# Clonidine as adjuvant for oxybuprocaine, bupivacaine or dextrorphan has a significant peripheral action in intensifying and prolonging analgesia in response to local dorsal cutaneous noxious pinprick in rats

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## Abstract

The aim of the study was to evaluate co-administration of clonidine with oxybuprocaine (ester type), bupivacaine (amide type) or dextrorphan (non-ester or non-amide type) and to see whether it could have a peripheral action in enhancing local anesthesia on infiltrative cutaneous analgesia in rats. Cutaneous analgesia was evaluated by a block of the cutaneous trunci muscle reflex (CTMR) in response to local dorsal cutaneous noxious pinprick in rats. The analgesic effect of the addition of clonidine with oxybuprocaine, bupivacaine or dextrorphan by subcutaneous injection was evaluated. On an $ED_{50}$ basis, the rank of drug potency was oxybuprocaine > bupivacaine > dextrorphan ($P$<0.01). Mixtures of clonidine (0.12 μmol) with oxybuprocaine, bupivacaine or dextrorphan ($ED_{50}$ or $ED_{95}$) extended the duration of action and increased the potency on infiltrative cutaneous analgesia. Among these drugs, the addition of clonidine to bupivacaine (amide type) elicits the most effective cutaneous analgesia. Clonidine at the dose of 0.12 and 0.24 μmol did not produce cutaneous analgesia. Oxybuprocaine showed more potent cutaneous analgesia than bupivacaine or dextrorphan in rats. Co-administration of oxybuprocaine, bupivacaine or dextrorphan with clonidine increased the potency and duration on infiltrative cutaneous analgesia. The addition of clonidine to bupivacaine (amide type) elicits more effective cutaneous analgesia than oxybuprocaine (ester type) or dextrorphan

(non-ester or non-amide type).

**Key Words:** Clonidine; Oxybuprocaine; Bupivacaine; Dextrorphan; Infiltrative Cutaneous Analgesia

Oxybuprocaine (benoxinate), an ester type local anesthetic, produces the dose-related cutaneous analgesia [16] and spinal anesthesia [17]. In a previous study we have shown that systemic toxicity following intravenous oxybuprocaine and proxymetacaine occurred later compared to equipotent doses of bupivacaine, an amide-linkage local anesthetic [16]. Dextrorphan, a non-ester or non-amide type local anesthetic, has a local anesthetic effect on infiltrative cutaneous analgesia, [7] spinal or sciatic nerve blockades [4, 15]. In addition, intravenous equipotent analgesic dose of dextrorphan is better tolerated to elicit cardiovascular and central nervous system toxicity than bupivacaine [8]. Furthermore, oxybuprocaine or dextrorphan showed a long-acting local anesthetic effect similar to that provided by bupivacaine [4, 17].

Clonidine is a frequently used adjuvant to local anesthetics. The analgesic effects of clonidine when administered epidurally or intrathecally have been showed and are due to its α2-adrenoreceptor properties [10, 11]. The benefit of clonidine when added to local anesthetics for peripheral nerve blockades is less clear, although it is widely believed that clonidine decreases postoperative analgesic requirement or improves the quality and duration of local anesthetic blockade [22]. Several experiments are focused on the benefit of clonidine added to amino-amide local anesthetic for nerve blocks [22]. The aim of the study was to evaluate co-administration of clonidine with oxybuprocaine, bupivacaine or dextrorphan on infiltrative cutaneous analgesia and to

see whether it could have a peripheral action in enhancing the quality and duration of local anesthesia after a single subcutaneous injection.

Two hundred and sixteen male Sprague-Dawley rats weighting 200-250 g were obtained from the National Laboratory Animal Centre (Taipei, Taiwan), and housed in a climate controlled room, with food and water available *ad libitum* up to time of testing. The climate- controlled room was maintained at 22˚C 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 (IACUC) of China Medical University, Taichung, Taiwan and conformed to the recommendations and policies of the International Association for the Study of Pain (IASP).

