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ORIGINAL ARTICLE

Naloxone prolongs cutaneous nociceptive block by lidocaine in rats

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ABSTRACT

We aimed to investigate the local anesthetic properties of naloxone alone or as an adjunct for the local anesthetic lidocaine. After the block of the cutaneous trunci muscle reflex (CTMR) with drugs delivery by subcutaneous infiltration, cutaneous nociceptive block was tested on the rats □ backs. We demonstrated that naloxone, as well as lidocaine, elicited cutaneous analgesia dose-dependently. The relative potency in inducing cutaneous analgesia was lidocaine [22.6 (20.1 – 25.4) μmol/kg] > naloxone [43.2 (40.3 – 46.4) μmol/kg] ($P < 0.05$). On an equianesthetic basis [50% effective dose (ED_{50}), ED_{25}, and ED_{75}], naloxone displayed a greater duration of cutaneous analgesic action than lidocaine ($P < 0.01$). Coadministration of lidocaine (ED_{95} or ED_{50}) and ineffective-dose naloxone (13.3 μmol/kg) intensifies sensory block ($P < 0.01$) with prolonged duration of action ($P < 0.001$) compared with lidocaine (ED_{95} or ED_{50}) alone or naloxone (13.3 μmol/kg) alone on infiltrative cutaneous analgesia. The preclinical data showed that naloxone is less potent than lidocaine as an infiltrative anesthetic, but its analgesic duration was longer than that of lidocaine. Furthermore, naloxone prolongs lidocaine analgesia, acting synergistically for nociceptive block.

INTRODUCTION

Naloxone, an opioid receptor antagonist, is currently considered an agent with a wide margin of safety (~10 mg) for a long clinical history of successful practice [1]. Furthermore, naloxone is administrated to combine with buprenorphine for the maintenance treatment (to help prevent the intravenous abuse) and for treating opioid-induced respiratory depression or (ultra) rapid detoxification in opioid-dependent patients [1]. In addition, naloxone is a competitive antagonist for the μ, κ, and δ opioid receptors and also blocks the action of σ-agonists at opioid sites [2,3]. Interestingly, naloxone blocks the sodium currents [4].

Essentially, the block of the voltage-dependent sodium channels, which is one of the molecular mechanisms of local anesthesia, succeeds spinal/epidural anesthesia, skin infiltration analgesia, and peripheral nerve blockade [5,6]. Moreover, surgery and postincisional pain controls are commonly practiced following the local anesthetic injections [7,8]. Unfortunately, this method is restricted by the short durations of anesthesia and analgesia [9]. The long-lasting local anesthetic bupivacaine is used for infiltration [10], but it indeed causes apparent cardiovascular toxicity [8,11]. Afterward epinephrine [12], clonidine [13], or naloxone [14] as an adjuvant was added to local anesthetic preparations for the purpose of prolonged anesthetic duration.

Based on a previous report that naloxone blocks sodium channels expressed in Xenopus oocytes [4], we hypothesized that naloxone may produce a local anesthetic effect. We aimed to investigate the local anesthetic effect of naloxone and its potential role as an adjunct to lidocaine in a rat model of infiltrative cutaneous analgesia. We demonstrated that naloxone produces dose-relatedly cutaneous/local anesthesia, while the addition of an ineffective dose of naloxone to lidocaine solution increases the quality and duration of subcutaneous infiltration of local anesthesia.

MATERIALS AND METHODS

Animals

Male adult Sprague Dawley rats weighing 220–270 g were obtained from the National Cheng Kung University (Tainan, Taiwan) and were housed in the Animal Housing Facilities. They were kept in clear plastic cages in groups of two under a 12-h light/dark cycle (light on at 7 AM) with constant temperature (22 °C) and humidity (50%) and free access to food and water throughout the studies. All experiments were approved *via* the animal investigation committee of the same university and were conducted in accordance with the policies and recommendations of the International Association for the Study of Pain (IASP). For consistency, a trained examiner, who was blinded to the treatment groups, was responsible for handling the rats and examining the local anesthetic effect.

