Emulsified Isoflurane Enhances Thermal Transient Receptor Potential Vanilloid-1 Channel Activation–mediated Sensory/Nociceptive Blockade by QX-314

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ABSTRACT

Background: QX-314 produces nociceptive blockade, facilitated by permeation through transient receptor potential vanilloid-1 (TRPV1) channels. TRPV1 channel can be activated by noxious heat and sensitized by volatile anesthetics. The authors hypothesized that emulsified isoflurane (EI) could enhance thermal TRPV1 channel activation–mediated sensory/nociceptive blockade by QX-314.

Methods: Rats were perineurally injected with QX-314 (Sigma-Aldrich Co. Ltd. Shanghai, China) alone or QX-314 combined with EI, followed by heat exposure on the injection site. The tail-flick and tail-clamping tests were used to assess sensory and nociceptive blockade, respectively; a sciatic nerve block model was used to assess motor and sensory blockade. Effects of EI on thermal activation of TRPV1 channels were evaluated on rat dorsal root ganglia neurons by whole-cell patch-clamp recordings.

Results: Heat exposure enhanced sensory/nociceptive blockade by QX-314 in rat tails, but not motor blockade in sciatic nerve block model. QX-314 alone or QX-314 + 42°C produced no nociceptive blockade. QX-314 + 48°C produced 100% nociceptive blockade with duration of 12.5 ± 2.0 h (mean ± SEM). By adding 2% EI, QX-314 + 42°C produced 80% nociceptive blockade with duration of 8.1 ± 1.9 h, which was similar to the effect of QX-314 + 46°C (7.7 ± 1.1 h; $P = 0.781$). The enhancement of heat on sensory/nociceptive blockade of QX-314 was prevented by TRPV1 channel antagonist. The temperature thresholds of TRPV1 channel activation on dorsal root ganglia neurons were significantly reduced by EI.

Conclusions: Thermal activation of TRPV1 channels enhanced long-lasting sensory/nociceptive blockade by QX-314 without affecting motor blockade. The addition of EI reduced temperature thresholds for inducing long-lasting sensory/nociceptive blockade due to QX-314. **(ANESTHESIOLOGY 2014; 121:280-9)**

CURRENTLY used local anesthetics produce nociceptive, sensory, motor, and sympathetic blockade. Long-lasting selective nociceptive blockade is potentially useful for postoperative analgesia and treatment of chronic pain. Local anesthetics with nociceptive-selective blockade will extend their clinical application. Although many attempts have been carried out,[1–5] none of them is perfect.[6–9]

QX-314, a quaternary derivative of lidocaine, produces long-lasting regional anesthesia with slow onset and low efficacy because of its permanent positive charge.[10,11] The positive charge of QX-314 impairs its ability to cross membrane of neurons or nervous fibers. Transient receptor potential vanilloid-1 (TRPV1) channel is exclusively expressed on nociceptor of peripheral nervous system and can be activated by capsaicin, proton, and noxious heat.[12–15] Recently, it has been demonstrated that the activation of TRPV1 channels by capsaicin[16] or acid solution[17] could specifically deliver QX-314 into sensory neurons (nociceptor), producing a rapid onset and long-lasting nociceptive-selective blockade.[16–21] However, regional injection of capsaicin or acid solution might induce injuries.[13,22,23] Noxious heat is also able to activate TRPV1 channels,[12] and volatile anesthetics including isoflurane have been found to decrease thresholds of TRPV1 channel activation.[24,25] Regional injection of volatile anesthetics might be harmful to local tissues. Emulsified

What We Already Know about This Topic

- QX-314, a permanently charged lidocaine analog, penetrates nerve fibers poorly, but can pass through the capsaicin-gated ion channel (transient receptor potential vanilloid-1) present on C fibers when the channel is stimulated
- The effects of emulsified general anesthetics and heat to open transient receptor potential vanilloid-1 channels and allow selective blockade by QX-314 have not been adequately studied *in vivo*

What This Article Tells Us That Is New

- In rats, local heat and emulsified isoflurane enhanced peripheral nerve block from locally applied QX-314, and the combination of moderate warmth plus isoflurane was as great as noxious heat

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isoflurane (EI), a novel emulsified preparation of isoflurane, is able to produce regional anesthesia without tissue damages.[26–29] Thus, EI might facilitate nonnoxious heat to activate TRPV1 channels, enhancing sensory/nociceptive-selective blockade by QX-314.

The current study was designed to test our hypothesis that EI could enhance thermal TRPV1 channel activation–mediated sensory/nociceptive blockade by QX-314.

Materials and Methods

With the approval of the Institutional Animal Experimental Ethics Committee of Sichuan University (Chengdu, Sichuan, China), Sprague–Dawley rats (weighing 250 to 300 g) were used. The rats were housed in cages on a 12-h light–dark cycle with free access to water and food. Rats were randomized using a SPSS-generated random number assignment in each experiment (SPSS Inc., Chicago, IL). For behavioral assessment of rats, observers were blinded to the treatments and group assignment of rats.

Emulsified isoflurane, a mixture of liquid isoflurane and 30% Intralipid, was prepared in our laboratory[30]; 8% (v/v) EI 100 ml contained 8 ml pure liquid isoflurane; 30% (w/v) Intralipid (30% soybean oil, 1.2% lecithin, 1.7% glycerin, water, and sodium hydroxide; pH = 8) was provided by the Sino-Swed Pharmaceutical Co. Ltd. (Wuxi, Jiangsu, China). Pure liquid isoflurane was provided by Abbott Pharmaceutical Co. Ltd. (Shanghai, China). QX-314, capsaicin, and capsazepine were purchased from Sigma-Aldrich Co. Ltd. (Shanghai, China). Lidocaine was purchased from Shanghai Fortune Zhaohui Pharmaceutical Co. Ltd. (Shanghai, China).

Tail Nerve Block Model

Tail nerve block model of rats was established as previously reported.[31] In brief, study solution (100 μl) was injected next to bilateral tail nerves. Nociceptive blockade was evaluated by tail-clamping test using an alligator clip (10 A, type 85, length 2 to 1/8 inch; Newark Electronics, Dublin, CA) with cutoff time at 10 s. Nociceptive blockade (tail-clamping blockade) was defined as no aversive response to tail-clamping stimulus.

