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Abstract: The aim of this study was to evaluate the local anesthetic effect of nisoxetine on infiltrative cutaneous analgesia. After rats were injected subcutaneously with nisoxetine, dose—response curves were constructed. The cutaneous analgesic effect of nisoxetine or MK-801 (dizocilpine) was compared with lidocaine, a traditional local anesthetic. We found that nisoxetine and MK-801 acted like lidocaine and elicited dose-related cutaneous (local) analgesia. The relative potency was nisoxetine > MK-801 > lidocaine ($P < 0.01$) on infiltrative cutaneous analgesia. On an equianalgesic doses (20% effective dose [ED20], ED50, and ED80), nisoxetine produced longer action of cutaneous analgesia than that of lidocaine or MK-801 ($P < 0.01$). Coadministration of nisoxetine or lidocaine with MK-801 showed an additive effect on infiltrative cutaneous analgesia. Neither local injection of a large dose of nisoxetine, MK-801 or lidocaine in the thigh area produced cutaneous analgesia (data not shown). In conclusion, nisoxetine had a local anesthetic effect on infiltrative cutaneous analgesia with durations of actions longer than that of lidocaine or MK-801. That N-methyl-D-aspartate receptors may not contribute to the cutaneous (local) analgesic effect of nisoxetine or lidocaine.

Nisoxetine produces local but not systemic analgesia against cutaneous nociceptive stimuli in the rat

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ABSTRACT

The aim of this study was to evaluate the local anesthetic effect of nisoxetine on infiltrative cutaneous analgesia. After rats were injected subcutaneously with nisoxetine, dose—response curves were constructed. The cutaneous analgesic effect of nisoxetine or MK-801 (dizocilpine) was compared with lidocaine, a traditional local anesthetic. We found that nisoxetine and MK-801 acted like lidocaine and elicited dose-related cutaneous (local) analgesia. The relative potency was nisoxetine > MK-801 > lidocaine ($P < 0.01$) on infiltrative cutaneous analgesia. On an equianalgesic doses (20% effective dose [ED_{20}], ED_{50}, and ED_{80}), nisoxetine produced longer action of cutaneous analgesia than that of lidocaine or MK-801 ($P < 0.01$). Coadministration of nisoxetine or lidocaine with MK-801 showed an additive effect on infiltrative cutaneous analgesia. Neither local injection of a large dose of nisoxetine, MK-801 or lidocaine in the thigh area produced cutaneous analgesia (data not shown). In conclusion, nisoxetine had a local anesthetic effect on infiltrative cutaneous analgesia with durations of actions longer than that of lidocaine or MK-801. That *N*-methyl-D-aspartate receptors may not contribute to the cutaneous (local) analgesic effect of nisoxetine or lidocaine.

Key Words: nisoxetine; MK-801; lidocaine; additive effect; cutaneous analgesia

1. Introduction

Nisoxetine, a potent inhibitor of norepinephrine reuptake (Yokogawa et al., 2002), has been known to treat for affective disorders (Mongeau et al., 1997) and suppress the nicotine-evoked increase of hippocampal noradrenaline release in a dose-dependent manner (Bolden-Watson and Richelson, 1993; Wong et al., 1995) by influencing the function of nicotinic acetylcholine receptors (Hennings et al., 1999). In addition, nisoxetine shows an inhibition of the fast tetrodotoxin (full)-sensitive inward Na^+ currents in rat superior cervical ganglia (Hennings et al., 1999). The blockade of Na^+ channels is an essential activity of local anesthetics (Fozzard et al., 2005). With this activity, local anesthetics produce infiltrative cutaneous analgesia, spinal/epidural anesthesia, and peripheral neural blockades (Fozzard et al., 2005). Because nisoxetine has a Na^+ channel blocking effect (Hennings et al., 1999), theoretically, it may have a local anesthetic effect, e.g., cutaneous (local) analgesia. However, this was never tested.