Benoxinate (oxybuprocaine) HCl, bupivacaine HCl, dextrorphan tartrate, and clonidine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in 0.9% NaCl.

Three experiments were performed. In experiment 1, the time course of oxybuprocaine (1.20, 0.60, 0.15, 0.03 μmol), bupivacaine (1.80, 0.45, 0.30, 0.23 μmol), dextrorphan (9.00, 6.00, 3.00, 0.60 μmol), and clonidine (0.24, 0.12 μmol) on infiltrative cutaneous analgesia was evaluated (n = 8 rats for each dose of each drug). In experiment 2, the %MPE (percent of maximum possible effect), duration, and area

under curves (AUCs) of drug ($ED_{50}$ or $ED_{95}$) or co-administration of drug ($ED_{50}$ or $ED_{95}$) and clonidine (0.12 μmol) were tested on infiltrative cutaneous analgesia (n = 8 rats for each dose of each drug). In experiment 3, after the above experiments, one control group was performed to rule out the possibility of systemic effect of drugs on infiltrative cutaneous analgesia. Animals (n = 8 rats for each dose of each drug) received subcutaneous injection (the region of rats' thigh) of testing drug (oxybuprocaine, bupivacaine or dextrorphan) at a dose of $2\times ED_{95}$ or clonidine at a dose of 0.24 μmol.

On the day before the subcutaneous injection, the hair on the rats' dorsal surface of the thoracolumbar region (10×6 $cm^2$) was mechanically shaved. The subcutaneous injection of drug was performed as reported previously [6, 16]. In brief, drug dissolved in 0.6 mL was injected subcutaneously using 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 one minute after subcutaneous injection. For consistency, one experienced investigator (Dr. Chen Y.W.) who was blinded to the drugs injected was responsible for assessing the cutaneous analgesic effect.

Animals were handled daily up to 7 days to minimize the stress on the rats

during the experiment and generally improve their experimental performance before the experiment. Cutaneous analgesia was evaluated by the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back produced by twitches of the lateral thoracospinal muscle in response to local dorsal cutaneous stimulation after drug injections [6, 16]. 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 produce the standardized nociceptive stimulus (19±1 g). After observing an animal normal reaction to pinpricks 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. Cutaneous analgesic effect of each drug was assessed 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 at 0, 2 and 5 min after injection, every 5 min after injection for the first 30 min afterwards, then again every 10 min after injection for 30-60 min, and every 15-30 min thereafter until the CTMR fully recovered from the block. During the test, the maximum blockade in a time course of cutaneous analgesia of the drug was described as the %MPE. The 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) [6, 16].

After subcutaneously injecting the rats with four doses of each drug ($n$ = 8 for each dose of each drug), dose-response curves were obtained by the % MPE for each dose of each drug. The value of 50% or 95% effective dose ($ED_{50}$ or $ED_{95}$), defined as the dose that caused 50% or 95% cutaneous analgesic effect, respectively, was obtained by a SAS NLIN analysis (SAS Institute Inc., Carey, NC) [5, 19].

Data are presented as mean ± SD (range) or $ED_{50}$ and $ED_{95}$ values with 95% confidence interval (95% CI). Data were analyzed by the Student's t-test or 1-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 injections of oxybuprocaine, bupivacaine, and dextrorphan produced dose-dependent cutaneous analgesia in rats (Fig. 1; [8, 16]). Clonidine alone at the doses of 0.12 and 0.24 µmol (data not shown) showed no cutaneous analgesia. The $ED_{50}$s and $ED_{95}$s of drugs constructed from Figure 1 were demonstrated in Table 1. On an $ED_{50}$ basis, the rank of drug potency was oxybuprocaine > bupivacaine > dextrorphan (Table 1).