Sensory blockade was assessed by tail-flick test using a tail-flick analgesic meter (Tail-Flick Unit 7360; Ugo Basile, Comerio, Italy) 1 min after each tail-clamping test. The intensity and cutoff time of radiant heat were set at 90 s (parameter of the equipment) and 10 s, respectively. Tail-flick latency was the time from onset of radiant heat to tail-flick response. Baseline tail-flick latency was measured before drug injection. Sensory blockade was standardized by calculating the percentage of maximum possible effect (%MPE) according to the formula[27]:

$$\%\mathrm{MPE} = \frac{\left(\begin{array}{l}\text{Tail-flick latency}_{\text{post-treatment}}\\ -\text{Tail-flick latency}_{\text{baseline}}\end{array}\right)}{\left(\begin{array}{l}\text{(Cutoff time)}\\ -\left(\text{Tail-flick lateny}_{\text{baseline}}\right)\end{array}\right)} \times 100\%$$

To test uncomfortable sensation of heat exposure alone, naive rats were exposed to water bath at temperature of 40°, 42°, 44°, 46°, or 48°C (10 rats for each temperature), respectively. The percentage of rats that developed aversive reactions and the time latency from exposure of water bath to aversive reactions were recorded. In 48° and 46°C groups, all the rats showed aversive reactions and average latencies were 10.1 ± 0.9 and 17.7 ± 1.8 s, respectively. In 44° and 42°C groups, aversive reactions were found in 8 of 10 and 4 of 10 rats, and their average latencies increased to 25.6 ± 2.9 and 40.3 ± 3.3 s, respectively. In 40°C group, no aversive reaction was found in all the study rats. On the basis of this preliminary test, we decided to test the effect of heat exposure on sensory/nociceptive blockade of QX-314 in the range from 40° to 48°C.

To explore the effect of heat exposure on sensory/nociceptive blockade by QX-314, 100 rats were randomized into one of the following 10 groups (10 rats per group): 1% lidocaine alone, 1% QX-314 alone, 1% QX-314 + 0.05% capsaicin,[16] normal saline + 48°C, 1% QX-314 + 48°C, 1% QX-314 + 46°C, 1% QX-314 + 44°C, 1% QX-314 + 42°C, 1% QX-314 + 75 μg/ml capsazepine[19] + 48°C, and 1% QX-314 + 75 μg/ml capsazepine + 46°C.

To investigate the effect of EI on sensory/nociceptive blockade by QX-314, another 70 rats were randomized into one of the following seven groups (10 rats per group): 1% QX-314 + Intralipid + 42°C, 1% QX-314 + 0.5%EI + 42°C, 1% QX-314 + 1%EI + 42°C, 1% QX-314 + 2%EI + 42°C, 1% QX-314 + 4%EI + 42°C, 1% QX-314 + 4%EI + 75 μg/ml capsazepine + 42°C, and 1% QX-314 + 4%EI + 40°C.

For application of heat exposure in the aforementioned groups, rat tails were immerged into water bath for 1 min after injection. During this period, the tail was taken out from water bath for 5 s if evasive movements observed. Onset of nociceptive or sensory blockade was the time from end of injection (or end of heat exposure if applied) to tail-clamping blockade and to %MPE reaching 50%, respectively. Duration of nociceptive or sensory blockade was defined as the time from blockade onset to recovery of aversive responses to tail-clamping test and to %MPE less than 50%, respectively.

Sciatic Nerve Block Model

With inhalation of 2% isoflurane, an incision approximately 0.5 cm (randomly on left or right limb) was made at popliteal fossa of rats to expose sciatic nerve. Eighty rats were randomized into one of the following eight groups (10 rats per group): 1% lidocaine alone, 1% QX-314 alone, 1% QX-314 + 0.05% capsaicin, normal saline + 46°C, 1% QX-314 + 46°C, 1% QX-314 + 44°C, 1% QX-314 + 2%EI + 44°C,

and 1% QX-314 + Intralipid + 44°C. Injection volume was 150 μl. Heat exposure was applied by an insulated electric heating wire that inserted near sciatic nerve and maintained for 1 min. The temperature of sciatic nerve was monitored by locally embedded electrodes (MLA 1212 Micro-Hook Electrodes, PowerLab data-acquisition system; AD Instruments, Bella Vista, New South Wales, Australia).

Sensory blockade in rat foots was measured by foot-flick test (Plantar test 37370; Ugo Basile). The intensity and cut-off time of radiant heat were set at 70 s (parameter of the equipment) and 10 s, respectively. Foot-flick latency was the time from onset of radiant heat to foot-flick response or obvious aversive reflex (if motor function impaired). Baseline foot-flick latency was measured before incision. Sensory blockade was standardized by calculating the %MPE as previously described in tail nerve block model. Onset of sensory blockade was the time from recovery of normal activity to %MPE reaching 50% and duration was the time from %MPE reaching 50% to %MPE less than 50%.

Motor blockade was evaluated as described in the study by Lim *et al.*[10] Rats were placed on an inverted mesh. The injected limb that unable to climb on the inverted mesh was regarded as motor blockade. Onset and duration of motor blockade were the time from recovery of normal activity to loss of ability to hang on the inverted mesh and to regain the ability, respectively.

Sensory and Histological Evaluations after Nerve Block

Twenty-four hours after complete recovery from nerve block, tail-flick latencies of the rats received tail nerve block and foot-flick latencies of the rats received sciatic nerve block were measured, respectively. All the rats that received water bath were checked for blister or injury on tail skin.

