

Suitability of Goat Tracheal Muscle Preparation for Evaluating Substances with Bronchodilator Activity

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Short Communication

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ABSTRACT

Bronchial asthma is a disease of airways that is characterized by hyper responsiveness of the tracheobronchial tree to a multiplicity of stimuli. Testing of drug action on tracheobronchial smooth muscle should be initially done with tracheal or bronchial tissue in an organ bath by conventional methods. The present study was done to evaluate the suitability of goat tracheal muscle preparation for screening the drugs or substances with bronchodilator activity. Goat trachea was collected from the slaughter house from a freshly slaughtered goat. A series of dose responses with different doses of histamine were obtained till the ceiling dose was obtained. Varying doses of theophylline anhydrous which was prepared by dissolving in warm distilled water were added to the bath and allowed to act. It was observed that theophylline anhydrous (5×10^{-3}) solution used antagonized histamine induced contraction responses ($p < 0.001$). The goat tracheal muscle preparations are easier to handle, prepare and also seems to be more sensitive than guinea pig tracheal chain with consistent and dependable responses. This preparation can be used for screening variety of plant extracts or products to evaluate the bronchodilator activity. The goat tracheal preparation is very easily available and sacrificing laboratory animals in large numbers can be avoided for evaluation of substances with bronchodilator property.

INTRODUCTION

Bronchial asthma is a disease of airways that is characterized by hyper responsiveness of the tracheobronchial tree to a multiplicity of stimuli ^[1]. A galaxy of drugs and measures has been employed to treat these patients. Although most of the patients respond well to currently available treatment, 5–10 % with severe disease responds poorly ^[2]. Scientific workers are presently engaged in the search for safe, acceptable, effective and inexpensive bronchodilator from the extract of herbs and plants commonly grown in India. Such herbs and plants have been used since ages for the treatment of bronchial asthma.^[1]The synthesis of an ideal bronchodilator could be guided by screening the extracts of herbs. Testing of drug action on tracheobronchial smooth muscle should be initially done with tracheal or bronchial tissue in an organ bath by conventional methods. This has few advantages like, relatively small amount of the test material is required and the effect is tested directly without the factors like absorption, metabolism, excretion or interference due to nerve reflexes ^[3]. A number of in vitro preparations have been used by various workers for the study of bronchodilator drugs ^[4]. These researchers used muscle obtained from large animals. A tracheal chain can prepared from smaller laboratory animal like Guinea pig by sectioning the trachea into circular rings and connecting the rings in chain fashion with loops of silk thread ^[5]. But trachea itself is very short and imbedded in the surrounding tissue requiring elaborate dissection. So though a classical preparation requires skill to prepare and is not sensitive for many agonists ^[4]. On the contrary goat tracheal muscle preparation are easier to handle and to prepare. It is also seems more sensitive than guinea pig tracheal chain ^[2].

The pharmacological action of histamine on tracheobronchial muscle varies on different species. The rodents, dog and man respond with bronchoconstriction [6]. Many studies have been conducted to find out the species variations and the nature of receptors for goat trachea [7]

The normal pharmacological responses of goat tracheal muscle to not only histamine but also to 5-HT, acetylcholine and catecholamine and their known antagonists have been worked out by some of the authors [4,7,8].

In addition to airway inflammation, asthmatics commonly exhibit bronchial hyperactivity. The concentration of histamine needed to produce a 20% increase in airway resistance is only 1%–2% of equally effective concentration in healthy control subjects [9]

Histamine produces highly variable effects on the airway smooth muscles of mammalian species. There are studies done to detect the nature of receptors present in the goat tracheal muscle. The goat trachea was found to contain H₁ (excitatory), scanty population of H₂ (inhibitory), 5-HT and also muscarinic excitatory receptors, since the Acetylcholine induced contractions were effectively blocked by atropine [10].

Histologically the trachea and bronchi possess a common type of cartilage and muscle and pharmacologically both trachea and bronchi react in the same way [11].

The antagonist can be assayed against the spontaneous contraction or spasmogen induced contraction where a spasm is induced usually with a standard agonist like histamine and an antagonist is added into the bath to bring about relaxation [3].

The present study was carried out to find out the suitability of goat tracheal chain preparation for the assay of an antagonist against spasmogen induced contraction on tracheal muscle. bronchodilator activity.

METHODOLOGY

Goat trachea was collected from the slaughter house from freshly slaughtered goat and was immediately transferred to a thermostat flask containing cold Krebs's Hansleit solution (4°C). The trachea was kept in Krebs's Hansleit solution at 4°C in the refrigerator until used on the next day.

Goat trachea was cut transversely between the segments of cartilage, so as to give a number of rings of tracheal muscle. The smooth muscle portion with small cartilaginous ends was separated from the rest of the ring. One end of the trachealis muscle was attached to the bent portion of the tissue holder cum aerator tube and the other to the lever writing on a smoked drum, so as to suspend the tissue in a 40 ml organ bath containing Krebs's Hansleit solution at 37°C and aerated with mixture of oxygen (95%) and carbon dioxide (5%). The load on the lever was 0.5gm. A preliminary period of 30 minutes was allowed for stabilization of the preparation.