When drugs at the dose of $ED_{50}$ were co-injected with clonidine (0.12 µmol),

complete sensory blockade (100% MPE) in the oxybuprocaine (8 of 8 rats) or bupivacaine (8 of 8 rats) group occurred, but not in the dextrorphan (5 of 8 rats) group (Fig. 2 and Table 2). Subcutaneous injection of clonidine alone at the dose of 0.12 µmol showed no cutaneous analgesia (Fig. 2). Compared with drugs at the dose of $ED_{50}$ alone, drugs ($ED_{50}$) co-injected with 0.12 µmol clonidine, the AUCs were increased in the oxybuprocaine, bupivacaine, and dextrorphan groups ($P<0.05$). The %MPE, duration and AUCs of drugs at the dose of $ED_{50}$ with clonidine were greater ($P<0.05$) than drugs ($ED_{50}$) without clonidine in Table 2. The rank in intensifying complete block time, full recovery time, and AUCs of the co-administration of drug ($ED_{50}$) and clonidine was bupivacaine = ocybuprocaine > dextrorphan (Table 2; $P<0.05$) when compared with drug ($ED_{50}$) alone.

At the dose of $ED_{95}$, oxybuprocaine, bupivacaine, and dextrorphan elicited 92%, 90%, and 88% sensory blockade, respectively, in Figure 2 and Table 3. Subcutaneous injection of clonidine alone at the dose of 0.12 µmol demonstrated no cutaneous analgesia (Fig. 2). After drugs at the dose of $ED_{95}$ were co-injected with clonidine (0.12 µmol), oxybuprocaine, bupivacaine, and dextrorphan caused 100%, 100%, and 100% sensory blockade (100% MPE), respectively (Fig. 2 and Table 3). The %MPE, duration and AUCs of drugs at the dose of $ED_{95}$ with clonidine were greater ($P<0.05$) than drugs ($ED_{95}$) without clonidine in Table 3. The rank in increasing complete block

time, full recovery time, and AUCs of the co-administration of drug ($ED_{95}$) and clonidine was bupivacaine > ocybuprocaine > dextrorphan (Table 3; $P<0.05$).

Neither the thigh subcutaneous injection of clonidine (0.24 μmol) nor thigh subcutaneous injections of oxybuprocaine, bupivacaine, and dextrorphan ($2ED_{95}$) demonstrated cutaneous analgesia, loss of motor activity or sedation (data not shown). All rats recovered completely after each subcutaneous injection of drug.

In this report we showed for the first time that oxybuprocaine was a more potent local anesthetic on infiltrative cutaneous analgesia than dextrorphan. Clonidine dramatically improves the sensory blocking effect and duration of oxybuprocaine, bupivacaine, and dextrorphan. The benefit rank of adding clonidine to local anesthetics is bupivacaine > oxybuprocaine > dextrorphan.

The clinical relevance of this prolonged sensory blockade, though useful in certain situations, may be limited overall, when considering the higher incidence of motor blockade caused by clonidine [9]. Hypotension may be an important problem when using clonidine as adjuvant in neuraxial anesthesia or in pain regimens [2, 9]. Motor blockade following central neuraxial block and sedative effects of clonidine should be limited. Given these dose-limiting central side effects, clonidine may be beneficial to apply peripherally. In addition, both scientific and clinical studies have also provided evidence for the mechanism of action of clonidine as a local anesthetic

additive, as well as suggesting local anesthetic-like properties of clonidine itself [3, 13]. Therefore, this study evaluated the adding clonidine to local anesthetics on infiltrative cutaneous analgesia in rats. We found that co-administration of clonidine (0.12 μmol) with oxybuprocaine, bupivacaine or dextrorphan prolonged duration of action and enhanced cutaneous analgesia. Our data agree with those results, which showed that clonidine has a significant peripheral action in enhancing local anesthetic duration on subcutaneous co-infiltration with lidocaine [23].

