Dextrorphan for Prolonged Skin Infiltration Anesthesia by Adding Epinephrine in Rats

Kuan-Ting Chen[1], Jhi-Joung Wang[2] and Yu-Wen Chen[*,2,3]

[1]*Department of Anesthesiology, Chi Mei Medical Center, Tainan, Taiwan*

[2]*Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan*

[3]*Department of Physical Therapy, China Medical University, Taichung, Taiwan*

Abstract: Dextrorphan could be a local anesthetic, while adding epinephrine to the local anesthetics prolonged their duration of action. Here we investigated whether epinephrine as adjuvant could prolong the local anesthetic effect of dextrorphan. The cutaneous analgesic effect of the addition of epinephrine (5 μg/mL) to dextrorphan was assessed in rats following the blockade of cutaneous trunci muscle reflex through subcutaneous injection of drugs. We showed that subcutaneous dextrorphan elicited dose-dependent cutaneous analgesia. Co-administration of epinephrine (5 μg/mL) with dextrorphan at 50% effective dose (ED_{50}) or ED_{95} had a longer duration than dextrorphan (ED_{50} or ED_{95}) alone, respectively. This study indicated for the first time that the mixtures of epinephrine with dextrorphan increased the duration as an infiltrative anesthetic.

Keywords: Dextrorphan, Epinephrine, Cutaneous trunci muscle reflex, Duration, Infiltrative cutaneous analgesia.

INTRODUCTION

Epinephrine has been known to be a vasoconstrictor, and therefore prolongs the action of local anesthetics [1-3]. For instance, adding epinephrine (5 μg/mL; 1:200,000) to lidocaine or bupivacaine solution was as effective as the higher doses to induce vasoconstriction during ear surgery and prolong analgesic duration of subcutaneous infiltration in human skin [4]. The concentrations of epinephrine should be selected seriously to prevent certain side effects, while the optimal doses of epinephrine may be vary based on the kind of the local anesthetics and the site of drug injection [1, 4, 5].

Dextrorphan is a Na^+ channel blocker [6] and has the characteristics of local anesthetics [7-12]. Interestingly, dextrorphan exhibited a long-acting local anesthetic effect similar to that provided by bupivacaine [11]. At an equipotent analgesic dose, dextrorphan shows better tolerated to cause central nervous system and cardiovascular system toxicity compared to bupivacaine [7]. No study revealed epinephrine at a common used dose of 1:200,00, prolonged the duration of skin infiltration anesthesia with dextrorphan to date. Therefore, the purpose of the study was to investigate cutaneous analgesia after subcutaneous injection of dextrorphan alone or co-administration of dextrorphan with epinephrine (5 μg/mL).

*Address correspondence to this author at the Department of Physical Therapy, China Medical University, No.91 Hsueh-Shih Road, Taichung 40402, Taiwan; Tel: 886-4-22053366, Ext. 7313; Fax: 886-4-22065051;
E-mail: cywhwok@mail.cmu.edu.tw

MATERIALS AND METHODS

Animals

These experimental protocols were approved by the Institutional Animal Care and Use Committee of China Medical University (Taichung, Taiwan), and conformed to the recommendations and policies of the International Association for the Study of Pain (IASP). Male Sprague-Dawley rats weighting 210-260 g were obtained from the National Laboratory Animal Center (Taipei, Taiwan), and housed in groups of two in a climate controlled room maintained at 21°C with approximately 50% relative humidity in the Animal Center of China Medical University (Taiwan). Lighting was on a 12-h light/dark cycle (light on at 6:00 AM), with food and water available *ad libitum* up to the time of experiment.

Drugs

Dextrorphan tartrate and (±)-epinephrine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were freshly dissolved in 0.9% NaCl (saline) before subcutaneous injections.

Experimental Designs

Three experiments were carried out. In experiment 1, the dose-response curve of dextrorphan (6.7, 11.2, 20.0, 48.0 μmol/kg) as infiltrative cutaneous analgesia was evaluated (n = 8 rats for each dose of each drug). In experiment 2, the %MPE (percent of maximal possible effect), duration, and area under the curves (AUCs) of dextrorphan at 50% effective dose (ED_{50}) or

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ED_{95} alone or co-administration of dextrorphan at ED_{50} or ED_{95} with epinephrine (5 μg/mL; 1:200,000) were tested on infiltrative cutaneous analgesia (n = 8 rats for each dose of each drug). Subcutaneous injection of saline (vehicle) produced no cutaneous analgesia. In experiment 3, two control groups were tested to rule out the possibility of systemic effect of drugs on infiltrative cutaneous analgesia. One group (n = 8 rats for each drug) received intraperitoneal injection of dextrorphan at a dose of $2\times ED_{95}$; another group (n = 8 rats for each drug) received intraperitoneal injection of co-administration of epinephrine (5 μg/mL) and dextrorphan at ED_{95}.