The local anesthetic lidocaine is thought not only to block Na^+ channels (Yanagidate and Strichartz, 2007) but also to interact with various receptors (Muth-Selbach et al., 2009). Also, there is a study to show that lidocaine, a well studied Na^+ channel blocker, decreases experimental pain behaviors via NMDA receptors (Muth-Selbach et al., 2009). Therefore, the aim of this study was to

investigate the cutaneous (local) analgesic effect of nisoxetine when compared with lidocaine, a common used local anesthetic. Furthermore, a selective non-competitive NMDA antagonist (MK-801) was used to evaluate lidocaine- or nisoxetine-elicited analgesia involves actions at the NMDA receptors.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats 5–6 weeks of age (200-250g) were obtained from the National Laboratory Animal Centre, Taipei, Taiwan. Then animals were housed in groups of three, with food and water freely available until the time of testing. The climate controlled room maintained at 22 ℃with approximately 50% relative humidity on a 12-h light/dark cycle (6:00 AM–6:00 PM). The experimental protocols were approved by the Institutional Animal Care and Use Committee of China Medical University, Taiwan, and conformed to the recommendations and policies of the International Association for the Study of Pain (ISAP).

2.2. Drugs

Nisoxetine HCl, (+)-MK-801 hydrogen maleate, and lidocaine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were freshly prepared in saline (0.9% NaCl) as solution before drug injections.

2.3. The experimental protocol

Five experiments were carried out. In experiment 1, the dose-response curves of nisoxetine, MK-801, and lidocaine on infiltrative cutaneous analgesia were evaluated. In experiment 2, the cutaneous analgesic effect of nisoxetine was compared with that of MK-801 or lidocaine at the same dose of 3.00 μmol. In experiment 3, at equipotent

doses (ED_{20}, ED_{50}, and ED_{80}), the duration of drug action on infiltrative cutaneous analgesia was obtained and compared. In experiment 4, the cutaneous analgesic effect of coadministration of nisoxetine (1.50 μmol) and MK-801 (2.24 μmol) was compared with nisoxetine (1.50 μmol) or MK-801 (2.24 μmol) alone. The cutaneous analgesic effect of coadministration of lidocaine (6.05 μmol) and MK-801 (2.24 μmol) was compared with lidocaine (6.05 μmol) or MK-801 (2.24 μmol) alone. In experiment 5, one control group was further added into the study to rule out the possibility of systemic effect of drugs on infiltrative cutaneous analgesia. Rats (n=8 rats for each group) received subcutaneous injection of testing drug (nisoxetine, MK-801 or lidocaine) in the thigh area with a dose of $2ED_{80}$.

2.4. Infiltrative cutaneous analgesia

Before drug injection, rats were handled daily up to 7 days to domesticate them with the investigator, the experimental environment, and the specific experimental procedures. On the day before subcutaneous injections, the hair on the rats' dorsal surface of the thoracolumbar region (6×10 cm^2) was mechanically removed. Subcutaneous injections of drugs were performed as reported previously (Chen et al., 2011b; Chen et al., 2011c). In brief, the drugs were subcutaneously injected 0.6 mL via a 30-gauge needle in unanesthetized rats at the dorsal surface of the thoracolumbar region. After subcutaneous injection, a circular elevation of the skin, a wheal,

approximately 2 cm in diameter occurred. The wheal was marked with ink within 30 seconds after injection. For consistency, one experienced investigator who was blinded to the drugs injected was responsible for evaluating the cutaneous analgesic effect. The drugs were prepared and injected by another investigator.

2.5. Neurobehavioral evaluation

The cutaneous (local) analgesic effect was evaluated via the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back produced (Chen et al., 2011a; Hung et al., 2010). 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 perform the standardized nociceptive stimulus (19±0.5 g). After observing an animal's normal reaction to pinpricks applied outside the wheal and on the contralateral side, we applied six pinpricks with a frequency of 0.5-1.0 Hz inside the wheal and scored the number to which the rat failed to react. The cutaneous anesthetic effect of each drug was evaluated quantitatively as the number of times the pinprick failed to elicit a response, with, for example, the complete absence of six responses was defined as complete nociceptive block (100% of possible effect; 100% PE). The test of six pinpricks was applied 5 min before drug injection, 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. The maximum value of PE was presented as percent

of maximal possible effect (% MPE) during the test. The duration of action of each drug was defined as the time from drug injection (i.e., time=0) to full recovery of CTMR (no anesthetic effect was found or 0% MPE recorded) (Chen et al., 2008).