The kymograph was set to move at the speed of 0.1 cm/min. The baseline was recorded on the smoked cylinder for 5 minutes and at the end of 5th minute 0.1ml of agonist histamine was added and allowed to act for 5 minutes. At the end of 5 minutes kymograph was stopped and tissue was washed with fresh Krebs's Hansleit solution and 3–4 washes were given whenever necessary. 15 minutes cycles were followed including 5 minutes for the recovery of the tissue. A series of dose related responses with different doses of histamine were obtained till the ceiling dose was obtained. Varying doses of theophylline anhydrous which was prepared by dissolving in warm distilled water were added to the bath and allowed to act for 3 minutes. The response to the sub maximal dose of histamine was noted in the presence of theophylline anhydrous solution. The heights of contraction due to histamine before and after each addition of theophylline were measured and tabulated. Percentage reduction in the height of the contraction was calculated.

RESULTS

Isolated goat tracheal muscle preparation exhibited notable contractions to histamine in concentrations 0.1–6.4 µg/ml. Histamine dose response curve (DRC) was plotted in absence and in presence of theophylline anhydrous solution. It was observed that theophylline anhydrous (5X10⁻³) solution used antagonized histamine induced contraction responses significantly (p<0.001)(Table No 1)

DISCUSSIONS

The goat tracheal muscle preparation is an ideal one for the study of agonists as well as antagonists and can be prepared with ease and is also more sensitive though slightly higher concentrations were used by A.K.Nagchaudhuri [4]. These preparations were found to give consistent results for 8–10 hours [11] Inhibition of histamine induced contraction was linearly related to the log concentration of

theophylline solution added to the bath which was also observed in a study by Dinesh K et al [2]. It was found that the response of the tissue was not consistent during rainy season, while the other seasons were favorable for conducting experiments.

It is possible that a given drug may act differently on bronchioles and larger bronchi or the trachea. The available evidence indicates that the trachea and larger bronchi react much alike to drugs because of close anatomical and physiological association which exists between tracheal and bronchial muscle [11].

Regarding the type of antagonism between histamine and theophylline, it is nonspecific which does not necessarily involve receptor occupation and may occur at any step from drug receptor interaction to the effector system which results in a response [9].

This study was conducted using drugs with proven bronchodilator activity to demonstrate that the goat tracheal preparation gives consistent and dependable responses. This preparation can be used for screening variety of plant extracts or products in the similar way to evaluate the bronchodilator activity [1, 2]. It is noteworthy that goat trachea is very easily available and one can avoid sacrificing laboratory animals in large numbers for evaluation of substances with bronchodilator property. In the wake of banning of laboratory animal dissection and dire need for adaptation of alternative methods to animal experimentation, this study gives an insight into using an inexpensive, simple method for drug screening.

Table1: Effect of antagonist (bronchodilator) theophylline anhydrous on histamine induced contraction of isolated goat tracheal chain preparation

Dose (10µgmL ⁻¹) histamine concentration	Maximum contraction (%)	
	control	Theophylline anhydrous
0.1	22.65±1.32	10.42±0.814*
0.2	37.24±1.87	18.80±0.886*
0.4	48.14±1.79	24.82±0.816*
0.8	62.60±1.91	36.19±0.924*
1.6	86.17±3.1	44.18±1.64*
3.2	91.07±2.04	46.60±1.91*
6.4	100±2.14	55.28±1.31*

* p<0.001, d f=5

REFERENCES

1. Jitendra YN, Vikas VS, Vittal ND, Mangesh DK, Vilas MA, Minal RN. Antiasthmatic activity of marketed Ayurvedic polyherbal Formulation. J Phram Research. 2009;2(9):1385–86.
2. Dinesh Kumar, Bhujbal SS, Patil PS, Buge PV, In Vitro activities of stem bark of methanolic extract of *Ailanthus Exscela* Roxb. In the management of asthma. Int Pharmacol. 2010:1–6
3. Burn JH. Estimation of spasmolytic activity, In: Practical Pharmacology,Oxford; Blackwell Scientific Publication; 17–19; 1956.
4. Nagchaudhari AK, Lahiri SC. Use of goat trachea for an isolated tracheal chain preparation. Indian J Pharmacol. 1974;6:149–51.
5. Staff of the Department of Pharmacology, University of Edinburgh., Experiments with other smooth muscle preparations, In: Pharmacological Experiments on isolated preparations, Second Edition; Edinburgh London And Newyork; Churchill Livingstone; p.100–103;1970
6. Aitken MM, Sanford J, The effect of drugs on bovine tracheobronchial and pulmonary Vascular tissue. Brit J Pharm.1970; 38: 443–44.
7. Kulashrestha S, Misra SS, Sharma AL, Sharma P, Singhal D. Response of Goat Trachea to some autonomic drugs. Indian J Pharmacol. 1983;15(2):107–10
8. Chand N, Eyre. Classification and Biological distribution of histamine receptor subtypes. Agents and actions. 1975;5:277–95.
9. Bradley J Udem. Pharmacotherapy of asthma, Chapter 27,IN:Lawrence L Brunton,Lazo S,Keith L.Parker – Editors, Goodman and Gillman’s Pharmacological basis of Therapeutics, 11th edition, Newyork; McGraw–Hill; p.717–36, 2006.
10. Durant GJ, Ganellin CR, Parsons ME. Chemical differentiation of histamine Hi–and Hz–receptors agonists. J Med Chem. 1975;18: 905.909.
11. Castillo E, De–Beer S. Guinea – Pig tracheal chain preparation. J Pharmacol Exp Therapeut. 1947; 90:104–09