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ORIGINAL ARTICLE

Application of quinidine on rat sciatic nerve decreases the amplitude and increases the latency of evoked responses

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Abstract
Purpose Multi-modality electrophysiological techniques were performed to assess the effects of quinidine on peripheral nerve conduction.
Methods Twenty-seven rats were treated with 1, 3, and 5 μmol quinidine in 0.1 ml 5 % glucose. The mixed-nerve somato-sensory evoked potential (M-SSEP), dermatomal-SSEP (D-SSEP), and compound muscle action potentials (CMAP) were evoked and recorded. After positioning Gelfoam strips saturated with quinidine and 5 % glucose around the left and right sciatic nerves, potentials were measured at baseline, immediately after treatment, every 15 min for the 1st hour, and every 30 min for the next 3 h. After 2 weeks, the walking behaviors and potentials were again analyzed and myelinated fibers in the sciatic nerve were counted.
Results Quinidine applied directly to sciatic nerves reduced the amplitude and prolonged the latency in SSEPs and CMAP, compared to baseline and the contralateral right limbs (controls). This persisted for at least 4 h. After 2 weeks, electrophysiological tests and walking behavior showed no significant difference between the controls and experimental limbs. There was also no difference in the number of myelinated fibers in the sciatic nerves.

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Conclusions Quinidine decreases amplitude and prolongs latency in the sciatic nerve in a dose-related manner without local neural toxicity.

Keywords Quinidine · Sciatic nerve · Somato-sensory evoked potential · Compound muscle action potentials

Introduction

Quinidine is categorized as a class IA antiarrhythmic drug because it slows the rapid upstroke of cardiac action potential by blocking the inward sodium current and by prolonged re-polarization through potassium channel blockade [1, 2]. Like lidocaine and other class I antiarrhythmic drugs, it acts on voltage-gated sodium channels (VGSCs) by inhibiting ionic current and by blocking conductive cell signal transmission [3, 4]. However, unlike other local anesthetics that bind preferentially during the inactive stage of sodium channels, quinidine binds in the open stage [5]. Thus, despite similarities between antiarrhythmic drugs such as quinidine and lidocaine, their local anesthetic effects in nerve blockade differ substantially.

Quinidine not only depresses myocardial contraction but also blocks the conductive velocity of the His bundle and Purkinje fibers [6–9]. At high concentrations, it produces dose-dependent reductions in conduction velocity in Purkinje fibers, papillary muscles, and ventricular muscles [10, 11]. However, studies on the electrophysiological effects of quinidine on cardiac conduction and contractile systems have not elucidated its effects on peripheral nerves. In a literature review, there was only one report on the local anesthetic properties of quinidine, and it showed that the subcutaneous infiltration of quinidine produces a dose-related analgesic effect [12].

This study tested the hypothesis that quinidine exerts local anesthetic effects on sensory and motor nerve conduction on exposed sciatic nerves through spinal somato-sensory evoked potential (SSEPs) and compound muscle action potentials (CMAP). The results show that quinidine produces a dose-related decrease in amplitude and increase in latency of mixed-nerve and dermatomal somato-sensory evoked potential (M-SSEP and D-SSEP), and CAMP. Further electrophysiology studies at 2 weeks show no difference between the experimental and control limbs (Fig. 1).

Materials and methods

Animal preparation

The 27 adult Wistar rats (24 treated, three naïve; weight, 200–320 g) were caged separately at the experimental animal center at 21° ± 0.5 °C with a 12/12 light/dark cycle and with free access to food and water. The Institutional Animal Care and Use Committee of Cheng Kung University, Tainan, Taiwan approved the study (IACUC Approval No. 96031). All of the animals were treated in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