2.6. The 50% effective dose (ED_{50})

After rats were injected with 4-5 different doses of each drug (n = 8 for each dose of each drug) subcutaneously, dose-response curves were constructed. The curves were then fitted using SAS Nonlinear (NLIN) Procedures (version 9.1; SAS Institute, Cary, NC), and the values of ED_{50}, defined as the doses that caused 50% blockades, were obtained (Chen et al., 2010; Leung et al., 2010). The ED_{20} and ED_{80} of drugs were obtained using the same curve fitting (SAS Nonlinear analysis) that was used to derive the ED_{50}. Furthermore, the area under curves (AUCs) of nociceptive/sensory blockades of drugs was estimated using Kinetica version 2.0.1 (InnaPhase Corporation, Philadelphia, PA).

2.7. Statistical analysis

Data are presented as mean ± SEM or ED_{50} values with 95% confidence interval (95% CI). The differences in potencies (ED_{50}s) (Table 1) between medications and the full recovery time, %MPE, and AUCs of drugs (Table 2) were evaluated by 1-way analysis of variance (ANOVA) and then the pairwise Tukey's honestly significant difference (HSD) test. The differences in durations (Fig. 3) among drugs were

evaluated by 2-way ANOVA followed by the pairwise Tukey's HSD test. SPSS for Windows (version 17.0) was used for all statistical analyses. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Dose-dependent effects of nisoxetine, MK-801, and lidocaine on infiltrative cutaneous analgesia

The nisoxetine and MK-801, as well as local anesthetic lidocaine produced dose-dependent effects of cutaneous analgesia in rats (Fig. 1). The ED_{50}s of drugs are shown in Table 1. The relative potency of these drugs was found to be nisoxetine > MK-801 > lidocaine (P<0.01 for the differences between drugs; Table 1). All rats recovered completely after each subcutaneous injection.

3.2. The cutaneous analgesic effects of nisoxetine, MK-801, and lidocaine

Nisoxetine at the dose of 3.0 μmol showed 96% of blockades (% MPE) with duration of action of about 146 min (Fig. 2). At the same given dose, MK-801 elicited 65% of blockades (% MPE) with duration of action of about 22 min. Lidocaine at 3.0 μmol displayed 10% of blockades (% MPE) with duration of action of about 4 min. The full recovery time and AUCs of cutaneous analgesic effect of nisoxetine are significantly greater than those of lidocaine or MK-801 (P<0.001 for the differences between drugs; Fig. 2 and Table 2).

On an equipotent basis (ED_{20}, ED_{50}, and ED_{80}), the blockade duration for nisoxetine was longer than that for lidocaine or MK-801 on infiltrative cutaneous analgesia (P<0.01 for the differences between drugs; Fig. 3). Also, subcutaneous

injection of drugs ($2ED_{80}$) in the thigh area produced no cutaneous analgesia, sedation or loss of motor activity (data not shown).

3.3. Co-administration of nisoxetine or lidocaine with MK-801

The co-administration of nisoxetine with MK-801 produced similar %MPE to the aggregate of nisoxetine alone and MK-801 alone on infiltrative cutaneous analgesia (Fig. 4A and Table 3). The co-administration of lidocaine with MK-801 also showed similar results (Fig. 4B and Table 3). These results reported that co-administration of nisoxetine or lidocaine with MK-801 produced an additive effect on infiltrative cutaneous analgesia (Tallarida, 2001).

4. Discussion

Our study showed that nisoxetine, MK-801, and lidocaine elicited dose-related cutaneous (local) analgesia. Nisoxetine was more potent and longer drug action at producing cutaneous analgesia than lidocaine or MK-801. Coadministration of MK-801 with nisoxetine or lidocaine displayed an additive effect on infiltrative cutaneous analgesia.

