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Short communication

Clonidine intensifies memantine cutaneous analgesia in response to local skin noxious pinprick in the rat

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ABSTRACT

Background: The purposes of this study were to evaluate the co-administration of clonidine with memantine and to determine whether it has a peripheral action in intensifying cutaneous analgesia.
Methods: Cutaneous analgesia was examined through inhibition of the cutaneous trunci muscle reflex in response to the local noxious pinprick in rats. Effect of the added subcutaneous clonidine to memantine on infiltrative cutaneous analgesia was assessed and compared with the local anesthetic lidocaine.
Results: On the 50% effective dose (ED_{50}) basis, the rank of drug potency was memantine [4.05 (3.95–4.18) μmol] > lidocaine [5.81 (5.70–5.98) μmol] ($p < 0.01$). Clonidine at a dose of 0.12 μmol did not elicit cutaneous analgesia. Mixtures of clonidine (0.12 μmol) with drug (memantine or lidocaine) at ED_{50} or ED_{95} prolonged the duration of action and enhanced the potency as infiltrative cutaneous analgesia. Clonidine enhanced the lidocaine cutaneous analgesia in which had a better effect than added to memantine.
Conclusions: Our resulting data showed that memantine displayed more potent cutaneous analgesia than lidocaine. Co-administration of memantine or lidocaine with clonidine increased the potency and duration of the cutaneous analgesia. Clonidine intensified the effects of lidocaine promoting cutaneous analgesia than added to memantine.

Introduction

Memantine is the first novel class of Alzheimer's disease drugs whose therapeutic practice is linked to its characteristic of blocking N-methyl-D-aspartate glutamate receptors [1,2]. Due to the inhibition of tetrodotoxin-resistant Na^+ currents [3], memantine also produced a local anesthetic effect in rats [4,5]. Infiltration anesthesia through a local anesthetic injection is used for managing laparoscopic surgery [6] and postoperative pain relief after inguinal hernia repair [7], because it is relatively free of side effects [8]. However, this technique has a short duration of anesthesia or analgesia [9].

Research has been shown that memantine elicited skin infiltrative analgesia in a dose-related fashion [4] and its block duration was similar to that of the long-term local anesthetic bupivacaine [5]. Moreover, at the equianesthetic doses, intravenous memantine tolerated better to induce the cardiovascular system and central nervous system toxicity than bupivacaine [5]. The clinical value of memantine is worthy of being studied.

In general, clonidine is a commonly-used adjuvant to the local anesthetic agents. Moreover, the clonidine-analgesic mechanism goes through its α2-adrenoreceptor properties when administered intrathecally or epidurally [10,11]. Our previous study demonstrated that clonidine enhanced the sensory blocking effect and duration of bupivacaine [12]. It is widely suggested that clonidine improves the potency and duration of the local anesthetic blockade and diminishes the postoperative analgesic requirement [13]. However, the administration of clonidine impacting toward the local anesthetic memantine for peripheral nerve block remains unclear.

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Therefore, the purposes of this study were to assess the effect of co-administration of clonidine with memantine and to determine whether it produces a peripheral action in intensifying the quality and duration of cutaneous analgesia, when compared with lidocaine. The local anesthetic lidocaine was used as a control agent.

Materials and methods

Animals

The experimental protocols were approved by the Institutional Animal Care and Use Committee of China Medical University (Taichung, Taiwan) in accordance to the recommendations and policies of the International Association for the Study of Pain (IASP). One hundred and seventy-six male Sprague-Dawley rats, each weighing 200–250 g, were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and kept in the animal housing facilities at China Medical University, with controlled humidity (approximately 50% relative humidity), room temperature (22 °C), and a 12 h on/12 h off light/dark cycle (light on at 6:00 AM).

Drugs

Memantine HCl, lidocaine HCl monohydrate, and clonidine HCl were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in saline (0.9% NaCl) before subcutaneous injection.

Groups and design

Three experiments were performed. In experiment 1, the cutaneous analgesia of memantine (12.0, 6.0, 3.0, and 1.5 μmol) and lidocaine (15.0, 7.5, 5.0, and 3.0 μmol) in a dose-related fashion was performed ($n = 8$ for each group). In experiment 2, the %MPE (percent of maximal possible effect), duration, and area under the curves (AUCs) of the drug (ED_{50} or ED_{95}) alone or co-administration of the drug (ED_{50} or ED_{95}) and clonidine (0.12 μmol) were evaluated on infiltrative cutaneous analgesia ($n = 8$ for each group). Subcutaneous injection of clonidine at 0.12 μmol elicited no cutaneous analgesia. In experiment 3, two control groups were tested to rule out the possible systemic effect of drugs on cutaneous analgesia. One group ($n = 8$ for each group) received intraperitoneal injection of drugs (memantine or lidocaine) at 2× ED_{95} or clonidine at 0.24 μmol. Another group ($n = 8$ for each group) received intraperitoneal injection of co-administration of clonidine (0.12 μmol) and drug (memantine or lidocaine) at ED_{95}.

