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A Modified in vitro Method for StudyingTracheal Smooth Muscle Response to Drugs

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Background: Vascular or nasal mucosal strips have been successfully used to study smooth muscle contractility in vitro. Using the trachea of the rat, we developed a simple and rapid in vitro technique for testing the effects of drugs that induce tracheal constriction or relaxation. **Methods:** We used our preparation to test the effectiveness of acetylcholine and methacholine as tracheal contraction drugs and the effectiveness of verapamil as a tracheal relaxing drug. A 5 mm long portion of rat trachea was submersed in 30 ml Kreb's solution in a muscle bath at room temperature. Changes in tracheal contractility in response to the application of parasympathetic mimetic agents were measured using a transducer connected to a Pentium III computer equipped with polygraphy software. **Results:** Addition of parasympathetic mimetics to the incubation medium caused the tracheal to contract in a dose-dependent manner. Addition of verapamil, a relaxation agent, induced a relaxation response only when the preparation had been pretreated with a tracheal constricting agent such as methacholine. Responses to verapamil were dose-dependent. **Conclusion:** The degree of drug-induced tracheal contraction or relaxation is dose-dependent. This method could prove useful for studying the effects of drugs on tracheal smooth muscle activity.

Key words: trachea, smooth muscle, in vitro study

INTRODUCTION

Vascular or nasal mucosal strips have been used to study smooth muscle contractility in vitro[1,2]. We used rat tracheas to develop a simple in vitro technique to test the effects of drugs that induce tracheal constriction or relaxation. Our technique was based on a previously described method[3,4] in which 10 mm strips of rabbit trachea are suspended in a tissue bath containing 20 ml of Kreb's solution. One end of the strip is attached to a steel plate and the other to an isometric transducer and a steel plate. A passive tension of 8 g is applied to the strips.

A simple and rapid test is needed for screening parasympathetic mimetic agents and potential tracheal contraction agents and for identifying agents that affect tracheal smooth muscle directly. Development of such a test may help to resolve the inconsistencies in responses of the trachea to drugs in vivo.

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MATERIALS AND METHODS

All chemical reagents were obtained from Sigma (St. Louis, MO, USA). We tested acetylcholine and methacholine as tracheal contraction drugs and verapamil as a tracheal relaxation drug. Eight rats were anesthetized by intraperitoneal administration of pentobarbital (45 mg/kg) and two pieces of trachea about 5 mm in length were removed from each rat. This study was approved by an animal experiment review board (LACUC-05-158). The tracheal specimen was mounted using two steel plates and submersed in a 30 ml muscle bath at room temperature. The bath (Fig. 1) was filled with 30 ml Kreb's solution consisting of (mmol/L) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 10.0.

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 0.5 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). Preliminary tests showed that a tracheal strip immersed in the bath solution used for subsequent experiments did not

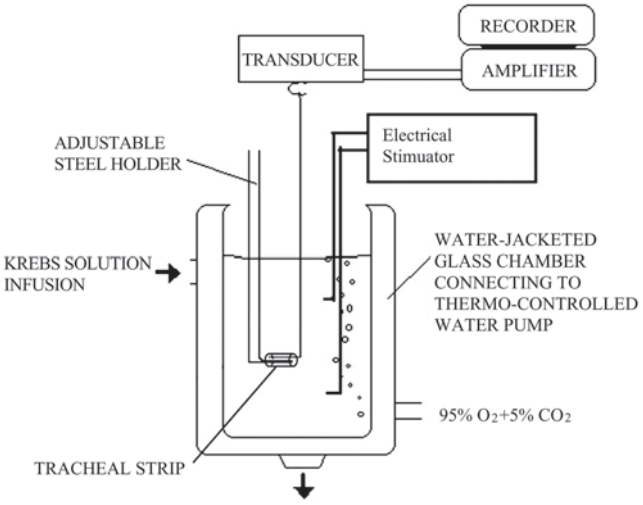

Fig. 1 Schematic diagram of measurement of tension in isolated rat tracheal smooth muscle.

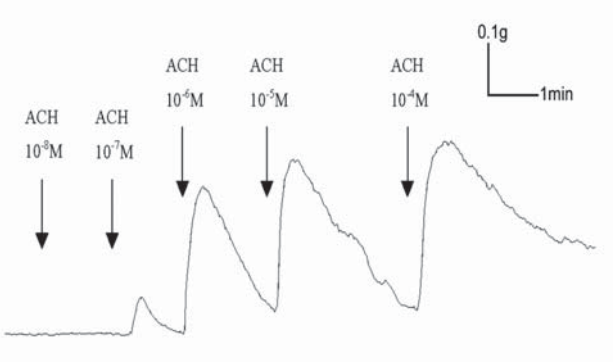

Fig. 2 Tension changes in the rat trachea after the application of various acetylcholine (ACH) concentrations. Basal tension was 0.5 g.