Lidocaine is a local anesthetic agent that produces neural blockade via a direct blocking effect on the voltage-gated Na^+ channels of the nervous tissues (Fozzard et al., 2005; Yanagidate and Strichartz, 2007). Because nisoxetine has a Na^+ channel blocking effect (Hennings et al., 1999), theoretically it may have a local anesthetic effect. In this study, we did find that nisoxetine has a local anesthetic effect on infiltrative cutaneous analgesia in rats. Furthermore, MK-801, a potent non-competitive antagonist of the N-methyl-d-aspartate (NMDA) receptor, elicited dose-related cutaneous analgesia, and we speculated that the local anesthetic property of MK-801 is due to its characteristic of Na^+ channel blockade (Halliwell et al., 1989).

We showed that nisoxetine, lidocaine, and MK-801 have local anesthetic effects as infiltrative cutaneous analgesia. Furthermore, nisoxetine was more potent at producing cutaneous analgesia when compared with lidocaine or MK-801 (Fig. 1. and

Table 1). Nisoxetine had almost 1.5- and 4.0-folds higher potency than MK-801 and lidocaine on infiltrative cutaneous analgesia, respectively. Combined administration of a dose (ED_{50}) of MK-801 with nisoxetine or lidocaine displays an additive analgesic effect. The cutaneous analgesic effects of adding MK-801 to nisoxetine or lidocaine are similar to the combinations of other anesthetics with nisoxetine or lidocaine. Our study suggested that these drugs act in a similar manner, for example Na^+ channel blockades.

The long-acting local anesthetics are frequently practiced for surgery and the management of postoperatic pain (Hung et al., 2009; Job et al., 1979). In this study, nisoxetine produced longer duration of action than lidocaine or MK-801 at the same dose of 3.0 μmol (Fig. 2 and Table 2). Additionally, the duration of action caused by nisoxetine was longer than that caused by lidocaine or MK-801 at equianalgesic doses (ED_{20}, ED_{50}, and ED_{80}) (Fig. 3). An extra experiment was added to the study to rule out the possibility of systemic effects by drugs. Systemic administration of a large dose of the test drugs produced no cutaneous analgesia. These results supported the local action of testing drugs on skin and nisoxetine elicits local (cutaneous) but not systemic analgesia.

Meanwhile, it remains unclear whether nisoxetine cause toxicity to the subcutaneous or neuronal tissues. In this study, all rats recovered completely after

experiments. Our data suggest that nisoxetine may have features that make it a valuable alternative cutaneous analgesia, although the possibility of nerve (tissue) damage from nisoxetine injection remains an open question for further investigations.

This preclinical study reported that nisoxetine had a local anesthetic effect as infiltrative cutaneous analgesia in rats. Nisoxetine elicited more potent and longer action than lidocaine or MK-801 in providing cutaneous analgesia. NMDA receptors did not involve in the cutaneous analgesic effect of nisoxetine or lidocaine.

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Table 1. The 50% effective doses (ED_{50}s), ED_{20}s, and ED_{80}s of nisoxetine, MK-801, and lidocaine on infiltrative cutaneous analgesia in rats

Drug	ED_{20} (95% CI)	ED_{50} (95% CI)	ED_{80} (95% CI)
Nisoxetine	0.47 (0.38–0.57)	1.50 (1.40–1.63)	2.73 (2.54–3.02)
MK-801	1.20 (1.10–1.33)	2.24 (2.13–2.36)	4.37 (4.16–4.78)
Lidocaine	3.51 (3.35–3.79)	6.05 (5.83–6.43)	9.35 (9.18–9.66)

ED_{50}s of drugs (μmol) were obtained from Figure 1. CI = confidence interval. The potency of drug (ED_{50}) was nisoxetine > MK-801 > lidocaine (P<0.01, for each comparison).

Table 2. The percent of maximal possible effect (%MPE), time to full recovery, area under curves (AUCs) of drugs on infiltrative cutaneous analgesia in rats

Drug	%MPE	Time to full recovery	AUCs (%min)
Nisoxetine	96±4***	146±25***	8126±1729***
MK-801	65±5†††	22±3†††	775±164†††
Lidocaine	10±7	4±2	60±40
Saline	–	–	–

The %MPE, duration, and AUCs for nisoxetine, MK-801, and lidocaine (mean±SEM) at the same dose of 3.00 μmol (n = 8). Saline group was used as a control. Symbols (***) indicate $P < 0.001$ when nisoxetine compared with lidocaine or MK-801. Symbols (†††) indicate $P < 0.001$ when MK-801 compared with lidocaine.

