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Addition of Dexmedetomidine to QX-314 Enhances the Onset and Duration of Sciatic Nerve Block in Rats

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Abstract

An experimental set-up was designed to observe whether adding dexmedetomidine to QX-314 would enhance the onset and duration of sensory and motor function in a rat sciatic nerve block model. Fifty-six Sprague-Dawley rats received unilateral sciatic nerve blocks with 0.2 ml of 35 mM QX-314 alone, dexmedetomidine (5.3 μM [$1 \mu\text{g}\cdot\text{kg}^{-1}$], 26.4 μM [$5 \mu\text{g}\cdot\text{kg}^{-1}$], 52.8 μM [$10 \mu\text{g}\cdot\text{kg}^{-1}$]) alone, or a combination of the two. Thermal nociception and motor function were assessed by a blinded investigator, sciatic nerves and perineural tissues were harvested at 14 days after injection. In addition, we examined the effects of these solutions on compound action potentials in isolated frog sciatic nerves. Dexmedetomidine added to QX-314 enhanced the onset and duration of thermal nociception block and motor block ($P < 0.05$) without aggravating histopathological injuries. Furthermore, 52.8 μM dexmedetomidine added to 35 mM QX-314 showed less inflammation than QX-314 alone at 14 days ($P = 0.003$). Dexmedetomidine plus QX-314 was shown to dose-dependently reduce the compound action potentials relative to QX-314 alone ($P < 0.05$). It was concluded that coadministration of QX-314 with a clinical-dose of dexmedetomidine produced a synergistic anesthetic effect to enhance the effect of sciatic nerve block.

Keywords: dexmedetomidine, QX-314, nerve block, drug combination

Introduction

The conventional long-acting local anesthetics such as ropivacaine, produce short-term analgesia for postoperative pain relief, particularly in the early stage. There are only a few drugs that can provide long-lasting analgesia. For example, Exparel (Pacira Pharmaceuticals, Inc., California, USA), a liposomal bupivacaine, can produce postoperative analgesia for up to three days as reported. However, intravenous opioid drugs are needed in conjunction, bringing about many side effects. Furthermore, it is generally known that QX-314, a quaternary lidocaine derivative, provides long-lasting anesthesia with slow onset and low efficacy owing to its positive charge (Kosugi et al. 2010; Lim et al. 2007). It has been shown that QX-314 can be administered together with capsaicin or acid solution to produce a nociceptor selective, long-lasting rat sciatic nerve block (Binshtok et al. 2007; Liu et al. 2011). However, local injection of these additives might cause severe neurotoxicity. There is therefore an increased interest to find new or adjuvant drugs that will provide long-lasting analgesia with minimal side effects (Caterina et al. 1997; Rukwied et al. 2007).

Dexmedetomidine, a potent α_2 -adrenoceptor agonist, has been shown to increase the analgesic effects of local anesthetics (Esmaoglu et al. 2010; Gupta et al. 2011). Animal studies demonstrated that dexmedetomidine prolongs the duration of sensory and motor blocks when added to local anesthetics without aggravating the nerve and muscle injuries (Ali Erdogan et al. 2013; Brummett et al. 2008; Brummett et al. 2009). The efficacy of dexmedetomidine plus local anesthetics for peripheral nerve blocks or intrathecal anesthesia in human studies has also been established (Safari et al. 2016). We previously observed that a combination of dexmedetomidine and QX-314 enhanced the duration of nerve blockade in rats (data not presented).

Taken together, we hypothesized that adding dexmedetomidine to QX-314 would enhance the effectiveness of QX-314-mediated sciatic nerve block while not aggravating the tissue toxicity. Further, we were also interested in investigating whether dexmedetomidine alone or added to QX-314 can affect action potential conduction *in vitro*. Hence we recorded compound action potentials (CAPs) from isolated frog sciatic nerves.

