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ORIGINAL ARTICLE

Effects of Bupivacaine on the Isolated Rat Tracheal Smooth Muscle

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Background: Bupivacaine is an amide-linked local anesthetic that has been used in subcutaneous infiltration, epidural and peripheral nerve block for surgery over three decades. Several studies have suggested that inhaled local anesthetics can reduce respiratory reflexes. To assess the direct actions of bupivacaine on airway smooth muscle we used our preparation to examine the effectiveness of bupivacaine on isolated rat trachea.

Methods: A 5-mm-long portion of rat trachea was mounted in Krebs solution at 37 °C. Changes in tracheal contractility in response to a parasympathetic mimetic agent and electrical stimulation were measured using a transducer connected to a Pentium III computer equipped with polygraphy software. The following assessments were done: (1) effect of bupivacaine on the resting tension of tracheal smooth muscle; (2) effect of bupivacaine on contraction caused by exposure to 10^{-6} M methacholine as a parasympathetic mimetic; (3) effect of bupivacaine on electrically induced contraction of tracheal smooth muscle.

Results: Bupivacaine had a negligible effect on the basal tracheal tension as the concentration increased. Bupivacaine inhibited the tracheal smooth muscle contraction in response to methacholine and electrical stimulation in a concentration-dependent manner.

Conclusion: The study indicates that bupivacaine might inhibit cholinergic neurotransmission and have an anti-spasmic effect on the trachea, in that it inhibited the contractions elicited by electrical field stimulation and a depolarizing agent.

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 adrenergic activation.[8] The effect of bupivacaine topically applied to the trachea has not been well explored. In the present study, we used a simple *in vitro* technique to investigate the direct effects of bupivacaine on the contractile response of isolated rat tracheal smooth muscle to methacholine and electric field stimulation (EFS).

2. Methods

2.1. Study design

This study was approved by the Animal Experiment Review Board (IACUC-08-146). All chemical reagents were obtained from Sigma (St Louis, MO, USA) and were of the highest purity available. Adult rats were anesthetized using intraperitoneal pentobarbital

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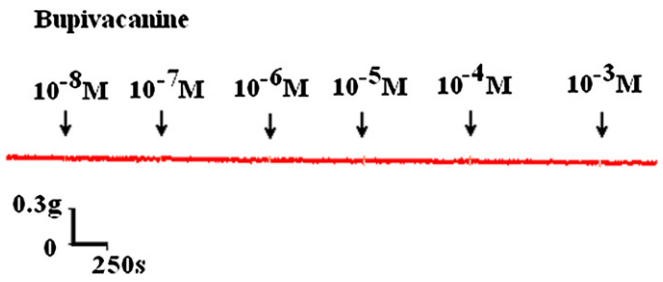

Figure 1 Effect of bupivacaine on non-stimulated tracheal smooth muscle.

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 $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25.0 mM $NaHCO_3$, 10.0 mM glucose) at 37°C aerated with 5% CO_2 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 immersed in the bath solution used for subsequent experiments did not contract when basal tension was applied. Prior to drug addition 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.[3] 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^{-4} 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^{-8} 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 final concentrations. The peak tension of the tracheal strip induced by EFS was not changed following the addition of bupivacaine to 10^{-8} 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