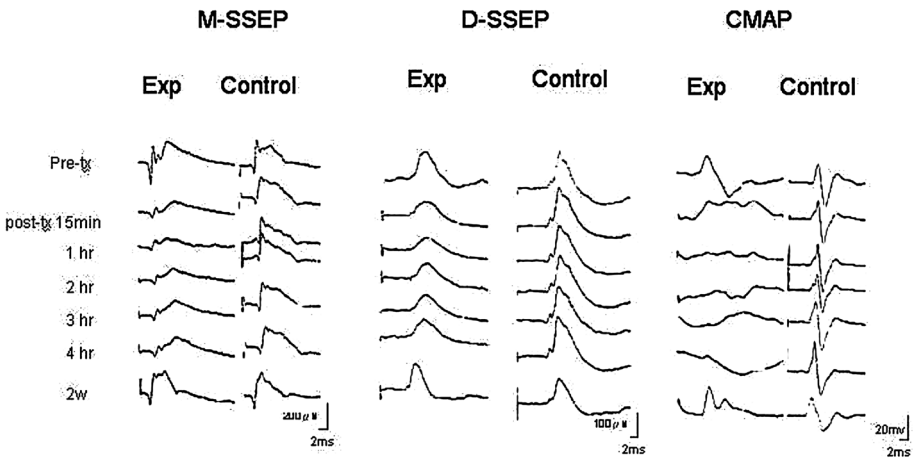

Fig. 1 Comparing the typical graphs of bilateral ascending M-SSEP and D-SSEP, descending CMAP, and the effects of quinidine 1 μmol (Q_1) during a 4-hour period. Characteristic waveforms for control (*right*) and experimental (*left*) groups show small downward waves and large upward waves in mixed-nerve-elicited spinal somato-sensory evoked potential (M-SSEP), dermatomal SSEP (D-SSEP). The CMAP produced a large and stable upward wave with consistent amplitude and latency. In group Q_1, quinidine (1 μmol in 0.1 ml) was placed on the sciatic nerve for 30 min and waveforms were recorded at 15 min, at 1, 2, 3, and 4 h, and at 2 weeks. *Pre-tx* pre-treatment; *Post-tx* post-treatment

Anesthesia

The animals were anesthetized with Citosol (thiamylal sodium, 50 mg/kg intra-peritoneal injection; Shin-Lin Ltd, Taoyuan, Taiwan). The anesthesia was maintained at a constant depth by applying appropriate doses of Citosol (10 mg/kg per dose) while observing the withdrawal reflex elicited by pinching the tail. The animals were pre-medicated with Gentamycin (8 mg/kg, intra-muscular; Yung Shin Pharmaceutical Industrial Co., Ltd., Taichung, Taiwan) via the right femoral vein. A 22-gauge catheter was cannulated into the ipsilateral femoral artery to provide continuous heart rate and blood pressure monitoring (CARDIOCAP, CM-104-28-01; Datex, Helsinki, Finland). A rectal thermometer (Portable Hybrid Recorder, model 3087; Yokogawa Hokushin Electric, Tokyo, Japan) was inserted in each rat to measure body temperature, which was maintained at approximately 37 °C with a warm water mattress and heating lamp.

Techniques for stimulating and recording evoked potentials

Surgical procedures were performed with the rat in a prone position with the hips extended and the head fixed in a stereotactic frame. The surgical procedures were performed under aseptic conditions and under 1.5× loupe magnification. After making a longitudinal incision in the back from the mid-thoracic to the upper lumbar spine, the bilateral para-vertebral muscles were retracted to reveal the thoraco-lumbar inter-spinous ligament. Before setting up the electrophysiological monitoring system, radiographs were taken with a metal marker to confirm the correct recording position. Two different electrophysiological surveillance systems were then used to record three kinds of evoked potential.

Ascending evoked potentials elicited from bilateral lower extremities

Spinal somato-sensory evoked potentials (SSEPs) were recorded by bipolar needle electrodes. After placing the cathode in the thoraco-lumbar inter-spinous ligament, the anode was placed 1 cm proximally to the cathode [13, 14]. Mixed-nerve-elicited SSEPs (M-SSEPs) were stimulated by electrodes placed under each sciatic nerve immediately proximal to the bifurcation in the peroneal branch with the cathode 3 mm proximal to the anode after dorsal incision in each thigh, with a 1.5-cm segment of each sciatic nerve exposed. Dermatomal SSEPs (D-SSEPs) were stimulated by a pair of subcutaneous needle electrodes with an inter-electrode distance of 2 mm in the lateral aspect of the forefoot, which corresponded to the L_5 dermatomal field. The duration of squared pulse impulses was 0.2 ms and the intensity was 5 times that of the visible potential threshold. Stimulations were applied 20 times at a rate of 5 impulses per second. The recording was filtered for data within a range of 10–5,000 Hz. The recording time was 20 ms.

Descending compound muscle action potential (CMAP)

The CMAP was recorded by monopolar myographic needle electrodes, placed in the lateral belly of the gastrocnemius muscle. All acquisition parameters were similar to those for SSEPs except the stimulation rate, which was decreased to 1/s, and the linear range of the filter, which was 1–2,000 Hz. A ground electrode was placed subcutaneously between the stimulus and the recording site. At least three sequential single-sweep runs (i.e., without averaging) with similar waveforms were recorded to confirm the consistency of the responses.

Direct quinidine applications on the sciatic nerve

The 24 rats were randomly divided into three groups with eight rats in each group. Groups Q_1, Q_3, and Q_5 received quinidine (quinidine gluconate salt, Sigma Chemical CO., St Louis, MO) 1, 3, and 5 μmol, respectively, in 5 % glucose 0.1 ml. The sciatic nerve was exposed by making an incision from the left sciatic notch to the distal thigh. The subcutaneous tissue was bluntly dissected to expose the biceps femoris. The sciatic nerve was freed from its investing fascia. The procedure was then repeated on the right side. Two small strips of Gelfoam (0.6×1.0 cm^2) (Pharmacia-Upjohn, Kalamazoo, MI) soaked with quinidine were placed under and over the left sciatic nerve for 30 min. The control data were obtained from the right limb, which was treated with Gelfoam soaked in 5 % glucose. The SSEPs and CMAP were recorded at baseline, immediately after quinidine treatment, then every 15 min thereafter for 1 h, then every 30 min thereafter for 3 h. The animals were allowed to recover and then kept separately for 2 weeks. After performing behavioral examinations, electrophysiological examinations were performed with the animals under intra-peritoneal anesthesia.