Methods

Animals

The study was approved by the Institutional Animal Experimental Ethics Committee of Sichuan University with the approval number: 2015014A. All the animals were purchased from the Experimental Animal Center of Sichuan province, adult male Sprague-Dawley (SD) rats and frogs (*Rana catesbiana* of either sex). The handling and experimental procedures were consistent with the Guide for the Care and Use of Laboratory Animals (Vol. 1, 2nd ed., 1993, from the Canadian Council on Animal Care).

Drug preparation

Commercially available dexmedetomidine hydrochloride (Jiangsu Hengrui Medicine Co. Ltd., Jiangsu, China) was made up to the concentrations of 5.3 μ M, 26.4 μ M, 52.8 μ M with 0.9% normal saline, and with or without 35 mM QX-314 (Sigma-Aldrich Co. Ltd., Shanghai, China). This concentration of QX-314 was based on a preliminary study (Supplemental material, Table 1), 35 mM was the minimal effective concentration to block sciatic nerve completely (data not shown). According

to the average weight (250 g) of rats, the doses of dexmedetomidine were approximately equal to 1 $\mu\text{g}\cdot\text{kg}^{-1}$, 5 $\mu\text{g}\cdot\text{kg}^{-1}$ and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ and were the clinically common doses (Brummett et al. 2009). The pH of these solutions, ranging between 6.2 and 7.2, was assumed to buffer quickly around the tissue.

Experiment 1: Sciatic nerve block in rats

Groups

The rats were housed on a 7:00 AM to 7:00 PM light-dark cycle and had unlimited access to food and water. All rats used in the experiment were used to handling. They had a minimum of seven days to adapt to the neurobehavioral facility before commencing the experiment. Fifty-six rats ($n = 8\cdot\text{group}^{-1}$) were divided into seven groups and treated with QX-314 (35 mM) alone or dexmedetomidine (5.3 μM , 26.4 μM , 52.8 μM) with or without QX-314 (35 mM). At the time of administration, the rats weighed approximately 240 to 262 g. The experimental design was that of a double-blinded procedure.

Sciatic nerve block model

Rats were briefly anesthetized by inhalation 2-3% isoflurane in oxygen, and the midpoint of landmarks (greater trochanter and ischial tuberosity) in the left hind limb were localized. A 26-gauge injection syringe was introduced to the ischiatic notch, then 0.2 mL of the test drug was injected once in contact with the bone (Gerner et al., 2008; Thalhammer et al. 1995). The right limb was always kept as a blank control to which no drugs were administered.

Neurobehavioral assessment

The assessment was performed at the following intervals after injection: 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h until both the sensory and motor values were up to the baselines.

Thermal nociception was investigated by using a modified hot plate test (Shankarappa et al. 2012). The fixed anatomic area of hind paws were exposed to a 56°C hot plate (model RB-200 hotplate analgesia metre; Chengdu Technology & market Cos. Ltd., Chengdu, China) in sequence (left then right). The time of latency until paw withdrawal (PWL) was recorded by a stopwatch. The investigator could remove the paw to avoid empyrosis or hyperalgesia if the $PWL > 12$ s (the cut-off value). $PWL > 6$ s were considered successful nerve block. The onset time of thermal nociception block was investigated as the $PWL > 6$ s from baseline. The duration of effective nociception blockade was calculated as the time greater than or equal to a value of 6 s.

Motor function was estimated by measuring the postural extensor thrust (PET) of the left hind limb (Shankarappa et al. 2012). The rats were suspended over a balance and the maximum force that resisted contact of a digital platform (model HZT-B5000; Huazhi scientific instrument Co. Ltd., Fujian, China) by the heel was measured. The following score standards were used: 0, no block, $PET = \text{baseline}$; 1, minimal block, $50\% \leq PET < 100\%$ of baseline; 2, moderate block, $20 \text{ g} \leq PET < 50\%$ of baseline; 3, complete block, $PET < 20 \text{ g}$. A score higher than 2 was considered a successful nerve block. The duration of effective motor function blockade was calculated as the time greater than or equal to score 2.

