J Anesth (2015) 29:809–812
DOI 10.1007/s00540-015-2026-8

SHORT COMMUNICATION

Effects of levobupivacaine on isolated rat tracheal smooth muscle

Hung-Chi Chang[1,2] · Shin-Yan Chen[1] · Yu-Feng Huang[1] · Feng-Lin Liu[1] · Yih-Giun Cherng[1,3] · Hsing-Won Wang[4,5]

Received: 22 September 2014 / Accepted: 30 April 2015 / Published online: 21 May 2015

Abstract Levobupivacaine has been developed as a safer alternative to bupivacaine because of its reduced systemic toxicity. However, the effect of directly delivering levobupivacaine into tracheal smooth muscle has not been adequately explored. We performed this study to determine the in vitro effects of levobupivacaine on isolated rat tracheal smooth muscle. A portion of rat trachea 5 mm in length was mounted in 30 ml of Krebs solution in a muscle bath at 37 °C. The following effects of levobupivacaine were assessed: (1) the effect on tracheal smooth muscle resting tension ($n = 6$), (2) the effect on contraction caused by 10^{-6} M methacholine ($n = 6$) and (3) the effect on electrically induced tracheal smooth muscle contractions ($n = 6$). Levobupivacaine caused dose-dependent relaxation in the trachealis muscle precontracted with 10^{-6} M methacholine. Contraction inhibition was statistically significant when 10^{-5} and 10^{-4} M levobupivacaine were applied, compared with the contraction inhibition that occurred in the control groups ($p < 0.01$). A high dose of levobupivacaine also decreased the spike contraction induced by electrical field stimulation. This study indicated that high concentrations of levobupivacaine might antagonize the cholinergic receptors and inhibit parasympathetic function of the trachea.

Keywords Levobupivacaine · In vitro study · Smooth muscle · Trachea · Electrical field stimulation

Y.-G. Cherng and H.-W. Wang contributed equally to this work.

✉ Hsing-Won Wang
w0512n@ms15.hinet.net

Yih-Giun Cherng
stainless@s.tmu.edu.tw

1 Department of Anesthesiology, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan

2 Graduate Institute of Translational and Interdisciplinary Medicine, National Central University, Taoyuan, Taiwan

3 Department of Anesthesiology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

4 Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

5 Department of Otolaryngology, Shuang Ho Hospital, Taipei Medical University, No. 291, Jhongjheng Rd., Jhonghe, Taipei 23561, Taiwan

Levobupivacaine, an amide-linked local anesthetic, has been developed as a safer alternative to bupivacaine because of reduced systemic toxicity. Its vasoconstrictive activity and long-acting effects may be advantageous for topical applications or infiltrative anesthesia. Clinical trials have demonstrated that topical levobupivacaine is more effective than lidocaine in producing postoperative analgesia after nasal surgery [1]. Nebulized lidocaine is a useful therapy to reduce airway reactivity in patients with chronic asthma [2]. However, inhaled lidocaine may cause vagally mediated reflex bronchoconstriction, most likely by stimulating irritant receptors within the airways [3]. No study has examined how levobupivacaine affects hyperreactive airways in either animals or humans. The effect of directly delivering levobupivacaine into tracheal smooth muscle has not been adequately explored. Therefore, we use a simple in vitro model to investigate the effects of levobupivacaine on the contractile response of isolated rat tracheal smooth muscle to methacholine and electrical field stimulation (EFS).

This study was approved by the Institutional Animal Care and Use Committee of Taipei Medical University (approval number: LAC-2013-0098). The equipment and process were designed based on our previous studies [4–6].

Eighteen rats were anesthetized by intraperitoneal administration of pentobarbital (45 mg/kg), and two pieces of trachea approximately 5 mm in length were removed from each rat. Each specimen was mounted using 2 steel plates and immersed in a 30-ml muscle bath of Krebs solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$ and 10.0 mM glucose) at 37 °C aerated with 5 % CO_2 balanced in oxygen. The upper side of the tracheal strip was attached to a Grass FT-03 force displacement transducer (AstroMed, West Warwick, USA) by using a steel plate and a 3–0 silk ligature. The other side of the strip was fixed to a steel plate attached to the bath. A passive tension of 0.3 g was applied to the strips, and subsequent changes in tension were continually recorded using Chart V4.2 software (PowerLab, ADInstruments, Colorado Springs, USA).

Methacholine can induce a dose-dependent contraction of a tracheal strip, and a concentration of 10^{-6} M results in a significant degree of contraction [4]. Before drug assays were conducted, isolated tracheal samples were equilibrated in the bath solution for 30 to 45 min. We used 10^{-6} M methacholine as a contraction agent for the trachea. All drugs were administered by adding a defined volume of stock solution to the tissue bath solution.