Morphological analysis and quantification of the sciatic nerve

Segments of the left sciatic nerve were examined by light microscopy in naïve rats ($n = 3$) and in Q_5 rats ($n = 3$) on POD_{2W}. A 2-cm segment of the sciatic nerve was measured following full exposure and sharply dissected. The excised segment was pinned on a silicone pad with the same dimension before fixation. The segment was then immersed in fixative solution (0.1 M phosphate buffered 2 %

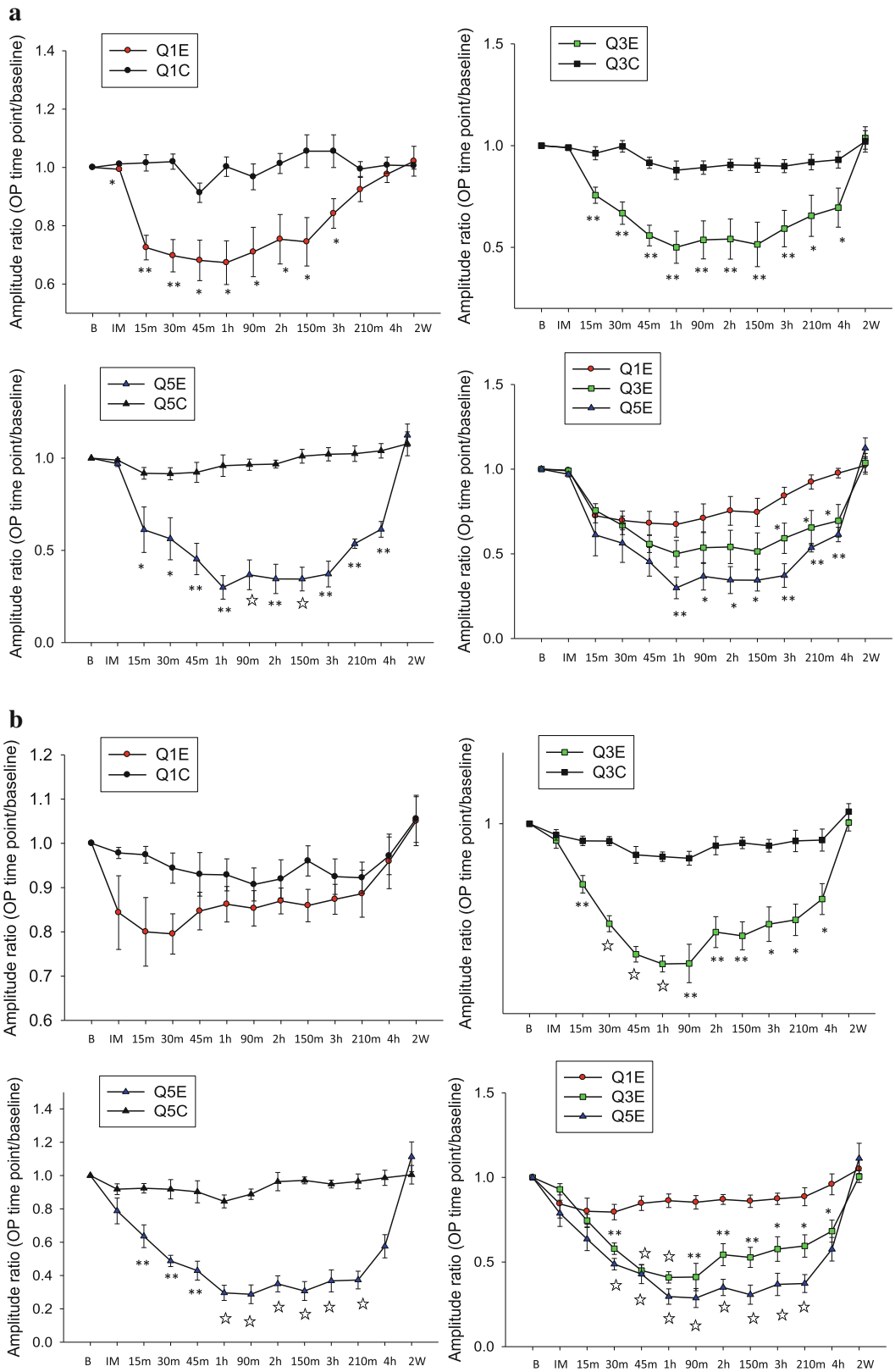

Fig. 2 Application of quinidine on the sciatic nerve reduced the amplitude ratio (each time point EP amplitude/baseline EP amplitude) of evoked potentials (EP). Statistical analysis (paired *t*-test) of (**a**, **b**) SSEPs and **c** CMAP revealed significant differences between the left [treated (QE)] and right [control (QC)]. Comparison at each time point after quinidine administration revealed dose-related reductions in amplitude ratio ($Q_1E < Q_3E < Q_5E$), by one-way ANOVA. *Error bars* represent SE.* $p < 0.05$; ** $p < 0.01$; $^{\star}p < 0.001$