The neurobehavioral evaluation was repeated three times at each point and calculated as the mean value to increase accuracy.

Experiment 2: Tissue harvesting and histology

To evaluate the pathology of the tissue, rats from Experiment 1 were euthanatized with 2 ml propofol at 14 days after drug injection. The sciatic nerves and adjacent tissues (2 cm long at the site of injection) were harvested and stained with haematoxylin-eosin following standard techniques (Padera et al. 2008). Briefly, samples were fixed in 10% neutral formalin for one day and transferred to an ethanol solution for dehydration. They were subsequently embedded in paraffin, cut by length (4 μ m) and stained with haematoxylin and eosin. The histopathological examinations were analyzed by a pathologist, blinded to drug treatment. The scoring standard has previously been described for inflammation (0–4) and myotoxicity (0–6) (Padera et al. 2006). The former is decided by the degree of inflammatory corpuscle. The scores assessing the degree of muscle injury are: 0, normal tissue; 1, perifascicular internalisation; 2, deep internalisation (over five cell layers); 3, perifascicular regeneration; 4, deep regeneration; 5, hemifascicular regeneration; and 6, holofascicular regeneration.

Experiment 3: Recording the CAPs from isolated frog sciatic nerves

Frogs (40-65 g) were pithed and dissected. Thirty-six sciatic nerves (4-5 cm long) were obtained from the lumbar plexus to the knee and then put into a Ringer's solution. Ringer's solution was made dissolving 6.50 ± 0.02 g NaCl, 0.14 ± 0.01 g KCl, 0.12 ± 0.01 g CaCl_2 , 0.20 ± 0.01 g NaHCO_3 and 0.01 g NaH_2PO_4 in one litre of distilled water (pH 7.0 ± 0.1). This solution was stirred at 300 rpm with a magnetic stirrer (MS-H280-Pro; SCILOGEX LLC, Berlin, CT 06037 USA) to keep the homogeneity of the Ringer's solution around the isolated sciatic nerves. They were subsequently randomly divided into six groups for further treatment: 35 mM QX-314

alone, 26.4 μ M and 52.8 μ M dexmedetomidine alone, or 5.3 μ M, 26.4 μ M and 52.8 μ M dexmedetomidine with 35 mM QX-314. All the drugs dissolved in Ringer's solution instead of 0.9% normal saline. The pH of the test solutions was adjusted to 7.0 ± 0.1 with HCl or NaOH (Katsuki et al. 2006).

Before the start of the experiment, when the frog sciatic nerves were soaked in the Ringer's solution for 20 min (Kosugi et al. 2010), the CAPs were recorded every five minutes to set the baseline values. Action potentials from the isolated frog sciatic nerves were recorded by the BL-420F biological signal acquisition and analysis system (Techman Software Co. LTD, Chengdu, China), using the air-gap method². The parameter settings were: frequency, 100 Hz; duration of rectangular pulses, 0.1 ms; voltage, 1 V. Measurements were recorded quickly (15 s at the most) after being soaked into the test solutions for the following intervals: 5 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min. Each frog sciatic nerve fiber was only used once to evaluate the effect of a test drug on CAPs. All the measurements were collected at room temperature (24-27 °C, regulated by an air-conditioner).

Statistical Analysis

The data were analyzed using SPSS 22.0 (IBM SPSS Inc., Chicago, USA). The neurobehavioral data and histopathologic scores are presented as medians with interquartile ranges because they are not normally distributed (the assumption of a normal distribution was confirmed using the Shapiro-Wilk test). To assess the statistical significance of neurobehavioral examinations between QX-314 and QX-314 plus 5.3 μ M dexmedetomidine at each time point, nonparametric analysis was used. The Mann–Whitney U test with Bonferroni's correction ($\alpha = 0.05/6$) was used for multiple comparisons of duration of effective block, and Bonferroni's correction (α

= 0.05/10) was completed for multiple comparisons of histopathologic examinations. For Experiment 3, data of CAPs are shown as mean $\pm$ standard error of mean (SEM). A repeated-measures ANOVA evaluated the effects of relative CAP amplitude between groups. The statistical significance was established at $P < 0.05$.

