Materials and Methods) to allocate 90 drug-naïve female CD-1 mice weighing 20 to 30 g (Charles Rivers Laboratories, Inc., Wilmington, MA) to the various groups.

Mouse Tail-flick Test

We modified the mouse tail-flick model of Grant et al.6 for use as a test for sensory blockade as we have previously described.2 In brief, habituated mice were placed

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§ Available at www.randomizer.org; accessed August 1, 2008.
inside vented restraining tubes with their tails protruding outside for bilateral tail injections (20 μl each side) as well as for multiple tail-flick tests in the hot water bath. The tail injections were made near the base of the tail, and only the distal tip (approximately 1.5 cm) of the tail was submerged in hot water. This meant that a distance of more than 5 cm separated the sites of tail injection and heat exposure. With a maximal hot water exposure of 5 s, a tail-flick latency less than 3 s identified normal responders for study entry before the injection of local anesthetic. We defined the onset of sensory blockade as when the tail response time first reached 4 s and the offset as when the tail response time later decreased to less than 4 s, each on two consecutive tests.

**Blinded TRPV1 Agonist Experiment**

First, 10 mice were subjected to unblinded pilot testing of the tail injection method. Lidocaine (2%, 40 μl; n = 5) was used as a positive control, and capsaicin (0.1%, 40 μl; n = 5) was used as a negative control. Next, 20 drug-naïve mice were randomized in lines of four to either the control group of QX-314 (2.5%, 40 μl; n = 10) or the treatment group of QX-314 combined with capsaicin (0.1%, 40 μl total volume; n = 10). A slightly greater concentration of QX-314 was chosen in comparison to our previous study because we had changed from the chloride to the bromide salt. Hot water tail-response time was measured at 1, 3, 5, 10, 15, 20, 25, 30, 45, and 60 min after the injection, at which time each animal was removed from its restraining tube. Temporary restraint was again used every hour for further measurements of tail-flick latency until the sensory block decreased as described in the paragraph above. Onset time and offset time of local anesthesia were obtained for each mouse.

**Blinded TRPV1 Antagonist Experiment**

After dose-response testing of a range of capsazepine concentrations (1, 10, 25, 50, and 100 μg/ml; 40 μl; n = 5 per group), 50 μg/ml was chosen as the concentration for the antagonist experiment. First, capsazepine alone was studied in an unblinded pilot test as a negative control (50 μg/ml; 40 μl; n = 5). Next, 45 drug-naïve mice were randomized in lines of five to one of the following five groups: (1) Intralipid® (20%) alone as a vehicle control; (2) QX-314 (2.5%) as a positive control; (3) QX-314 combined with the agonist capsaicin (0.1%); (4) QX-314 combined with the agonist capsaicin as well as the antagonist capsazepine; and (5) QX-314 combined with just the antagonist capsazepine (40 μl; n = 9 per group). After the injection, hot water tail-response time was measured at 1, 3, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min.

**Drugs and Chemicals**

Capsaicin, N-(2,6-dimethylphenyl carbamoylmethyl)trithethylammonium bromide (QX-314), lidocaine HCl, and NaCl were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). Capsazepine was procured from Cayman Biochemicals (Ann Arbor, MI). Intralipid® 20% (a stabilized emulsion containing soybean oil, egg phospholipids, and glycerol) was obtained from Baxter Corporation (Toronto, Ontario, Canada). All drug solutions were prepared on the day of injection. Capsaicin (1 mg/ml), capsazepine (50 μg/ml), and QX-314 (25 mg/ml) were dissolved in sterile Intralipid® from previously unopened packages. Lidocaine (20 mg/ml) was dissolved in a NaCl solution (0.9% in distilled water). We have expressed concentrations as mass-volume percentages (mass of solute in g per 100 ml of the resulting solution).

**Statistical Analysis**

Statistical analysis was performed by using Prism version 4 software (GraphPad, San Diego, CA). For onset/offset times, comparison of two groups was made using the Mann-Whitney test, and comparison of three or more groups was made using the Kruskal-Wallis test followed by Dunn’s post test. "Survival" fractions of categorical time-to-event data were calculated using the Kaplan-Meier method, with comparison of curves using the log-rank test. Categorical data were compared with Fisher’s exact test. Statistical tests were two-tailed, and differences were considered significant at P < 0.05. We have expressed data as median (interquartile range [IQR]), and n represents the sample size.