In this study, cutaneous analgesia (AUC) of co-administration of drug (oxybuprocaine, bupivacaine and dextrorphan) at the dose of $ED_{95}$ and clonidine (0.12 μmol) was approximately 3.7-, 4.2-, and 2.9-folds greater than drug alone, respectively. Our results also demonstrated that clonidine as an adjuvant for oxybuprocaine, bupivacaine, and dextrorphan increased the potency of drug on infiltrative cutaneous analgesia. Furthermore, cutaneous analgesia (AUC) of co-administration of drug (oxybuprocaine, bupivacaine and dextrorphan) at the dose of $ED_{50}$ and clonidine (0.12 μmol) was approximately 7.7-, 8.2-, and 5.3-folds greater than drug alone, respectively. Coadministration of bupivacaine with clonidine extended longer duration on infiltrative cutaneous analgesia than oxybuprocaine or dextrorphan. It has been mentioned that clonidine, clinically added to preparations of local anesthetics, prolonged the duration of action by 3 possible mechanisms. First,

clonidine may cause local vasoconstriction, [10, 27] thus decreasing local anaesthetic spread and removal around nerves [10, 21]. Secondly, clonidine blocked C and Aδ fibres [3, 12] as a consequence of an increase in $K^+$ conductance in isolated neurons, [1, 20] thus intensifying local anesthetic conduction block [12]. Thirdly, spinal clonidine combined with local anesthetics [21, 25] or used in peripheral nerve blockades [12, 23] intensifies and prolongs anesthesia.

A single subcutaneous injection of clonidine (1.2 mg/kg) resulted in delayed tactile hypersensitivity 24–34 h after clonidine administration in rats [24]. Our study displayed that subcutaneous injection of clonidine (0.12–0.24 µmol; 0.14–0.28 mg/kg), an α2-adrenoreceptor agonist, did not produce any cutaneous analgesia to local dorsal cutaneous noxious pinprick. Our previous experiment showed that dextrorphan with epinephrine produced an additive effect on infiltrative cutaneous analgesia, [7] but adding clonidine to dextrorphan elicited a supra-additive effect as a cutaneous analgesic (Fig. 2). It may be explained that α1-adrenoceptor agonists (e.g. epinephrine and phenylephrine) at these doses between 0.003 – 1.47 µmol can mainly act by mixed subtypes of α1-adrenoceptor to induce the local anesthetic activity [26]. In this study, we used the pinprick with a frequency of 1 Hz [14, 18] which is impossible that repeated pinpricks may change, in fact decrease the response to stimulus.

In summary, oxybuprocaine produced more potent cutaneous analgesia than dextrorphan on an $ED_{50}$ basis, and the effect of clonidine on infiltrative local anesthesia with oxybuprocaine, bupivacaine or dextrorphan has a significant peripheral action in intensifying and prolonging local anesthetic effects. On an equipotent basis ($ED_{50}$ or $ED_{95}$), bupivacaine (amide type) showed more effective cutaneous analgesia than oxybuprocaine (ester type) or dextrorphan (other type) when adding clonidine.

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**Table 1.** The 50% effective doses ($ED_{50}$s) and $ED_{95}$s of drugs on infiltrative cutaneous analgesia

| Drug | $ED_{50}$s ( 95% CI ) | $ED_{95}$s ( 95% CI ) |
|---|---|---|
| Oxybuprocaine | 0.23 (0.19 – 0.28) | 1.29 (1.23 – 1.39) |
| Bupivacaine | 0.66 (0.55 – 0.78) | 1.97 (1.88 – 2.09) |
| Dextrorphan | 2.47 (2.03 – 3.00) | 8.45 (8.33 – 8.68) |

$ED_{50}$s and $ED_{95}$s of drugs (μmol) were obtained from Figure 1. CI = confidence interval. Potencies of drugs ($ED_{50}$s) were oxybuprocaine > bupivacaine > dextrorphan ($P$<0.01, for each comparison) using 1-way ANOVA followed by pairwise Tukey's HSD test.

