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Co-administration of memantine with epinephrine produces a marked peripheral action in intensifying and prolonging analgesia in response to local skin pinprick in rats

Yu-Wen Chen[a,b,1], Jann-Inn Tzeng[c,d], He-Jia Pan[a], Ching-Hsia Hung[e,*], Yu-Chung Chen[f,1], Jhi-Joung Wang[a]

[a] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[b] Department of Physical Therapy & Graduate Institute of Rehabilitation Science, China Medical University, Taichung, Taiwan
[c] Department of Food Sciences and Technology, Chia Nan University of Pharmacy and Science, Jen-Te, Tainan City, Taiwan
[d] Department of Anesthesiology, Chi-Mei Medical Center, Yong Kang, Tainan City, Taiwan
[e] Institute & Department of Physical Therapy, National Cheng Kung University, Tainan, Taiwan
[f] Division of Physical Therapy, Department of Physical Medicine and Rehabilitation, Cheng Hsin General Hospital, Taipei, Taiwan

HIGHLIGHTS

- Epinephrine produced more potent cutaneous analgesia than memantine or lidocaine.
- Epinephrine increased the potency of memantine and lidocaine on cutaneous analgesia.
- Epinephrine prolonged the duration of cutaneous analgesia of memantine and lidocaine.

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ABSTRACT

The purpose of this study was to examine the effect of epinephrine as adjuvant for memantine or lidocaine as an infiltrative anesthetic. Using a rat model of cutaneous trunci muscle reflex (CTMR), we evaluated the effects of adding epinephrine to memantine or lidocaine on infiltrative cutaneous analgesia. Lidocaine, a known local anesthetic, was used as control. We found that epinephrine, memantine, and lidocaine produced a dose-dependent local anesthetic effect as infiltrative cutaneous analgesia. On a 50% effective dose (ED_{50}) basis, the relative potencies were epinephrine [0.012 (0.006–0.020) μmol] > memantine [4.010 (3.311–4.988) μmol] > lidocaine [6.177 (5.333–7.218) μmol] ($P < 0.05$ for each comparison). Mixtures of epinephrine (2.7 nmol or 13.7 nmol) with drugs (memantine or lidocaine) at ED_{50} or ED_{95}, respectively, enhanced the potency and prolonged the duration of action on infiltrative cutaneous analgesia. Intraperitoneal injection of co-administration of drugs (memantine or lidocaine) at ED_{95} with epinephrine (13.7 nmol) produced no cutaneous analgesia (data not shown). Epinephrine, memantine, and lidocaine were shown to have local anesthetic effects as infiltrative cutaneous analgesia. Epinephrine increased the duration and potency of memantine and lidocaine as an infiltrative anesthetic.

Memantine, a noncompetitive *N*-methyl-D-aspartate receptors antagonist, is the first in a novel class of Alzheimer's disease drugs clinically [27,30]. Recently, we showed that memantine elicited local analgesia against skin nociceptive stimulus, was more potent than lidocaine [7], and produced a similar duration of action when compared with a long-acting local anesthetic bupivacaine [11]. Moreover, intravenous equianesthetic dose of memantine displayed better tolerated to cause cardiovascular system and central nervous system toxicity than bupivacaine [11]. We presume that memantine is a local anesthetic [7,11] whose therapeutic utility is linked to their ability to inhibit tetrodotoxin-resistant Na^+ currents [2].

Injection of long-acting local anesthetics for surgery and postoperative pain control is frequently performed [19]. It has been well established that epinephrine is often added to a local anesthetic to extend the duration of central and peripheral neuraxial blocks [28], whereas epinephrine itself at a dose of 0.137 μmol (1:20,000) elicited a transient and complete block of infiltrative cutaneous

* Corresponding author. Tel.: +886 6 2353535x5939; fax: +886 6 2370411.
E-mail address: chhung@mail.ncku.edu.tw (C.-H. Hung).
[1] Contributed equally to this work.

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analgesia in rats [10]. It is important to restrict the dosage of epinephrine seriously to prevent side effects while optimal concentrations of epinephrine may vary based on the anesthetic drug and its injection site [15,23,28]. We suggested that the addition of epinephrine could intensify and prolong memantine cutaneous analgesia. The purpose of this study was to assess the local anesthetic potency and duration of memantine and lidocaine after the addition of epinephrine. Lidocaine, a common local anesthetic, was used as a control.

The experimental protocols were approved by the Institutional Animal Care and Use Committee of China Medical University (Taichung, Taiwan) and conducted according to IASP ethical guidelines [31]. Male Sprague-Dawley rats, each weighing 200 to 250 g, were purchased from the National Laboratory Animal Centre (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-hour (6:00 AM to 6:00 PM) light/dark cycle.

Memantine HCl, lidocaine HCl monohydrate, and (±)-epinephrine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in saline (0.9% NaCl) before experiments.

Three investigations were performed. In investigation 1, cutaneous analgesia of memantine (10.8, 7.2, 3.3, 0.9 μmol), epinephrine (78.3, 13.7, 3.9, 2.7 nmol), and lidocaine (12.0, 9.0, 6.0, 3.0 μmol) in a dose-related fashion was examined ($n = 8$ for each group). Epinephrine (2.7 nmol) and saline elicited no cutaneous analgesia. These doses of epinephrine were chosen according to our previous study [10]. In investigation 2, the %MPE (percent of maximal possible effect), duration, and area under the curves (AUCs) of drug (ED_{50} or ED_{95}) alone or co-administration of drug (ED_{50} or ED_{95}) with epinephrine (2.7 nmol or 13.7 nmol) were evaluated on infiltrative cutaneous analgesia ($n = 8$ for each group). In investigation 3, two control groups were further tested to rule out the possibility of systemic effect of drugs on infiltrative cutaneous analgesia. One group ($n = 8$ for each group) received intraperitoneal injection of testing drug (memantine or lidocaine) at a dose of $2 \times ED_{95}$ or epinephrine at a dose of 13.7 nmol (1:200,000); another group ($n = 8$ for each group) received intraperitoneal injection of co-administration of epinephrine (13.7 nmol) with drug (memantine or lidocaine) at ED_{95}.