Results

Experiment 1: The effect of sciatic nerve block in rats

Rats were tested for both thermal nociception and motor function blockades by using the hotplate and postural extensor test, respectively. The onset time of thermal nociception block was approximately 2 h for 35 mM QX-314, but 30 min when QX-314 was administered together with three doses of dexmedetomidine (Fig. 1A). Addition of dexmedetomidine to 35 mM QX-314 showed similar effects on motor function block (Fig. 1B). Thermal nociception and motor function measured at individual time points from 15 min to 10 h after injection showed significant differences when comparing 5.3 μ M dexmedetomidine added to 35 mM QX-314 and 35 mM QX-314 alone (Fig. 1, C-D, $P < 0.05$). But the effects of sciatic nerve block (both sensory and motor function) were not statistically significant at anytime point between the three concentrations of dexmedetomidine/QX-314 ($P > 0.05$).

Duration of effective thermal nociception and motor function blockades were prolonged when 35 mM QX-314 was coadministered with dexmedetomidine (5.3 μ M, 26.4 μ M, 52.8 μ M), compared with QX-314 alone (Fig. 2, $P < 0.0083$). Similarly, there were no statistically significant differences between the three doses of dexmedetomidine/QX-314 ($P > 0.0083$).

In addition, concentrations of 5.3 μ M, 26.4 μ M and 52.8 μ M dexmedetomidine

exhibited no effective sciatic nerve block (Fig. 3, A, C, E). The right limbs (negative controls, without drug administered) of the three groups of QX-314 plus dexmedetomidine did not produce analgesia or motor block (Fig. 3, B, D, F). For all the rats, the time to resumption of righting reflex was less than 15 min. Therefore, there was little systemic analgesic effect of dexmedetomidine.

Experiment 2: Tissue Harvesting and Histology

In all cases, tissues had a benign appearance, with mild or moderate inflammation at 14 days. Rats injected with one of the three concentrations of dexmedetomidine alone showed no or little inflammation. Compared with the positive control (samples of 23 mM bupivacaine; obtained from preliminary study), microscopic examination of the tissues injected with 35 mM QX-314 revealed similar inflammatory reaction ($P > 0.005$). Dexmedetomidine (52.8 μM) administered alongside 35 mM QX-314 alleviated the inflammation relative to 35 mM QX-314 alone (Fig. 4A, $P = 0.003 < 0.005$). All sections showed no apparent injury of muscle (Fig. 4B).

Experiment 3: The CAPs of isolated frog sciatic nerve

As shown in the Figure 5A, soaking the frog sciatic nerve fibers in Ringer's solution containing dexmedetomidine (26.4 μM or 52.8 μM) did not change the peak amplitudes of CAPs, and the relative CAPs amplitudes showed no significant differences between the two solutions during soaking for 60 min.

Adding dexmedetomidine (5.3 μM , 26.4 μM or 52.8 μM) to 35 mM QX-314 inhibited the peak amplitudes of CAPs (Fig. 5, B-C). There were statistically significant differences between all four treatment groups ($P < 0.05$).

Discussions

In this study, we demonstrated that 35 mM QX-314 alone produced short-acting effective thermal nociception block. We furthermore demonstrated that the addition of dexmedetomidine to QX-314 greatly enhanced the effect on nerve blockade of both thermal nociception and motor function in a rat sciatic nerve blockade model, and attenuated the inflammatory response when 52.8 μ M dexmedetomidine was added. Dexmedetomidine is therefore thought to be a good additive to QX-314 to produce long-lasting local anesthesia in the future. Our data also showed that the administration of dexmedetomidine in combination with QX-314 inhibited the CAPs of frog sciatic nerve fibers dose-dependently *in vitro*. This suggests that dexmedetomidine can affect the conduction of action potentials at the local nerve level.

