Cutaneous analgesia and systemic toxicity of carbetapentane and caramiphen in rats

Ching-Hsia Hung[a] , Ph.D., Chin-Chen Chu[b], M.D., Ph.D., Yu-Chung Chen[c], M.S., Kuo-Sheng Liu[d], Ph.D., Yu-Wen Chen[b,e,]*, Ph.D., Jhi-Joung Wang[b], M.D., Ph.D.

[a] Institute & Department of Physical Therapy, National Cheng Kung University, Tainan, Taiwan;

[b] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan;

[c] Division of Physical Therapy, Department of Physical Medicine and Rehabilitation, Cheng Hsin General Hospital, Taipei, Taiwan;

[d] Graduate Institute of Pharmaceutical Science, Chia Nan University of Pharmacy & Science, Tainan, Taiwan;

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

1. **Ching-Hsia Hung, Ph.D.**

   Title: Associate Professor

   Affiliation: National Cheng Kung University

   Email: chhung@mail.ncku.edu.tw

   Role: This author helped design the study, conduct the study, and analyze the data

   Conflicts: Ching-Hsia Hung reported no conflicts of interest

   Attestation: Ching-Hsia Hung has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

2. **Chin-Chen Chu, M.D., Ph.D.**

   Title: Assistant Professor

   Affiliation: Chi-Mei Medical Center

   Email: chinchen.chu@gmail.com

   Role: This author helped conduct the study and analyze the data

   Conflicts: Chin-Chen Chu reported no conflicts of interest

   Attestation: Chin-Chen Chu has seen the study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for

archiving the study files

3. Yu-Chung Chen, M.S.
   Title: P.T.
   Affiliation: Cheng Hsin General Hospital
   Email: kogochen@yahoo.com.tw
   Role: This author helped design the study, conduct the study, and analyze the data
   Conflicts: Yu-Chung Chen reported no conflicts of interest
   Attestation: Yu-Chung Chen has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

4. Kuo-Sheng Liu, Ph.D.
   Title: Assistant Professor
   Affiliation: Chia Nan University of Pharmacy & Science
   Email: lanceliu@mail.chna.edu.tw
   Role: This author helped conduct the study and analyze the data
   Conflicts: Kuo-Sheng Liu reported no conflicts of interest
   Attestation: Kuo-Sheng Liu has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

5. Yu-Wen Chen, Ph.D.
   Title: Associate Professor
   Affiliation: China Medical University (current affiliation: Chi-Mei Medical Center)
   Email: cywhwok@mail.cmu.edu.tw
   Role: This author helped design the study, conduct the study, analyze the data, and write the manuscript
   Conflicts: Yu-Wen Chen reported no conflicts of interest
   Attestation: Yu-Wen Chen has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

6. Jhi Joung Wang, M.D., Ph.D.
   Title: Professor
   Affiliation: Chi-Mei Medical Center

Email: 400002@mail.chimei.org.tw

Role: This author helped conduct the study and analyze the data

Conflicts: Jhi Joung Wang reported no conflicts of interest

Attestation: Jhi Joung Wang has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files

* Address correspondence and reprint requests to: Yu-Wen Chen, PhD, Department of Physical Therapy, China Medical University, No.91 Hsueh-Shih Road, Taichung 40402, Taiwan

Tel: 886-4-22053366 ext 7327

Fax: 886-4-22065051

E-mail: cywhwok@mail.cmu.edu.tw

ABSTRACT

Background: Although caramiphen produces spinal anesthesia, caramiphen and carbetapentane have never been tested as infiltrative cutaneous analgesia. The aim of this study was to compare cutaneous analgesia of caramiphen and carbetapentane with bupivacaine and evaluated their central nervous system (CNS) and cardiovascular (CV) toxicity.

Methods: After the blockade of cutaneous trunci muscle reflex with subcutaneous drug injections in rats, we evaluated the local anesthetic effect of carbetapentane and caramiphen on infiltrative cutaneous analgesia. Following continuous intravenous infusion of equipotent doses of bupivacaine, carbetapentane, caramiphen, and saline, we observed mean arterial blood pressure (MAP) and heart rate (HR) and monitored the onset time of seizure, apnea, and impending death.

Results: Carbetapentane and caramiphen acted like bupivacaine and elicited cutaneous analgesia in a dose-related fashion. On a 50% effective dose (ED_{50}) basis, the ranks of potencies were bupivacaine (1.78 [1.52 – 2.07]) > carbetapentane (2.53 [2.38 – 2.77]) > caramiphen (3.60 [3.41 – 3.99]) ($P < 0.01$). At equianalgesic doses (ED_{25}, ED_{50}, ED_{75}), the duration caused by carbetapentane or caramiphen was similar to that caused by bupivacaine. Under equipotent doses, the infusion time of carbetapentane or caramiphen required to cause seizure, apnea, and impending death

was longer than that of bupivacaine ($P < 0.05$). The decline in MAP and HR was slower with carbetapentane or caramiphen when compared with bupivacaine ($P < 0.01$ for the differences) at equipotent doses.

