Effects of Bupivacaine on the Isolated Rat Tracheal Smooth Muscle

Ying-Nan Chang 1, Chih-Hung Wang 1, Chi-Chung Wu 1, Hsing-Won Wang 1,2 *

1 Department of Otolaryngology-Head and Neck Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan
2 Graduate Institute of Clinical Medicine and Department of Otolaryngology, Taipei Medical University-Shuang Ho Hospital, Taipei, Taiwan

1. Introduction

Vascular or nasal mucosal strips have been used to study smooth muscle contractility in vitro. 1,2 Effects of drugs on the airway have been examined using a rat tracheal smooth muscle preparation because this tissue has a resting tone and pharmacological responses similar to those of the human airway. We developed a simple and rapid test for identifying agents that affect tracheal smooth muscle directly. 3 The development of such a test could help resolve the inconsistencies observed in trachea responses to drugs in vivo.

Bupivacaine is an amide-linked local anesthetic that is usually used for infiltration, nerve block, epidural and intrathecal anesthesia. Bupivacaine acts by binding to the sodium channels and blocking sodium influx into nerve cells, which prevents depolarization of the nerve fibers. Some reports have shown that aerosol inhalation of local anesthetics can reduce cough, inflation and deflation reflexes, and facilitate tracheal intubation during surgical operations. 4–6 Intravenous administration of lidocaine or bupivacaine has been reported to attenuate bronchial hyperreactivity in awake humans. 7 It has been demonstrated that bupivacaine or lidocaine applied topically to the smooth muscle of isolated rat tail arteries can inhibit vascular contraction in response to methacholine. 8–10

1878-3317/$ – see front matter Copyright © 2011, Taipei Medical University. Published by Elsevier Taiwan LLC. All rights reserved.
administration (45 mg/kg) and two pieces of trachea each ~5 mm long were removed from each rat. Each specimen was mounted using two steel plates and immersed in a bath containing 30 mL of Krebs solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄·7H₂O, 25.0 mM NaHCO₃, 10.0 mM glucose) at 37°C aerated with 5% CO₂ balanced in oxygen as described.³ The upper side of the tracheal strip was attached to a Grass FT-03 force displacement transducer (AstroMed, West Warwick, RI, USA) 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 recorded continuously using Chart V4.2 software (PowerLab, ADInstruments, Colorado Springs, CO, USA). Tests showed that a tracheal strip continuously using Chart V4.2 software (PowerLab, ADInstruments, Colorado Springs, CO, USA). Tests showed that a tracheal strip immersed in the bath solution used for subsequent experiments did not contract when basal tension was applied. Prior to drug administration or electrical stimulation, isolated trachea samples were equilibrated in the bath solution for 30–45 min.

2.2. Stimulation of tracheal smooth muscle

An earlier study showed that methacholine can induce a dose-dependent contraction of a tracheal strip and a concentration of 10⁻⁶ M resulted in significant contraction.¹ In view of the toxicity of the drug and fatigue of the tracheal smooth muscle, we used 10⁻⁶ M methacholine as a tracheal constricting drug. All drugs were administered by adding a defined volume of stock solution to the bath.

EFS (50 V, 5 Hz, pulse duration 5 milliseconds, trains of stimulation for 5 seconds) was applied to the tracheal strip with two wire electrodes parallel with the tissue and connected to a direct current stimulator (Grass S44, Quincy, MA, USA). We used an interval of 2 minutes between stimulation periods to allow recovery from the response. Stimulation was applied to the trachea strips at 37°C.

2.3. Assessment of effects of bupivacaine

To determine the cumulative dose–response relationships, bupivacaine was added in increasing concentrations without washout between additions of the drug. Sufficient time was allowed to obtain the maximal effect of each dose. The effects of bupivacaine on (1) tracheal smooth muscle resting tension, (2) contraction caused by 10⁻⁶ M methacholine as a parasympathetic mimetic and (3) electrically induced tracheal smooth muscle contraction were assessed. Six tracheal strips were used for each experiment and one untreated strip served as a control.

2.4. Statistical analysis

Drug concentrations are expressed as the concentration in the 30 mL of bath solution. Data are presented as mean ± SD. Differences between mean values were compared using one-way analysis of variance. The statistically significant difference was set at p < 0.05.

3. Results

The degree of contraction or relaxation in tracheal strips was determined from the tension applied to the transducer. The addition of bupivacaine to the bath did not significantly alter the resting tension in non-stimulated tracheal strips (Figure 1). Tracheal contraction induced by methacholine was easily detected and the tissue remained in a contracted state until the drug was rinsed from the tissue. When bupivacaine was cumulatively applied to the steady state of 10⁻⁶ M methacholine-induced contraction, a concentration-dependent tension reduction occurred (Figure 2). At 10⁻⁸ M bupivacaine, the tension was 98.2% ± 2.6% of control values; at 10⁻⁶ M bupivacaine the tension was 56.7% ± 10.3% and at 10⁻³ M bupivacaine the tension was −13.7% ± 13.8% (Figure 3). The contraction induced by methacholine was totally abolished at 10⁻³ M bupivacaine. The inhibition of contraction was statistically significant (p < 0.05) at 10⁻⁴ M and 10⁻³ M bupivacaine compared to that at 10⁻⁶ M bupivacaine and the control.