Dexmedetomidine was first proposed as an adjuvant to lidocaine for intravenous regional anesthesia to improve the quality of perioperative analgesia without causing sideeffects by Memis (Memis et al. 2004). In addition, a combination of high-concentration dexmedetomidine and bupivacaine has been shown to significantly increase the duration of bupivacaine-induced antinociception in rat sciatic nerve blockade without neurotoxicity (Brummett et al. 2009). Indeed, previous research and clinical studies have demonstrated the efficacy when dexmedetomidine was added to frequently-used local anesthetics. For example, a single-centre, prospective, randomized, three-blind and controlled trial (Fritsch et al. 2014), showed that the addition of dexmedetomidine to ropivacaine for interscalene blocks increased the duration of nerve block and improved postoperative pain without side effects or neurological complication (reference needed). Mahendru (Mahendru et al. 2013) concluded that intrathecal dexmedetomidine can prolong both the motor and sensory

block as well as maintain the hemodynamic stability when compared to clonidine.

QX-314, a quaternary lidocaine derivative, has recently gained interest because of its potential application in prolonged or sensory-selective regional anesthesia (Binshtok et al. 2007). In our study, 35 mM QX-314 only produced about two hours of effective thermal nociception block with mild inflammation and muscle injury, as reported previously (Shankarappa et al. 2012). Shankarappa demonstrated that long-acting anesthesia is achieved only with a high concentration of QX-314 (70 mM), resulting in associated severe tissue toxicity making it thereby inappropriate for clinical use. Since then efforts have been made to minimize its neurotoxicity and capitalize on the long-lasting anesthetic properties of QX-314. Some researchers (Binshtok et al. 2007) reported how QX-314 can block sodium channels in the existence of capsaicin, a transient receptor potential cation channel subfamily V member 1 (TRPV1) agonist. Binshtok (Binshtok et al. 2009) used lidocaine instead of capsaicin to delivery QX-314 into nociceptors through TRPV1 channels. Sagie and Kohane (Sagie and Kohane 2010) demonstrated that certain doses of surfactants (sodium octyl sulfate, octyltrimethylammonium bromide, Tween 20) coadministered with QX-314 can produce long-acting and sensory-selective nerve block. Here we found that dexmedetomidine may have a similar effect.

There are many probable mechanisms of action for dexmedetomidine improving QX-314-mediated nerve block, such as a direct action in the peripheral nerve, centrally-mediated analgesia, vascular constriction by α_2 -adrenoceptor, or as a TRP channel opener. In the present study, rats displayed unilateral blocks with a blank control paw, standing for central analgesia, so the effects were predominately at the peripheral nerve level. Also, the isolated frog sciatic nerve experiment showed that low concentrations of dexmedetomidine (26.4 μ M and 52.8 μ M) did not affect the

CAPs. This is in agreement with Kosugi (Kosugi et al. 2010), showing that only high-concentration dexmedetomidine reduced the peak amplitude of CAPs ($IC_{50} = 400 \mu M$). However, our data showed a dose-dependent effect on the inhibition of the peak amplitude of CAPs when administering dexmedetomidine with QX-314. This observation can evidence the effect of dexmedetomidine on peripheral nerve block. Brummett (Brummett et al. 2011) demonstrated that the analgesic effect of dexmedetomidine can be reversed by pretreating with an hyperpolarization-activated current enhancer, not an α_2 -adrenoceptor antagonist. Considering QX-314's characteristics, the prolongation of nerve block may reflect a dexmedetomidine-induced entry of QX-314 into the peripheral nerve through pathways such as TRP channels (Binshtok et al. 2007). Regional injection of capsaicin, acid solution, local anesthetics, emulsified isoflurane or heat exposure can deliver QX-314 into sensory neurons by activating TRPV1 channels to provide a rapid onset and long-acting nociceptor-selective block (Binshtok et al. 2007; Liu et al. 2011; Romanovsky et al. 2009; Zhou et al. 2014; Brenneis et al. 2014). However, so far no research has suggested that dexmedetomidine could open a TRP channel. Therefore, further experiments are needed to clarify the exact mechanism underlying perineural administration of dexmedetomidine in combination with QX-314 for prolonged local anesthesia.