EFS (5 Hz, 5-ms pulse duration at a voltage of 50 V, 5-s trains of stimulation) was applied to the tracheal strip with 2 wire electrodes that were parallel to the tissue and connected to a direct-current stimulator (Grass S44, Quincy, USA). An interval of 2 min was imposed between each stimulation period to allow for recovery from the response. Stimulation was applied to the tracheal strips at 37 °C.

The following effects of levobupivacaine were assessed: (1) the effect on tracheal smooth muscle resting tension ($n = 6$), (2) the effect on contraction caused by 10^{-6} M methacholine ($n = 6$), and (3) the effect on electrically induced tracheal smooth muscle contractions ($n = 6$). In each experiment, one untreated strip served as the control.

Concentrations of the drugs are expressed as concentrations used in the 30-ml bath solution. Data are presented as mean values and standard deviations (SDs) and statistical significance was tested using a one-way ANOVA with Bonferroni's post hoc test; P values less than 0.01 were considered significant.

The degree of contraction or relaxation of the tracheal strips was based on the tension applied to the transducer. Levobupivacaine exerted a negligible effect on the basal tension of the trachea as the concentration increased (not shown).

When levobupivacaine was cumulatively applied to the steady state of 10^{-6} M methacholine-induced contraction, the trachea relaxed (Fig. 1a). When 10^{-8} M levobupivacaine was used, the tension was 94.8 ± 1.1 % of the control value, and when 10^{-5} and 10^{-4} M levobupivacaine

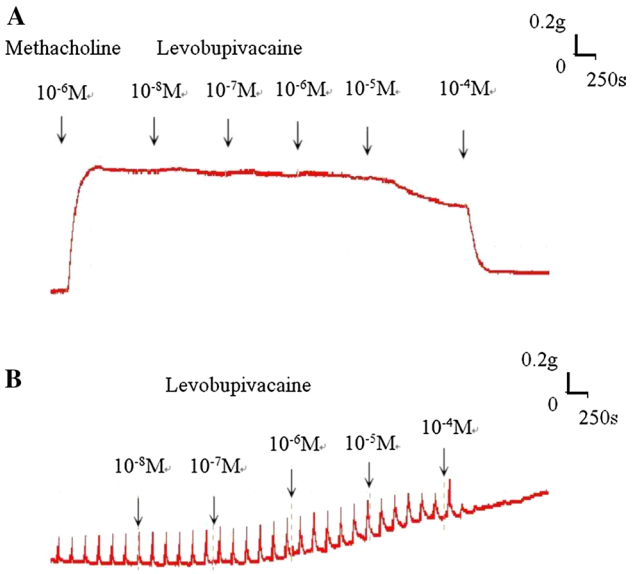

Fig. 1 **a** Original recording of the effects of levobupivacaine on 10^{-6} M methacholine-induced contraction of rat trachea ($n = 6$). **b** Original recording of the effects of levobupivacaine on electrically induced tracheal smooth muscle contractions ($n = 6$)

were used, the tensions were 56.7 ± 4.2 and 13.7 ± 5.7 %, respectively (Fig. 2a). Contraction inhibition was statistically significant when 10^{-5} and 10^{-4} M levobupivacaine were applied, compared with the contraction inhibition that occurred when the 10^{-8} M levobupivacaine was used and that which occurred in the control group ($P < 0.01$).

Levobupivacaine also inhibited the spike contraction induced by EFS (Fig. 1b). The peak tension of the tracheal strip evoked by EFS after adding 10^{-8} M levobupivacaine was 100 ± 0.0 %, whereas the peaks were 37.2 ± 3.3 % after adding 10^{-5} M levobupivacaine. The spike contraction was completely inhibited by 10^{-4} M levobupivacaine (Fig. 2b). The peak tension values of the tracheal strip evoked by EFS when 10^{-5} and 10^{-4} M levobupivacaine were used were significantly lower than that of the control group ($P < 0.01$).

Although in vitro assays for investigating tracheal responses to local anesthetic have been developed [7, 8], our method provides distinct advantages. The epithelium layer plays an essential role in modulating the basal tone and reactivity of smooth muscle. Because the method of preparing isolated trachea used in our experiments involved maintaining intact rings without damaging the epithelium or mucosa, our model adequately represents tracheal responses to the test agents.