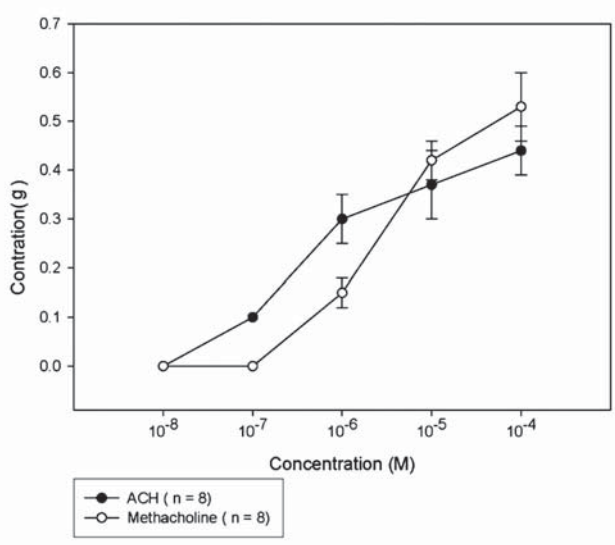

Fig. 3 Different drug effects on the tension of rat's trachea. Results were mean±SD (n=8). Both tracheal contractions induced by 10^{-6} M and 10^{-4} M were statistically significant between acetylcholine (ACH) and methacholine.

contract when basal tension was applied. Before drug assays were conducted, isolated tracheas were equilibrated in the bath solution for 15-30 min, during which continuous aeration with a mixture of 95% O_2 and 5% CO_2 was applied. Stepwise increases in the amount of drugs used were employed to study contraction or relaxation responses of tracheal strips. All drugs were administered by adding a defined volume of stock solution to the tissue bath solution.

Concentrations of drugs are expressed as concentrations present in the 30 ml bath solution. Data were presented as mean values and standard deviations (SDs). Differences between mean values were compared using Student's t-test. Differences were assumed to be significant at $P < 0.01$.

RESULTS

The degree of contraction or relaxation of tracheal strips was estimated from the tension applied to the transducer. Tracheal contraction induced by a small dose of acetylcholine was easily detected (Fig. 2) and the tissue remained in a contracted state until the drug was rinsed from the tissue.

Contractile responses to another parasympathomimetic agent, methacholine, are shown in Fig. 3. Tracheal mucosa was slightly less sensitive to methacholine than acetylcholine when present in low concentrations. The degree and rate of tissue contraction induced by both agents was dose dependent (Fig. 3). Addition of the muscular relaxation agent, verapamil, on its own elicited no response, but resulted in relaxation of the trachea when introduced after the addition of a constricting agent such as methacholine (Fig. 4). Low doses of verapamil resulted in a slight decrease in contraction and higher doses relaxed the trachea more quickly.

DISCUSSION

Although in vitro assays for monitoring tracheal responses to drugs have been developed by other groups[3-6], they all have disadvantages. In such assays, a tracheal mucosa strip measuring 8 mm×20 mm is attached to an isometric transducer and suspended in a tissue bath containing 30 ml of Kreb's solution. This is a relatively

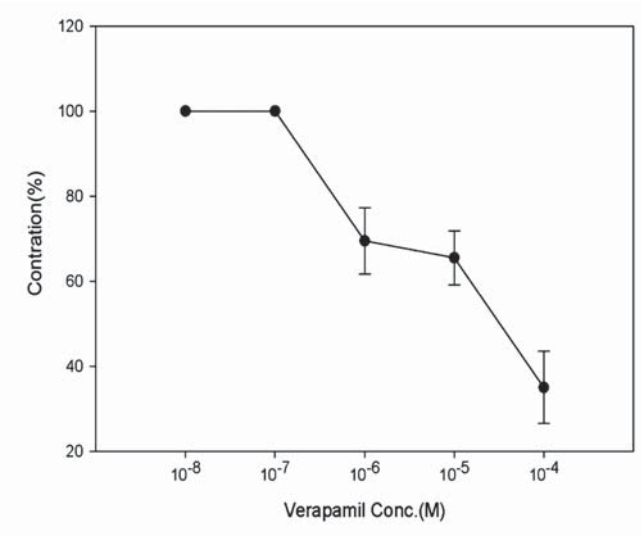

Fig. 4 Effects of verapamil on 5 x10^{-6} M methacholine-induced contraction (contraction area was calculated at 100%) of rat's trachea. Results were mean ± SD (n = 6).

invasive procedure as it requires several centimeters of trachea; sufficient quantities of trachea are difficult to obtain from rats. Our test only requires a few millimeters of trachea, which is excised as an intact ring of trachea without permanent injury to the animal. An intact tracheal ring is an important component of our technique. Previous authors have used tracheal smooth muscle strips to conduct drug tests[3-6]. Our test is simpler and more robust than the tests in which tracheal rings are destroyed. An intact tracheal ring is much more representative of a physiological situation than smooth muscle strips.

