ORIGINAL ARTICLE

Comparison between the effects of Captopril and Lisinopril on Bradykinin-induced contraction in tracheal smooth muscle of guinea pig

JAVARIA ARSHAD MALIK[1], WAQAR AHMED SIDDIQUI[2], SEHRISH ZAFAR[3]

ABSTRACT

Background: ACE inhibitors are used for the treatment of hypertension and congestive heart disease may induce hyper-reactivity of the airways which may be associated with the accumulation of bradykinin and other inflammatory mediators present in the airways.

Aim: To compare the effect of captopril with lisinopril on bradykinin-induced tracheal smooth muscle contraction of guinea pig trachea.

Methods: A Comparative controlled in-vitro experimental study was carried out on the tracheal smooth muscle strips of guinea pig pretreated with indomethacin (10^{-6}M), phentolamine (10^{-5}M) and propranalol (10^{-6} M) to remove the effect of endogenous Catecholamin and prostaglandins. The activity of trachealis smooth muscle was noted through the Isometric Force Displacement Transducer on a Four Channel Oscillograph. Cumulative concentration-response relationship was displayed by adding successive concentrations of bradykinin on the tracheal strips starting with 22µg to 132 µg/dl.

Results: Bradykinin produced concentration dependent reversible contraction of isolated tracheal smooth muscle. The mean value of response achieved with 132 µg/dl of bradykinin in the presence of captopril was 51.33±2.79 and in the presence of lisinopril was 38.17±2.94. These ACE inhibitors shifted the concentration response curves of bradykinin to left and upward. On comparison among themselves it was observed that lisinopril as compared to captopril produced less enhancement of bradykinin-induced contraction of guinea pig tracheal smooth muscle.

Conclusion: Lisinopril produce less enhancement of bradykinin induced tracheal smooth muscle contraction then captopril.

Keywords: ACE inhibitors, cough, Bradykinin, Captopril, lisinopril, guinea pig trachea

INTRODUCTION

Angiotensin-converting-enzyme inhibitor (ACE inhibitor) are used for the treatment of hypertension and congestive heart failure. These drug cause vasodilatation, hypovolemia, results in decreasing blood pressure and decreased myocardial oxygen demand. Their major action is to inhibit the angiotensin-converting enzyme, of the renin–angiotensin–aldosterone system[1]. It is proposed that ACE inhibitor through blockade of angiotensin I conversion, limits the generation of angiotensin II, causes the reduction of vasoconstriction and water and sodium retention[2].

Commonly used ACE inhibitors are captopril, lisinopril, enalapril and ramipril. Captopril is a Sulfhydryl-containing agent and Lisinopril is a Dicarboxylate-containing agent. Studies suggest that these ACE inhibitors may be useful not only in the treatment of essential hypertension, chronic heart failure but also in nephropathy[3,4]. Their adverse effect include hypotension, fatigue, headache, cough, hyperkalemia and renal impairment[5]. .ACE inhibitors might increase inflammation-related pain, due to increase of bradykinin (a vasodilator peptide)[6]. Dry, irritant cough is reported in about 15% hypertensive patients due to accumulation of bradykinin and its degradation by ACE inhibitor[7].

The exact mechanism of ACE inhibitor-induced cough is not known. However, it is proposed that bradykinin (BK) is degraded by ACE drugs and precipitated in the upper respiratory tract and bring airway smooth muscle contraction carried out via tachykinins released from C-fiber endings[8]. Lately it is thought that bradykinin is converted to inactive metabolites by ACE, therefore inhibition of this ACE enzyme leads to raised levels of bradykinin, which causes cough via bronchoconstriction. The incidence of ACE inhibitor-induced cough has been reported to be in the range of 5 to 35% among patients treated with these agents[9].

This fact has been now proved in many aspects that all ACE Inhibitors are not equivalent[10]. ACE inhibitors differ in chemical structure[11]. The structures of captopril, enalapril and lisinoprill were compared in

[1,2]Assistant professor Pharmacology, CMH Lahore Medical & Dental College, Lahore
[3]Senior Demonstrator Pharmacology,
Correspondence to Dr. Javaria Arshad Malik, Email: drjavaria@yahoo.com, cell: 0321-5003757

a study. The analysis revealed that thiol group of captopril and carboxylate group of lisinopril have direct interaction with the zinc ion present on the active site of ACE enzyme. Other difference are also observed at other sites of binding pocket[12].

Captopril has been shown to sensitizes bronchial smooth muscles and this sensitization may be a cause of dry cough[13], and lisinopril shows antifibrotic effect due to inhibition of synthesis of angiotensin II that causes stimulation of fibroblast proliferation and collagen synthesis. It is therefore protect the paraquat (herbicide)-induced lung fibrosis[14]. ACE inhibitors are usually characterised by flat dose-response curves whereas lisinopril is shows a linear dose-response curve[15].

It is found that both captopril and lisinopril are well tolerated but lisinopril produced better fall in diastolic blood pressure[16] . However a study found that lisinopril seem to well-tolerated with efficacy on once-daily dosing better that captopril[17]. Recently it is found that Captopril initiators had the highest rate of mortality (117.8/ 1,000,000 person) as compared to other ACE inhibitors like lisinopril, ramipril and enalapril (54.3-79.4/1,000,000 person) but it should be confirm by further studies[18].