Two days after complete recovery from nerve block, tail nerves of rats from 1% QX-314 + 4%EI + 42°C group and sciatic nerves of rats from 1% QX-314 + 2%EI + 44°C group were harvested for histological evaluation. Hematoxylin–eosin staining and solochrome cyanine (Sigma-Aldrich Co. Ltd.) staining[32] were applied. Expression of CD-68 (macrosialin) on sciatic nerves was evaluated with anti-macrophage antibody (1:600; AbD Serotec Ltd., Oxford, United Kingdom). The procedure of immunohistochemistry and positive expression of CD-68 on sciatic nerve (long-term infusion of 0.75% bupivacaine) was performed as described in the study by Yang *et al.*[32] All the histological images were taken by DPTO camera coupled with a BX51 microscope (Olympus, Tokyo, Japan) and evaluated by a trained pathologist blinded to the treatments of rats.

Electrophysiological Recording

The isolation procedure of dorsal root ganglia (DRG) neurons was modified as previously described.[16] Rats were anesthetized by ketamine/xylazine (40/15 mg/kg) and DRG was removed and digested in collagenase I (2 mg/ml) for 80 to 90 min and in trypsin 0.5 mg/ml for 10 min. Small DRG neurons (<25 μm) were selected for recording. For TRPV1 channel current recording,[33] holding potential was −60 mV and temperature of extracellular solution was increased from 28° to 42°C. The extracellular solution contained 110 mM of NaCl, 26 mM of $NaHCO_3$, 5.6 mM of KCl, 1 mM of NaH_2PO_4, 0.1 mM of $CaCl_2$, 5 mM of $MgCl_2$, and 11 mM of glucose, pH 7.40. The pipette electrode solution contained 140 mM of KCl, 10 mM of EGTA, 1 mM of $MgCl_2$, and 10 mM of HEPES, pH 7.30. Electrode resistance was 3 to 5 MΩ. Capsaicin at 1 μM[16] and capsazepine at 5 μM[13] were used. Isoflurane was diluted from EI stock (500 mM, approximately 6.4%) with extracellular solution and applied locally to DRG neurons through a perfusion pipette (diameter 0.2 mm). The concentration of isoflurane was determined by local sampling of the perfusate at the site of the recording pipette tip and analyzed by gas chromatography.

Voltage-gated sodium channel currents were recorded using the conventional whole-cell patch-clamp technique. Holding potential was −90 mV. The extracellular solution contained 110 mM of NaCl, 26 mM of $NaHCO_3$, 5.6 mM of KCl, 1 mM of NaH_2PO_4, 0.1 mM of $CaCl_2$, 5 mM of $MgCl_2$, 20 mM of tetraethylammonium, and 11 mM of glucose, pH 7.40. The pipette electrode solution contained 140 mM of CsCl, 5 mM of EGTA, 1 mM of $MgCl_2$, 10 mM of HEPES, and 3 mM of MgATP, pH 7.30. Currents were sampled at 20 kHz and filtered at 1 to 3 kHz using an Axon 200B amplifier (Axon/Molecular Devices, Sunnyvale, CA). Temperature of extracellular solution was increased from 28° to 42°C, and sodium channel currents were recorded in the following five groups (n = 5 to 6): control (extracellular solution or extracellular solution + 30% Intralipid), 5 mM QX-314,[16] 0.6 mM EI, 5 mM QX-314 + 0.6 mM EI, and 5 mM QX-314 + 0.6 mM EI + 5 μM capsazepine.[13]

Statistical Analysis

SPSS version 16.0 (SPSS Inc.) was used in all the statistical analysis except for particular mention. By our preliminary test (n = 3 to 4) about difference of sensory blockade duration between QX-314 alone and QX-314 + capsaicin (5.2 ± 2.3 *vs.* 12.8 ± 5.7 h), calculated minimal sample size was 6.87 ($\alpha = 0.05$; $\beta = 0.10$). Thus, sample size of 10 was chosen in the current study, which was also similar to the sample size reported in previous studies.[18,19] Latency data (onset time, latency, and duration) were expressed as mean ± SEM. Kruskal–Wallis test followed by *post hoc* of Dunn test (software Prism 6.0; GraphPad, San Diego, CA) was applied for comparison of latency data among groups. The whole "survival curves" of tail-clamping blockade and motor blockade was compared by the Kaplan–Meier method with log-rank test among groups.

Inhibition of sodium channel currents was expressed as mean ± SEM of inhibitory percentage. ANOVA followed by Bonferroni *post hoc* test was used for comparison among groups. Two-way ANOVA (groups and temperatures as principal factors) was applied for comparison in heat-mediated inhibition of sodium channel currents. Arrhenius plot[34] was used to calculate the relation between temperatures and

sodium channel currents, by which heat-mediated inhibition of sodium channel currents illustrated two thermal response phases. Clampfit 10.0 (Axon/Molecular Devices), Origin 8.0 (OriginLab Corp., Northampton, MA), and Excel 2010 (Microsoft, Redmond, WA) were used for data analysis, figure preparation, and curve fitting. Statistical tests were two-tailed and *P* value less than 0.05 was considered as statistically significant.

Results

QX-314 Alone Produced Sensory but Not Nociceptive Blockade in Rat Tails

No rats developed nociceptive blockade in 1% QX-314 alone group (fig. 1 and table 1). For these rats, onset of sensory blockade was 25.0 ± 2.2 min and maximal %MPE of sensory blockade was 82 ± 11% (table 1). Sensory blockade duration of 1% lidocaine alone and 1% QX-314 alone was similar (3.0 ± 0.3 *vs.* 4.5 ± 0.4 h; *P* = 0.074; table 1).

Sensory/Nociceptive Blockade by QX-314 in Rat Tails Enhanced by Thermal TRPV1 Channels Activation

Normal saline + 48°C did not produce any sensory or nociceptive blockade (table 1). All the rats in 1% QX-314 + 48°C group developed nociceptive blockade, which was similar to 1% lidocaine alone and 1% QX-314 + capsaicin groups (fig. 1 and table 1). Duration of nociceptive blockade in 1% QX-314 + 48°C group was significantly longer than that in 1% QX-314 + capsaicin group (12.5 ± 2.0 *vs.* 5.9 ± 0.7 h; *P* = 0.019; fig. 1). For 1% QX-314 + 46°C group, nociceptive blockade was found in 9 of 10 rats and last for 7.7 ± 1.1 h (*P* = 0.198 *vs.* 1% QX-314 + 48°C). Nociceptive blockade was observed only in 1 of 10 rats in 1% QX-314 + 44°C group and its duration of sensory blockade was significantly shorter than that in 1% QX-314 + 48°C and 1% QX-314 + 46°C groups (*P* < 0.001). No enhancement was found when temperature decreased to 42°C (table 1).