Table 3. The percent of maximal possible effect (%MPE) of co-administration of nisoxetine or lidocaine with MK-801 on infiltrative cutaneous analgesia in rats

	%MPE
Nisoxetine with MK-801	
Nisoxetine	50±12
Nisoxetine+MK-801	86±4
MK-801	56±7
Lidocaine with MK-801	
Lidocaine	52±11
Lidocaine+MK-801	96±2
MK-801	50±10

Values are mean±SEM. The doses for injections were ED_{50} (50% effective dose) for a single drug or ED_{50} for drugs in combination. The values of % MPE were derived from Fig. 4A and B after calculation.

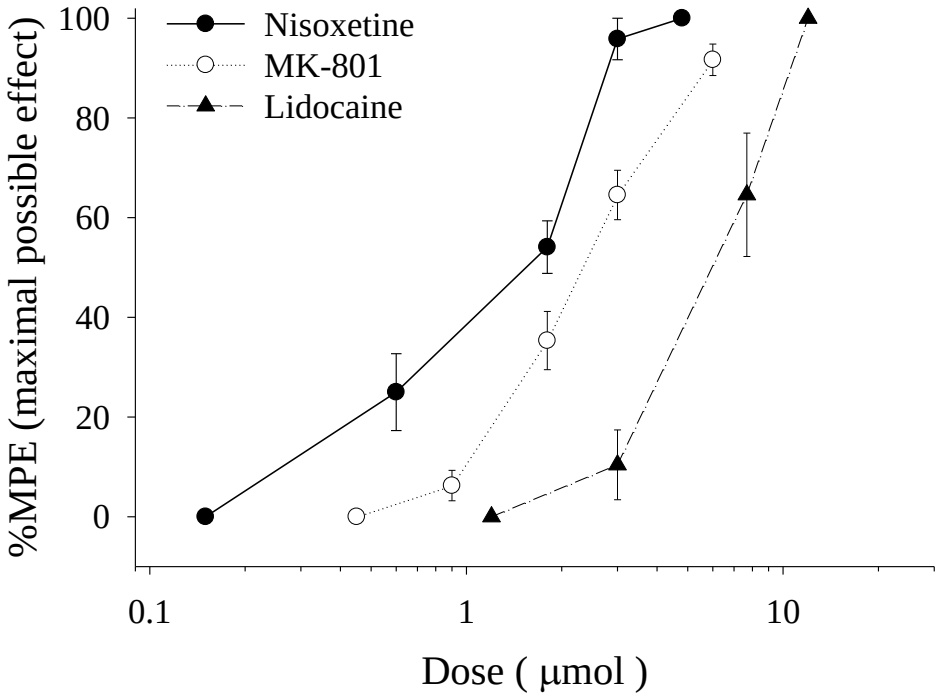

%MPE (maximal possible effect)
100
80
60
40
20
0
Nisoxetine
MK-801
Lidocaine
0.1
1
10
Dose (μmol)

Fig. 1.

