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研究課題名(和文) Pre-emptive Delivery of Pain Specific Local Anaesthetic (QX-CAP) to Prevent Endodontic Postoperative Pain

研究課題名(英文) Pre-emptive Delivery of Pain Specific Local Anaesthetic (QX-CAP) to Prevent Endodontic Postoperative Pain

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研究成果の概要(和文)：カブサイシンと併用することにより感覚神経だけを麻酔できるQX-314を用いて、根管治療における疼痛軽減を検討した。下顎切歯を切削し歯髄を露出した後、根管治療を施したラットでは、シヤムおよび未処置ラットと比較して、三叉神経脊髄路核尾側亜核(Vc)にc-Fos発現が増加していた。根管治療後、オトガイ神経支配領域にQX-CAPを注入すると、Vcにおけるc-Fos発現が減少することが確かめられた。このことは、根管治療の痛覚によるニューロン活動がQX-CAP注入によって減少することを示しており、根管治療後の疼痛軽減にQX-CAPが有効である可能性を示唆していると考えられる。

研究成果の学術的意義や社会的意義

Anaesthetizing the nerves that supply the dental pulp of a tooth using the combination of a permanently charged sodium channel blocker (QX-314) and a TRPV1 agonist (capsaicin; QX-CAP) prior to endodontic procedure may reduce the pain during treatment of the dental pulp like root canal treatment.

研究成果の概要(英文)：We have investigated the possibility of using the combination of a permanently charged sodium channel blocker (QX-314) and a TRPV1 agonist (capsaicin; QX-CAP) in reducing postoperative pain after endodontic treatment. We have made an animal model to mimic endodontic treatment procedure. The dental pulp of the rat's lower central incisor was exposed using dental drill and stimulated with an endodontic file to mimic the endodontic treatment procedure. A neuronal marker, c-Fos, expression increased in the trigeminal subnucleus caudalis (Vc) following the endodontic procedure compared to that of sham and naive rats, indicating increase of neuronal activity in the Vc due to the endodontic procedure. QX-CAP injection near the mandibular and mental nerve areas reduced the number of c-Fos expressing neurons in the Vc in rats with endodontic procedure, indicating reduction of endodontic procedure-induced neuronal activation by the QX-CAP injection.

研究分野：Dentistry

キーワード：Qx-314 Capsaicin Endodontic pain Trigeminal nerve c-Fos TRPV1

様式 C - 19、F - 19 - 1、Z - 19 (共通)

## 1 . 研究開始当初の背景

Local anesthetics (LAs) are widely used for blocking pain while retaining consciousness. LAs block the generation and propagation of action potentials by blocking voltage-gated sodium channels at the intra-neuronal site. Commercially available LAs (e.g., lidocaine) have little or no selectivity to nerve fibers. Therefore, although the goal of LAs is to block pain, the administration of LAs also produces numbness due to the blocking of pressure and touch receptors, immobility due to the blocking of motor axons, and low blood pressure due to the blocking of autonomic (sympathetic) nerve fibers. For example, during dental surgery procedures, LAs may cause numbness of the lips, cheeks and tongue, and partial immobility of the tongue. While this may be acceptable during surgery, specific blocking of only the pain sensation would be desirable. Sensory-selective local anesthesia has long been an important goal for local anesthetic development.

In our previous research we, we found that the combination of a permanently charged sodium channel blocker QX314 and capsaicin (QX-CAP) attenuated neuropathic pain (NP) for a long period following injury to inferior alveolar nerve, a branch of trigeminal nerve (Zakir et al. PLoS One, 2012). When QX-314 combines with capsaicin (a TRPV1 activator), it opens the TRPV1 channel, allowing QX-314 to enter nociceptive neurons (Binshtok et al. Nature, 2007). In this study, we investigated the possibility of using the QX-CAP in reducing postoperative pain after endodontic treatment. Endodontic postoperative pain is a common problem faced by dentists in their clinical practice which showed neuropathic pain like symptoms (Nixdorf et al. J Endod. 2010). Prolong activation of pulpal nerves during endodontic procedure (like root canal treatment) may lead to central sensitization (plastic changes in second order neurons in the brainstem) responsible for occurrence of pain after endodontic procedures. Blockade of surge of neuronal activity for long time after endodontic procedures may prevent central sensitization. Long lasting QX-CAP anesthetic has the potential to block the neural surge for longer period which may reduce the central sensitization thereby may prevent/reduce the post-endodontic neuropathic like pain.