**Table 2.** The %MPE, duration, and AUCs of drug ($ED_{50}$) or co-administration of drug ($ED_{50}$) and clonidine (0.12 µmol)

| | %MPE | Duration (min) | | AUCs (%MPE×min) |
|---|---|---|---|---|
| | | Complete blockade Time | Full Recovery Time | |
| *Drug ($ED_{50}$)* | | | | |
| Oxybuprocaine | 50 ± 9 | NEVER | 31 ± 6 | 926 ± 242 |
| Bupivacaine | 52 ± 11 | NEVER | 41 ± 11 | 1035 ± 464 |
| Dextrorphan | 50 ± 13 | NEVER | 37 ± 9 | 916 ± 361 |
| *Drug ($ED_{50}$) + Clonidine* | | | | |
| Oxybuprocaine | 100 ± 0[a] | 40.5 ± 6.5[b] | 118 ± 12[ab] | 7101 ± 561[ab] |
| Bupivacaine | 100 ± 0[a] | 46.3 ± 7.4[b] | 128 ± 20[ab] | 8484 ± 606[ab] |
| Dextrorphan | 93 ± 9[a] | 2.1 ± 2.7 | 97 ± 8[a] | 4821 ± 590[a] |
| *Clonidine alone* | 0 | NEVER | 0 | 0 |

Percent of maximum possible effect (%MPE), duration of action, area under curves (AUCs) for drug ($ED_{50}$) or co-administration of drug ($ED_{50}$) and clonidine at 0.12 µmol (n = 8 in all groups) on infiltrative cutaneous analgesia (mean ± SD). NEVER: the complete blockade was never obtained. The symbol (a) indicates $P < 0.05$ when drug alone compared with the co-administraion of drug and clonidine using a student's *t* test. The symbol (b) indicates $P < 0.05$ when dextrorphan compared with oxybuprocaine or bupivacaine using 1-way ANOVA followed by pairwise Tukey's HSD test. Clonidine was at the dose of 0.12 µmol.

**Table 3.** The %MPE, duration, and AUCs of drug ($ED_{95}$) or co-administration of drug ($ED_{95}$) and clonidine (0.12 µmol)

| | %MPE | Duration (min) | | AUCs (%MPE×min) |
|---|---|---|---|---|
| | | Complete blockade Time | Full Recovery Time | |
| *Drug ($ED_{95}$)* | | | | |
| Oxybuprocaine | 92 ± 9 | 3.1 ± 4.1 | 62 ± 12 | 2894 ± 882 |
| Bupivacaine | 90 ± 14 | 4.4 ± 6.1 | 75 ± 25 | 3390 ± 1003 |
| Dextrorphan | 88 ± 12 | 2.3 ± 1.3 | 66 ± 14 | 2915 ± 751 |
| *Drug ($ED_{95}$) + Clonidine* | | | | |
| Oxybuprocaine | 100 ± 0[a] | 70.5 ± 22.5[ab] | 155 ± 25[ab] | 10812 ± 1727[ab] |
| Bupivacaine | 100 ± 0[a] | 113.0 ± 15.5[abc] | 180 ± 0[abc] | 14097 ± 596[abc] |
| Dextrorphan | 100 ± 0[a] | 44.8 ± 13.3[a] | 127 ± 16[a] | 8360 ± 1108[a] |
| *Clonidine alone* | 0 | NEVER | 0 | 0 |

Percent of maximum possible effect (%MPE), duration of action, area under curves (AUCs) for drug ($ED_{95}$) or co-administration of drug ($ED_{95}$) and clonidine at 0.12 µmol (n = 8 in all groups) on infiltrative cutaneous analgesia (mean ± SD). NEVER: the complete blockade was never obtained. The symbol (a) indicates $P < 0.05$ when drug alone compared with the co-administraion of drug and clonidine using a student's *t* test. The symbol (b) indicates $P < 0.05$ when dextrorphan compared with oxybuprocaine or bupivacaine using 1-way ANOVA followed by pairwise Tukey's HSD test. The symbol (c) indicates $P < 0.05$ when oxybuprocaine compared with bupivacaine using 1-way ANOVA followed by pairwise Tukey's HSD test.