Compared with 1% QX-314 + 48°C and 1% QX-314 + 46°C groups, addition of capsazepine to 1% QX-314 before

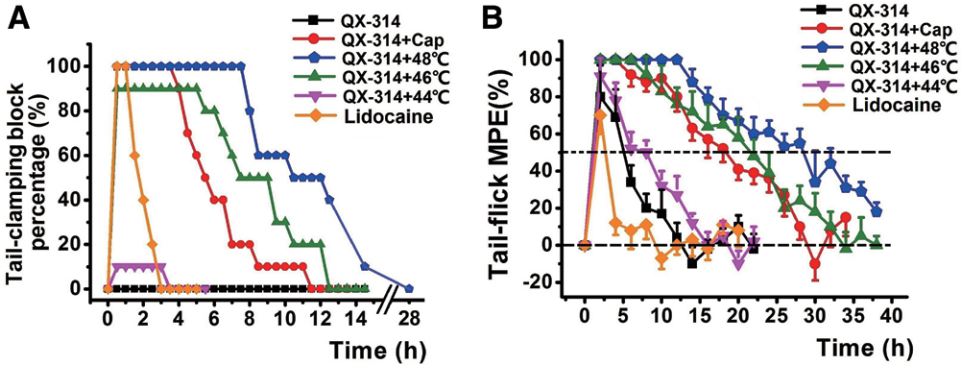

Fig. 1. Sensory/nociceptive blockade of QX-314 enhanced by heat exposure in rat tails. "Cap" indicates capsaicin. QX-314 and lidocaine at concentration of 1% were used. (*A*) Nociceptive blockade was evaluated by tail-clamping test. (*B*) Sensory blockade was evaluated by tail-flick test and calculated by percentage of maximum possible effect (%MPE). Effect of 1% QX-314 + 46°C was similar to the effect of 1% QX-314 + capsaicin. 1% QX-314 + 42°C produced no nociceptive blockade (not shown in this figure). Treatment of normal saline + 48°C did not exert any sensory or nociceptive blockade in rat tails (not shown in this figure). All the results of this figure are listed in table 1.

Table 1. Heat Exposure Enhanced Sensory/Nociceptive Blockade in Rat Tails

	Nociceptive Blockade			Sensory Blockade		
Groups	Rate (%)	Onset (min)	Duration (h)	Max (%MPE)	Onset (min)	Duration (h)
1% Lidocaine	100	2.2 ± 0.2	1.8 ± 0.6	100 ± 0	1.0 ± 0.0*	3.0 ± 0.3
QX alone	0	N/A	N/A	82 ± 11	25.0 ± 2.2	4.5 ± 0.4
QX + capsaicin	100	9.8 ± 1.2	5.9 ± 0.7	100 ± 0	6.8 ± 1.8*	17.2 ± 1.8*
NS + 48°C	0	N/A	N/A	25 ± 9*	N/A	N/A
QX + 48°C	100	2.9 ± 0.4	12.5 ± 2.0	100 ± 0	1.7 ± 0.3*	29.5 ± 3.8*
QX + 46°C	90	3.8 ± 0.3	7.7 ± 1.1	100 ± 0	2.8 ± 0.4*	20.2 ± 2.5*
QX + 44°C	10	35	3.0	92 ± 7	19.5 ± 2.5	7.7 ± 0.6*
QX + 42°C	0	N/A	N/A	85 ± 13	23.8 ± 2.7	4.9 ± 0.8
QX + CPZ + 48°C	60	14.5 ± 0.9	3.3 ± 0.9	90 ± 8	10.9 ± 1.7*	8.4 ± 0.7*
QX + CPZ + 46°C	10	30	2.5	85 ± 12	19.1 ± 2.4	6.3 ± 0.8

Sample size was 10 in each group. The data were expressed as mean ± SEM.

* *P* < 0.05, comparing with 1% QX-314 alone group.

CPZ = capsazepine; %MPE = percentage of maximum possible effect; N/A = no action; NS = normal saline; QX = 1% QX-314.

water bath at the same temperatures, nociceptive blockade rate and duration of nociceptive/sensory blockade significantly decreased while onset of nociceptive/sensory blockade delayed (table 1).

EI Enhanced Thermal TRPV1 Channel Activation–mediated Sensory/Nociceptive Blockade by QX-314 in Rat Tails

No nociceptive blockade was found in the rats from 1% QX-314 + Lipid + 42°C group (table 2). With concentrations of EI increasing from 0.5 to 4%, nociceptive and sensory blockades were enhanced (fig. 2 and table 2). For the rats in 1% QX-314 + 0.5%EI + 42°C and 1% QX-314 + 1%EI + 42°C groups, nociceptive blockade was found in 6 of 10 and 8 of 10 rats, respectively, and their sensory blockade durations were 7.2 ± 1.5 and 10.8 ± 1.2 h, respectively. Eight of 10 rats developed nociceptive blockade in 1% QX-314 + 2%EI + 42°C group and its sensory blockade duration was 18.7 ± 1.8 h, which was similar to that in 1% QX-314 + capsaicin group (17.2 ± 1.8 h; $P = 0.971$). For the rats in 1% QX-314 + 4% EI + 40°C group, nociceptive blockade was observed in 3 of 10 rats and sensory blockade duration was 6.1 ± 0.5 h, which was significantly longer than that of 1% lidocaine alone (3.0 ± 0.3 h; $P = 0.015$) and 1% QX-314 alone (4.5 ± 0.4 h; $P = 0.038$).