Conclusions: Carbetapentane and caramiphen were similar to bupivacaine at producing durations of cutaneous analgesia but were less likely than bupivacaine to induce CNS and CV toxicity.

Key words: Carbetapentane, Caramiphen, Bupivacaine, Infiltrative cutaneous analgesia, Systemic toxicity

INTRODUCTION

Carbetapentane, a non-opioid antitussive agent, has been known to have atropine-like and effectively suppresses acute cough due to common upper respiratory infections.[1] Another known non-opioid antitussive, caramiphen, was first introduced into the therapy for diseases of the basal ganglia [2] and was available as an antitussive agent in Europe since 1950.[3] Recently, it has been shown that the antagonism of N-Methyl-D-aspartate receptor activation and the facilitation of $GABA_A$ receptor activation by caramiphen in the basolateral amygdale may play an important role in the anticonvulsive and neuroprotective properties of caramiphen. [4] In addition, caramiphen has been shown to have a local anesthetic effect on spinal anesthesia in rats.[5]

The injections of local anesthetics are used for infiltration anesthesia of skin incision sites for laparoscopic surgery [6] and to provide postoperative pain relief after vaginal hysterectomy and inguinal hernia repair.[7] However, the technique is limited by the short duration of analgesia or anesthesia.[8] For this reason, bupivacaine is chosen for infiltration anesthesia because of its longer duration of effective analgesia.[9] Recently, we showed that the long duration caused by caramiphen [5] was similar to that caused by bupivacaine [10] on spinal anesthesia in rats. Furthermore, carbetapentane and caramiphen, two non-opioid antitussive agents, have similar

chemical structures. However, cutaneous analgesia following subcutaneous injection of carbetapentane and caramiphen has not been evaluated.

The local anesthetics, despite physical or chemical differences, all have central nervous system (CNS) toxicity and cardiovascular (CV) toxicity.[10-13] Although some of them may have less toxicity to the CNS or CV system, however, the differences are minor. This may be explained due to their similar chemical structures.[11] Before carbetapentane and caramiphen are applied in clinical practice, the toxicity of these drugs should be tested. There are no studies evaluating the systemic toxicity of carbetapentane and caramiphen; it is known that bupivacaine induces significant CV toxicity.[14,15] In this study, we compared cutaneous analgesia of carbetapentane and caramiphen with that of bupivacaine. Furthermore, we also evaluated the systemic toxicity of drugs by infusing equipotent doses of these three drugs.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighting 240-290g were obtained from the National

Laboratory Animal Centre (Taipei, Taiwan), and then were housed in groups of three,

with food and water freely available until the time of the study. The room temperature

was controlled at 22℃ with approximately 50% humidity and a 12-h light/dark cycle

(6:00 a.m. – 6:00 p.m.). The experimental procedures 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).

Drugs

Carbetapentane citrate salt and bupivacaine HCl were purchased from

Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Caramiphen edisylate was

purchased from Rarechemical Co. (R288810, USA). All drugs were freshly solved in

saline (0.9% NaCl) before the subcutaneous injections or intravenous infusion.

Experimental protocols

The protocol was divided into two parts. In Part I, the cutaneous analgesic effect

of different doses of bupivacaine (7.0, 4.0, 2.7, 2.0, 1.6, 1.3, 0.8 $\mu mol \cdot kg^{-1}$),

carbetapentane (10.1, 8.0, 5.3, 2.7, 2.0, 1.3, 1.1 $\mu mol \cdot kg^{-1}$), and caramiphen (16.0,

13.3, 8.0, 5.3, 2.7, 2.0, 1.3 $\mu mol \cdot kg^{-1}$) was performed (n = 8 rats for each dose of each drug). According to the dose-response curves, bupivacaine at 8.0 $\mu mol \cdot kg^{-1}$, carbetapentane at 11.4 $\mu mol \cdot kg^{-1}$, and caramiphen at 16.0 $\mu mol \cdot kg^{-1}$ also tested to determine the equivalent potencies of these drugs; meanwhile, the full recovery time (duration) of carbetapentane, caramiphen, and bupivacaine was evaluated at equianalgesic doses (ED_{25}, ED_{50}, ED_{75}) (n = 8 for each dose of each drug). In Part II, time to cause toxicity (seizures, apnea, and cardiac arrest), mean arterial blood pressure (MAP), and heart rate (HR) were evaluated after equipotent doses of the drugs (bupivacaine, carbetapentane, and caramiphen) were infused into the rat (n = 8 rats for each dose of each drug). Saline group (n = 8 rats) was used as a control.