Drugs

Naloxone HCl dihydrate and lidocaine HCl monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Drugs were freshly prepared in saline (0.9% NaCl) before subcutaneous injection.

Infiltrative cutaneous analgesia

Subcutaneous injection was performed as previously described [15,16]. After the rats' hairs on the dorsal surface of the thoracolumbar region (6 cm × 10 cm) were shaved mechanically, a total volume of 0.6 mL drug was subcutaneously injected using a 30-gauge needle (BD) in unanesthetized animals. A cut end of an 18-gauge needle was attached to the von Frey hair (No. 15; Somedic Sales AB, Stockholm, Sweden) that can execute a standardized pinprick (19 ± 1 g). Cutaneous analgesia was characterized by cutaneous trunci muscle reflex (CTMR) responses. CTMR was described as the reflex movement of the back skin produced by the lateral thoracic-spinal muscle twitches in response to the local dorsal skin pinprick [17,18]. After observing the rat's CTMR from the pinprick applied on the contralateral side and outside the wheal, six pinpricks were applied at six different sits within a wheal (a frequency of 2 Hz). The agent's cutaneous analgesia was assessed quantitatively as the number of times the stimulation failed to produce the CTMR. For example, the absence of any CTMR following six pinpricks was characterized as full sensory/nociceptive blockade (100% of possible effect; 100% PE), which was defined as followed:

$$\%\ \mathrm{PE} = ((\text{number of pinpricks that elicited no response})/6) \times 100\%$$

Each of the drug's action duration was recorded from injection (e.g., time=0) to the complete recovery of block (0% PE). The percentage of maximum possible effect (%MPE) was defined as the maximum blockade in the time course of drug □ s action. Furthermore, the area under the curve (AUC) of drug action was calculated by Kinetica version 2.0.1 (InnaPhase Corporation, Philadelphia, PA) [19,20]. The dose-related curves were obtained from the %MPE of each dose of each drug after animals were injected with different doses of each drug subcutaneously. Each curve was fitted *via* the SAS nonlinear (NLIN) procedures (version 9.1, SAS Institute, Cary, NC). The 50% effective dose (ED_{50}) was characterized as a dose that elicited 50% sensory block [21,22].

Experimental designs

Animals were randomly separated into three experiments ($n = 8$ rats for each dose of each group). In experiment 1, the potencies of naloxone (13.3, 26.7, 40.0, 53.3, 160.0 μmol/kg) and lidocaine (4.4, 13.3, 26.7, 36.7, 62.2 μmol/kg) in inducing cutaneous analgesia were performed. According to our previous study, lidocaine (9 μmol) produced 40% nociceptive/sensory block [23]. Therefore, we tested naloxone at 40.0 μmol/kg (9 μmol), and other doses were performed randomly. Then, the effects of naloxone at 160.0 μmol/kg, lidocaine at 62.2 μmol/kg, and saline (vehicle) were compared to each other. In experiment 2, on an equianalgesic basis (ED_{25}, $ED_{50,}$ and ED_{75}), the duration of naloxone was compared to that of lidocaine. The ED_{25}, $ED_{50,}$ and ED_{75} were obtained by the dose–response curves which was fitted *via* the SAS nonlinear (NLIN) procedures. In experiment 3, to assess the supra-additive effects of naloxone and lidocaine, the cutaneous analgesic effect of the mixture of

naloxone (13.3 μmol/kg) with lidocaine (ED_{50} or ED_{95}) was compared to naloxone (13.3 μmol/kg) alone or lidocaine (ED_{50} or ED_{95}) alone.

Statistical analyses

Data are presented as means±SEM. or EDs with 95% confidence interval (95% CI). All statistical analyses were performed using SPSS for Windows (version 17.0, SPSS, Inc, Chicago, IL, USA). The values were evaluated by 1-way or 2-way analysis of variance (ANOVA) followed by pairwise Tukey's honest significance difference (HSD) test or Student's *t*-test, and statistical significance was set at $P < 0.05$.