We found that perineural administration of $52.8 \mu M$ dexmedetomidine with $35 mM$ QX-314 alleviated the inflammation when compared to $35 mM$ QX-314 alone. Similarly, the inflammatory reaction of bupivacaine decreased when coadministered with $120.6 \mu M$ dexmedetomidine (Brummett et al. 2009). Further, recent research (Huang et al. 2014) demonstrated that $120.6 \mu M$ dexmedetomidine can have an anti-inflammatory effect by reducing inflammatory cytokines through inhibiting the

translocation of activated nuclear factor- κ B to the nucleus.

There were some limitations to this study. First, the sensory measure in our experiment was limited to the reaction to a heat stimulus. Other types of nociception like mechanical stimulation could be tested. Second, although the isolated frog sciatic nerve study could show the direct dose-dependent effect of dexmedetomidine plus QX-314 on peripheral nerve block, this is an *in vitro* study. It therefore cannot mimic a two-drug mechanisms of action *in vivo*, for a large fraction of drugs will be metabolized or not even reach the nerve fibres in rodent studies. Further studies will be required to fully elucidate the exact mechanism of action of dexmedetomidine.

In summary, this animal study verified the hypothesis that dexmedetomidine can enhance the onset and duration of QX-314-induced anesthesia in rat sciatic nerve block, and inhibit the peak amplitudes of CAPs in frog sciatic nerves *in vitro*. Furthermore, adding 52.8 μ M dexmedetomidine to QX-314 can alleviate the perineural inflammatory response 14 days after drug administration. Accordingly, dexmedetomidine may be a good adjuvant to QX-314 for long-acting anesthesia.

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Figure captions

Fig. 1. Time courses of sciatic nerve block after injection of 35 mM QX-314 with or without three concentrations of dexmedetomidine in rats ($n = 8 \cdot \text{group}^{-1}$). (A) The PWLs were enhanced when treated with 35 mM QX-314 plus 5.3 μM , 26.4 μM and 52.8 μM dexmedetomidine. (B) 35 mM QX-314 could not effectively block the motor function of rats' sciatic nerves, but coadministration of QX-314 with dexmedetomidine resulted in effective motor blockade that lasted for 5-8 h. (C-D) From post-drug 15 min to 10 h, statistically significant differences were found at individual time points of both thermal nociception and motor function blockade between 5.3 μM dexmedetomidine/35 mM QX-314 combination and 35 mM QX-314 alone. Data are shown as median and 25th percentile. The dotted lines (PWL = 6 s; Motor score = 2) mean the boundaries between effective and ineffective blockades, above being effective blockades. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Abbreviations: PWL, paw withdrawal latency; DEXM, dexmedetomidine.

Fig. 2. Duration of effective block between 35 mM QX-314 with or without dexmedetomidine at different concentrations (5.3 μM , 26.4 μM and 52.8 μM , $n = 8 \text{ rats} \cdot \text{group}^{-1}$). Dexmedetomidine added to 35 mM QX-314 significantly prolonged the duration of effective thermal nociception blockade (A) and motor blockade (B) when compared with 35 mM QX-314 alone, but not in a dose-dependent fashion when comparing the groups of three concentrations of dexmedetomidine/QX-314 ($P > 0.0083$ and detailed P values noted in the figure). The centre line is the median, the lower and upper boundaries are the 25th and 75th percentiles, and the error bars are the minimum and maximum. # $P < 0.05/6$ (0.0083). Abbreviation: DEXM, dexmedetomidine.