Subcutaneous injection

Animals were handled daily for a week to minimize the stress on the rats during the experiment and generally improved their experimental performances. On the day before the injection, the hair on the rats' dorsal surface of the thoracolumbar region (10 cm × 6 cm) was mechanically shaved. The subcutaneous injection procedure was performed as previously reported [14,15]. In brief, the drugs, which were dissolved in saline, were injected subcutaneously in un-anesthetized rats at the dorsal surface of the thoracolumbar region by using a 30-gauge needle. The entire injection volume was 0.6 mL. After the subcutaneous injection, a wheal occurred approximately 2 cm in diameter as a circular elevation of the skin. It was marked with ink within 1 min after injection. The cutaneous analgesia was assessed by the cutaneous trunci muscle reflex (CTMR) of subcutaneous muscles in response to the pinpricks. CTMR is a reaction from the noxious skin stimulus involving the local contraction of skeletal muscle beneath the skin with parallel movements of the nearby skin over the rat's dorsum [5,16].

Cutaneous analgesia

A von Frey filament (No. 15; Somedic Sales AB, Stockholm, Sweden), to which the cut end of an 18-gauge needle was affixed, was used to produce the standardized nociceptive stimulation (19 ± 1 g) without producing skin damage. After observing a normal reaction to pinpricks applied outside the wheal and on the contralateral side, we applied six pinpricks with a frequency of 1 Hz inside the wheal and selected the number to which the rat failed to react. The cutaneous analgesia of each drug was calculated quantitatively as the number of times the pinprick failed to elicit a response. For example, the complete absence of six responses demonstrated to be a full nociceptive block (100% of possible effect; 100% PE) [17,18].

For consistency, an experienced investigator, who was blinded to inject the drugs, was responsible for the neurobehavioral examinations. The six-pinprick test was applied at 0, 2 and 5 min after injection. First, the test was assessed every 5 min after the injection for the first 30 min. Second, it happened again every 10 min after the injection for 30–60 min, and then every 15–60 min until the CTMR fully recovered from the block. During the test, the maximal blockade during the time course of cutaneous analgesia of the drug was described as the %MPE. The duration of the drug action started from the injection (i.e., time = 0) to the full recovery moment of CTMR (no analgesic effect or 0% MPE) [19,20].

The 50% effective dose (ED_{50}) and AUCs

After subcutaneously injecting the rats with four doses of each drug ($n = 8$ for each dose of each drug), the dose–response curves were obtained from the %MPE for every drug dose. The values of ED_{50} and ED_{95}, as defined as the dose that caused 50% and 95% cutaneous analgesic effect, respectively, were obtained by a SAS NLIN analysis (SAS Institute Inc., Cary, NC, USA) [21–23]. The AUCs of the sensory block of drugs were obtained by using the Kinetica version 2.0.1 (InnaPhase Corporation, Philadelphia, PA, USA) program.

Statistical analysis

Data in Table 1 are presented as ED_{50} or ED_{95} values with 95% confidence interval (95% CI) and analyzed by the one-way analysis of variance (ANOVA) followed by the pairwise Tukey's honest significance difference (HSD) test. The values in Tables 2 and 3 are displayed as mean ± SEM with a secondary data spread shown even though a normal distribution was not assumed. The comparisons between the drug alone and the co-administration of drug with clonidine in between memantine and lidocaine groups were mentioned in each group, using nonparametric statistics (Mann–Whitney U test). A statistical software, SPSS for Windows (version 17.0, SPSS, Inc, Chicago, IL, USA), was used, and a p value less than 0.05 was considered statistically significant.

Table 1
The 50% effective doses (ED_{50}s) and ED_{95}s of memantine and lidocaine as infiltrative cutaneous analgesic.

Drug	ED_{50}s (95% CI)	ED_{95}s (95% CI)
Memantine	4.05 (3.95–4.18)	12.1 (11.0–13.7)
Lidocaine	5.81 (5.70–5.98)	20.3 (19.3–21.6)

ED_{50}s and ED_{95}s of drugs (μmol) were obtained from Fig. 1. CI, confidence interval. The potency of drug (ED_{50}) was memantine > lidocaine ($p < 0.01$), for each comparison using one-way ANOVA followed by pairwise Tukey's HSD test.