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The inhibitory effect of locally injected dexmedetomidine on carrageenan-induced nociception in rats ☆

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ABSTRACT

Recent studies showed that the administration of dexmedetomidine relieved hyperalgesia in the presence of neuropathic pain. These findings have led to the hypothesis that the local administration of dexmedetomidine is useful for relieving acute inflammatory nociception, such as postoperative pain. Thus, we evaluated the inhibitory effect of locally injected dexmedetomidine on acute inflammatory nociception. Acute inflammatory nociception was induced by an intraplantar injection of 1% carrageenan into the hindpaws of rats, and dexmedetomidine was also injected combined with carrageenan. The paw withdrawal threshold based on von Frey filament stimulation was measured until 12 h after injection. We compared the area under the time-curve (AUC) between carrageenan and carrageenan with dexmedetomidine. To clarify that the action of dexmedetomidine was via α_2-adrenoceptors, we evaluated the effect of yohimbine, a selective antagonist of α_2-adrenoceptors, on the anti-nociception of dexmedetomidine. As the results, the intraplantar injection of carrageenan with over 10 μM dexmedetomidine significantly increased AUC, compared to that with only carrageenan injection. This effect of dexmedetomidine was reversed by the addition of yohimbine to carrageenan and dexmedetomidine. These results demonstrated that the locally injected dexmedetomidine was effective against carrageenan-induced inflammatory nociception via α_2-adrenoceptors. The findings suggest that the local injection of dexmedetomidine is useful for relieving local acute inflammatory nociception.

1. Introduction

In some types of surgery using local anesthesia, including dental surgery, the management of postoperative pain is usually dependent on the duration of the effect of the local anesthetic used. Actually, we often use analgesic agents, such as a non-steroidal anti-inflammatory drugs (NSAIDs), for relieving acute inflammatory pain after the discontinuation of the effect of local anesthetics in a clinical setting. However, the higher the dose of analgesic agents, the higher the incidence of side effects. Relief from acute postoperative pain with minimum side effects is an important requirement to ensure the quality of patient management in the perioperative period.

Dexmedetomidine, a selective α_2-adrenoceptor agonist, is known to have effects on sedation, analgesia, and the cardiovascular system via α_2-adrenoceptors in the central nervous system (CNS) (Kamibayashi and Maze, 2000). However, other novel actions have been reported over the last decade. We and another group demonstrated that dexmedetomidine enhanced the anesthetic potency of local anesthetics in combination in animals (Yoshitomi et al., 2008; Brummett et al., 2008), and we also indicated that the local anesthesia with dexmedetomidine enhanced the anesthetic potency in humans (Yamane et al., 2015).

Furthermore, it has been reported that α_2-adrenoceptor

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agonizts, including norepinephrine and clonidine, possess a potent anti-inflammatory capacity that reduces endotoxin- or operation-induced inflammatory responses in blood samples (Kim and Hahn, 2000; Maes et al., 2000), rats with spinal cord injury (Can et al., 2009), and septic rats (Taniguchi et al., 2004), inhibiting the production of inflammatory mediators. Our recent study (Sukegawa et al., 2014) also demonstrated the inhibitory effect of locally injected dexmedetomidine on carrageenan-induced edema, the accumulation of leukocytes, and COX-2 and TNFα production at the injection site, and suggested that it exhibits an anti-inflammatory effect against local acute inflammatory responses.

Additionally, recent studies showed that the epidural or intraperitoneal administration of dexmedetomidine could inhibit hyperalgesia in the presence of neuropathic pain (Kimura et al., 2012; Poree et al., 1998) and inflammation induced by carrageenan (Walker et al., 2005). Another study (Lee et al., 2013) demonstrated that locally injected dexmedetomidine attenuated the neuropathic pain induced by spinal nerve ligation via peripheral α_2-adrenoceptors in rats. Furthermore, clinical studies (Al-Metwalli et al., 2008; Cheung et al., 2011) showed that the local administration of dexmedetomidine had a postoperative analgesic effect on patients undergoing surgery. These findings have led to the hypothesis that the local administration of dexmedetomidine is useful for relieving acute inflammatory pain, such as postoperative pain. In this study, we investigated whether locally administered dexmedetomidine had anti-nociceptive effects against carrageenan-induced nociception in rats.

2. Materials and methods

The protocol of the present study was approved by the Animal Care and Use Committee of Okayama University.

2.1. Animals

We used male Sprague–Dawley rats, aged 8 weeks (weight: 250–340 g), which were obtained from Charles River Laboratories (Osaka, Japan). Rats were housed under a 12 h day/night cycle and provided with food and water ad libitum.