In rats and humans, acetylcholine released from parasympathetic nerve terminals is recognized as the most critical bronchoactive pathway in the airways [9]. The results derived from in vitro studies on the effects of local anesthetics on airways are conflicting. Clinically relevant concentrations of lidocaine caused guinea pig tracheal smooth muscle

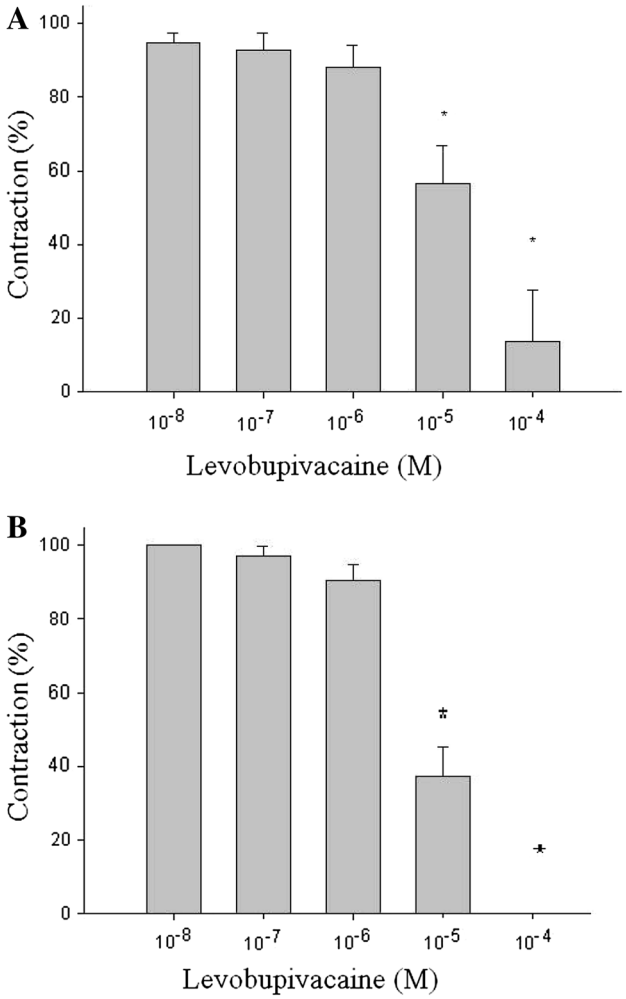

Fig. 2 **a** Effects of levobupivacaine on 10^{-6} M methacholine-induced contraction (contraction area calculated at 100 %, without levobupivacaine) of rat trachea. Contraction inhibition was statistically significant when 10^{-5} and 10^{-4} M levobupivacaine were applied, compared with the contraction inhibition that occurred in the control group. *Asterisk* $P < 0.01$ versus the control group. **b** Effects of levobupivacaine on electrically induced smooth muscle contractions (contraction area calculated at 100 %, without levobupivacaine) of rat trachea. The peak tension values of the tracheal strip evoked by electrical field stimulation when 10^{-5} and 10^{-4} M levobupivacaine were used were significantly lower than that of the control group. *Asterisk* $P < 0.01$ versus the control group

to contract [10]. Studies have used guinea pig trachealis to demonstrate that bupivacaine produces a biphasic response, exhibiting contraction at low concentrations and relaxation at high concentrations [11]. However, our recent studies have shown that these two local anesthetics did not lead to a significant change in resting tension [4, 5]. In this investigation, levobupivacaine exerted a minimal effect on the basal tension as the concentration increased. These results indicated that levobupivacaine did not exert a direct contracting effect.

The findings on the dose-dependent protective effects of levobupivacaine against methacholine-induced contraction are consistent with the results obtained using lidocaine and bupivacaine [4, 5, 7, 10, 12]. A first possibility is that levobupivacaine may antagonize the effect of cholinergic agents. However, lidocaine acts as a noncompetitive antagonist to cholinergic stimulation, and pretreatment with atropine did not modify the ability of lidocaine to inhibit hypertonic potassium contractions [13]. Moreover, lidocaine (10^{-4} M) enhanced basal cyclic adenosine monophosphate accumulation in bovine tracheal smooth muscle precontracted with methacholine [8]. The results supported the hypothesis that interaction with the muscarinic M2 receptor-mediated signaling pathway might contribute to relaxation. Another possible explanation may be an effect on calcium mobilization [9]. In porcine preparations, lidocaine inhibited both the acetylcholine-induced increase in calcium sensitivity of the contractile apparatus and the release of muscarinic M3 receptor-mediated intracellular calcium [12]. In addition, the relaxant responses to local anesthetics are partially attributable to the blocking of extracellular calcium influx through voltage-dependent calcium channels [7, 12]. We proposed that levobupivacaine could modulate intracellular calcium dynamics in the presence of methacholine. Further studies could be conducted to elucidate these possible mechanisms.