Part I - Infiltrative cutaneous analgesia

Subcutaneous injection and neurobehavioral examinations

A trained examiner who was blinded to the experimental groups was responsible for handling of animals and behavioral examinations. Infiltrative cutaneous analgesia was evaluated by the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back elicited by twitches of the lateral thoracospinal muscle in response to local dorsal cutaneous stimulation after drug injections.[16] In brief, the hair on the rats' dorsal surface of the thoracolumbar region (6 cm×10 cm) was mechanically shaved on the day before subcutaneous injection, and

then each drug solved in 0.6 ml saline was injected subcutaneously using a 30-gauge

needle into a naïve area of the shaved back of the un-anesthetized rats.[17,18] The wheal

within 2 cm diameter was marked with ink within 1 min after subcutaneous injection.

The cut end of an 18-gauge needle (a fresh regular bevel needle) was affixed to a von

Frey filament (no. 15; Somedic Sales AB, Stockholm, Sweden) to produce a

standardized noxious punctate mechanical stimulus (19±1 g) without making tissue

damage.

After observing a rat's normal reaction to stimuli applied outside the wheal and

on the contralateral side, we applied 6 stimuli at 6 different points within each wheal,

with a frequency of 0.5 to 1 Hz, and scored the number to which the rat failed to react.

The magnitude of cutaneous analgesia was described as the percent of possible effect

(% PE). For example, the absence of any response after 6 stimuli was defined as

complete nociceptive block (100% PE), which was calculated as follows:

% PE = (number of stimuli that provoked no response/6) ×100%

During the drug action, the maximum value of % PE was presented as percent of

maximal possible effect (% MPE). Each duration (full recovery time) of drug action

was defined as the time from drug injection (i.e., time=0) to full recovery of CTMR

(no analgesic effect or 0% MPE).[19]

The 50% effective dose (ED_{50}), ED_{25}, and ED_{75}

After injecting subcutaneously the rats with 7 different doses of each drug ($n = 8$

for each dose of each drug), dose-response curves were constructed. Then the curves

were fitted by SAS NLIN Procedures (SAS Institute Inc., Carey, NC, version 9.1),

and the value of 50% effective dose (ED_{50}), defined as the dose that caused 50%

cutaneous analgesia, was obtained.[20,21] The ED_{25} and ED_{75} of drugs were obtained by

the same SAS NLIN Procedures that were used to derive the ED_{50}.[22] Then the

blockade duration caused by each drug was performed at equianalgesic doses (ED_{25},

ED_{50}, ED_{75}) ($n = 8$ rats for each dose of each drug). Furthermore, the area under

curves (AUCs) of sensory blockades of drugs was estimated using Kinetica version

2.0.1 (InnaPhase Corporation, Philadelphia, PA).

Part II - Cardiovascular and neurological effects

On Day 1, rats were anesthetized with pentobarbital sodium (i.p.) at the dose of

50 mg·kg^{-1} and the right femoral artery and vein were cannulated with polyethylene

catheters (PE-50), which were filled with heparin saline (35 U/mL). The free end of

the catheter was threaded through a 18-gauge needle and then tunneled

subcutaneously. The catheter was cut with 5 cm protruding from the skin at the

midline in the posterior cervical area and sealed by heating it with a match and

compressing it with a hemostat.[17,23]

On Day 2, the rats were placed in a small cage with an open top to allow the

lines to reach the animal and prevent the animal from chewing on the lines. The tube

in the right femoral vein was connected to an infusion pump (Harvard Model 22

Infusion Pump, Harvard Apparatus Inc., Holliston, MA) for delivery of the drugs. The

tube in femoral artery was connected to a transducer, and MAP and HR were recorded

using a polygraph (MP36, BIOPAC Systems Inc, Goleta, CA, USA).[17,23] The

investigator (Dr. Chen) was blinded to the drugs under study. After intravenous

infusions of either 1) bupivacaine at 8.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, carbetapentane at 11.4

$\mu mol \cdot kg^{-1} \cdot min^{-1}$, orcaramiphen at 16.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ or 2) normal saline at a

rate of 400 $\mu L \cdot kg^{-1} \cdot min^{-1}$, the onset time of seizure, respiratory arrest, time to cause

impending death, MAP, and HR were evaluated.

The onset time of seizure was defined as the time when the first convulsion

occurred and respiratory arrest when apnea occurred for 15 seconds by observation of

chest movement. The time to impending death was defined as the time it took for the

HR decreased to 0 per minute.[17,23]

Statistical Analysis

Values are presented as mean±SEM or ED_{50} values with 95% confidence

interval (95% CI). The differences in baseline data, potencies (ED_{50}s), %MPE, full

recovery time, AUCs, and the time to cause toxicity between medications were

evaluated using one-way analysis of variance (ANOVA) and then the pairwise

Tukey's honestly significant difference (HSD) test. The differences in duration (Fig. 3)

among drugs were evaluated by two-way ANOVA followed by pairwise Tukey's HSD

test.   Analysis of variance with repeated measures followed by Duncan's

multiple-range test was used for post hoc multiple comparisons of means on MAP and

HR. SPSS for Windows (version 17.0) was used for all statistical analyses. Statistical

significance was set at $P < 0.05$.