RESULTS

Cutaneous analgesia of infiltration with naloxone in a dose-related fashion

Naloxone, as well as the local anesthetic lidocaine, demonstrated a dose-related effect in producing cutaneous analgesia in rats (*Figure 1a*). The ED_{25}, ED_{50}, and ED_{75} of lidocaine and naloxone are shown in *Table I*. On an equianesthetic (ED_{50}) basis, the relative potency was lidocaine greater than naloxone ($P < 0.05$; *Table I*). Naloxone at 160 μmol/kg reached its peak effect (100% MPE; *Figure 1b*) with an action duration of about 88 ± 8 min (*Table II*), whereas lidocaine (62.2 μmol/kg) reached its peak effect (100% MPE; *Figure 1b*) with an action duration of about 59 ± 5 min (*Table II*). The AUC of naloxone (4803 ± 278) was greater than that of lidocaine (3771 ± 381) ($P < 0.05$; *Table II*). The subcutaneous injection of saline (vehicle) did not elicit cutaneous analgesia (*Figure 1b*). On an equipotent basis (ED_{25}, ED_{50}, and ED_{75}), the nociceptive/sensory block duration produced by naloxone was greater than that produced by lidocaine ($P < 0.01$; *Figure 2*).

Cutaneous analgesia of subcutaneous infiltration of lidocaine combined with ineffective-dose naloxone

Subcutaneous infiltration with naloxone at the dose of 13.3 μmol/kg did not provoke cutaneous analgesic action (*Figure 3*). After lidocaine at ED_{95} was coadministrated with naloxone (13.3 μmol/kg), complete block of CTMR responses (8 of 8 rats) appeared (*Figure 3*). The full recovery time and AUCs of lidocaine (ED_{95}) combined with naloxone (13.3 μmol/kg) were greater ($P < 0.001$) than lidocaine (ED_{95}) alone or naloxone (13.3 μmol/kg) alone (*Table III*). Coadministration of lidocaine (ED_{50}) and naloxone (13.3 μmol/kg) exhibited a better sensory block (85% MPE, $P < 0.01$; AUC 2359 ± 184, $P < 0.001$) than the same dose of lidocaine alone (50% MPE; AUC 804 ± 229) or the same dose of naloxone alone (0% MPE; AUC 0 ± 0) in *Table III*. All animals recovered fully after each subcutaneous infiltration.