Before experiments, rats were handled daily up to 7 days to minimize the stress on the rats during the experiment and generally improve their experimental performance. On the day before the subcutaneous injection, the hair on the rats' dorsal surface of the thoracolumbar region ($10 \times 6\ cm^2$) was mechanically shaved. Subcutaneous injections of drugs were performed as reported previously [12,17]. In brief, drugs dissolved in saline were injected subcutaneously using a 30-ga needle in unanesthetized rats at the dorsal surface of the thoracolumbar region. The total volume of injection was 0.6 mL. In order to reduce the numbers of experimental animals used, the back of rat was further divided into left and right parts, either of which, after a washout period of 1 week, received one drug injection. After a subcutaneous injection, a wheal, a circular elevation of the skin, approximately 2 cm in diameter occurred. The wheal was marked with ink within 30 s after injection. Cutaneous analgesia was evaluated through the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back produced by twitches of the lateral thoracispinal muscle in response to local dorsal cutaneous stimulation [8,16].

A Von Frey filament (No.15; Somedic Sales AB, Stockholm, Sweden), to which the cut end of an 18-ga needle was affixed, was tested to elicit the standardized nociceptive stimulation (19 ± 1 g) without producing skin damage [5,13]. After observing a normal reaction to the pinprick applied outside the wheal and on the contralateral side, we applied six pinpricks with a frequency of 1 Hz inside the wheal and scored the number to which the rat failed to react. Each drug's cutaneous analgesia was evaluated quantitatively as the number of times the pinprick failed to produce a response, with, for instance, the complete absence of six responses was defined as complete nociceptive/sensory block (100% of possible effect; 100% PE). During the test, the maximal blockade in a time course of cutaneous analgesia of the drug was described as the %MPE.

For consistency, one experienced investigator who was blinded to the drugs injected was responsible for evaluating the cutaneous analgesia effect. The drugs were prepared and injected by another investigator. The test of six pinpricks was applied at 0, 2 and 5 min after injection, every 5 min after injection for the first 30 min afterward, then again every 10 min after injection for 30–60 min, and every 15–60 min thereafter until the CTMR completely recovered from the block. Each drug's duration of action was defined as the time from drug injection (i.e., time = 0) to full recovery of CTMR (no analgesic effect or 0%MPE) [4,9].

After subcutaneously injecting the rats with different doses of each drug, dose-response curves were constructed through the %MPE for each dose of each drug. The curves were then fitted by using the computer-derived SAS NLIN Procedures (SAS Institute Inc., Carey, NC), and the values of ED_{50} and ED_{95}, defined as the dose that caused 50% and 95% cutaneous analgesic effect, respectively, was obtained [6,22]. The AUCs were obtained by using *Kinetica version 2.0.1* (InnaPhase Corporation, Philadelphia, PA).

Data are presented as mean ± S.E.M. or ED_{50} and ED_{95} values with 95% confidence interval (95% CI). Data were analyzed by the Student's *t*-test or one-way analysis of variance (ANOVA) followed by pairwise Tukey's honest significance difference (HSD) 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.

Subcutaneous injection of memantine and epinephrine, as well as local anesthetic lidocaine elicited a dose-dependent effect of cutaneous analgesia. Epinephrine at 78.3 nmol produced 100% sensory/nociceptive blockade, whereas a low dose of epinephrine (2.7 nmol) and saline produced no cutaneous analgesia in rats (Fig. 1). The ED_{50}s and ED_{95}s of drugs were shown in Table 1. On an ED_{50} basis, the relative potency of drugs was found to be epinephrine > memantine > lidocaine (Table 1; $P < 0.05$).

Memantine and lidocaine at ED_{50} exhibited 52% and 54% of sensory blockade (%MPE), respectively, in Fig. 2. When drugs at ED_{50} were co-injected with epinephrine (2.7 nmol), complete nociceptive/sensory blockade (100% MPE) in the memantine (8 of 8 rats) group occurred, but not in the lidocaine (6 of 8 rats) group (Fig. 2). Drugs (ED_{50}) co-injected with epinephrine (2.7 nmol), the %MPE, time to full recovery, and AUCs were increased in the memantine and lidocaine groups ($P < 0.01$) compared with drugs at ED_{50} alone (Table 2). Furthermore, added epinephrine (13.7 nmol) to drugs (memantine or lidocaine) at ED_{50} prolonged drug nociceptive/sensory blockade (Fig. 2).

At ED_{95}, memantine and lidocaine showed 92% and 98% nociceptive/sensory blockade, respectively (Fig. 3). After drugs at ED_{95} were co-injected with epinephrine (2.7 nmol), both memantine and lidocaine produced 100% and 100% sensory blockade (100% MPE), respectively (Fig. 3). The %MPE, duration, and AUCs of drugs (ED_{95}) with epinephrine (2.7 nmol) were greater ($P < 0.05$) than drugs (ED_{95}) alone in Table 3. Moreover, added epinephrine (13.7 nmol) to drugs (memantine or lidocaine) at ED_{95} extended drug nociceptive/sensory blockade (Fig. 3). Neither intraperitoneal injection of epinephrine (13.7 nmol) combined with drugs (memantine or lidocaine) at ED_{95} nor intraperitoneal administration of a large dose of drugs ($2 \times ED_{95}$) produced cutaneous analgesia at 2–270 min after