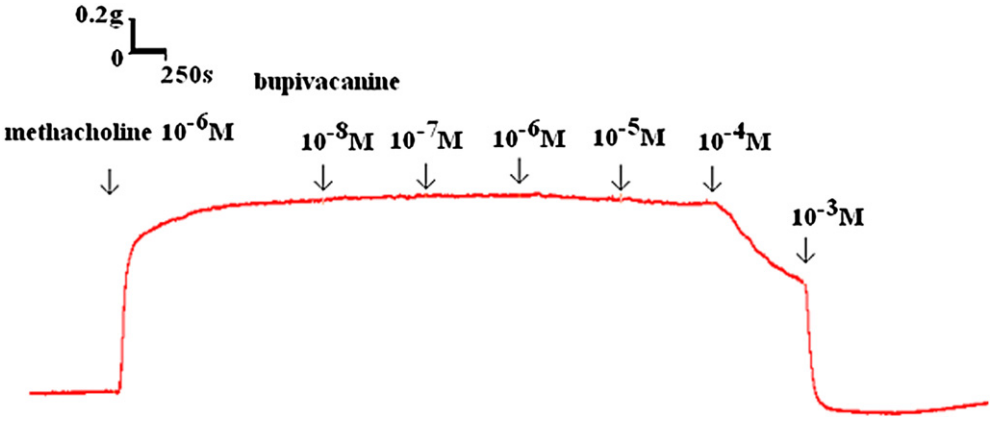

Figure 2 Representative time course of cumulative relaxation effect of bupivacaine on 10^{-6} M methacholine-induced rat tracheal contraction.

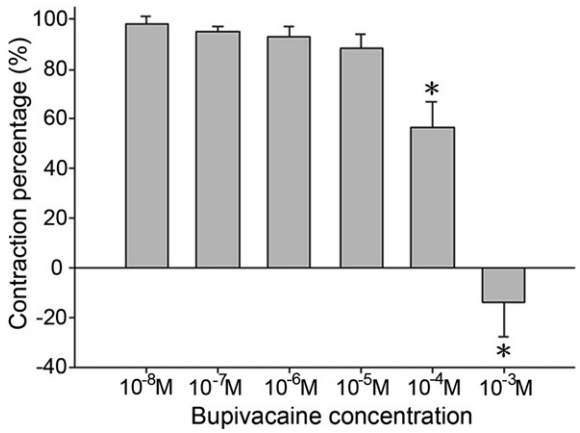

Figure 3 Concentration-dependent relaxation effect of bupivacaine on 10^{-6} M methacholine-induced tracheal contraction. *A p value <0.05 comparing 10^{-4}M–10^{-3}M bupivacaine with 10^{-8}M–10^{-5}M bupivacaine. Values are mean ± SD ($n = 6$).

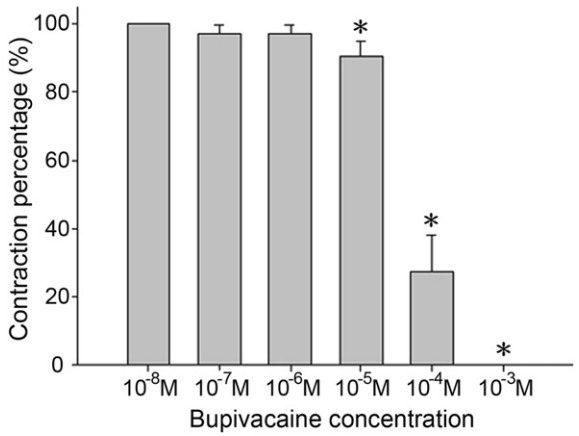

Figure 5 Concentration-dependent relaxation effect of bupivacaine on tracheal contraction produced by EFS. *A p value <0.05 comparing 10^{-5}M–10^{-3}M bupivacaine with 10^{-8}M–10^{-6}M bupivacaine. Values are mean ± SD ($n = 6$).

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

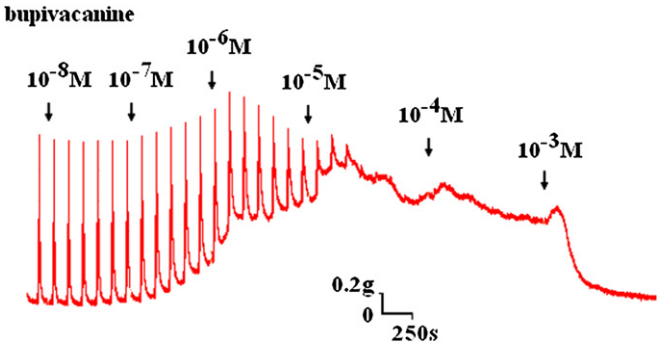

Figure 4 Representative time course of cumulative relaxation effect of bupivacaine on EFS-induced rat tracheal contraction.

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 antispasmic 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.

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