Heat Exposure and EI Enhanced Sensory but Not Motor Blockade in Sciatic Nerve Block

Normal saline + 46°C did not produce any sensory or motor blockade (table 3). Durations of sensory blockade in 1% QX-314 + 46°C group (146.0 ± 7.8 min) and 1% QX-314 + 2%EI + 44°C group (132.0 ± 10.2 min) were significantly longer than that in 1% QX-314 alone group (76.5 ± 7.2 min; $P < 0.001$). No significant change was found in rate and duration of motor blockade among 1% QX-314 alone group, 1% QX-314 + 46°C group, and 1% QX-314 + 2% EI + 44°C group (fig. 3 and table 3).

Sensory and Histological Observations after Nerve Block

None of the rats developed burn injury (blister) on tail skin after water bath. Tail-flick latency (table 4) of rats after tail nerve block and foot-flick latency (table 5) of rats after sciatic nerve block recovered to baselines. No edema or necrosis

Table 2. EI Enhanced Thermal TRPV1 Channel Activation–mediated Nociceptive/Sensory Blockade by QX-314

	Nociceptive Blockade			Sensory Blockade		
Groups	Rate (%)	Onset (min)	Duration (h)	Max (%MPE)	Onset (min)	Duration (h)
QX + Lipid + 42°C	0	N/A	N/A	80 ± 12	21.5 ± 2.2*	4.6 ± 0.7*
QX + 0.5%EI + 42°C	60	9.7 ± 1.3*	3.4 ± 1.9*	100 ± 0	7.7 ± 1.2*	7.2 ± 1.5*
QX + 1%EI + 42°C	80	8.4 ± 1.5*	3.8 ± 2.0*	100 ± 0	5.8 ± 1.1*	10.8 ± 1.2*
QX + 2%EI + 42°C	80	4.8 ± 0.3	8.1 ± 1.9	100 ± 0	2.9 ± 0.3	18.7 ± 1.8
QX + 4%EI + 42°C	90	3.8 ± 0.4	8.9 ± 2.3	100 ± 0	2.7 ± 0.3	20.7 ± 7.1
QX + 4%EI + CPZ + 42°C	20	20, 30	3.5, 2.5	79 ± 10	17.6 ± 2.0*	6.9 ± 0.8*
QX + 4 %EI + 40°C	30	12.8 ± 1.8*	3.1 ± 1.9*	85 ± 13	6.9 ± 1.4*	6.1 ± 0.5*

Sample size was 10 in each group. The data were expressed as mean ± SEM.

* $P < 0.05$ comparing with 1% QX + 4%EI + 42°C group.

CPZ = capsazepine; EI = emulsified isoflurane; Lipid = 30% Intralipid (Sino-Swed Pharmaceutical Co. Ltd., Wuxi, Jiangsu, China); %MPE = percentage of maximum possible effect; N/A = no action; QX = 1% QX-314; TRPV1 = transient receptor potential vanilloid-1.

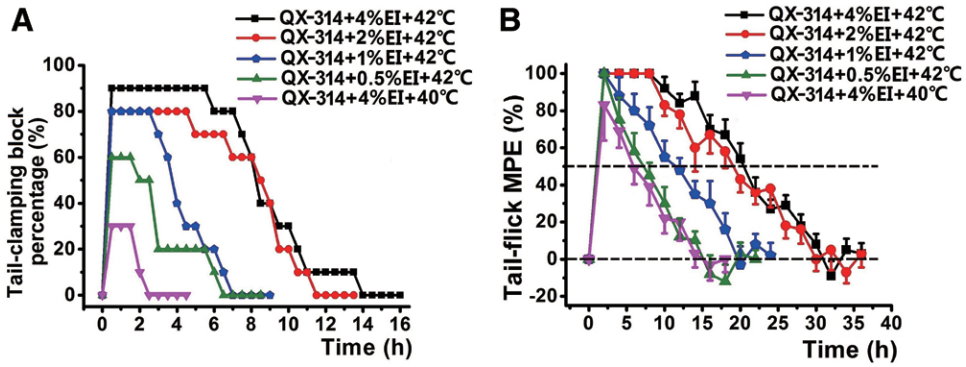

Fig. 2. Emulsified isoflurane (EI) decreased temperature thresholds for inducing long-lasting sensory/nociceptive blockade by QX-314. QX-314 at concentration of 1% was used. (*A*) Nociceptive blockade was evaluated by tail-clamping test. (*B*) Sensory blockade was evaluated by tail-flick test and calculated by percentage of maximum possible effect (%MPE). By adding EI, temperature threshold for inducing long-lasting sensory/nociceptive blockade was significantly decreased to approximately 42°C. Intralipid at 30% did not produce any enhancement (not shown in this figure). All the results of this figure are listed in table 2.

Table 3. Sensory and Motor Blockade in Sciatic Nerve Block

	Sensory Blockade			Motor Blockade		
Groups	Max (%MPE)	Onset (min)	Duration (min)	Rate (%)	Onset (min)	Duration (min)
1% Lidocaine	90±9	N/D	33.0±4.4*	100	N/D	33.5±3.4*
QX alone	79±9	3.5±1.7	76.5±7.2	30	0, 3, 5	65.0±5.0
QX + capsaicin	99±3	N/D	157.0±11.6*	30	0, 1, 3	85.0±8.7
NS + 46°C	30±11*	N/A	N/A	0	N/A	N/A
QX + 46°C	96±7	N/D	146.0±7.8*	30	0, 0, 3	75.0±5.1
QX + 44°C	88±7	1.9±0.8*	85.0±6.2	40	0, 0, 1, 3	56.3±5.8
QX + 2%EI + 44°C	92±10	N/D	132.0±10.2*	40	0, 0, 3, 3	57.5±5.3
QX + Lipid + 44°C	90±7	2.1±1.2*	87.5±5.7	30	0, 1, 3	70.0±4.8

Sample size was 10 in each group. The data were expressed as mean ± SEM, and onset of motor blockade was the raw data.

* $P < 0.05$ comparing with 1% QX-314 alone group.

CPZ = capsazepine; EI = emulsified isoflurane; Lipid = 30% Intralipid (Sino-Swed Pharmaceutical Co. Ltd., Wuxi, Jiangsu, China); %MPE = percentage of maximum possible effect; N/A = no action; N/D = not determined; NS = normal saline; QX = 1% QX-314.