Infiltrative Cutaneous Analgesia

An experienced investigator, who was blinded to the identity of the injected drugs, was responsible for assessing the cutaneous analgesia effect. Subcutaneous injections of drugs were carried out as reported previously [13, 14]. Cutaneous analgesia produced *via* various drugs was evaluated according to the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back elicited *via* twitches of the lateral thoracispinal muscle in response to local dorsal cutaneous stimuli [15, 16]. During the testing, the maximum value of %PE was presented as % MPE. CTMR testing was applied 5 min before injection of drugs to confirm normal responses, then every 5 min after injection for the first 30 min and every 10-15 min thereafter, until the CTMR fully recovered from the block (no more than 2 hours). 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, i.e. 0% MPE). To calculate the ED_{50} and ED_{95} of dextrorphan, rats were subcutaneously injected with 4 different doses of dextrorphan (n = 8 for each dose of each drug), and then the dose-response curve was constructed from the % MPE of each dose of each drug. The curve was then fitted by SAS Nonlinear (NLIN) Procedures (version 9.1, SAS Institute, Cary, NC), and the value of ED_{50} or ED_{95}, defined as the dose that caused 50% or 95% cutaneous analgesia, were obtained [17, 18]. The AUCs of sensory block of drugs were obtained using *Kinetica version 2.0.1* (InnaPhase Corporation, Philadelphia, PA).

Statistical Analysis

The data are presented as mean ± S.E.M. or ED_{50} and ED_{95} values with 95% confidence interval (95% CI). The difference in Table **2** among groups was evaluated by 2-sided Student *t* test with unequal variances. SPSS for Windows (version 17.0) was used for all statistical analyses. Statistical significance was set at $P < 0.05$.

RESULTS

Dextrorphan (6.7-48.0 μmol/kg) dose-dependently elicited cutaneous analgesia in rats (Figure **1**). The ED_{50} and ED_{95} of dextrorphan constructed from Figure **1** were displayed in Table **1**. At a dose of ED_{50}, dextrorphan had 50% sensory/nociceptive blockade in Figure **2A**. Subcutaneous injection of saline (vehicle) showed no skin/local analgesia (data not shown). After dextrorphan at the dose of ED_{50} was co-injected with epinephrine (5 μg/mL; 1:200,000), dextrorphan caused 93% nociceptive blockade (% MPE) (Figure **2A** and Table **2**). The %MPE, full recovery time and AUCs of dextrorphan at the dose of ED_{50} with epinephrine were greater ($P < 0.001$) than dextrorphan (ED_{50}) alone in Table **2**.

Table 1: The 50% Effective Dose (ED_{50}) and ED_{95} of Dextrorphan as Skin Infiltrative Anesthesia

Drug	ED_{50} (95% CI)	ED_{95} (95% CI)
Dextrorphan	14.1 (13.1 – 15.5)	30.4 (29.2 – 32.0)

The ED_{50} and ED_{95} of dextrorphan (μmol/kg) were calculated and obtained from Figure **1**. CI = confidence interval.

Table 2: The Percent of Maximal Possible Effect (%MPE), Duration, and Area under the Curves (AUCs) of Coadministration of Epinephrine (1:200,000) with Dextrorphan at 50% Effective Dose (ED_{50}) or ED_{95}

	%MPE	Complete Blockade Time (min)	Time to Full Recovery (min)	AUCs (%min)
ED_{50}				
Dextrorphan (DOR)	50 ± 7	—	35 ± 3	1006 ± 267
DOR + Epinephrine	93 ± 3***	—	79 ± 7***	5183 ± 681***
ED_{95}				
Dextrorphan (DOR)	94 ± 4	—	54 ± 4	3005 ± 422
DOR + Epinephrine	100 ± 0	29 ± 5	78 ± 7*	5034 ± 434**

The %MPE, duration of action, AUCs for coadministration of epinephrine with dextrorphan or dextrorphan alone (mean±SEM) (n = 8 in each group). The symbol (*,**,***) indicates $P < 0.05$, $P < 0.01$, $P < 0.001$ when coadministration of epinephrine with drug compared with drug alone using 2-sided Student *t* test with unequal variances.

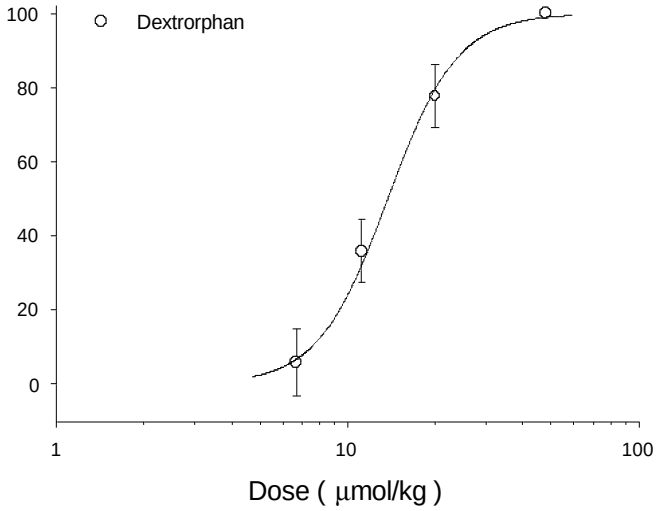

Figure 1: The dose-dependent curve of cutaneous anesthesia with dextrorphan (4 doses) in rats. Values are expressed as mean±S.E.M. For each group of the time course study, *n*=8 rats.