2.2. Agents

Lambda-carrageenan (carrageenan) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, USA) and used as a 1% (weight/volume) solution, diluted with physiological saline. Dexmedetomidine (Precedex^R) was purchased from Maruishi Pharmaceutical Co. (Osaka, Japan). Yohimbine hydrochloride (yohimbine), a selective antagonist of α_2-adrenoceptors, was purchased from Sigma-Aldrich (St. Louis, USA), and diluted with ultra-pure water.

2.3. Animal model of acute inflammatory nociception

Inflammatory nociception was induced by the injection of carrageenan at a volume of 50 μl into the hindpaws of rats with a 27-gauge needle under inhalation anesthesia with isoflurane. The degree of nociception after injection of the test solutions was evaluated by measuring the paw withdrawal threshold on applying mechanical stimulation with von Frey filaments (Touch Test^R Sensory Evaluators, North Coast Medical, Inc., Gilroy, USA). Rats were placed on a metal mesh floor in individual clear plastic cases. After adaptation to the environment, the plantar surface of the hindpaw was touched vertically with a series of von Frey filaments (0.4, 0.6, 1, 1.4, 2, 4, 6, 8, and 15 g). Each trial was started with 2 g for 2–3 s. A brisk withdrawal or flinching of the paw was considered a positive response. The 50% withdrawal threshold was determined using the up-and-down method (Chaplan et al., 1994). In the absence of a positive response to a filament, a stronger stimulus was applied, whereas, in the presence of a positive response, the next weaker stimulus was applied. The resulting pattern of positive and negative responses was tabulated using the convention: X=positive; O=negative and the 50% response threshold was interpolated using the following formula:

$$50\%\ \text{g threshold} = (10^{[Xf+\kappa\delta]})/10{,}000$$

where Xf=value (log units) of the final von Frey filament used, κ=tabular value for the pattern of positive/negative responses (Chaplan et al., 1994), and δ=mean difference (log units) between stimuli (here, 0.224).

The investigator who injected the test solutions was blinded to the solutions being administered, and the investigator who measured the withdrawal threshold was also blinded to them.

Before its commencement, we decided on the concentration of carrageenan to be used in this study based on a preliminary study. In the preliminary study, we confirmed the 50% paw withdrawal threshold until 12 h after the injection of carrageenan at 0.04%, 0.2%, 1%, and 5%, and then calculated the area under the withdrawal threshold time-curve (AUC) of each concentration of carrageenan. We decided to use the 1% concentration in the present study because the 50% effective concentration was 1.075% (Supplementary Figure).

2.4. Evaluation of effect of locally injected dexmedetomidine

Dexmedetomidine was subcutaneously injected at a final concentration of 1, 5, 10, or 100 μM into the hindpaws of rats, combined with 1% carrageenan. The 50% paw withdrawal threshold was evaluated 2, 4, 6, 8, and 12 h after the injection, then we calculated the area under the withdrawal threshold time-curve (AUC) of each test solution, and compared the results among the concentrations of dexmedetomidine. Furthermore, to ensure that the action of dexmedetomidine was mediated via α_2-adrenoceptors, we subcutaneously injected yohimbine at a final concentration of 10 μM, combined with 1% carrageenan or 1% carrageenan plus dexmedetomidine at 10 μM, and the effect of yohimbine on dexmedetomidine's action was evaluated. As a control, only 1% carrageenan was subcutaneously injected into the hindpaw of rats.

2.5. Evaluation of central effect of dexmedetomidine

In order to evaluate whether or not the effect of dexmedetomidine was due to its central effect, we subcutaneously injected saline or dexmedetomidine at 10 or 100 μM as the hindpaw injection into the backs of rats under inhalation anesthesia with isoflurane instead of injection into the hindpaw. At the same time, 1% carrageenan or 1% carrageenan with 10 μM yohimbine was subcutaneously injected into the hindpaws of rats. As a control, only 1% carrageenan was subcutaneously injected into the hindpaw of rats. The 50% paw withdrawal threshold was evaluated after the injection of 1% carrageenan into the hindpaw in the same way.

In order to evaluate the sedative effect of dexmedetomidine, the righting reflex was evaluated at the same time point as measuring the paw withdrawal threshold according to a previous study (Irifune et al., 2007). We tilted the beaker by hand to an angle of approximately 45° from a horizontal plane. The beaker was tilted three times at each recording time and righting reflex scores were evaluated according to the rating scale: a score of 0 indicated a normal righting reflex; +1 indicated that the mouse righted itself within 2 s on all three trials; +2 indicated that the