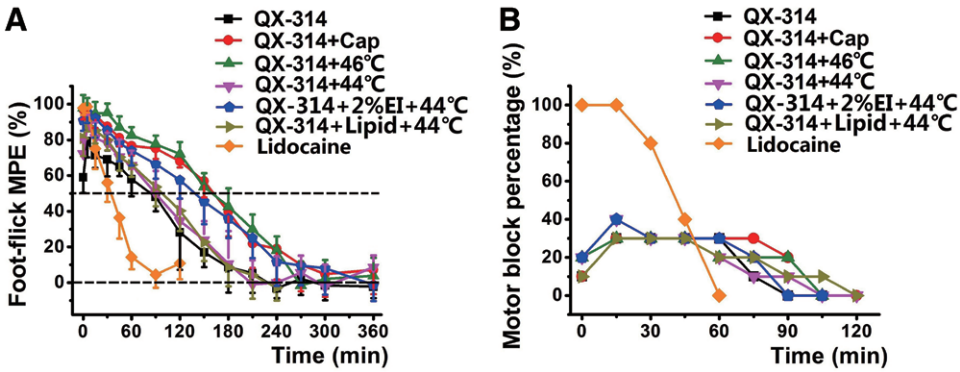

Fig. 3. Sensory and motor blockade by QX-314 was determined in sciatic nerve block model. "Cap" indicates capsaicin and "Lipid" indicates 30% Intralipid (Sino-Swed Pharmaceutical Co. Ltd., Wuxi, Jiangsu, China). QX-314 and lidocaine at concentration of 1% were used. (*A*) Sensory blockade was evaluated by foot-flick test and calculated by percentage of maximum possible effect (%MPE). (*B*) Motor blockade by QX-314 in sciatic nerve block model was shown. Motor blockade was not enhanced by heat exposure and/or emulsified isoflurane (EI) because similar motor blockade was found among all the groups except for 1% lidocaine. All the results of this figure are listed in table 3.

Table 4. Tail-flick Latencies of Rats before and after Tail Nerve Block

Groups	QX	QX + 48°C	QX + 46°C	QX + 44°C°C	QX + 4%EI + 42°C	QX + 2%EI + 42°C	QX + 1%EI + 42°C°C	QX + 0.5%EI + 42°C
Before (s)	2.9±0.2	2.9±0.2	2.7±0.2	2.9±0.3	2.7±0.2	2.7±0.2	3.0±0.2	2.7±0.2
After (s)	2.7±0.2	2.6±0.2	2.5±0.2	2.7±0.2	2.8±0.2	2.8±0.2	3.0±0.2	2.9±0.2

All the data were expressed as mean ± SEM.

EI = emulsified isoflurane; QX = 1% QX-314.

Table 5. Foot-flick Latencies of Rats before and after Sciatic Nerve Block

Groups	QX	QX + 46°C	QX + 44°C	QX + 2%EI + 44°C
Before (s)	4.0±0.2	4.1±0.2	4.2±0.2	4.3±0.2
After (s)	3.9±0.2	4.0±0.2	3.9±0.2	3.9±0.2

All the data were expressed as mean ± SEM.

EI = emulsified isoflurane; QX = 1% QX-314.

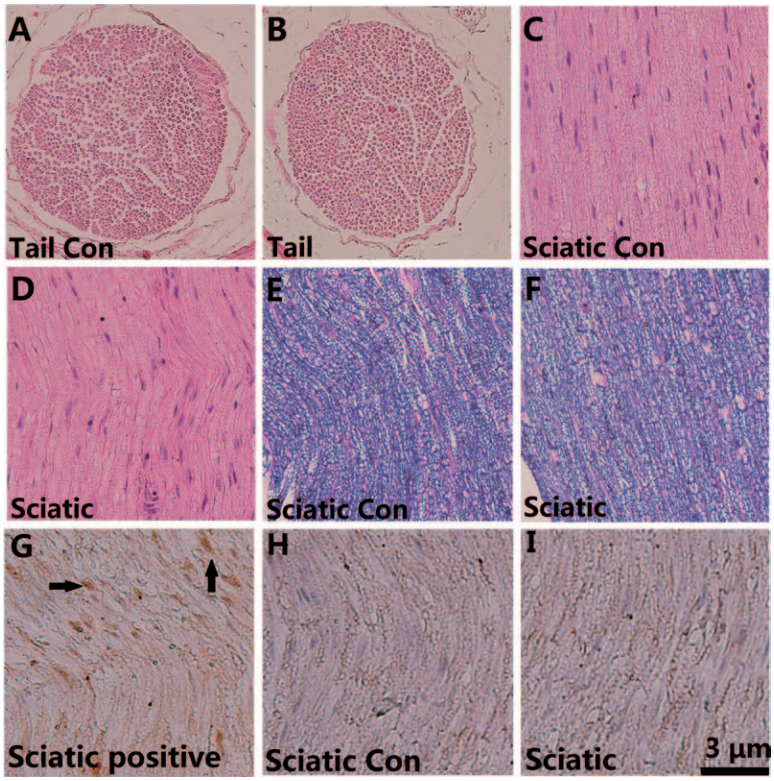

Fig. 4. Histological evaluation of tail and sciatic nerves after nerve block. (*A*) Normal tail nerves were evaluated by hematoxylin–eosin (HE) staining. (*B*) Tail nerves of rats from 1% QX-314 + 4%EI + 42°C group were stained by HE staining. (*C*) Normal sciatic nerves of rats were stained by HE staining. (*D*) Sciatic nerves of rats from 1% QX-314 + 2% EI + 44°C group were stained by HE staining. (*E*) Normal sciatic nerves were stained by solochrome cyanine staining. (*F*) Sciatic nerves of rats from 1% QX-314 + 2% EI + 44°C group were stained by solochrome cyanine staining. (*G*) Positive expression of CD-68 (macrosialin) (*arrows*) in sciatic nerves was shown by immunohistochemistry. (*H*) Expression of CD-68 in normal sciatic nerves was shown by immunohistochemistry. (*I*) Expression of CD-68 in sciatic nerves from 1% QX-314 + 2%EI + 44°C group was shown by immunohistochemistry. EI = emulsified isoflurane.