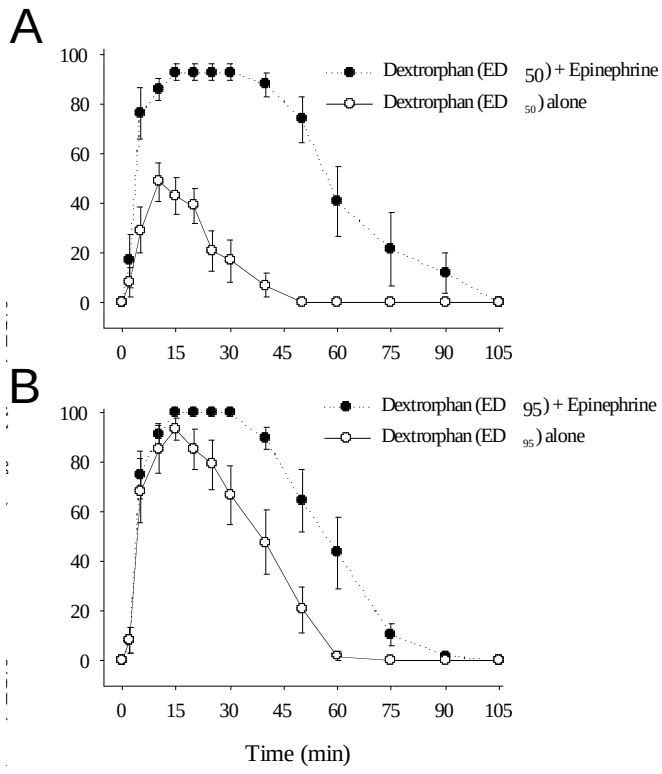

Figure 2: The addition of epinephrine with dextrorphan at ED_{50} (**A**) or ED_{95} (**B**) as infiltrative cutaneous analgesia in rats. Epinephrine was at the dose of 5 μg/mL. Values are expressed as mean±S.E.M.; n = 8 rats for each dose of each drug. The ED_{50} means 50% effective dose.

While dextrorphan at the dose of ED_{95} was co-injected with epinephrine (5 μg/mL; 1:200,000), complete sensory/nociceptive blockade (100% MPE) in dextrorphan (8 of 8 rats) group occurred (Figure **2B** and Table **2**). Compared with dextrorphan at the dose of ED_{95} alone, dextrorphan (ED_{95}) co-injected with epinephrine showed an increase in full recovery time and AUCs ($P < 0.05$; Table **2**). Also, neither intraperitoneal injection of dextrorphan at a dose of $2\times ED_{95}$ nor intraperitoneal injection of co-administration of epinephrine (5 μg/mL) and dextrorphan at ED_{95} produced cutaneous analgesia (data not shown). In our experiment, all rats recovered completely after subcutaneous injection.

DISCUSSION

We showed that dextrorphan elicited a local (skin) anesthetic effect in a dose-dependent fashion in rats. These results are in agreement with our previous experiments that dextrorphan exhibited skin infiltrative anesthesia [8-10]. Epinephrine as adjuvant for dextrorphan has a significant peripheral action in prolonging cutaneous analgesia in rats.

To obtain the ED_{50} or ED_{95}, we studied the dose-response curve of dextrorphan as infiltrative cutaneous analgesic, which is in agreement with that dextrorphan blocked Na^+ currents *in vitro* [6]. It has been shown that co-administration of 5 μg/mL epinephrine with lidocaine 1% exhibited as effective as the higher concentration of lidocaine alone at causing vasoconstriction for ear surgery [4]. In this experiment, combined administration of epinephrine (5 μg/mL) with dextrorphan (ED_{50} or ED_{95}) produces a prolonged analgesic effect. Injection of long-acting local anesthetics for surgery and postoperative pain control is frequently performed [19]. The addition of dextrorphan with epinephrine may be an option for long-term pain control after surgery while our results showed that epinephreine as adjuvant for dextrorphan had a prolonged cutaneous anesthesia in response to local dorsal cutaneous noxious pinprick in rats.

In summary, we exhibited that dextrorphan elicits local/cutaneous analgesia against skin nociceptive stimuli in a dose-related fashion. Co-administration of epinephrine and dextrorphan intensified and prolomged the cutaneous analgesic effect compared with dextrorphan alone.

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