## 2 . 研究の目的

In this study, our objectives were. 1) Making an animal model to mimic endodontic treatment procedure (ETP). 2) Understanding the pattern of neuronal activation in the spinal trigeminal sub-nucleus caudalis (Vc) and associated regions by the mimicked endodontic procedure. 3. Effect of pre-emptive delivery of QX-CAP anesthetic on neuronal activation by the endodontic procedure.

## 3 . 研究の方法

The study conducted in Sprague–Dawley (SD) rats. Ethical approval of study protocol obtained from Matsumoto Dental University Animal Ethics Committee. Four groups of rats were used.

1) **Experimental Groups: Naïve group:** This group received no treatment. **Sham group:** rats received superficial drilling on enamel of the left lower central incisor without exposing the dental pulp and needle prick into the tissues near the mandibular and mental nerves. **Rats recieved ETP:** rats received repeated stimulation of the exposed dental pulp. **QX-CAP anesthetic group:** rats received QX-CAP anesthetic before ETP.

2) **QX-CAP anesthetic:** QX-314 followed by capsaicin was injected as QX-CAP anesthetic 10 min prior to starting the drilling of the tooth. 100 µl QX-314 followed by 50 µl of 0.05% capsaicin was injected in the subcutaneous connective tissue near the mandibular nerve using a syringe.

Additionally, 50  $\mu$ l QX-314 followed by 25  $\mu$ l of 0.05% capsaicin was injected in the subcutaneous connective tissue near the mental nerve. QX-314 [N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide] (Sigma-Aldrich, USA) dissolved in normal saline. Capsaicin (Wako Pure Chemical Industries, Ltd., Japan) solution (0.05%) was prepared with ethanol, Tween 20, and normal saline.

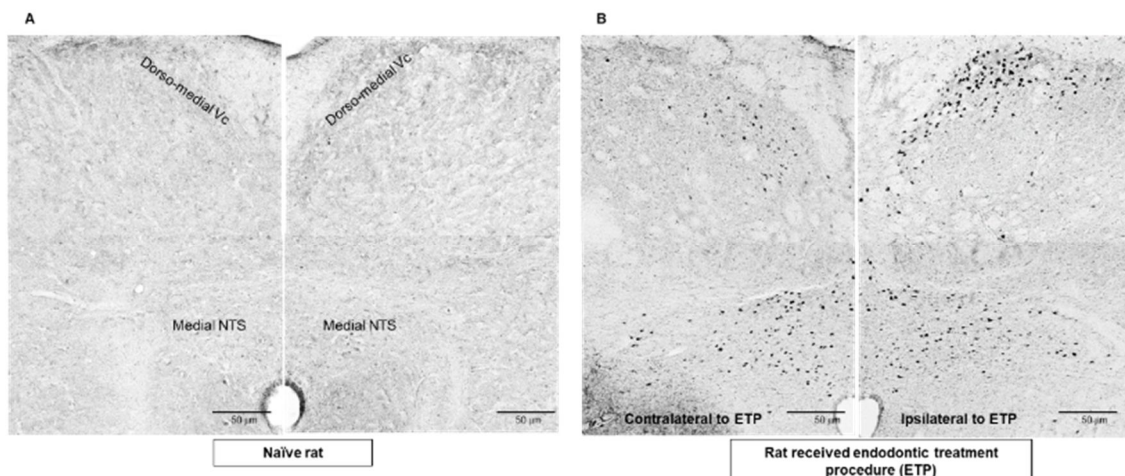
3) **Making of an animal model:** Under general anesthesia we exposed the dental pulp of the rat's lower central incisor using dental drill and then repeatedly stimulate the dental pulp using an endodontic file to mimic the ETP. 2 hours after the procedure, we checked whether this procedure increased the activity of neurons in Vc areas of the brainstem.