(fig. 4, A–D), demyelination (fig. 4, E–F), or increased expression of CD-68 (fig. 4, G–I) were found in study nerves.

EI Facilitated Thermal TRPV1 Channel Activation and Enhanced Thermal TRPV1 Channel Activation–mediated Inhibition of Sodium Channel Currents

Transient receptor potential vanilloid-1 channel currents were evoked above 42°C (fig. 5A) and completely inhibited by 5 μM capsazepine. With the addition of EI from 0.1 and 1.5 mM, the temperature thresholds for inducing TRPV1 channel currents were significantly decreased in a concentration-dependent manner (fig. 5B). Maximal enhancement was found when EI greater than 0.9 mM and no enhancement was found when EI less than 0.3 mM.

Sodium channel currents were not significantly inhibited by 5 mM QX-314 (recorded for 5 to 10 min after perfusion of study drugs), with inhibition of 18 ± 5% (fig. 6, A and B). When temperature of extracellular solution increased to 40°C, 5 mM QX-314 significantly inhibited sodium channel currents (fig. 6C). With the addition of 0.6 mM EI, sodium channel currents were completely inhibited by 5 mM QX-314 at 36°C. This heat-mediated inhibition of sodium channel currents by QX-314 + 0.6 mM EI could be prevented by 5 μM capsazepine. With Arrhenius plot,[34] thermal TRPV1 channel activation–mediated inhibition of sodium channel currents could be illustrated into two thermal response phases (fig. 6D).

Discussion

Long-lasting sensory/nociceptive-selective blockade is useful in clinical settings. This study confirmed our hypothesis that EI facilitated nonnoxious heat to activate TRPV1 channels and deliver QX-314 into nociceptor, producing a rapid onset and long-lasting sensory/nociceptive-selective blockade. In the current study, TRPV1 channel antagonist (capsazepine) prevented the enhancement of heat exposure and EI on sensory/nociceptive blockade of QX-314. Therefore, similar to capsaicin[16] and acid solution,[17] sensory/nociceptive blockade of QX-314 was enhanced through activation of TRPV1 channels.

Regional injection of capsaicin or acid solution might induce injuries[13,22,23] and temperature greater than 45°C could produce microscopic changes in skin, whereas temperature less than 45°C is safe even with prolonged exposure.[35]

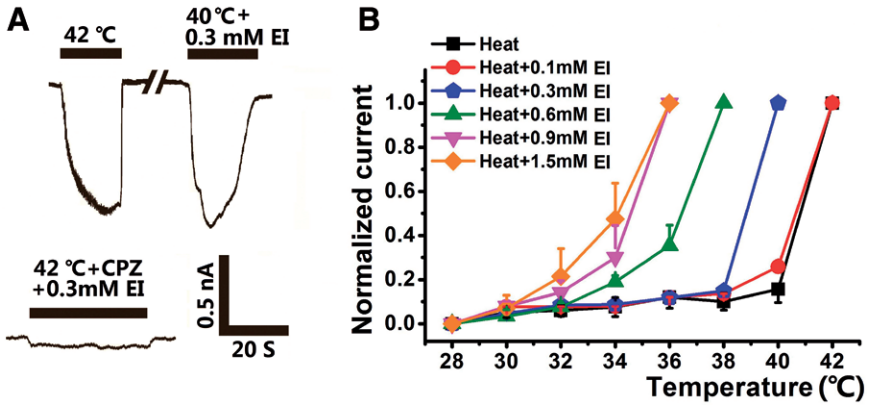

Fig. 5. Emulsified isoflurane (EI) could reduce temperature thresholds for inducing transient receptor potential vanilloid-1 (TRPV1) channel currents in dorsal root ganglia neurons. (*A*) TRPV1 channel currents were evoked at 42°C. With the addition of 0.3 mM EI, similar TRPV1 channel currents were evoked at 40°C. All the TRPV1 channel currents were inhibited by capsazepine (CPZ). (*B*) EI enhanced heat-evoked TRPV1 channel currents in a concentration-dependent manner.