RESULTS

Carbetapentane and caramiphen, as well as bupivacaine produced dose-dependent cutaneous analgesia in rats (Figure 1). The ED_{50}s of drugs are shown in Table 1. On the ED_{50} basis, the relative potency of these three drugs was found to be bupivacaine > carbetapentane > caramiphen ($P<0.01$; Table 1). At equipotent doses of 8.0 µmol · kg^{-1} for bupivacaine, 11.4 µmol · kg^{-1} for carbetapentane, and 16.0 µmol · kg^{-1} for caramiphen, all the local anesthetic drugs caused 100% blockade with durations of actions of 108±11, 124±11, and 115±12 min, respectively (Figure 2 and Table 2). At these given doses (Table 2), there were no significant differences among these three drugs for the %MPE, full recovery time, and AUCs. Saline elicited no cutaneous analgesic effects. At equianalgesic doses (ED_{25}, ED_{50}, ED_{75}), the block duration caused by carbetapentane or caramiphen was similar to that caused by bupivacaine, a long-acting local anesthetic (Fig. 3). All rats recovered completely after each subcutaneous injection.

The baseline data of body weight, MAP, and HR showed no significant differences among groups (Table 3). At equipotent doses, the times required to cause seizure, respiratory arrest, and impending death were longer in the carbetapentane ($P<0.05$) or caramiphen ($P<0.05$) group than in the bupivacaine group (Figure 4). There were no systemic toxicities in any of the animals in the saline group during the

infusion period. The HR and MAP displayed a tendency to decrease before CV

collapse (Figure 5) in all drug groups. The declines in MAP and HR were slower in

the carbetapentane ($P<0.01$) or caramiphen ($P<0.01$) group when compared with the

bupivacaine group (Figure 5). The rapidity of decline of the MAP and HR occurred in

the following order: bupivacaine > carbetapentane > caramiphen (Figure 5).

DISCUSSION

This study showed that carbetapentane and caramiphen displayed a dose-dependent local anesthetic effect on infiltrative cutaneous analgesia. The sensory/nociceptive block duration caused by carbetapentane or caramiphen was equal to that caused by bupivacaine, a long-acting local anesthetic. At equipotent doses, carbetapentane and caramiphen did not elicit systemic toxicity as quickly as bupivacaine.

Both carbetapentane and caramiphen have been known to treat coughing and related conditions clinically.[1,4,24,25] Recently, we demonstrated that caramiphen has a local anesthetic effect on spinal anesthesia.[5] Infiltrative cutaneous anesthesia is an acceptable choice for management of surgical anesthesia and postoperative pain, because it is relatively free of side effects.[15] In this study, we showed that carbetapentane and caramiphen had a local anesthetic effect on infiltrative cutaneous analgesia in a dose-related fashion. Although the pharmacological mechanisms of carbetapentane and caramiphen are largely unclear, inhibiting Na^+ currents may be one of the principle mechanisms of carbetapentane and caramiphen to hold local anesthetic effects, which is worth testing in the next study.

Our previous studies showed that the spinal blockades with caramiphen at 4.62 μmole/kg [5] were similar to those with bupivacaine at 3.1 μmole/kg [10] on spinal

anesthesia. In this recent experiment, bupivacaine has almost 1.4- and 2.0-fold greater

potencies than carbetapentane and caramiphen as infiltrative cutaneous analgesia,

respectively. There appears to be a uniformity of the comparative potencies of

caramiphen and bupivacaine with respect to cutaneous analgesia (Figure 1 and Table

1) and spinal anesthesia. Furthermore, the sensory block duration of carbetapentane or

caramiphen was similar to bupivacaine, a long-acting local anesthetic, on an

equianalgesic basis (ED_{25}, ED_{50}, ED_{75}). These findings suggest that there may be a

great potential for the use of carbetapentane and caramiphen as local anesthetics in the

clinical setting, provided that the CNS and CV toxicity is investigated.

Accidental intravenous injection of local anesthetics may cause CNS and CV

system toxicity and even result in death.[23,26] Through an animal model of local

anesthesia, we performed the local anesthetic effects of carbetapentane, caramiphen,

and bupivacaine as infiltrative cutaneous analgesia to determine the equipotent

analgesic doses of these drugs. At equipotent doses, we showed that infusion of

carbetapentane or caramiphen produced a delayed onset of CNS and CV toxicity

when compared with bupivacaine. However, the degrees of toxicities were the same

once toxicity occurred (Figure 4). Furthermore, we chose the animal model with the

spontaneously breathing rats, a clinical scenario when local anesthesia is practiced on

humans. In addition, we also noticed that the declines in MAP and HR were longer

with carbetapentane and caramiphen compared with bupivacaine. Overall, these results suggest that carbetapentane and caramiphen are less "toxic" and may feature a safer systemic toxicity profile than bupivacaine during continuous intravenous infusion.