Fig. 3. Thermal nociception blockade after injection of dexmedetomidine (5.3 μ M, 26.4 μ M and 52.8 μ M) alone or added to 35 mM QX-314 ($n = 8$ rats·group⁻¹). (A, C, E) Injection of three doses of dexmedetomidine alone did not produce effective thermal nociception blockade. (B, D, F) The analgesic effects of different concentrations of dexmedetomidine added to QX-314 were significantly greater in the operation paws than the control paws. Abbreviations: DEXM, dexmedetomidine; PWL, paw withdrawal latency.

Fig. 4. Results of haematoxylin-eosin staining at day 14 ($n = 8$ sections·group⁻¹). (A) In the inflammation score, there were no statistical differences between 23 mM bupivacaine (from the preliminary study as a positive control) and 35 mM QX-314 with or without dexmedetomidine (5.3 μ M and 26.4 μ M) ($P > 0.005$). But 52.8 μ M dexmedetomidine added to QX-314 showed less inflammatory response relative to QX-314 alone. (B) None of the samples showed obvious signs of degeneration or regeneration of muscle. * $P < 0.05/10$ (0.005).

Fig. 5. Recordings of compound action potentials (CAPs) from frog sciatic nerve fibres ($n = 6$ ·group⁻¹). (A) The relative peak amplitudes of CAPs from sciatic nerve fibres treated with dexmedetomidine at two concentrations for 60 min. Compared with baselines (the average values for 20 min before giving drugs), the relative CAPs amplitudes were not significantly changed by dexmedetomidine (26.4 μ M and 52.8 μ M). (B) Results of relative amplitudes of CAPs of QX-314 (35 mM) with or without dexmedetomidine (5.3 μ M, 26.4 μ M and 52.8 μ M). Statistical differences were found between both of four groups ($P < 0.05$). Typical CAPs of four groups are presented in

Figure 5C, showing post-drug 0 min (baselines, not being soaked with test solution), 15 min and 60 min, respectively. Data are shown as mean $\pm$ SEM.

Draft

Fig. 1. Time courses of sciatic nerve block after injection of 35 mM QX-314 with or without three concentrations of dexmedetomidine in rats ($n = 8$ •group-1).

81x57mm (600 x 600 DPI)

Fig. 2. Duration of effective block between 35 mM QX-314 with or without dexmedetomidine at different concentrations (5.3 μ M, 26.4 μ M and 52.8 μ M, n = 8 rats•group-1).

95x163mm (300 x 300 DPI)

Fig. 3. Thermal nociception blockade after injection of dexmedetomidine (5.3 μ M, 26.4 μ M and 52.8 μ M) alone or added to 35 mM QX-314 ($n = 8$ rats•group-1).

120x126mm (600 x 600 DPI)

Fig. 4. Results of haematoxylin-eosin staining at day 14 (n = 8 sections•group-1).

110x201mm (600 x 600 DPI)

Fig. 5. Recordings of compound action potentials (CAPs) from frog sciatic nerve fibres (n = 6•group-1).

152x138mm (300 x 300 DPI)

Supplemental material -- preliminary experiment

TABLE 1. Successful sciatic nerve blocks of different agents in preliminary experiment.

Agent	Concentration	n	Number of successful blocks	
	mmol/L		Sensory	Motor
Saline	/	8	0	0
Bupivaciane	23	8	8	8
QX-314	25	8	4	1
	35	8	8	8
	45	8	8	8

Each rat received a 0.2 mL solution injection on left leg. Sensory block was determined in the same anatomic area using a hotplate at a temperature of 56°C, successful sensory block was defined as the first time point when thermal latency was greater than 6 s. Motor block was measured as grams exerted in hind paw push-off on an upright balance using a digital platform balance, successful motor block was defined as the value was lower than 50% of baseline. At day 14, we harvested the adjacent nerve and muscle tissues of eight rats in group bupivacaine for haematoxylin-eosin staining to test the tissue reaction.