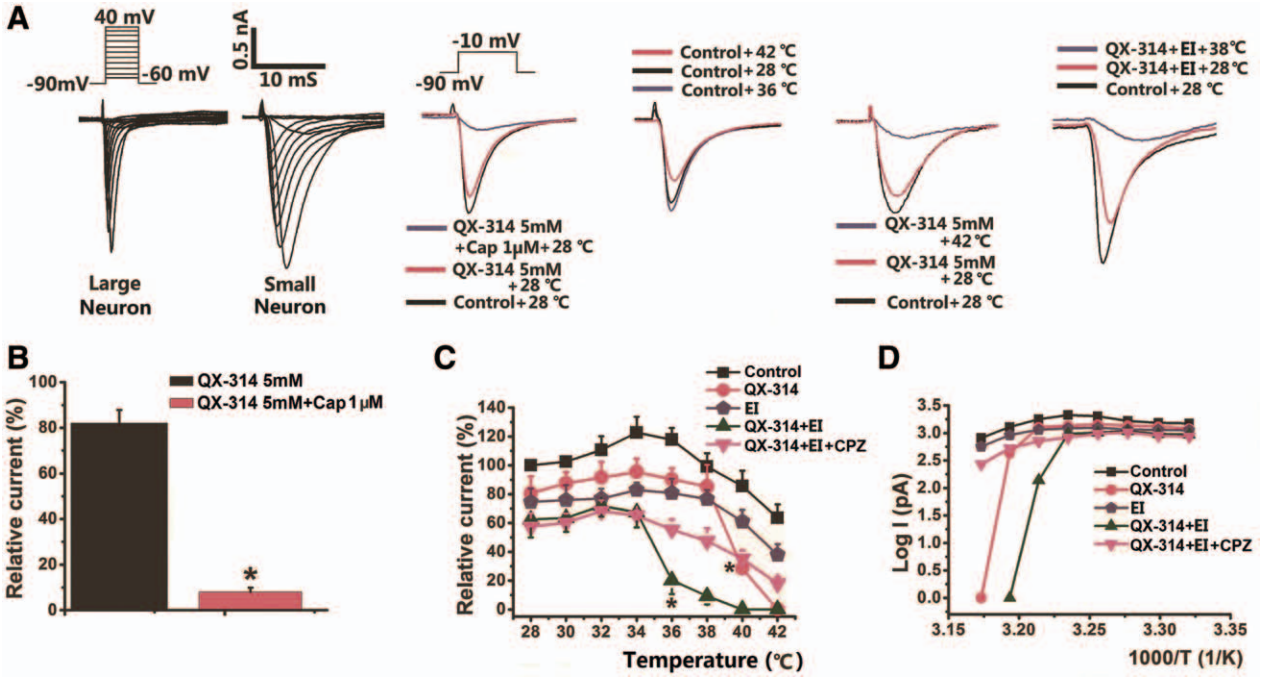

Fig. 6. QX-314 could inhibit sodium channel currents by thermal activation of transient receptor potential vanilloid-1 channels in dorsal root ganglia neurons. Sodium channel currents were recorded for 5–10 min after perfusion of study drugs. (*A* and *B*) Sodium channel currents were recorded on small dorsal root ganglia neurons. QX-314 at 5 mM significantly inhibited sodium channel currents with addition of capsaicin. (*C*) Sodium channel currents were significantly inhibited by 5 mM QX-314 when temperature of extracellular solution increased to 40°C. With addition of 0.6 mM emulsified isoflurane (EI), sodium channel currents were inhibited by QX-314 at lower temperatures. (*D*) The relation between temperatures and sodium channel currents was calculated by Arrhenius plot, by which heat-mediated inhibition of sodium channel currents illustrated two thermal response phases. EI at 0.6 mM could decrease the temperature threshold. CPZ = capsazepine.

In this study, we found that heat exposure at noxious 48° and/or 46°C significantly enhanced sensory/nociceptive blockade of QX-314, whereas heat exposure at 42°C did not enhance sensory/nociceptive blockade of QX-314. Thus, finding a way to reduce temperature thresholds for TRPV1 channel activation will be of clinical significance.

Volatile anesthetics including isoflurane have been found to decrease thermal thresholds of TRPV1 channel activation.[24,25] EI is a novel injectable anesthetic, which has been shown to be safe for producing regional anesthesia in various animal models.[26–29] In the current study, EI was found to facilitate nonnoxious thermal enhancement on sensory/nociceptive blockade of QX-314. No aversive response was found when rats received water bath at 40°C. However, no satisfied sensory/nociceptive blockade was found with 4% EI and 1% QX-314 at 40°C. Interestingly, QX-314 +

4% EI and QX-314 + 2% EI at nonnoxious 42°C produced similar sensory/nociceptive blockade as 1% QX-314 + 46°C without EI. Therefore, by adding EI to QX-314, the temperature thresholds for inducing long-lasting sensory/nociceptive blockade could be decreased under 45°C. Compared with QX-314 alone or lidocaine alone, QX-314 + EI plus nonnoxious temperature produced a faster onset, longer and more nociceptive-selective blockade. This finding may be clinically useful: First, 42° or 44°C can be easily applied without any hurt to patients; Second, long-lasting nociceptive-selective blockade is useful for the management of postoperative pain, chronic pain, and cancerous pain.

On the basis of the results of current study, EI enhanced sensory/nociceptive blockade of QX-314 by thermal TRPV1 channel activation. This is further supported by electrophysiology recording. With the addition of EI from 0.1 to 1.5 mM, the temperature thresholds for inducing TRPV1 channel currents were significantly decreased in a concentration-dependent manner. Maximal effect was found when EI greater than 0.9 mM and no enhancement was found when EI less than 0.3 mM. Compared with 0.1 mM EI, 0.9 mM EI reduced the temperature threshold by approximately 6°C. After adding 0.6 mM EI, sodium channel currents were inhibited when temperature higher than 36°C. When 4% EI added to 1% QX-314 *in vivo*, the temperature threshold for inducing long-lasting nociceptive-selective blockade was decreased by approximately 4°C. These data indicate that the effect of EI on thermal thresholds of TRPV1 channel activation is similar between animal study and electrophysiological recording. Compared with regional anesthetic effect of EI in previous studies, 0.9 mM EI (maximal effective concentration for reducing temperature thresholds of TRPV1 channel activation in current study) is similar to its IC_{50} (median inhibitory concentration) on sodium channel currents in DRG neurons[28] and 4% EI in animal study is also similar to EC_{50} (median effective concentration) of EI in regional anesthesia *in vivo*.[26–29]

The enhancement of EI was less effective in sciatic nerve block compared with that in tail nerve block. In sciatic nerve block, for low boiling point of isoflurane, exact concentration of EI should be lower than 2% because heat exposure was applied in incised tissue near sciatic nerve. In addition, sciatic nerve is bigger in diameter than tail nerve, with different types of nervous fibers.

There are still some limitations for the current study. First, an incision was made at popliteal fossa of the rats to expose sciatic nerve because it was difficult to warm sciatic nerve by water bath. The incision might affect behavioral evaluation. Second, higher concentrations of capsaicin might produce longer tail-clamping blockade by QX-314; however, we did not test the effect of capsaicin at higher concentrations because serious pungency or even nervous toxicity could be induced. Thus, it is difficult to quantitatively compare enhancement of heat exposure and capsaicin. Third, to further assure safety of heat exposure, QX-314, and EI on nerves, delayed outcomes such as neuropathic pain[23] are needed to be observed.

In summary, we found that thermal activation of TRPV1 channels enhanced long-lasting sensory/nociceptive blockade by QX-314 without affecting motor blockade. The addition of EI reduced temperature thresholds for inducing long-lasting sensory/nociceptive blockade due to QX-314.

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Competing Interests

The authors declare no competing interests.

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