In summary, our experiments reported that carbetapentane and caramiphen produced dose-dependent cutaneous analgesia and their block durations were similar to bupivacaine. Intravenous equipotent analgesic doses of carbetapentane and caramiphen are better tolerated to produce central nervous system and cardiovascular system toxicity than bupivacaine. The clinical relevance of these effects warrants further investigation.

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Table 1. The 50% effective dose (ED_{50}), ED_{25}, and ED_{75} of drugs on infiltrative cutaneous analgesia in rats

Drug	ED_{25}	ED_{50} (95% CI)	ED_{75}
Bupivacaine	1.38	1.78 (1.52 – 2.07)*#	2.44
Carbetapentane	1.82	2.53 (2.38 – 2.77)*	4.67
Caramiphen	2.27	3.60 (3.41 – 3.99)	6.49

EDs of drugs (μmol/kg) were obtained from Fig. 1. CI = confidence interval. Symbols (*,#) indicate $P<0.01$ when compared with caramiphen and carbetapentane, respectively, by using one-way ANOVA and pairwise Tukey's HSD test for paired comparisons.

Table 2. The percent of maximal possible effect (%MPE), full recovery time, and area under curves (AUCs) of bupivacaine at 8.0 μmol · kg^{-1}, carbetapentane 11.4 at μmol · kg^{-1} and caramiphen at 16.0 μmol · kg^{-1} as infiltrative cutaneous analgesia in rats

	%MPE	Full recovery time (min)	AUCs (%min)
Bupivacaine	100 ± 0	108 ± 11	7036 ± 762
Carbetapentane	100 ± 0	124± 11	7552±415
Caramiphen	100 ± 0	115 ± 12	6668 ± 806

The %MPE, duration of drug action, AUCs (mean±SEM) between bupivacaine, carbetapentane, and caramiphen were not significantly different.

Table 3. Baseline data are showed as mean±SEM

Variable	Saline	Bupivacaine	Carbetapentane	Caramiphen
Body Weight	271±10	264±8	259±11	266±9
MAP	106±4	105±4	100±3	100±4
HR	425±6	430±11	450±16	457±17