EFS activates nerve terminals within the preparation and induces the release of endogenous neurotransmitters, thereby triggering smooth muscle contraction. Because the frequency used in our study was within the physiological range of vagal stimulation (5−15 Hz), an EFS-induced spike contraction of the tracheal smooth muscle was believed to result from the stimulation of parasympathetic innervation. Lidocaine is effective in protecting against contractions evoked by the electrical stimulation of guinea pig trachealis muscle [13]. In a study of both dogs and rabbits, administering bupivacaine aerosol inhibited the bronchoconstriction produced by an electrical stimulation of a cut vagus nerve [14]. These phenomena may be explained by the depression of efferent nervous activity in the airways. Thus, this study demonstrated that high concentrations of levobupivacaine might attenuate nerve conduction and reflex arcs involved in the mediation of airway hyperreactivity.

The findings of this study can be compared with the results of previous studies that indicated that lidocaine and bupivacaine induce complete relaxation at a concentration of 10^{-3} M [4, 5]. The concentration of local anesthetic required to produce neural blockade was inversely related to its potency. Under our experimental conditions, the potency of levobupivacaine was greater than that of bupivacaine and lidocaine in promoting the relaxation of the airways muscle. The levobupivacaine findings suggested that its in vitro effects and clinical potency as a local anesthetic were not correlated because levobupivacaine exhibits

similar sensory and motor-blocking properties as bupivacaine does [15]. The most reasonable explanation for this may be related to differences in the effective tissue levels of local anesthetics. The penetration of levobupivacaine into the tracheal smooth muscle may be sufficiently deep to achieve high concentrations in airway tissue. Future studies focusing on the effects of tracheal smooth muscle and local anesthetic tissue concentrations may clarify these concerns.

In conclusion, our in vitro study indicated that high concentrations of levobupivacaine might antagonize the cholinergic receptors and inhibit parasympathetic function of the trachea.

Acknowledgments This work was not supported by any research grants or funding.

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Demiraran Y, Ozturk O, Guclu E, Iskender A, Ergin MH, Tokmak A. Vasoconstriction and analgesic efficacy of locally infiltrated levobupivacaine for nasal surgery. Anesth Analg. 2008;106:1008–11.
2. Hunt LW, Swedlund HA, Gleich GJ. Effect of nebulized lidocaine on severe glucocorticoid-dependent asthma. Mayo Clin Proc. 1996;71:361–8.
3. McAlpine LG, Thomson NC. Lidocaine-induced bronchoconstriction in asthmatic patients. Relation to histamine airway responsiveness and effect of preservative. Chest. 1989;96:1012–5.
4. Kao CH, Chu YH, Wang HW. Effects of lidocaine on rat's isolated tracheal smooth muscle. Eur Arch Otorhinolaryngol. 2010;267:817–20.
5. Chang Y-N, Wang C-H, Wu C-C, Wang H-W. Effects of bupivacaine on the isolated rat tracheal smooth muscle. J Exp Clin Med. 2012;4:62–5.
6. Chang H-C, Cherng Y-G, Hsu C-T, Liu M-C, Wang H-W. Effects of dexmedetomidine on the isolated rat tracheal smooth muscle. J Exp Clin Med. 2013;5:139–42.
7. Lautner RQ, Zapata-Sudo G, Sudo RT. Relaxation of tracheal smooth muscle independent on functional epithelium cells induced by lidocaine, bupivacaine and isomers in rats. Eur J Pharmacol. 2009;610:93–8.
8. Yunoki M, Nakahara T, Mitani A, Maruko T, Kubota Y, Sakamoto K, Ishii K. Lidocaine attenuates muscarinic receptor-mediated inhibition of adenylyl cyclase in airway smooth muscle. Eur J Pharmacol. 2003;470:65–71.
9. Yamakage M, Namiki A. Cellular mechanisms of airway smooth muscle relaxant effects of anesthetic agents. J Anesth. 2003;17:251–8.
10. Okumura F, Denborough MA. Effects of anaesthetics on guinea pig tracheal smooth muscle. Br J Anaesth. 1980;52:199–204.
11. Downes H, Loehning RW. Local anesthetic contracture and relaxation of airway smooth muscle. Anesthesiology. 1977;47:430–6.
12. Kai T, Nishimura J, Kobayashi S, Takahashi S, Yoshitake J, Kanaide H. Effects of lidocaine on intracellular $Ca^{2+}$and tension in airway smooth muscle. Anesthesiology. 1993;78:954–65.
13. Weiss EB, Anderson WH, O'Brien KP. The effect of local anesthetic, lidocaine, on guinea pig trachealis muscle in vitro. Am Rev Respir Dis. 1975;112:393–400.
14. Dain DS, Boushey HA, Gold WM. Inhibition of respiratory reflexes by local anesthetic aerosols in dogs and rabbits. J Appl Physiol. 1975;38:1045–50.
15. Cox CR, Checketts MR, Mackenzie N, Scott NB, Bannister J. Comparison of S(-)-bupivacaine with racemic (RS)-bupivacaine in supraclavicular brachial plexus block. Br J Anaesth. 1998;80:594–8.