**Results**

During the unblinded pilot tests, lidocaine (2%) produced sensory blockade of the mouse tail with an onset time of 1 min (IQR 1–3 min) and an offset time of 20 min (IQR 18–28 min) (n = 5). In contrast, neither capsaicin alone (0.1%; n = 5) nor capsazepine alone (50 μg/ml; n = 5) produced local anesthesia.

In the TRPV1 agonist study, all mice in the control group (QX-314 2.5% alone) and the treatment group (QX-314 with capsaicin 0.1%) developed a long-lasting and reversible sensory blockade of the tail. In addition, capsaicin reduced the onset time of local anesthesia by QX-314 (Mann-Whitney test, P = 0.007). For QX-314 without capsaicin, the onset time of sensory blockade was 23 min (IQR 15–30 min) (n = 10), whereas the QX-314 onset with capsaicin was 4 min (IQR 3–8 min) (n = 10). There was no difference, however, in the time to offset of local anesthesia for the two groups. For QX-314 without capsaicin, the offset time of sensory blockade was 300 min (IQR 285–375 min), whereas the QX-314 offset with capsaicin was 360 min (IQR 285–435 min). To illustrate the interaction between QX-314 and...
capsaicin, figure 1 shows the onset time (A) and offset time (B) of sensory blockade in the form of time-to-event curves.

In the TRPV1 antagonist study, all mice in the control group (QX-314 2.5% alone), the agonist group (QX-314% with capsaicin 0.1%) and the agonist-antagonist group (QX-314 with capsaicin plus capsazepine 50 µg/ml) developed long-lasting and reversible sensory blockade of the tail. Again, the onset time was reduced in the agonist group (P = 0.0003 for three group Kruskal-Wallis test, P < 0.001 for Dunn’s post test; n = 9), whereas the onset time was not different in the agonist-antagonist group compared to control (fig. 2). When QX-314 was injected with capsazepine but without capsaicin, however, there was a decrease in the number of mice that developed local anesthesia in comparison to all of the other groups (excluding vehicle controls; fig. 3A). After a 90-min observation period, 6 of 9 mice injected with QX-314 plus the TRPV1 antagonist had not developed sensory blockade (Fisher exact test, P = 0.009; fig. 3B). No mouse injected with Intralipid® alone as a vehicle control showed signs of sensory blockade (n = 9; not shown).

Since capsazepine prevented sensory blockade by QX-314, we then conducted additional experiments to exclude the possibility that repeated heat exposure of the distal tail mediated QX-314’s local anesthetic action. In a first set of experiments, we reduced the number of heat exposures in five drug-naive mice by measuring hot water tail-response time before tail injection and then once again 45 min later (compare with Blinded TRPV1 Agonist Experiment section in Materials and Methods). In these unblinded experiments, we found that sensory blockade was present in three of five mice at 45 min. Next, to determine if the preinjection heat exposure was required for QX-314 blockade, we randomized 12 drug-naive mice to either a control group (no preinjection heat exposure; n = 6) or an exposure group (preinjection heat exposure; n = 6) and delayed testing in both groups to 60 min. In this blinded comparison, we found no difference at 60 min; sensory blockade was present in four of six mice in each group. These results demonstrated that repeated exposure of the distal tail to noxious heat at a distance greater than 5 cm away from the site of injection was not required for local anesthetic action by QX-314.

Discussion

We have demonstrated in the mouse hot water tail-flick test that the TRPV1 agonist capsaicin can reduce the onset time of sensory blockade by QX-314 in vivo. Despite a reduction of the onset time by more than 75%,
however, capsaicin tested at a single concentration did not reduce the QX-314 offset time. As to the question of delay by a TRPV1 antagonist, we have shown that capsazepine injected without capsaicin can decrease QX-314’s efficacy and prevent sensory blockade. The coinjection of capsazepine and capsaicin on the other hand, tested at single concentrations, did not reduce or delay the QX-314 onset time. In addition, the repeated exposure of the distal tail to noxious heat at a distance greater than 5 cm away from the proximal site of QX-314 injection is not necessary for sensory blockade to occur.