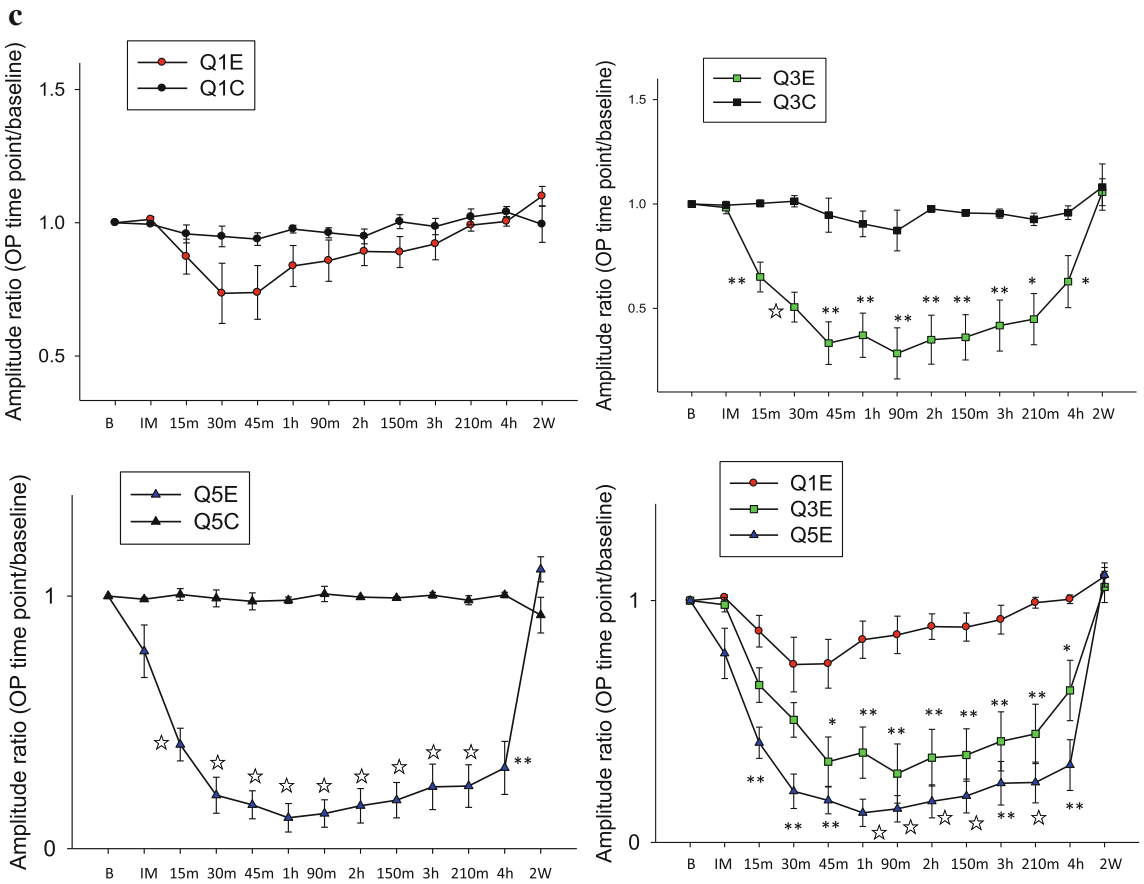

Fig. 2 continued

paraformaldehyde + 2.5 % glutaraldehyde, pH 7.4) for 15 min. The nerve segment was cut into 3 sections with a sharp razor. The middle portion of the 10-mm nerve segment was utilized for semi-thin section staining using toluidine blue. In the present study, the anatomical structures of biopsied nerves (myelinated nerve numbers in a restricted area, density of myelinated fibers: fibers in selected area/cross-sectional area) were calculated. The 10-mm-long sample of the left sciatic nerve segment was removed and then immersed in the same fixative solution overnight at 4 °C. The nerve segments were then post-fixed for 2 h in 1 % osmium tetroxide (Sigma, St. Louis, USA) in 0.1 M PBS. After rinsing twice with distilled water, the segments were immersed in 0.1 M PBS for 1.5 h. After dehydration with numerous quick alcohol passages (starting from 50 % × 2, 75 % × 2, 85 % × 2, 95 % × 2, to absolute ethanol × 2), the segments were left standing in the same ethanol concentrations for 10 min after each passage.

After dehydration, the segments were routinely processed for flat embedding in 100 % epoxy resin (Fluka; Switzerland) at 60 °C for 72 h. Segments were cut with a ultra-microtome (Reichert-Jung; Japan) to thicknesses of 1 μm in each transverse section, then stained with 0.5 % toluidine blue and examined by light microscopy (Olympus CKX41; Japan). Twelve sample units were collected in each group, 4 units of sampling in each rat. The animals underwent serial sectioning of the 10-mm sciatic nerve segment. A random sample was taken 0.1 mm from the segment edge. Using a method from Geuna et al. [15] to quantify the density and number of selected myelinated sciatic nerve fibers in a cross section, we identified tops of nerve fibers in the selected random areas. Briefly, 16 large sub-areas of equal size were delineated on the nerve cross-sectional profile. Each sub-area was then divided into 9 sub-fields, and one subfield per sub-area was randomly selected. The investigator then systemically jumped to sampling the same sub-fields in all of the other sub-areas.