## Legends to figures

**Figure 1.** Time courses of cutaneous analgesia of oxybuprocaine, bupivacaine, and dextrorphan on infiltrative cutaneous analgesia (four doses in each group) in rats. Values are expressed as mean ± SD; n = 8 rats for each dose of each drug.

**Figure 2.** The addition of clonidine (CL) with oxybuprocaine, bupivacaine or dextrorphan ($ED_{50}$ or $ED_{95}$) on infiltrative cutaneous analgesia in rats. Clonidine was at the dose of 0.12 μmol and produced no cutaneous analgesia. Values are expressed as mean ± SD; n = 8 rats for each dose of each drug. The $ED_{50}$ or $ED_{95}$ means 50% or 95% effective dose, respectively.

## References

[1] R. Andrade, G.K. Aghajanian, Opiate- and alpha 2-adrenoceptor-induced hyperpolarizations of locus ceruleus neurons in brain slices: reversal by cyclic adenosine 3':5'-monophosphate analogues, J. Neurosci. 5 (1985) 2359-2364.

[2] S. Bhatnagar, S. Mishra, S. Madhurima, M. Gurjar, A.S. Mondal, Clonidine as an analgesic adjuvant to continuous paravertebral bupivacaine for post-thoracotomy pain, Anaesth. Intensive Care 34 (2006) 586-591.

[3] J.F.t. Butterworth, G.R. Strichartz, The alpha 2-adrenergic agonists clonidine and guanfacine produce tonic and phasic block of conduction in rat sciatic nerve fibers, Anesth. Analg. 76 (1993) 295-301.

[4] Y.W. Chen, Y.C. Chen, C.N. Lin, C.C. Chu, M.T. Lin, J.J. Wang, C.H. Kao, The spinal anaesthetic effect of dextromethorphan, dextrorphan, and 3-methoxymorphinan, Eur. J. Pharmacol. 569 (2007) 188-193.

[5] Y.W. Chen, C.C. Chu, Y.C. Chen, J.J. Wang, C.H. Hung, The dose-dependent study of verapamil and diltiazem on spinal anesthesia in the rat, Neurosci. Lett. 482 (2010) 76-80.

[6] Y.W. Chen, K.S. Chu, C.N. Lin, J.I. Tzeng, C.C. Chu, M.T. Lin, J.J. Wang, Dextromethorphan or dextrorphan have a local anesthetic effect on infiltrative cutaneous analgesia in rats, Anesth. Analg. 104 (2007) 1251-1255.

[7] Y.W. Chen, K.S. Liu, J.J. Wang, W. Chou, C.H. Hung, Isobolographic analysis of epinephrine with bupivacaine, dextromethorphan, 3-methoxymorphinan, or dextrorphan on infiltrative anesthesia in rats: dose-response studies, Reg. Anesth. Pain Med. 33 (2008) 115-121.

[8] Y.W. Chen, J.J. Wang, T.Y. Liu, Y.C. Chen, C.H. Hung, Systemic dextromethorphan and dextrorphan are less toxic in rats than bupivacaine at equianesthetic doses, Can. J. Anaesth. 58 (2011) 55-61.

[9] X. Culebras, E. Van Gessel, P. Hoffmeyer, Z. Gamulin, Clonidine combined with a long acting local anesthetic does not prolong postoperative analgesia after brachial plexus block but does induce hemodynamic changes, Anesth. Analg. 92 (2001) 199-204.

[10] J.C. Eisenach, M. De Kock, W. Klimscha, alpha(2)-adrenergic agonists for regional anesthesia. A clinical review of clonidine (1984-1995), Anesthesiology 85 (1996) 655-674.

[11] N. Elia, X. Culebras, C. Mazza, E. Schiffer, M.R. Tramer, Clonidine as an adjuvant to intrathecal local anesthetics for surgery: systematic review of randomized trials, Reg. Anesth. Pain. Med. 33 (2008) 159-167.