Do these agonist and antagonist findings mean that TRPV1 activity rather than slow diffusion plays a critical role in mediating QX-314’s local anesthetic action? In our previous study of in vivo nociceptive, sensory, and motor blockade,2 QX-314’s efficacy was variable at concentrations less than 1% in all three models, consistent with a slow concentration-dependent diffusion through lipid membranes. However, the prevention of sensory blockade by the antagonist capsazepine alone raises the possibility that basal TRPV1 activity may mediate QX-314’s local anesthesia. This new hypothesis is supported by the observation that TRPV1 channels are tonically activated.

A critical role for TRPV1 activity is made uncertain, however, by our previous observations of motor blockade by QX-314 in the mouse.2 For example, sciatic nerve motor blockade was induced by QX-314 2% in approximately 6 min. Furthermore, we have recently found that lumbar intrathecal administration of a low concentration of QX-314 (approximately 0.25%) can produce motor blockade as well in a short time period in the mouse.9 TRPV1 channels are located on unmyelinated afferent fibers and sensory neurons; therefore, TRPV1 cannot be essential for QX-314 action. Could there be a non-TRPV1 pathway available to local anesthetics with a permanent positive charge that is nonselectively antagonized by capsazepine?

Many quaternary amines are capable of diffusing across lipid membranes. For example, tunicaine is a quaternary lidocaine derivative with a permanent positive charge like QX-314. And tunicaine reportedly produces long-acting motor blockade in the rat sciatic nerve model10 similar to that of QX-314. Other examples of positively charged quaternary amine drugs that must diffuse from the vascular compartment to their site of action include atracurium, pancuronium, bretylium, and glycopyrrolate.11

Although we believe that the fat emulsion Intralipid® acted as a therapeutically acceptable vehicle in the present experiments for the solubilization of capsaicin and capsazepine, it may not have provided a design advantage over other vehicles5 (see fig. 3 from Gerner et al.12). Typically, a solvent such as ethanol and an emulsifier such as the detergent Tween 20 are used in combination as a vehicle for capsaicin3 and capsazepine because they are insoluble in water. The fat emulsion Intralipid® was used a number of years ago to transport capsaicin,13 so we decided to use it here for capsazepine as well. Intralipid®, of course, is used as a parenteral formulation to administer lipid-soluble drugs such as propofol. In addition, Intralipid® has been used to inject solvents such as volatile anesthetics in the laboratory.15,16 Since Intralipid® can reportedly reverse excessive intracellular absorption of conventional local anesthetics,17 our vehicle may have restricted the diffusion of capsazepine in the three mice that developed sensory blockade after the coinjection with QX-314. Like conventional local anesthetics, capsaicin and capsazepine may have to diffuse through the neuronal membrane before interacting with an intracellular binding site on the TRPV1 channel.18

The suggestion that QX-314 in the presence of capsaicin can produce a “nociceptor-selective” nerve blockade mediated by TRPV1 activity5 has attracted significant therapeutic interest.19,12 However, we would conclude from our previous findings of nociceptive, sensory, and motor blockade2 that QX-314 may have a concentration-dependent “nociceptive-predominant” effect. Nonetheless, full concentration-response testing of sensory and motor blockade with capsaicin and QX-314 must be completed to determine if QX-314’s action is fundamentally different from conventional local anesthetics. Interestingly, an enhancement of motor blockade by TRPV1...
activity has been reported in an older study of capsaicin and the sodium channel blocker tetrodotoxin. Using sciatic nerve injections in the rat, tetrodotoxin produced motor blockade, and capsaicin did not. But coinjection of tetrodotoxin and capsaicin prolonged the tetrodotoxin motor blockade almost fourfold. Finally, it should be noted that lidocaine can activate the TRPV1 receptor in rat dorsal root ganglion cell culture and release inflammatory mediators. If TRPV1 activity proves to be a mechanism for local anesthetic-related neurotoxicity, the clinical role for TRPV1 agonists to decrease onset time of long-acting quaternary anesthetics may be uncertain.

In summary, we have confirmed in a sensory blockade model that QX-314, a quaternary lidocaine derivative, is a local anesthetic that produces a reversible nerve blockade with a slow onset and a very long duration. Capsaicin, a TRPV1 agonist, accelerates QX-314’s onset kinetics, whereas capsazepine, a TRPV1 antagonist, decreases QX-314’s nerve blocking efficacy. Although exogenous TRPV1 activation does not appear to be essential for QX-314’s local anesthetic efficacy, these observations raise the possibility that endovanilloids may modulate cell entry of QX-314.

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References