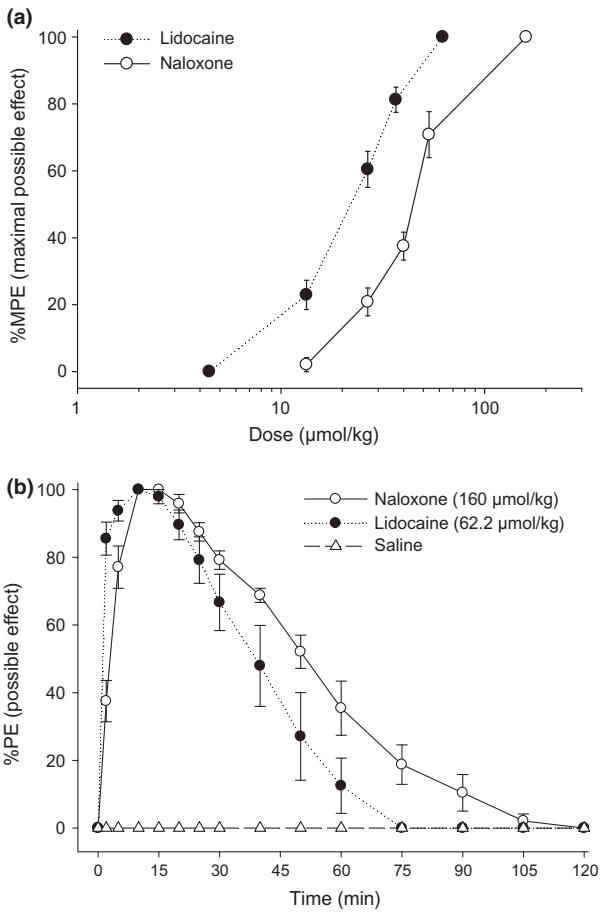

Figure 1 Inhibition of the cutaneous trunci muscle reflex (CTMR) in response to local skin pinpricks. *a*, the dose–response curves of lidocaine and naloxone at producing cutaneous analgesia. *b*, time courses of cutaneous analgesia of naloxone (160 μmol/kg), lidocaine (62.2 μmol/kg), and saline (vehicle). Subcutaneous injection of saline did not elicit cutaneous analgesic effect. Data are expressed as means±SEM. For each group of each study, $n = 8$ rats.

DISCUSSION

This present study for the first time demonstrated that naloxone provoked dose-dependent cutaneous analgesia. At the equipotent doses (ED_{75}, ED_{50}, and ED_{25}),

Table I The 25% effective doses (ED_{25}s), ED_{50}s, and ED_{75}s of lidocaine and naloxone.

Drug	ED_{25} (95% CI)	ED_{50} (95% CI)	ED_{75} (95% CI)	ED_{95} (95% CI)
Lidocaine	14.8 (14.4–15.5)*	22.6 (20.1–25.4)*	34.5 (34.0–35.4)*	50.3 (49.0–52.6)
Naloxone	31.2 (30.7–32.1)	43.2 (40.3–46.4)	59.8 (59.1–60.7)	–

The EDs (μmol/kg) were calculated from *Figure 1a*.

*$P < 0.05$ when compared to naloxone using a one-way ANOVA and pairwise Tukey's honest significance difference test for paired comparisons. CI = confidence interval.

Table II The area under the curves (AUCs), duration of action, and percent of maximal possible effect (%MPE) of lidocaine (62.2 μmol/kg), naloxone (160 μmol/kg), and saline (vehicle).

		Duration (min)		
	%MPE	Complete block time	Full recovery time	AUCs (% × min)
Naloxone	100 ± 0	12 ± 1	88 ± 8[b]	4803 ± 278[a]
Lidocaine	100 ± 0	14 ± 3	59 ± 5	3771 ± 381
Saline	–	–	–	–

Values (means±SEM) were obtained from *Figure 1b* ($n = 8$ rats for each dose of each drug). All the animals demonstrated full sensory/nociceptive block (100% MPE), whereas subcutaneous saline (vehicle) elicited no cutaneous analgesia. The symbols (a, b) indicate $P < 0.05$ and $P < 0.01$ compared to lidocaine.

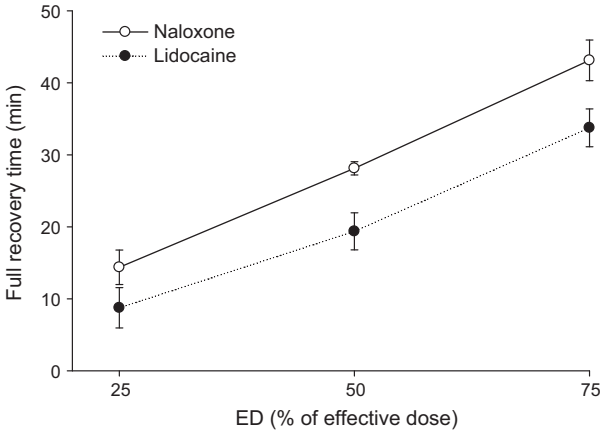

Figure 2 Full recovery time (duration) of drug's action on infiltrative cutaneous analgesia at 50% effective dose (ED_{50}), ED_{25}, and ED_{75} ($n = 8$ rats at each testing point). Data are expressed as means±SEM. The difference in duration was examined using 2-way ANOVA followed by pairwise Tukey's HSD test.

the duration of naloxone was greater than that of lidocaine in producing cutaneous analgesia. Coadministration of naloxone and lidocaine enhanced the cutaneous analgesic effect compared to either alone.