To count the myelinated axons in the selected sub-field, the top objects that fell within the dissector sub-field were sampled. The number of axons in the selected square areas of similar size was counted at 400× magnification. The proximal and distal sections were not examined. The sections were analyzed and recorded using a TissueFAXS System microscope and TissuQuest software

(TissueGnostics, Vienna, Austria). The total selected number of myelinated fibers was measured using Image J software.

Data analysis

Amplitude was measured from the peak to the trough of the major wave within the evoked response. Latency of the response was measured from the onset of the electrical shock artifact to the initial positive peak. In all three groups, latency and amplitude were measured and calculated at each time-point. For each time point, amplitude was compared with that before quinidine treatment. Since a 5–10 % latency prolongation is considered suspect in neuro-monitoring practice [16], and given the difficulty in accurately calculating conduction velocity, latency was analyzed as the percentage of change from the pre-treatment baseline value to the post-treatment value.

To compare the variable parameters of vital signs, the amplitude and latency of all recordings, and the nerve functional indices during and after quinidine administration, a paired *t*-test or one-way analysis of variance (ANOVA) with repeated measures was used for intra- and inter-group comparisons. Statistical significance was set at $p < 0.05$. All data were expressed as mean ± SE.

Results

Hemodynamic responses to quinidine were stable during the experiment and hemodynamic changes were not associated with quinidine concentration. All of the experimental rats tolerated the surgery without post-operative wound infections or complications. At 2 weeks post-treatment, no rats had died and none exhibited hind-limb paralysis.

Electrophysiological findings

Quinidine reduced the amplitude of evoked potentials

Baseline amplitudes for the right and left limbs were 173.3 ± 66.0 and 165.3 ± 63.7 μV, respectively, for M-SSEP, 18.2 ± 8.2 and 19.3 ± 8.9 μV, respectively, for D-SSEP, and 2,774.7 ± 1,009.3 and 2,808.7 ± 1,029.6 μV, respectively, for CMAP. Amplitude did not significantly differ between the left and right sides at baseline. The ratios of amplitude (mean ± SE) at each time interval to baseline are shown in a graph (Fig. 2). Direct application of quinidine on the sciatic nerve produced a dose-related decrease in amplitude at ascending SSEPs and descending CMAP when comparing baseline with other time points, or when comparing the experimental left limb to the right contra-lateral glucose-treated limb.

There was a dose-related decrease in amplitudes of M-SSEP, D-SSEP, and CMAP after quinidine was directly applied to the sciatic nerve (Fig. 2a–c) ($Q_1 < Q_3 < Q_5$). In groups Q_3 and Q_5, the effect of quinidine on the sciatic nerve persisted for more than 4 h because the SSEPs and CMAP values did not return to pre-treatment baseline levels.

Quinidine delayed the latency of evoked potentials

Baseline latency values for the right and left limbs were 1.2 ± 0.2 and 1.3 ± 0.1 ms, respectively, of M-SSEP; 2.6 ± 0.2 and 2.6 ± 0.3 ms, respectively, of D-SSEP; and 4.6 ± 0.3 and 4.7 ± 0.3 ms, respectively, of CMAP. The latencies of SSEPs and CMAP potentials after quinidine applications were increased compared to baseline and the contralateral side (Fig. 3, QE and QC). Throughout the 4-h experiment, the latencies were consistent and did not significantly differ in the contralateral right limb. In groups Q_3 and Q_5, quinidine increased the latency of the sciatic nerve over nearly 4 h. Nonetheless, in both SSEPs and CMAP, the amplitudes and latencies measured at 2 weeks did not significantly differ from those at baseline (Figs. 2, 3).

Myelinated fiber counts in the sciatic nerve

Transverse sections (1 μm) of the left sciatic nerves were examined by light microscopy in naïve rats and in Q_5 rats 2 weeks after the administration of quinidine. The average cross-sectional area of the selected sciatic nerve segment was 0.67 ± 0.08 mm^2 in naïve rats, 0.67 ± 0.04 mm^2 in Q_5 rats on the experimental side, and 0.66 ± 0.06 mm^2 in Q_5 rats on the control side. Myelinated fibers in each selected sub-field were counted and summed up in each transverse section (Fig. 4a, b). The average number of myelinated fibers in the 12 selected sections was 621.2 ± 84.5 in naïve rats (Fig. 4a), 593.7 ± 66.3 in Q_5 rats on the experimental side (Fig. 4b), and 635.8 ± 63.5 in Q_5 rats on the control side, with no significant difference among the groups ($p = 0.372$). There was also no significant difference in density of myelinated fibers (fibers in selected area/cross section area) between naïve and experimental Q_5 survival animals ($p = 0.236$) (Fig. 4c).