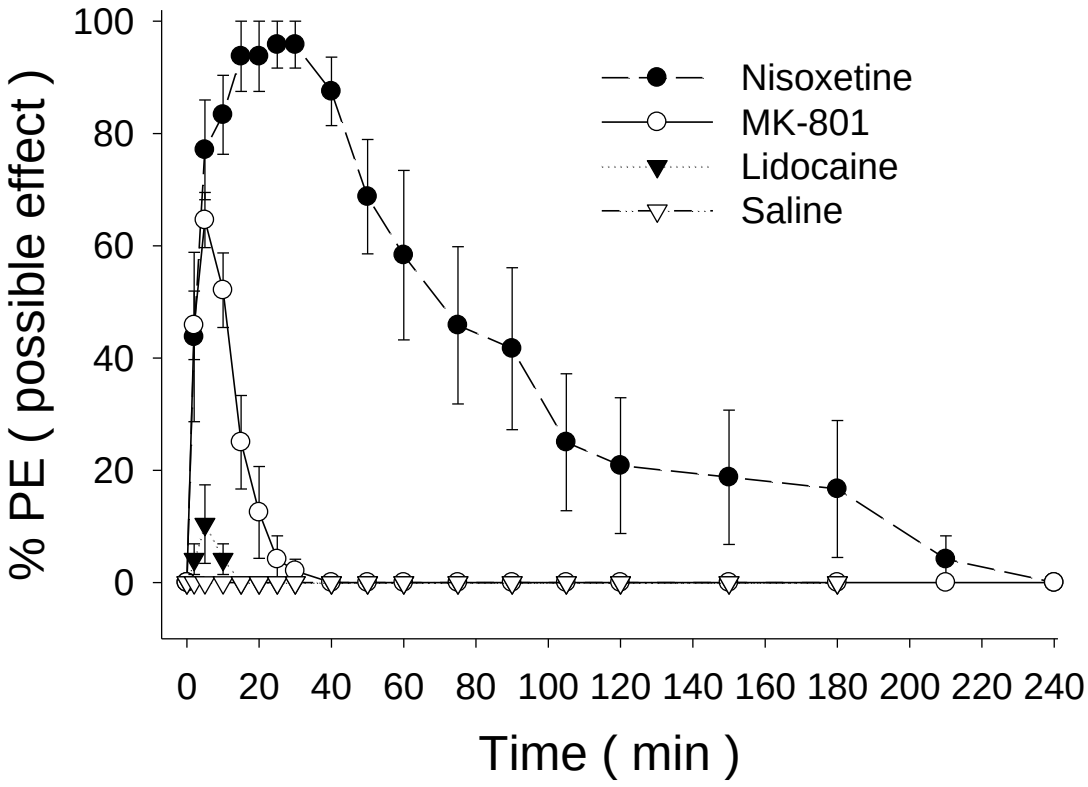

% PE (possible effect)
100
80
60
40
20
0
0 20 40 60 80 100 120 140 160 180 200 220 240
Time (min)
Nisoxetine
MK-801
Lidocaine
Saline

Fig. 2.