4) **Immunohistochemistry:** Two hours after ETP, the rat was deeply anesthetized with the same anesthesia and transcardially perfused sequentially with physiological saline followed by 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS). The brainstem was removed and then transferred to 30% sucrose in PBS for 2 to 3 days for cryoprotection. Frozen transverse cryotome sections (50  $\mu$ m) were collected on glass slides. After washing, the sections were placed in blocking solution (BLOXALL, Vector Laboratories, USA) for 30 min. They were incubated for 2 hrs. at room temperature in 5% normal goat serum (NGS) and 0.3% Triton X-100 in PBS and then incubated with an anti-c-Fos antibody (Abcam, USA; 1:25,00) diluted in 2.5% normal goat serum with 0.3% Triton X-100 in PBS for 24 hrs. Then the sections were incubated with biotinylated goat anti-rabbit-IgG (Vector Laboratories, USA) for 2 hrs at room temperature. Sections were then subjected to a peroxidase-conjugated avidin reaction (ABC Vectastain, Vector Laboratories, USA), and reaction products were visualized with a diaminobenzidine (DAB) substrate kit (ImmPACT DAB EqV, Vector Laboratories, USA). All sections were dehydrated in an alcohol series, cleared in xylene, and cover-slipped with synthetic mountant (Vector Laboratories, USA). Images were acquired by light microscopy, with a CCD camera attached to the microscope (Olympus, Japan). Transverse sections of the brainstem (-1.6 mm to 0.5 mm from obex) (Paxinos and Watson, 2007) were immunostained for c-Fos and examined bilaterally.

#### 4 . 研究成果

##### 1) **Animal model prepared to mimic ETP successfully activate brainstem neurons**

The animal model that we prepared to mimic ETP, successfully activate neurons in the trigeminal sub-nucleus caudalis (Vc) and associated nucleus tractus solitarius (NTS) areas. In the Vc, activated neurons were mainly observed in the dorso-medial regions (Figure 1). In the NTS, activated neurons were mainly observed in the medial NTS regions.