Discussion

Direct application of quinidine on the sciatic nerve produced a dose-related inhibition of both the ascending (M- and D-SSEP) and descending (CMAP) nerve conduction.

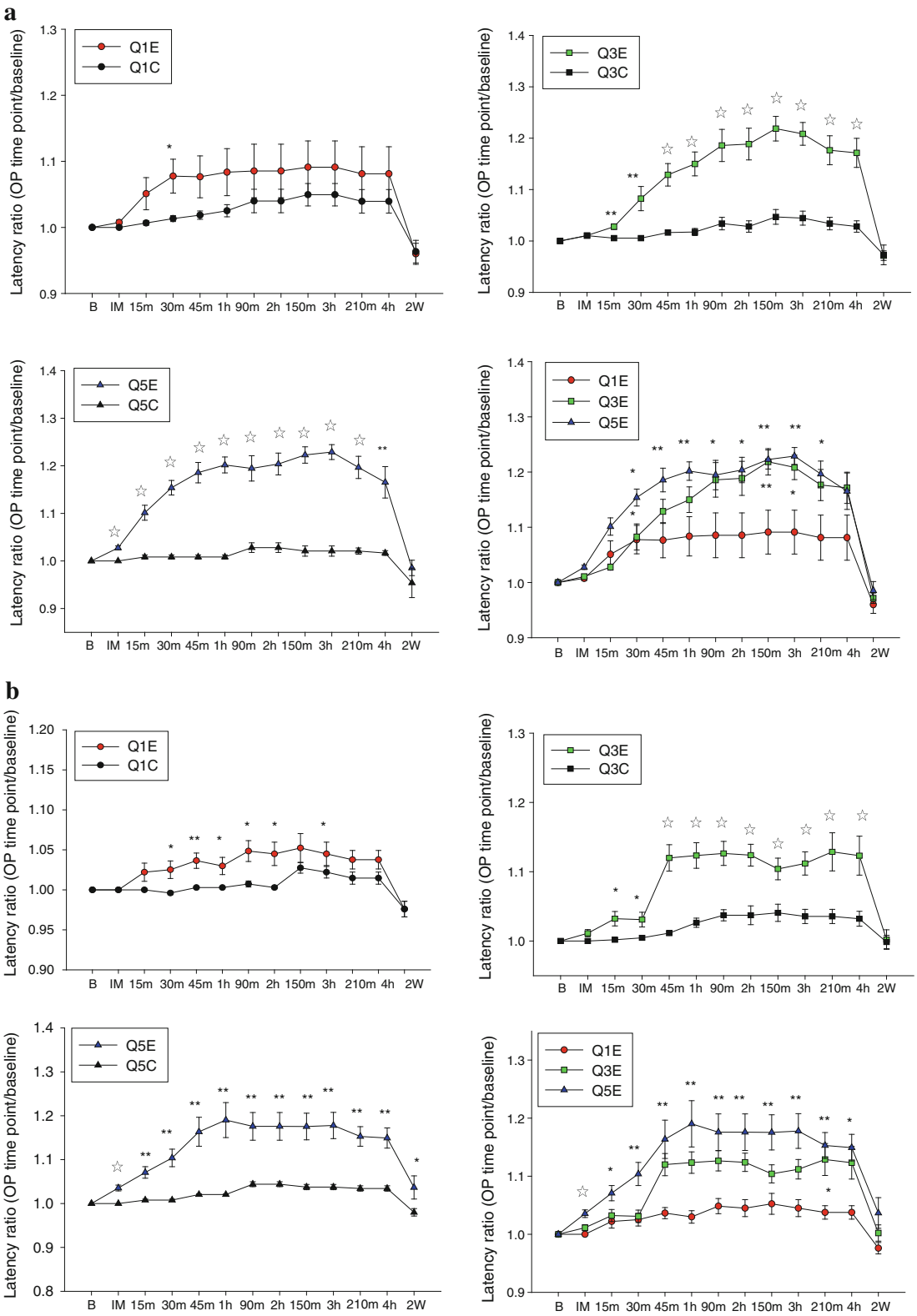

Fig. 3 Application of quinidine on the sciatic nerve increased the latency ratio of the evoked potential (EP) (EP latency at each time point/baseline EP latency before treatment). Analysis of SSEP and CMAP revealed significant differences in latency ratios between the left [treated (QE)] and right [control (QC)], by paired *t*-test. Comparing each time point after quinidine treatment revealed dose-related increases in latency ratio ($Q_1E < Q_3E < Q_5E$), by one-way ANOVA. Error bars represent SE. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{\star}p < 0.001$