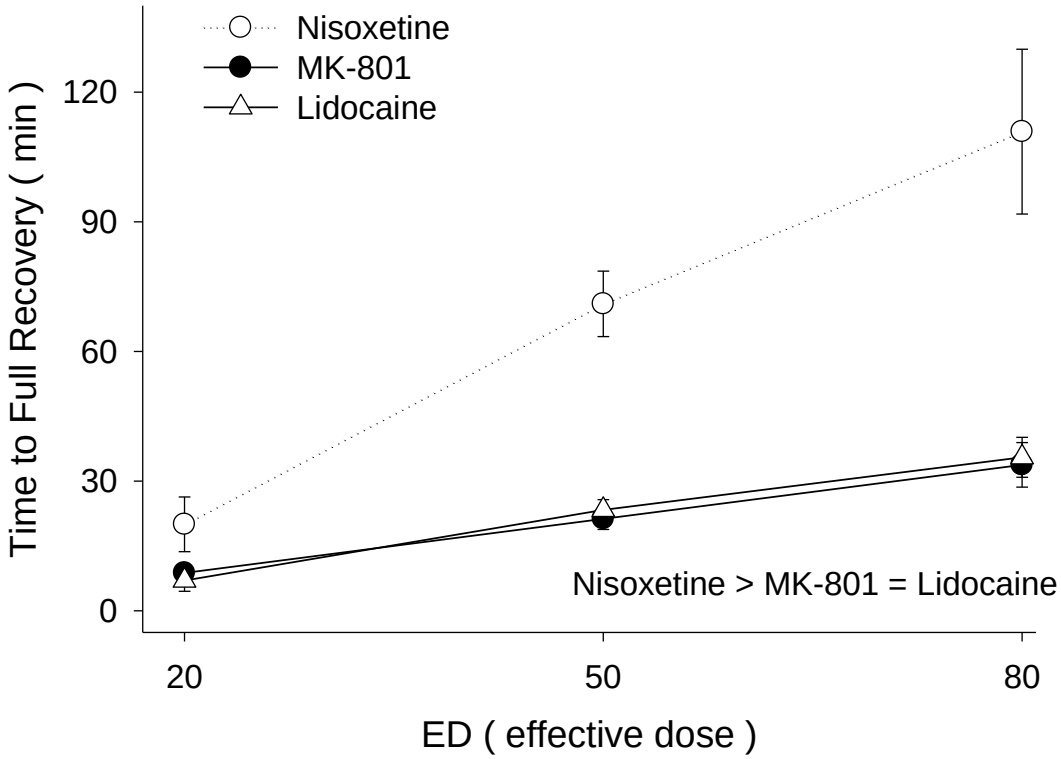
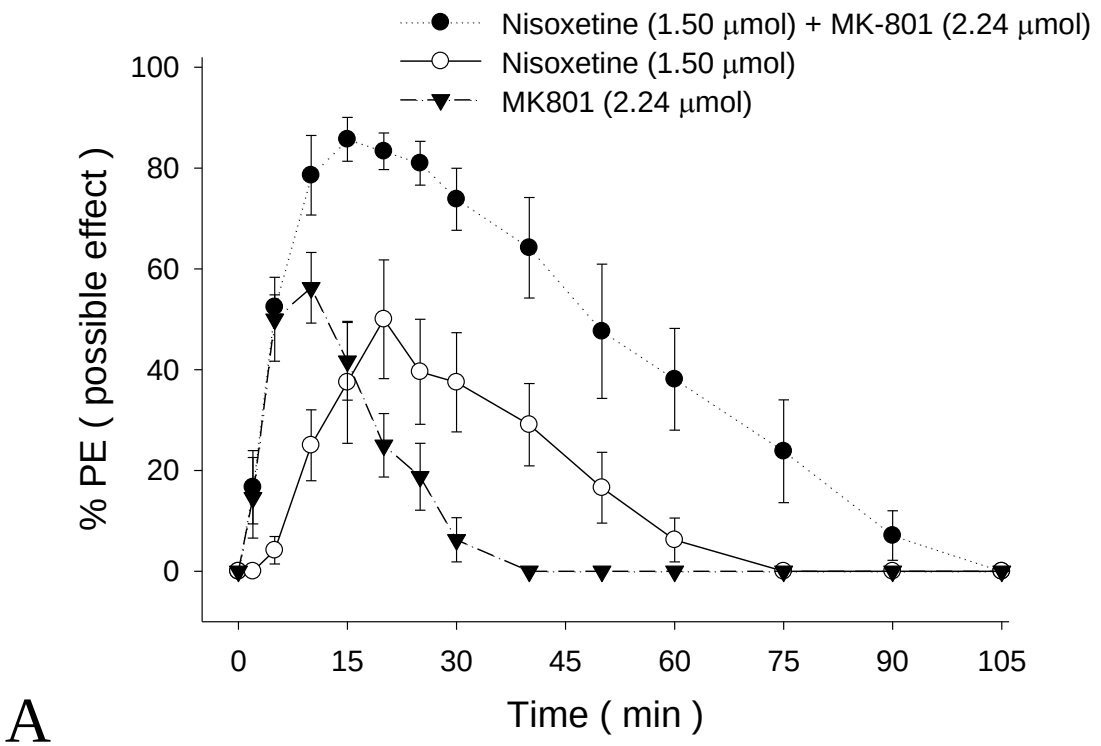
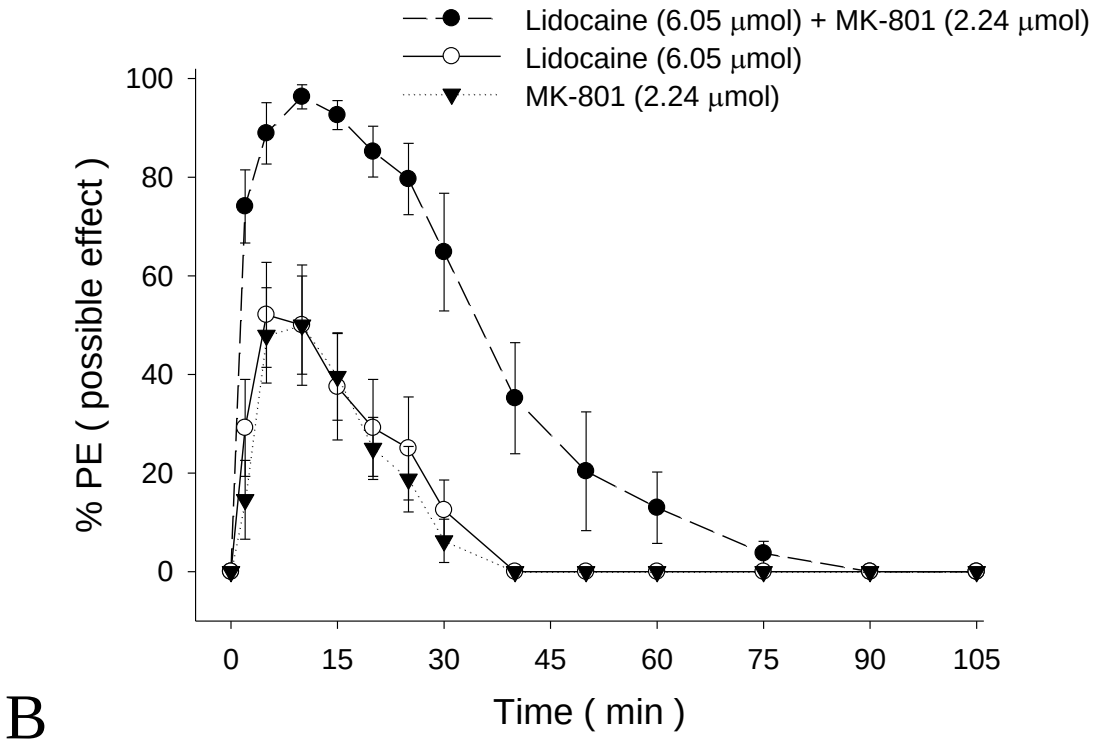