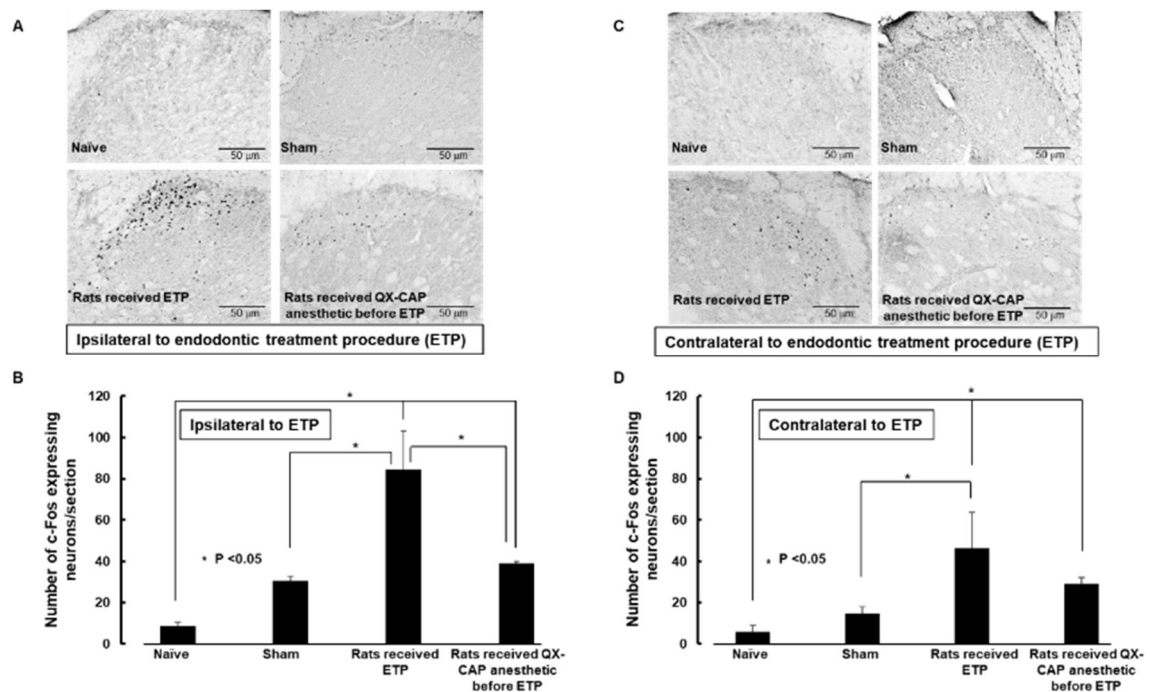


**Figure 1.** (A) Sections showing Vc and associated areas taken from naïve rats (rats that received no treatment). The number of neurons expressing c-Fos were very few. (B) Sections showing Vc and associated areas taken from rats that received endodontic treatment (ETP). A large number of neurons were activated by ETP (indicated by c-Fos expressions) in the Vc and NTS regions. Neurons were activated bilaterally, although, more neurons were activated ipsilateral to ETP specially in Vc areas compared to that of contralateral to ETP.

## 2) Pattern of neuronal activation and effect of prior local application of QX-CAP anesthetic near the nerves

### A. Vc regions

c-Fos expressing neurons were very few in the Vc of the naïve rats. In sham operated rats the number increased bilaterally but it was not significantly different than naïve rats (Figure 2). In the rats that received ETP, the number of c-Fos expressing neurons significantly increased bilaterally compared to naïve and sham operated rats (Figure 2). The number of c-Fos expressing neurons was more in ipsilateral side of the ETP compared to that of contralateral side, although the difference was not statistically significant. Prior application of QX-CAP anesthetic near the mandibular and mental nerve areas significantly reduced the number of c-Fos expressing neurons in the Vc, ipsilaterally (Figure 2). In the contralateral side, although QX-CAP anesthetic reduced the number of ETP-induced c-Fos expressing neurons, it was not statistically significant. The significant reduction of c-Fos expressing neurons in the Vc by prior application of QX-CAP anesthetic indicates that QX-CAP anesthetic can reduce ETP-induced neuronal activation in the Vc ipsilaterally (Figure 2).