It has been well accepted that local anesthetics provide the neural block through blocking the voltage-gated Na^+ channels within the nervous tissues [24]. Naloxone has been shown to block voltage-gated Na^+ channels [25,26], and therefore it may produce the local anesthetic effect. We did reveal that naloxone elicited a local anesthetic effect as an infiltrative anesthetic. This is in resemblance to a previous experiment that naloxone displayed a local anesthetic property by blocking the single C-fibers in Sprague Dawley rats [27].

Naloxone, an opioid receptor antagonist, is presently used as a drug in the treatment of detoxification for a long clinical history of successful practice [1]. Naloxone is also currently acceptable as a subcutaneous, intravenous, or intramuscular injection [28]. In general, infiltration anesthesia of skin incision sites was performed by injections of local anesthetics for laparoscopic surgery [29] and vaginal hysterectomy or postoperative pain relief following inguinal hernia repair [30]. In the present study, we showed that subcutaneous infiltration of naloxone produced a dose-dependent cutaneous analgesic effect. Lidocaine has nearly 1.9-folds greater potency than naloxone in provoking cutaneous analgesia.

The feature of 2-chloroprocaine (chloroprocaine) is a candidate to replace lidocaine for short procedures because it is a local anesthetic agent with a very short biological half-life [31]. Therefore, we compared the block duration of naloxone with that of lidocaine. The resulting data showed that naloxone had a lesser potency compared with lidocaine at producing cutaneous analgesic action, but naloxone elicited a longer duration of sensory blockade. Furthermore, the cutaneous analgesic effect (AUC) of naloxone had

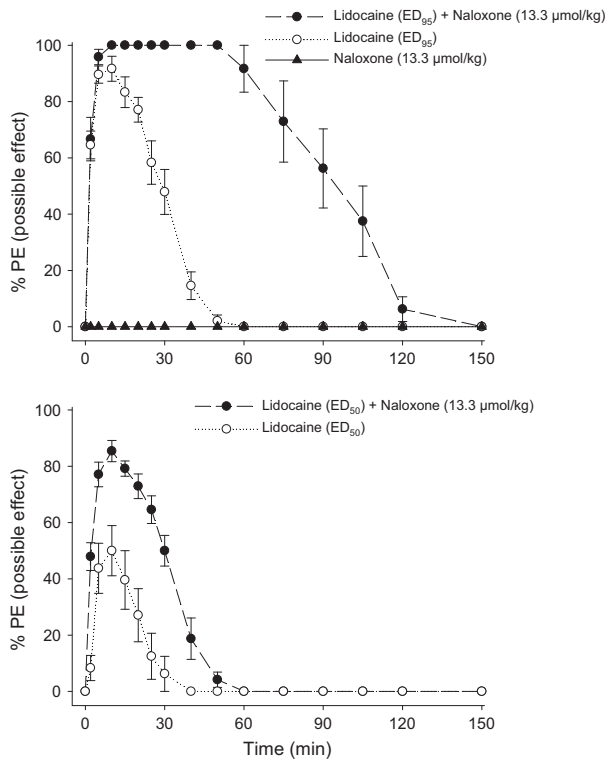

Figure 3 The time course of lidocaine (ED_{95} or ED_{50}) alone, naloxone (13.3 μmol/kg) alone, or coadministration of lidocaine (ED_{95} or ED_{50}) and naloxone (13.3 μmol/kg) on infiltrative cutaneous analgesia. Data are expressed as means±SEM. For each group of the time course study, $n = 8$ rats.

approximately 1.27 times higher than did lidocaine (*Table II*). Moreover, we also revealed that naloxone exhibited a longer duration of sensory/nociceptive block than lidocaine on an equipotent basis (ED_{75}, ED_{50} and ED_{25}) (*Figure 2*).

