Neuroscience Letters 654 (2017) 23–27

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Mexiletine co-injected with clonidine increases the quality and duration of cutaneous analgesia in response to skin pinpricks in the rat

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HIGHLIGHTS

- Subcutaneous mexiletine provoked dose-dependent cutaneous analgesia.
- Clonidine increased the potency of cutaneous analgesia by mexiletine.
- Mexiletine co-injected with clonidine prolonged the sensory block duration.

ARTICLE INFO

Article history:
Received 25 April 2017
Received in revised form 3 June 2017
Accepted 14 June 2017
Available online 16 June 2017

Keywords:
Clonidine
Mexiletine
Co-administration
Infiltrative cutaneous analgesia
Pinpricks

ABSTRACT

The goal of the experimental design was to assess the cutaneous analgesic effect of mexiletine by co-injection with clonidine. The effect of nociceptive block was evaluated according to the inhibition of the cutaneous trunci muscle reflex (CTMR) in response to skin pinpricks in rats. The dose-related analgesic effect of mexiletine alone or mexiletine co-administrated with clonidine was constructed after subcutaneous injection. Subcutaneous injections of mexiletine elicited dose-related cutaneous analgesia. Compared with mexiletine (1.8 μmol), adding clonidine to mexiletine (1.8 μmol) solutions for skin nociceptive block potentiated and prolonged the action ($p < 0.01$). Mexiletine (6 μmol) combined with clonidine extended the duration of cutaneous analgesia when compared with mexiletine (6 μmol) alone ($p < 0.01$). Co-administration of clonidine increases the potency and extends the duration of cutaneous analgesia by mexiletine, and the minimal dose of clonidine to intensify the analgesic effect is 0.06 μmol.

Mexiletine is one of the most commonly used class I B antiarrhythmic agents [7,43]. In most cases, it was mainly studied for the treatment of acute and chronic ventricular arrhythmias. Interestingly, mexiletine has been shown to block voltage-gated sodium channels [2], and therefore produces a local anesthetic effect [11,19,36]. Mexiletine was more potent than lidocaine on infiltrative cutaneous analgesia [36]. The nociceptive block duration caused by mexiletine was similar to that caused by the local anesthetic lidocaine at the equianesthetic doses [36].

Infiltrative administration of local anesthetics into the surgical site is commonly performed in the treatment of postincisional pain following inguinal hernia repair [35] or the procedure of laparoscopy [6] due to it is relatively deficient in side effects [24]. Unfortunately, it has the short-term duration of anesthesia or analgesia [5]. According to its α2-adrenoreceptor property and direct action on peripheral nerves [15,16,25], the use of clonidine to local anesthetic preparations has been frequently practiced for the purpose of improving the quality and extending the duration of local anesthesia [10]. However, the administration of clonidine impacting toward the antiarrhythmic agent mexiletine for cutaneous analgesia remains unclear. The objective of the present experiment

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http://dx.doi.org/10.1016/j.neulet.2017.06.022
0304-3940/

was to evaluate the potency and duration of mexiletine in combination with clonidine in a rat model of cutaneous trunci muscle reflex (CTMR) in response to skin pinpricks.

The experimental protocols were approved by the Institutional Animal Care and Use Committee of Chi Mei Medical Center (Tainan, Taiwan), and all the experimental procedures were followed the guidelines of the international association for the study of pain [42]. Male Sprague-Dawley rats, weighing 200–250 g, were obtained from the National Laboratory Animal Centre (Taipei, Taiwan). Animals were kept two-three per cage in the animal housing facility at Chi Mei Medical Center with controlled room temperature (23 ± 2 °C), and relative humidity approximately 40–60% under a natural light-dark cycle [12 h light/dark (7:00 A.M. to 7:00 P.M., light) cycle] with free access to food and water.

Mexiletine hydrochloride and clonidine hydrochloride were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and then were dissolved in normal saline before performing experiment.

A total of 88 rats were used in this study. Three specific experiments were designed. In experiment 1, the dose-related effects of mexiletine at 0.6, 1.8, and 6.0 μmol were constructed (n = 8 for each group). The dose rate chosen was according to the previous study [36]. In experiment 2, the %MPE (percent of maximal possible effect), duration of action, and area under the curves (AUCs) of drug (1.8 or 6.0 μmol) alone or the mixture of drug (1.8 or 6.0 μmol) with clonidine (0.0006, 0.006, or 0.06 μmol) were compared on infiltrative cutaneous analgesia (n = 8 for each group). Subcutaneous injection of saline vehicle or clonidine (0.0.6 μmol) did not elicit cutaneous analgesic effects. In experiment 3, two control groups were performed to eliminate the possibility of systemic effect of drugs from local skin analgesia. One group (n = 8 for each group) underwent intraperitoneal administration of mexiletine (6.0 μmol) combined with clonidine (0.06 μmol). Another group (n = 8 for each group) underwent intraperitoneal administration of mexiletine at 6.0 μmol or clonidine at 0.06 μmol.

Cutaneous analgesia in response to skin pinpricks was evaluated after subcutaneous injection of drugs. The procedures of subcutaneous injection were performed based on the previous report [21,38]. Simply speaking, 0.6 ml solution was subcutaneously injected by using a 30-gauge needle in conscious rats. Before injection, the hair on the rats' dorsal surface of the thoracolumbar region (10 cm × 6 cm) was mechanically shaved. After subcutaneous injection, a round elevation, approximately 2 cm in diameter, was formatted on the surface of the skin. Then, mark the wheal with ink within 1 min after injection.