Nisoxetine
MK-801
Lidocaine
Time to Full Recovery (min)
120
90
60
30
0
20
50
80
ED (effective dose)
Nisoxetine > MK-801 = Lidocaine

Fig. 3.


Nisoxetine (1.50 μmol) + MK-801 (2.24 μmol)
Nisoxetine (1.50 μmol)
MK801 (2.24 μmol)
% PE (possible effect)
Time (min)
0
20
40
60
80
100
0
15
30
45
60
75
90
105

A


Lidocaine (6.05 μmol) + MK-801 (2.24 μmol)
Lidocaine (6.05 μmol)
MK-801 (2.24 μmol)
% PE (possible effect)
Time (min)
0
20
40
60
80
100
0
15
30
45
60
75
90
105

B

Fig. 4.

Figure Legends

Fig. 1. The dose—response curves of nisoxetine, MK-801, and lidocaine on infiltrative cutaneous analgesia in rats (*n* = 8 at each testing point). Data are shown as mean±SEM.

Fig. 2. Time courses of cutaneous analgesia of nisoxetine, MK-801, and lidocaine at the same dose of 3.0 μmol in rats. The saline group is as the control. Values are expressed as mean±SEM. Each testing point of the time course study contained eight rats.

Fig. 3. Time to full recovery (duration) of drug effect on infiltrative cutaneous analgesia at doses of ED_{20}, ED_{50}, and ED_{80} (*n* = 8 at each testing point). Data are mean±SEM. The differences in duration were evaluated using 2-way ANOVA and then the pairwise Tukey's HSD test.

Fig. 4. The time course (A) of nisoxetine at 1.50 μmol, MK-801 at 2.24 μmol or coadministration of nisoxetine at 1.50 μmol and MK-801 at 2.24 μmol on infiltrative cutaneous analgesia in rats. The time course (B) of lidocaine at 6.05 μmol, MK-801 at 2.24 μmol or coadministration of lidocaine at 6.05 μmol and MK-801 at 2.24 μmol on infiltrative cutaneous analgesia in rats. Values are expressed as mean±SEM. For each group of the time course study, n=8 rats.