To deal with the short duration of anesthesia or analgesia [9], the long-acting local anesthetic bupivacaine [10] was employed or an adjuvant (i.e., epinephrine or clonidine) [12,13] was added to local anesthetic preparations. Interestingly, we showed that combined injection of the subthreshold-dose naloxone with lidocaine displays a supra-additive analgesic effect. In association with a recent study, adding a low dose of naloxone (100 ng) to 30 mL of 0.5% bupivacaine (150 mg) prolonged the duration of nerve block during the supraclavicular brachial plexus block [14]. The sensory block duration in patients received 30 mL bupivacaine with 100 ng naloxone was almost 1.6-folds longer than that in patients received 30 mL bupivacaine alone [14]. This dose of naloxone is less than 0.1% of that used in the present study. In our experiment, the value of AUCs in lidocaine (ED_{50}) combined with naloxone (13.3 μmol/kg) group was almost 2.9-folds higher than that in lidocaine (ED_{50}) group alone. We suggest that a higher dose naloxone provokes a better effect in prolong local anesthetic action.

Cumulative evidence shows that opioids induce inhibitory and/or excitatory modulation of action potentials in sensory neurons [32]. In human studies, a low dose of epidural naloxone enhances analgesia as well as reduces opioid side effects [33–35]. Interestingly, a larger dose of naloxone elicited hyperalgesia, whereas a small dose resulted in paradoxical analgesia [36–38]. In an emergency intra-operative setting, adding a low-dose naloxone intensified morphine analgesic effects by releasing endorphins or probably relocating endorphins from their receptors [39]. Furthermore, naloxone in low doses has been shown to enhance the analgesic effect of morphine probably owing to increasing the reuptake of excitatory amino acids from the synaptic cleft [40,41]. This phenomenon may elucidate the potential use of naloxone in human.

Table III The area under the curves (AUCs), duration of action, and percent of maximal possible effect (%MPE) of drug alone or the mixture of two drugs.

		Duration (min)		
	%MPE	Complete block time	Full recovery time	AUCs (%MPE × min)
Lidocaine (ED_{95}) + Naloxone (13.3 μmol/kg)	100 ± 0	65 ± 6	116 ± 9[b]	8706 ± 693[b]
Lidocaine (ED_{50}) + Naloxone (13.3 μmol/kg)	85 ± 4[a]	–	48 ± 3[b]	2359 ± 184[b]
Lidocaine (ED_{95})	94 ± 4	–	46 ± 3	2430 ± 115
Lidocaine (ED_{50})	50 ± 9	–	26 ± 3	804 ± 229
Naloxone (13.3 μmol/kg)	–	–	–	–

Data are expressed as means ± SEM. ($n = 8$ in each group) and constructed from *Figure 3*. The symbols (a, b) indicate $P < 0.01$ and $P < 0.001$ when lidocaine (ED_{95} or ED_{50}) alone compared with the coadministration of lidocaine (ED_{95} or ED_{50}) and naloxone (13.3 μmol/kg).

The limitation of this experiment is that naloxone has high affinity for opioid receptors. It could be investigated that the local anesthetic property of naloxone mediated by its affinity for the opioid receptor. Moreover, ED_{50} of naloxone for cutaneous analgesia is nearly 43 μmol/kg, which corresponds to nearly 15 mg/kg. This dose is extremely high, because we always administer naloxone of less than 1 mg to antagonize the effect of opioids. Systemic adverse effects induced by this high dose of naloxone or the possibility of neurotoxicity from naloxone administration remains an open question for further experiments. We cannot exclude the duration of local anesthesia from local tissue pathological damage; however, it is noteworthy that we did not detect behavioral abnormalities or apparent side effects after injection.

CONCLUSIONS

The preclinical data showed that naloxone provoked cutaneous analgesia dose-dependently. The block duration induced by naloxone was longer than that induced by lidocaine as an infiltrative anesthetic. Naloxone was useful as an adjuvant for the local anesthetic lidocaine at provoking cutaneous analgesia.

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CONFLICT OF INTERESTS

No conflict of interests reported.

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