The results of our experiments should be interpreted within the context of the test materials used. Although it is difficult to determine which tissue component of the trachea was responsible for drug-induced contraction, the nature of specific tissues and their responses to specific drugs provide some indication. Firstly, the tracheal strips used in our study were crude preparations that contained cartilage and tracheal smooth muscle (Fig. 5). The smooth muscle of the trachea appeared to be the main tissue component responsible for contraction as the other components (epithelium, glands, connective tissue, nerves, and cartilage) did not contract to a significant extent.

Because the method involves cross contraction, changes in tension were caused by radial contraction of the tracheal ring. Responses to drugs and electrical stimulation have been verified for similar preparations[4-7]. However, the contractile response observed was probably an aggregate

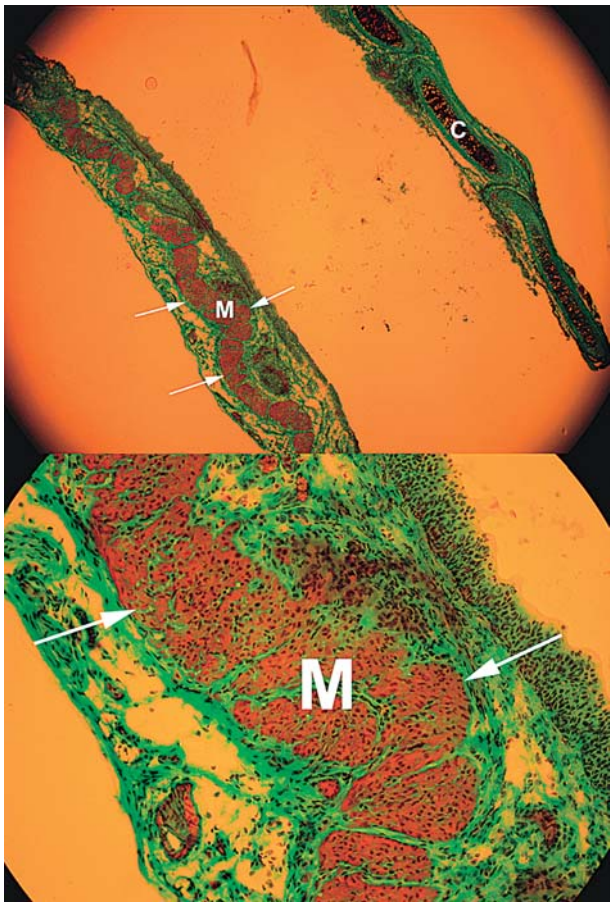

Fig. 5 Upper: A longitudinal section of rat's trachea was shown. The muscles were indicated by arrows. C indicated cartilage. (Papanicolaou's staining, x25); Bottom: Higher power view of muscle (m). (x200)

of the responses of various types of muscle tissue. As the epithelium may release factors that affect tracheal smooth muscle, it is possible that some drugs affect tracheal smooth muscle indirectly via changes to the epithelium. Secondly, the isolated tracheal preparations used in our experiments were excised from rats without damaging the endothelium or smooth muscle. It is therefore reasonable to assume that tracheal responses to test agents in our study are comparable to those observed after application of a spray to the trachea during an asthma attack.

Both contracting agents tested are commonly used. It is noteworthy that drug-induced relaxation of tissue was dependent on prior partial contraction of smooth muscle using acetylcholine or methacholine. It should thus be possible to assay the effects of common drugs and agents supposedly responsible for relieving asthma. Verapamil, a calcium channel blocker, reduced methacholine-induced contraction[2].

We still have yet to examine the effects of changes in the composition of Kreb's solution, storage conditions, and other drugs on our preparation. Further research with this preparation could provide additional insight into the mechanism of drug-related control of tracheal smooth muscle tension. This preparation may have clinical applications as it is simple and quick.

ACKNOWLEDGMENTS

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REFERENCES

1. Beny J, Pacicca C. Bidirectional electrical communication between smooth muscle and endothelial cells in the pig coronary artery. Am J Phisiol 1994;266,H1465-H1472.
2. Ichimura K, Jackson RT. Calcium, calcium blockers, and nasal smooth muscle. Arch Otolaryngol 1983;109: 593-597.
3. Bratton DL, Tanaka DT, Grunstein MM. Effects of temperature on cholinergic contractility of rabbit airway smooth muscle. Am J Phy 1987;63:1933-1941.
4. Gonzalez O, Santacana GE. Effect of low temperature on tracheal smooth muscle contractile and relaxing responses evoked by electrical field stimulation. Phy Res 2001;20:237-243.
5. Yau KI, Ko FN, Chien CH. Effects of prokinetic agents on contractile responses to electrical field stimulation of isolated guinea pig trachea. J Formos Med Assoc 1999;98:567-572.
6. Yau KI, Hwang TL. The nonadrenergic noncholinergic system can modulate the effect of prokinetic agents on contractile response of isolated guinea pig trachea segments to electrical field stimulation. J Formos Med Assoc 2002;101:695-699.