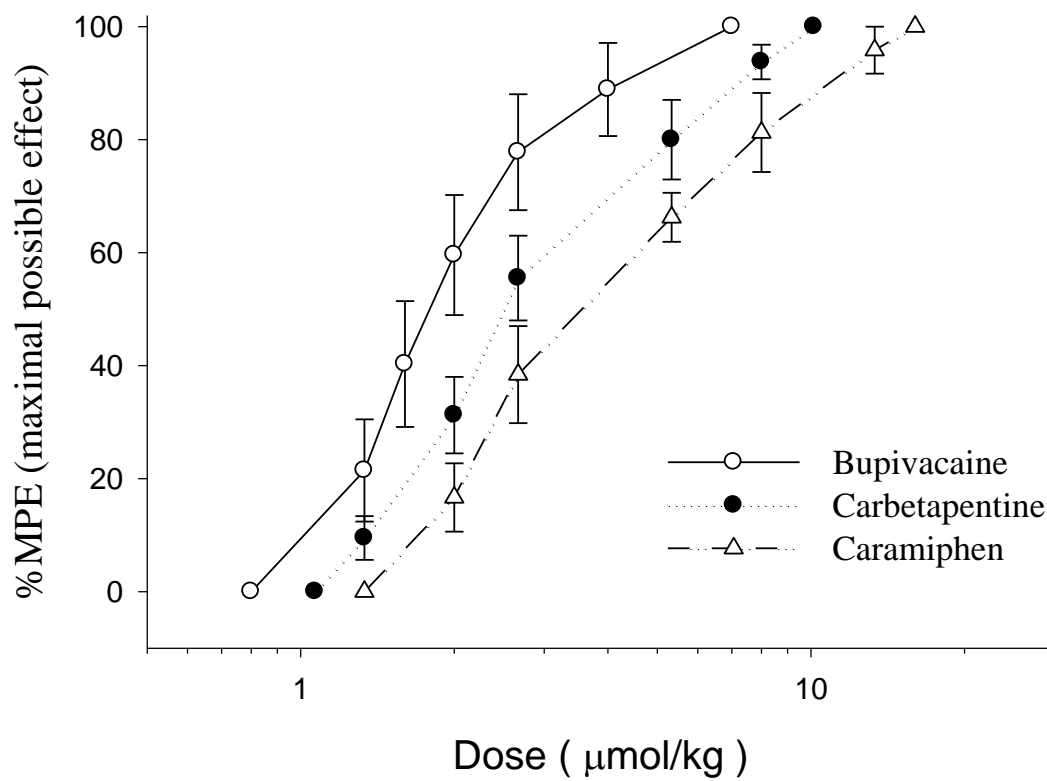There were no significant differences among the groups for these variables. MAP = mean arterial blood pressure; HR = heart rate.