The tracheal strips contracted in response to EFS at 5 Hz. After the contraction reached a steady state, bupivacaine was added to the bath to different concentrations. The peak tension of the tracheal strip induced by EFS was not changed following the addition of bupivacaine to 10⁻⁶ M; whereas at 10⁻⁵ M, 10⁻⁴ M and 10⁻³ M bupivacaine, the peak tension was 90.4% ± 4.5%, 27.2% ± 10.6% and 0% of the control, respectively. Bupivacaine also

---

**Figure 1** Effect of bupivacaine on non-stimulated tracheal smooth muscle.

**Figure 2** Representative time course of cumulative relaxation effect of bupivacaine on 10⁻⁶ M methacholine-induced rat tracheal contraction.
decreased the spike contraction induced by EFS (Figure 4). The spike contraction was totally inhibited at $10^{-3}$ M bupivacaine (Figure 5). The peak tension values of the tracheal strip evoked by EFS at $10^{-5}$ M, $10^{-4}$ M and $10^{-3}$ M bupivacaine were significantly lower ($p < 0.05$) than that observed for $10^{-8}$ M bupivacaine.

4. Discussion

Many in vitro assays have been used for investigating tracheal responses to local anesthetics or other drugs. In those assays, the tracheal preparation was opened by cutting through the cartilage rings or the tracheal mucosa was removed or only tracheal smooth muscle strips were used.9–13 All of these procedures violate the normal physiology in vivo. The tracheal strips used in our examination were excised as an intact trachea ring without damaging the mucosa or smooth muscle; i.e., they were crude preparations that contained mucosa, cartilage and tracheal smooth muscle. The intact tracheal ring is an important component of our study. Our test was much more robust and representative of a physiological situation than tests in which the tracheal rings were destroyed. It was therefore reasonable to assume that the tracheal response to the test drug in our investigation was comparable to those observed after application of a spray to the trachea during an asthma attack or stimulation.

In this study, neither low nor high concentrations of bupivacaine caused the non-stimulated tracheal strip to contract. This suggested that aerosol administration of bupivacaine might not elicit bronchospasm in patients with bronchial hyper-reactivity.

It was noteworthy that the tracheal strip relaxation induced by bupivacaine was dependent on the prior smooth muscle contraction induced by methacholine. This finding implied that this drug might be effective in relieving asthma or bronchospasm. Bupivacaine, a long-duration local anesthetic that could reduce methacholine-induced contraction of smooth muscle, is known as a sodium channel blocker but how the sodium channel blocker affects the tracheal smooth muscle remains unclear. Although it has been demonstrated that lidocaine relaxes airway smooth muscle directly by decreasing the intracellular concentration of Ca$^{2+}$; the exact mechanism requires further exploration.11

EFS-induced contraction of canine nasal mucosa disappeared after ipsilateral cervical sympathetic ganglionectomy. The EFS-induced spike contraction of nasal mucosa was proven from the stimulation of sympathetic innervation.14 Under these conditions, tracheal smooth muscle spike contraction in response to the EFS in the present study was considered to result from the stimulation of parasympathetic innervation. The frequency of stimulation (5 Hz) used is within the physiologic range of parasympathetic nerve activity. Thus, the anesthetic effects under these experimental conditions could reflect an important mode of action in the intact organism. In this study, the EFS-induced contraction of trachea smooth muscle was decreased progressively as the concentration of bupivacaine was increased. This suggests that the amide-linked anesthetic antagonized the parasympathetic innervation in the tracheal smooth muscle contraction and might have an antispasmodic effect on the tracheal smooth muscle in patients with asthma or prone to bronchospasm. Bupivacaine, like other local anesthetics, can inhibit action potential by blocking the sodium channel, which is thought to contribute to inhibition of neural transmission during electrical stimulation.

Several studies have characterized the action of local anesthetic agents on tracheal smooth muscle reactivity and have shown that bupivacaine can induce relaxation of tracheal smooth muscle contraction induced by cholinergic agents, an action that was partially dependent on Ca$^{2+}$ influx into the muscle cells.15,16 Aside from the anticholinergic effect, our study showed that bupivacaine inhibited tracheal smooth muscle responsiveness to electrical stimulation. Thus, the relaxant effect of bupivacaine was caused by depression of neural response to EFS in addition to antagonism of the cholinergic agents.
In conclusion, our *in vitro* study revealed concentration-dependent bupivacaine attenuation of the tracheal contractions induced by EFS and by a depolarizing agent in a manner. These findings imply that bupivacaine might inhibit cholinergic neurotransmission and have an anti-spasmic effect on the trachea.

**References**