[12] D.M. Gaumann, P.C. Brunet, P. Jirounek, Clonidine enhances the effects of lidocaine on C-fiber action potential, Anesth. Analg. 74 (1992) 719-725.

[13] D.M. Gaumann, P.C. Brunet, P. Jirounek, Hyperpolarizing afterpotentials in C fibers and local anesthetic effects of clonidine and lidocaine, Pharmacology 48 (1994) 21-29.

[14] A. Haderer, P. Gerner, G. Kao, V. Srinivasa, G.K. Wang, Cutaneous analgesia after transdermal application of amitriptyline versus lidocaine in rats, Anesth. Analg. 96 (2003) 1707-1710.

[15] C.H. Hou, J.I. Tzeng, Y.W. Chen, C.N. Lin, M.T. Lin, C.H. Tu, J.J. Wang, Dextromethorphan, 3-methoxymorphinan, and dextrorphan have local anaesthetic effect on sciatic nerve blockade in rats, Eur. J. Pharmacol. 544 (2006) 10-16.

[16] C.H. Hung, K.S. Liu, D.Z. Shao, K.I. Cheng, Y.C. Chen, Y.W. Chen, The systemic toxicity of equipotent proxymetacaine, oxybuprocaine, and bupivacaine during continuous intravenous infusion in rats, Anesth. Analg. 110 (2010) 238-242.

[17] C.H. Hung, J.J. Wang, Y.C. Chen, C.C. Chu, Y.W. Chen, Intrathecal oxybuprocaine and proxymetacaine produced potent and long-lasting spinal anesthesia in rats, Neurosci. Lett. 454 (2009) 249-253.

[18] M.A. Khan, P. Gerner, G. Kuo Wang, Amitriptyline for prolonged cutaneous analgesia in the rat, Anesthesiology 96 (2002) 109-116.

[19] S. Minkin, K. Kundhal, Likelihood-based experimental design for estimation of ED50, Biometrics 55 (1999) 1030-1037.

[20] R. Murphy, J.E. Freedman, Morphine and clonidine activate different K+ channels on rat amygdala neurons, Eur. J. Pharmacol. 415 (2001) R1-3.

[21] T. Nishikawa, S. Dohi, Clinical evaluation of clonidine added to lidocaine solution for epidural anesthesia, Anesthesiology 73 (1990) 853-859.

[22] D.M. Popping, N. Elia, E. Marret, M. Wenk, M.R. Tramer, Clonidine as an adjuvant to local anesthetics for peripheral nerve and plexus blocks: a meta-analysis of randomized trials, Anesthesiology 111 (2009) 406-415.

[23] J.N. Pratap, R.K. Shankar, T. Goroszeniuk, Co-injection of clonidine prolongs the anesthetic effect of lidocaine skin infiltration by a peripheral action, Anesth. Analg. 104 (2007) 982-983.

[24] A. Quartilho, H.P. Mata, M.M. Ibrahim, T.W. Vanderah, M.H. Ossipov, J. Lai, F. Porreca, T.P. Malan, Jr., Production of paradoxical sensory hypersensitivity by alpha 2-adrenoreceptor agonists, Anesthesiology 100 (2004) 1538-1544.

[25] A. Rochette, O. Raux, R. Troncin, C. Dadure, R. Verdier, X. Capdevila, Clonidine prolongs spinal anesthesia in newborns: a prospective dose-ranging study, Anesth. Analg. 98 (2004) 56-59.

[26] J.P. Shieh, C.C. Chu, J.J. Wang, M.T. Lin, Epinephrine, phenylephrine, and

methoxamine induce infiltrative anesthesia via alpha1-adrenoceptors in rats, Acta. Pharmacol. Sin. 30 (2009) 1227-1236.

[27] T. Sjoberg, L. Norgren, K.E. Andersson, S. Steen, Comparative effects of the alpha-adrenoceptor agonists noradrenaline, phenylephrine and clonidine in the human saphenous vein in vivo and in vitro, Acta Physiol. Scand. 136 (1989) 463-471.