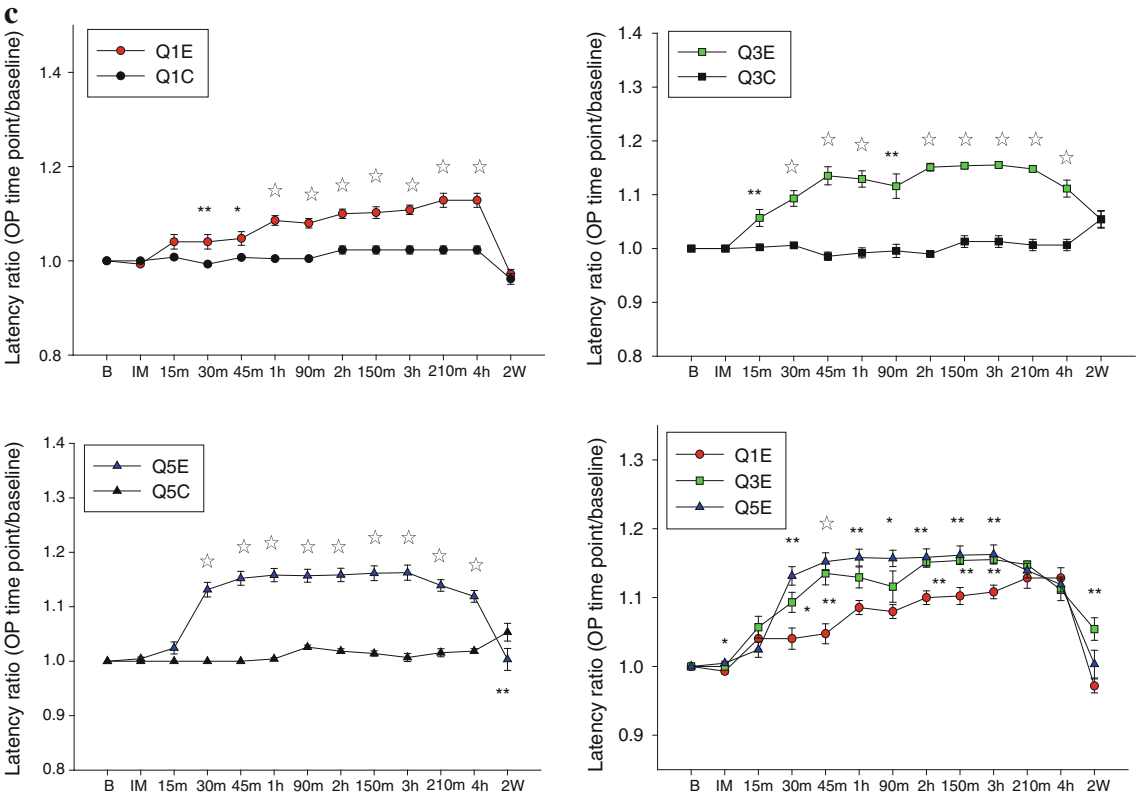

Fig. 3 continued

The suppression persisted for over 4 h in groups Q_3 and Q_5. Since the potentials recorded for the glucose-treated contralateral limbs revealed no significant change in amplitude or latency, the possibility of systemic effects of quinidine were excluded. The reversible depressant effect on SSEPs and CMAP in the sciatic nerves demonstrated that quinidine interrupted nerve conduction temporarily in the treated limbs. At 2 weeks post-treatment, the experimental hind limb showed no difference in terms of SSEPs and CMAP, and in walking track with the contralateral hind limb. In addition, there were comparable average numbers of myelinated axons in the sciatic nerve between group Q_5 and naïve rats. These results confirmed that direct application of quinidine on the sciatic nerve caused a dose-related depression of SSEPs and CMAP and exerted a local anesthetic effect on the peripheral nerve.

Local anesthetics inhibit nerve impulse transmission by blocking VGSCs. At least nine variants of α-subunits of VGSCs ($Nav_{1.1}$ to $Nav_{1.9}$) are distributed in various tissues and organs. Generally, $Nav_{1.1}$ and $Nav_{1.2}$ are distributed in the brain, $Nav_{1.4}$ in the skeletal muscles, $Nav_{1.5}$ in the cardiac muscles, and $Nav_{1.1}$, $Nav_{1.6}$, $Nav_{1.7}$, $Nav_{1.8}$, and $Nav_{1.9}$ in the peripheral nervous system [17–19]. Since quinidine is a kind of anti-arrhythmia sodium channel blocker that effectively treats rapid heart rate, it likely blocks VGSCs in peripheral nerves. Leszczynska and Kau [20] reported that injecting quinidine hydrochloride (0.8 μmol; 0.05 ml) into the popliteal space of the hind limb in mice led to a local anesthetic effect by loss of motor function. Revenko et al. [21] found that quinidine inhibited sodium and potassium currents in the Ranvier node of frog myelinated nerve fibers when investigated by means of the voltage clamp technique. More recently, a rat model of subcutaneous infiltration in Tzeng et al. [12] showed that subcutaneous application of quinidine 1 and 1.72 μmol produces cutaneous analgesic effects for 38 and 50 min, respectively. The current study extends these studies by confirming the local anesthetic properties of quinidine and by demonstrating its dose-related effects on peripheral nerve conduction.