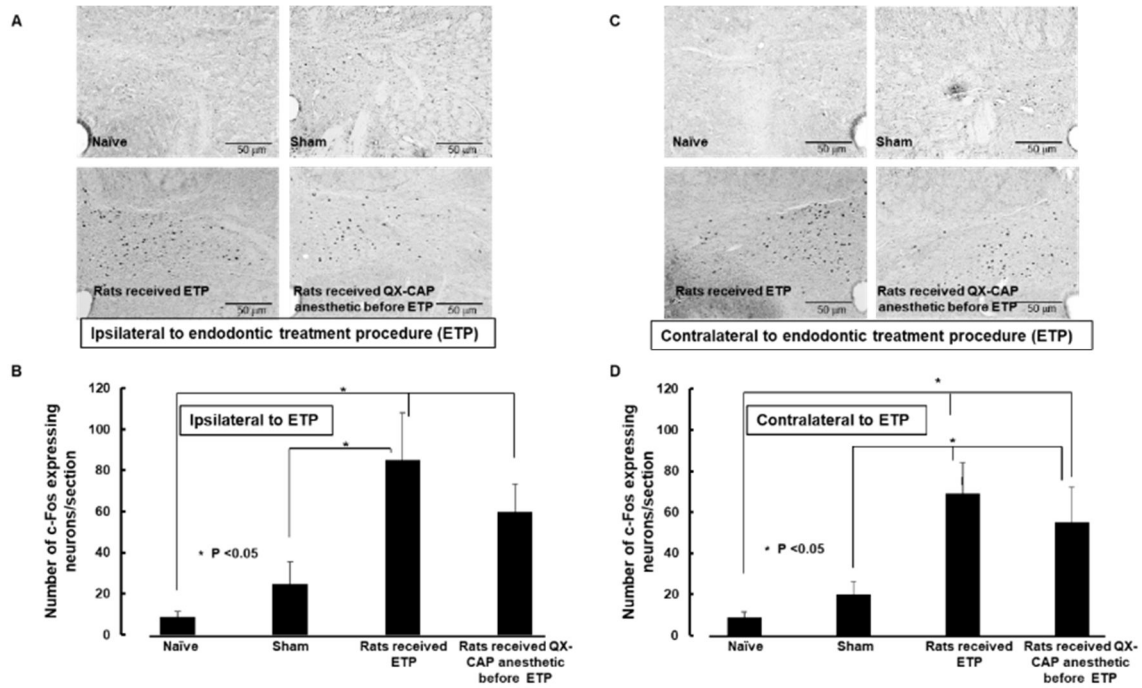


**Figure 2.** (A) Sections showing Vc regions taken from different rat groups ipsilateral to ETP. (B) Bar graph shows the average number of c-Fos expressing neurons per section ipsilateral to ETP for different rat groups. The number of c-Fos expressing neurons significantly ( $p < 0.05$ ) increased in the rats that received ETP compared to sham/naïve rats. The number of c-Fos expressing neurons significantly reduced in rats that received QX-CAP anaesthetic prior to ETP. (C) Sections showing Vc regions taken from different rat groups contralateral to ETP. (D) Bar graph shows the average number of c-Fos expressing neurons per section contralateral to ETP for different rat groups. The number of c-Fos expressing neurons increased in the rats

that received ETP compared to sham/naïve rats. The number of c-Fos expressing neurons reduced in rats that received QX-CAP anaesthetic prior to ETP although it was not statistically significant.

## B. NTS regions

c-Fos expressing neurons were very few in NTS of the naïve rats. In sham operated rats the number increased bilaterally but it was not significantly different than naïve rats (Figure 3). In the rats that received ETP, the number of c-Fos expressing neurons significantly increased bilaterally compared to naïve and sham operated rats (Figure 3). Prior application of QX-CAP anesthetic near the mandibular and mental nerve areas reduced the number of c-Fos expressing neurons in the NTS, bilaterally, however, the difference was not statistically significant (Figure 3).



**Figure 3.** (A) Sections showing NTS regions taken from different rat groups ipsilateral to ETP. (B) Bar graph shows the average number of c-Fos expressing neurons per section ipsilateral to ETP for different rat groups. The number of c-Fos expressing neurons significantly ( $p < 0.05$ ) increased in the rats that received ETP compared to sham/naïve rats. The number of c-Fos expressing neurons reduced in rats that received QX-CAP anaesthetic prior to ETP, however, the difference was not statistically significant. (C) Sections showing NTS regions taken from different rat groups contralateral to ETP. (D) Bar graph shows the average number of c-Fos expressing neurons per section contralateral to ETP for different rat groups. The number of c-Fos expressing neurons increased in the rats that received ETP compared to sham/naïve rats. The number of c-Fos expressing neurons reduced in rats that received QX-CAP anaesthetic prior to ETP, however, it difference was not statistically significant.

## 考察

The above findings suggest that endodontic treatment procedure (ETP) activate neurons in the Vc and NTS regions of the brainstem. Local application of QX-CAP anaesthetic near the nerves prior to ETP reduce the number of neuronal activation. Therefore, this anaesthetic can be used during the endodontic treatments.

## 5. 主な発表論文等

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〔図書〕 計0件

〔産業財産権〕

〔その他〕

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6. 研究組織

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