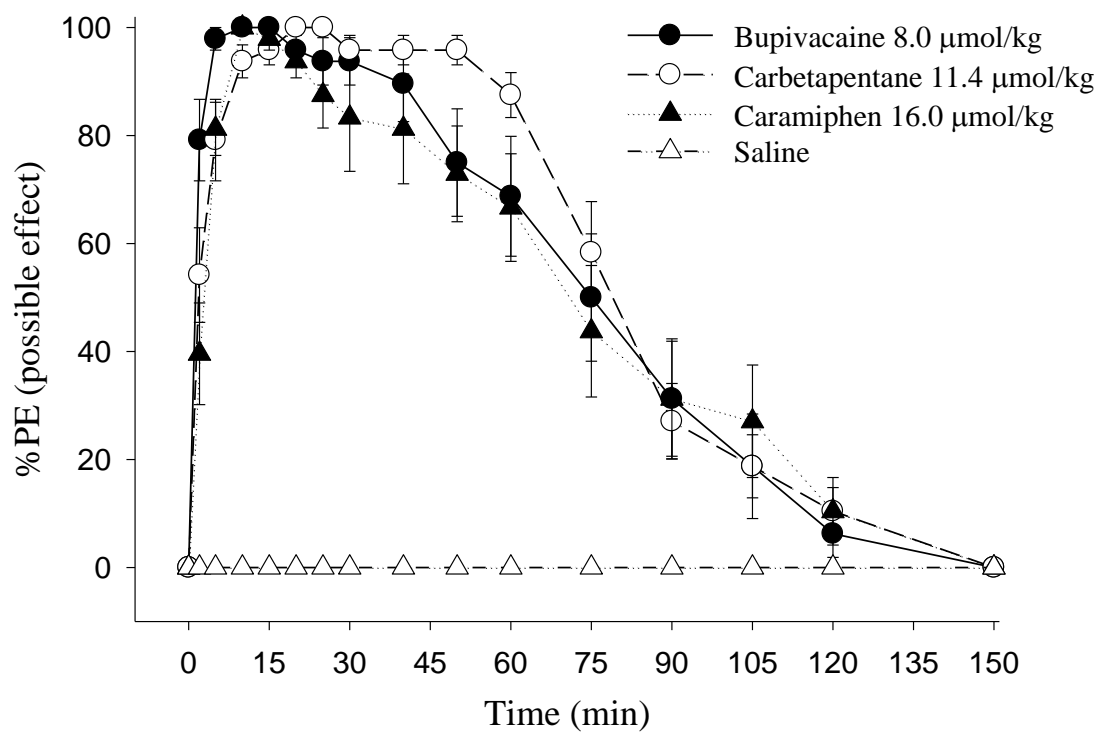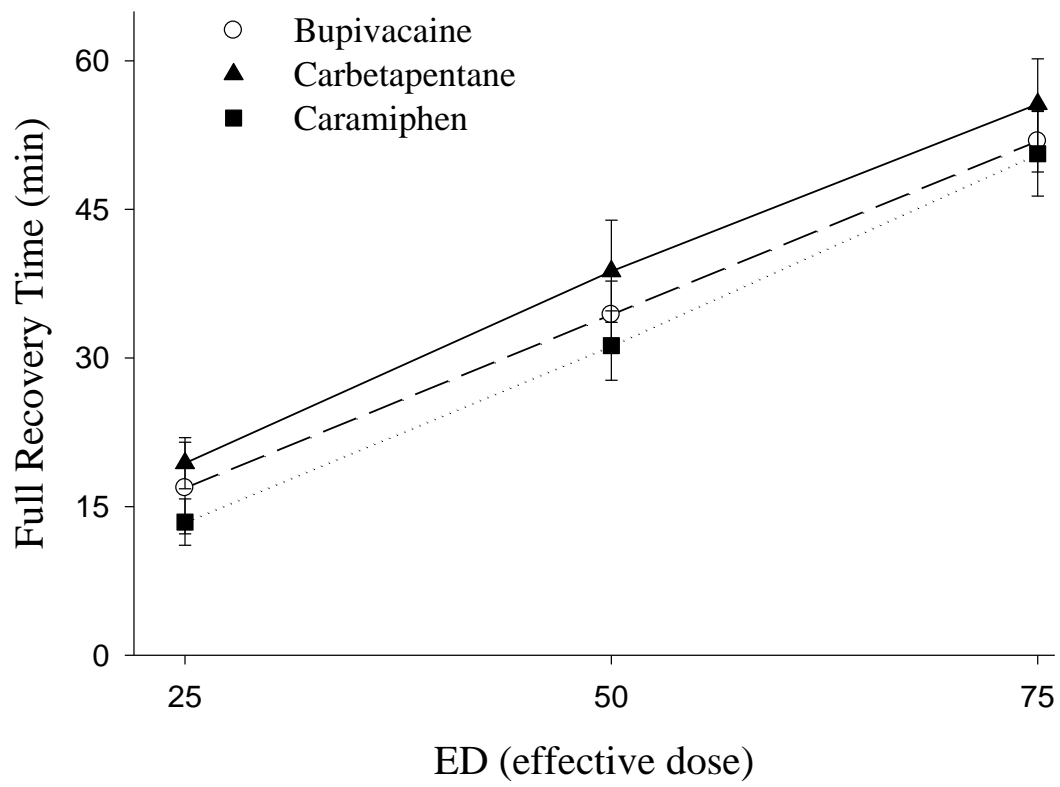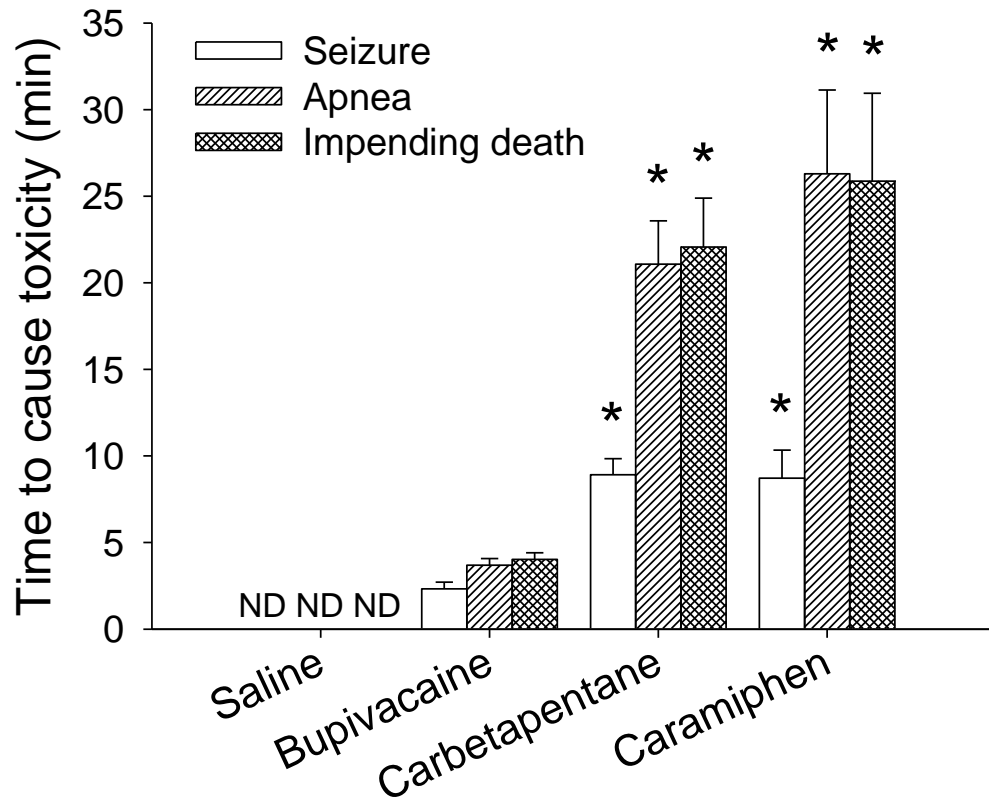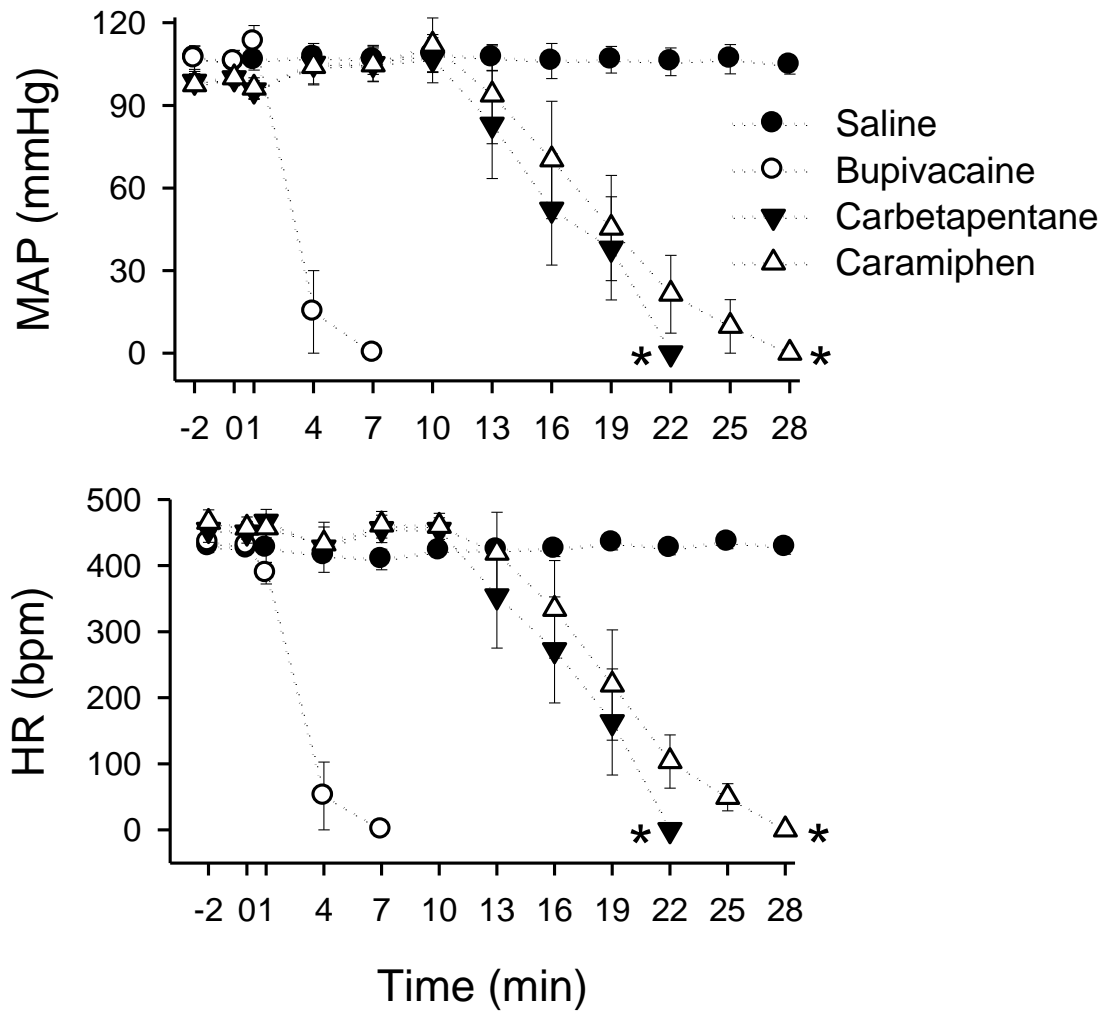
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