The quality of cutaneous analgesia was based on the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back produced by twitches of the lateral thoracospinal muscle in response to local dorsal cutaneous stimulation [20,40]. A cut end of the 18-gauge needle attached to a von Frey filament (No.15; Somedic Sales AB, Stockholm, Sweden), which could produce a standardized nociceptive stimulus (19 ± 1 g), was used to elicit the CTMR responses. After the observation of the CTMR reaction to pinpricks outside the wheal and on the contralateral side, six pinpricks inside each wheal were tested. The nociceptive block (percent of possible effect; %PE) was defined as the number of the pinpricks that the rat failed to react, with, for example, the complete absence of six responses was recorded as complete nociceptive block (100% of possible effect; 100% PE) [9,37]. The maximal blocking effect was presented as the percentage of maximal possible effect (%MPE), and the full recovery time was defined as the period from subcutaneous injection to complete recovery of the CTMR reactions. The observation of six pinpricks was carried out at 0, 2 and 5 min after injection, every 5 min after injection for the first 30 min, followed by every 10 min for 30–60 min after injection and every 15–30 min afterwards until the CTMR fully recovered from the block. The AUCs of the nociceptive block were calculated by using the Kinetica version 2.0.1 (InnaPhase Corporation, Philadelphia, PA) program [12,23]. A researcher, who was in charge of evaluating the cutaneous analgesic effect, was blind to each drug injection.

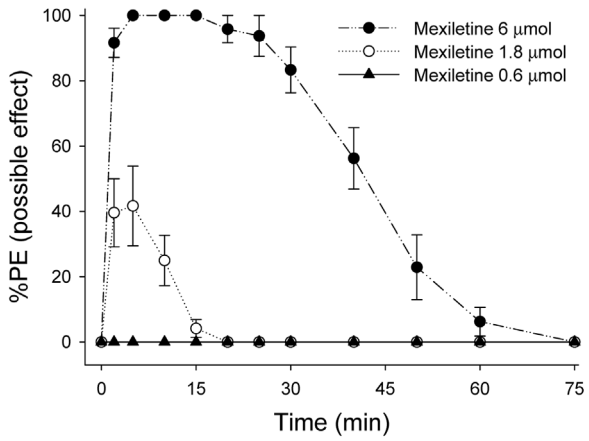

Fig. 1. Time courses of cutaneous analgesia after three-dose treatment of mexiletine. Mexiletine at 6 μmol produced complete (100%) nociceptive block. Experimental data are presented as mean ± SEM; n = 8 rats for each drug dose.

Data are expressed as mean ± SEM. The duration, AUCs and% MPE among the groups were analyzed using one-way ANOVA followed by Tukey's honest significant difference (HSD) test for paired comparisons. The F-values together with their associated degrees of freedom (treatment and residual) are reported as F (df of treatment/residual). SPSS for Windows (version 17.0, SPSS Inc., Chicago, IL, USA) was used for statistics and *P* value less than 0.05 was considered significant.

Subcutaneous injections of mexiletine elicited dose-dependent cutaneous analgesia in rats (Fig. 1). Subcutaneous mexiletine at 1.8 μmol showed 36% blockade (%MPE), whereas mexiletine at 6 μmol exhibited 100% blockade, as depicted in Fig. 1. When mexiletine (1.8 μmol) co-administrated with clonidine (0.0006, 0.006, or 0.06 μmol), the sensory block (%MPE) reached 89–92% (Fig. 2A). Saline vehicle showed no cutaneous analgesic effects (Fig. 2A). Mexiletine at 6 μmol achieved complete nociceptive block (Fig. 2B), whereas clonidine at 0.06 μmol alone manifested no analgesic effect at all (Fig. 2B). After mexiletine (6 μmol) co-injected with clonidine (0.0006, 0.006, or 0.06 μmol), complete (100% MPE) sensory block occurred (Fig. 2B).

The %MPE, duration of action, and AUCs of each drug alone and in combination with clonidine are presented in Table 1. Compared with mexiletine (1.8 μmol) alone, mexiletine (1.8 μmol) co-administrated with clonidine (0.0006, 0.006, or 0.06 μmol) increased the values of%MPE [$F_{(3/28)} = 11.79$; $p \leq 0.01$], full recovery time [$F_{(3/28)} = 25.89$; $p \leq 0.01$], and AUCs [$F_{(3/28)} = 20.94$; $p \leq 0.01$] (Table 1). Mexiletine (6 μmol) combined with clonidine (0.006 or 0.06 μmol) increased the values of complete block time [$F_{(3/28)} = 16.06$; $p \leq 0.01$], full recovery time [$F_{(3/28)} = 14.56$; $p \leq 0.01$], and AUCs [$F_{(3/28)} = 18.64$; $p \leq 0.01$] (Table 1) when compared with mexiletine (6 μmol) alone. Neither intraperitoneal administration of mexiletine (6.0 μmol) combined with clonidine (0.06 μmol) nor intraperitoneal administration of mexiletine at 6.0 μmol or clonidine at 0.06 μmol provoked the cutaneous analgesic effect. All of the rats recovered completely after each injection.

In the current study, mexiletine elicited a dose-related effect on infiltrative cutaneous analgesia. The resulting data were in agreement with the previous report that mexiletine produced dose-dependent effects of nociceptive block [36]. Furthermore, clonidine (0.006–0.06 μmol) enhanced the potency and prolonged the dura-