Earlier studies have indicated that these well-established electrophysiological techniques are sufficiently sensitive for assessing how neural conduction is affected by chemical or physical change [13, 22]. In the present study, directly applying quinidine (1 μmol) to the left sciatic nerve does not significantly change the amplitude or latency of either ascending or descending conduction in the experimental limb, compared to pre-treatment baseline or to the untreated contralateral limb. However, dosages of 3 and 5 μmol both significantly decrease the amplitude and delay conduction. This study is the first to demonstrate the electrophysiological effect of quinidine on peripheral nerves in a rodent model. Further analysis of

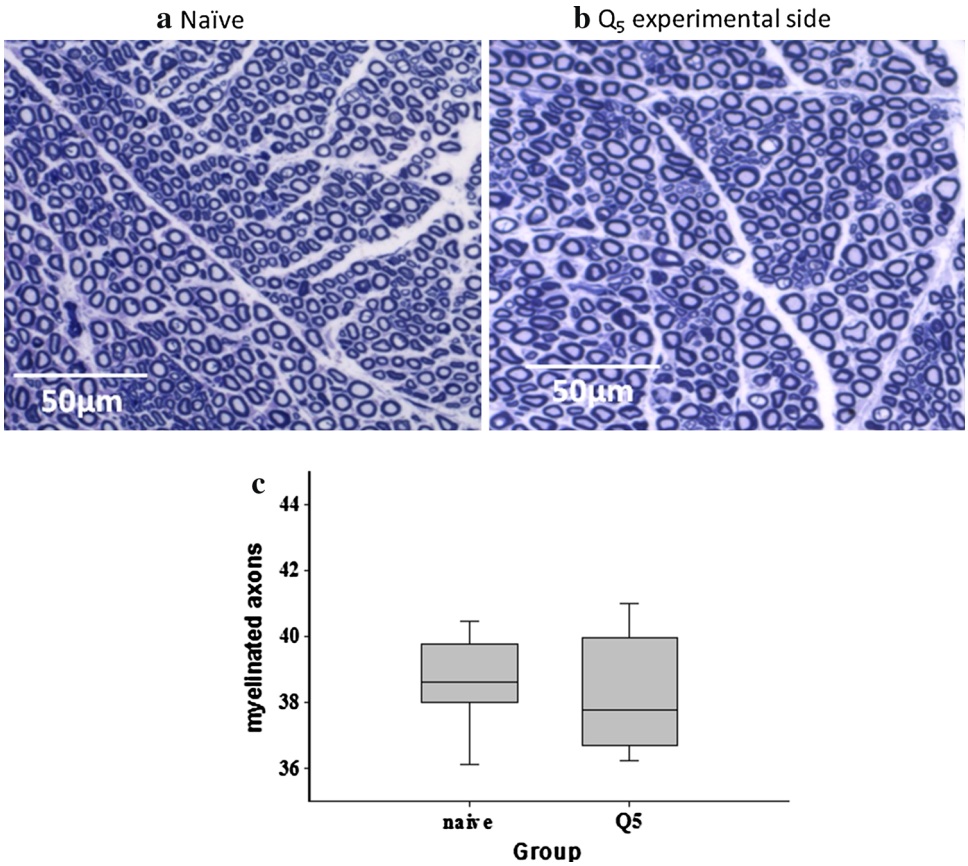

Fig. 4 Comparable density of myelinated fibers between naïve and Q_5 rats. **a** Transverse sections (1 μm) of the left sciatic nerve were examined by light microscopy in 12 sub-fields. Myelinated fibers in naïve rats, **b** Q_5 rats of the experimental side. *Bar* 50 μm. **c** Comparable density of myelinated fibers (fibers in selected area/cross-section area;/mm^2)

electrophysiology and functional change after 2 weeks also revealed no adverse effects of quinidine on the sciatic nerve.

Quinidine not only inhibits sodium channels but suppresses delayed rectifier potassium channels [23]. Blocking delayed rectifying potassium channels apparently alleviates neuronal excitation [24] and augments the effects of local anesthesia [25]. Although the classification of quinidine as a local anesthetic requires further verification of its blocking effects on voltage-dependent sodium channels, this study confirms that quinidine, when directly applied to the sciatic nerve, provides a dose-related conduction blockade. This blocking effect on both sensory and motor conduction is comparable to that produced by local anesthetics [12, 20] and supports the hypothesis that quinidine may also be suitable as a local anesthetic, which has significant clinical relevance. Although quinidine is mainly used as an antiarrhythmic agent rather than as a local anesthetic or a K channel blocker, it may be a candidate for treating chronic neuropathic pain.

Conclusions

Quinidine, when directly applied to an exposed sciatic nerve, produces a dose-related decrease in amplitude and latency prolongation in D-SSEP, M-SSEP, and CAMP. This suppression is transient and occurs without local neural toxicity. Aside from being a sodium channel blocker acting on cardiac arrhythmia, quinidine may also function as a local anesthetic on peripheral nerves. It may be a potential alternative to local anesthetic when treating chronic pain.

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