FIGURE LEGENDS

Fig. 1. The dose—response curves of bupivacaine, carbetapentane, and caramiphen as infiltrative cutaneous analgesia in rats (n = 8 at each testing point). Data are shown as mean±SEM.

Fig. 2. Time courses of bupivacaine (8.0 μmol/kg), carbetapentane (11.4 μmol/kg), caramiphen (16.0 μmol/kg), and saline (vehicle) on infiltrative cutaneous analgesia in rats. Data are presented as mean±SEM; each group, n=8.

Fig. 3. Full recovery time (duration) of cutaneous analgesia of bupivacaine, carbetapentane, and caramiphen at doses of ED_{25}, ED_{50}, and ED_{75} ($n = 8$ at each testing point) in rats. Values are expressed as mean±SEM. The differences in duration were evaluated by using two-way ANOVA followed by pairwise Tukey's HSD test.

Fig. 4. Time to cause toxicity of equipotent bupivacaine, carbetapentane, and caramiphen at the onset of seizure, respiratory arrest, and time to cause impending death. Saline group was not detected (ND) the toxicity symptoms. The symbol (*) indicates $P < 0.05$ when carbetapentane or caramiphen compared with bupivacaine. Data are presented as mean±SEM; each group, n=8.

Fig. 5. Mean arterial blood pressure (MAP) and heart rate (HR) change during infusion of either 1) bupivacaine at $8.0 \ \mu mol \cdot kg^{-1} \cdot min^{-1}$, carbetapentane at 11.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, or caramiphen at $16.0 \ \mu mol \cdot kg^{-1} \cdot min^{-1}$ or 2) normal saline in the

volume of $400 \ \mu L \cdot kg^{-1} \cdot min^{-1}$ (the same volume given to the animals in the drug

group) as infusion; 0 min is the start of infusion. Infusion was stopped when the time

to cause impending death was reached. The symbol (*) indicates $P < 0.01$ for

carbetapentane or caramiphen compared with bupivacaine. Data are presented as

mean±SEM; each testing group contained eight rats.