ACE inhibitors have been extensively used in the treatment of hypertension, but the comparative effectiveness regarding their ability to enhance bradykinin-induced tracheal smooth muscle contraction is seldom reported.

Study was designed to compare the effect of captopril with lisinopril on bradykinin-induced tracheal smooth muscle contraction of guinea pig trachea.

MATERIALS AND METHODS

Chemicals: Bradykinin acetate and Phentolamine Hydrochloride was purchased from Sigma Chemical Co, USA. Captoril Disulfide and Lisinopril Dihydrate was kindly provided by Chemo S.A .Lugano Brach, Hetero Drug Limited and Tanabe/Seiyaku Japan respectively. Indomethacin Acetate by Shanghai-Chang-Hua industry limited China, and Propranalol Hydrochloride by Changzhou Yabang Pharmaceutical Company All other chemicals used were purchased from local commercial sources. Solutions and dilutions of all drugs were prepared in the distilled water.

Experimental Procedure: Experiments performed were compiled with the rulings of the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council, and were approved by the PCGS committee for research, the National University of Science and Technology Islamabad Pakistan (NUST). Guinea pigs of either sex, of the Dunkin Hartley variety (500 to 600g) were housed at the animal house of the Army Medical College, Rawalpindi, NUST University, at room temperature. They were given tap water *ad libitum* and a standard diet. The guinea pigs were killed by cervical dislocation after approval of method by ethical committee. The tracheal tube was taken out and cut into rings 2–3 mm wide, each containing about two cartilages. The tissue preparation was mounted to an isolated tissue bath of 50 ml, capacity containing Kreb's Henseleit solution at 37º C and was aerated with oxygen continuously. The tissue was allowed a period of equilibration for 45 minutes against an imposed tension of two grams. A tension of one gram was applied to the tracheal strips continuously throughout the experiments. The trachealis muscle activity was measured with an Isometric Force Displacement transducer (Harvard model no 72-4494) and was recorded on Four Channel Oscillograph (Harvard model no 50-9307). After the equilibration period tracheal muscle preparation was incubated for 15 minutes with indomethacin (10^{-6}M), with phentolamine (10^{-5}M) and with propranalol (10^{-6}M) to eliminate the effect of endogenous prostaglandins and catecholamines. These drugs were added simultaneously in all the experiments and after 15 minutes experiments were started with this preparation.

In group I, after the preincubation period with the baseline tension of 1 gram cumulative concentration-response curves of bradykinin was obtained using concentrations 22, 44, 66, 88, 110 and 132µg/dl. When the plateau was achieved with the first concentration of bradykinin, then the subsequent dose was added to the tissue bath without washing the previous concentration.

In group II, cumulative concentration-response curve of bradykinin was obtained using the same concentrations of bradykinin as in the previous experiments in the presence of captopril 10^{-5} concentration.

In group III, cumulative concentration-response curve of bradykinin was obtained using the same concentrations of bradykinin as in the previous experiments in the presence of lisinopril10^{-5} concentration.

In group IV, cumulative concentration-response curve of captopril was obtained using concentrations 1, 1.5, 2, 2.5 and 3µM of captopril in the presence of fixed concentration of bradykinin 66µg/dl. This concentration of bradykinin has been chosen which causes consistent and submaximal effects, enabling us to observe potentiation or inhibition of contraction. Maximum response of smooth muscle contraction with captopril 3 µM concentration was taken as hundred percent and effects with lisinopril were compared to that.

In group V, cumulative concentration-response curve of lisinopril was obtained using concentrations 1, 1.5, 2, 2.5 and 3 µM of lisinopril in the presence of fixed concentration of bradykinin 66µg/dl. Six experiments were performed in the same way to get six recordings in all the five groups.

Statistical analysis

The results were expressed as Means±Standard deviation. The arithmetic means of amplitudes of contractions and S.D were calculated using SPSS version 18. In order to find the significance of the difference between two observations 'student t test' was used. P value <0.05 was considered significant.

RESULTS

Captopril enhanced the amplitude of tracheal contraction from mean value of 7.7mm to 35.6mm Semi logarithm concentration-response curve of bradykinin in the presence of captopril shifted to the left and upwards.

lisinopril at 10^{-5} M concentration enhances the amplitude of tracheal contraction from mean value of 7.7mm to 29.1mm. The concentration response curve of bradykinin in the presence of lisinopril was shifted to the left and upwards.

In comparison of Control Group I (Bradykinin) and Group II (Captopril+Bradykinin) The mean values of response produced by each concentration of bradykinin used, compared between Group I and Group II were found statistically significant (P<0.05).

In comparison of Control Group I (Bradykinin) and Group III (Lisinopril+Bradykinin) The mean values of response produced by each concentration of bradykinin used compared between Group I and Group III were found statistically significant *(P <0.05).*

In comparison of Group II (Captopril + Bradykinin) and Group III (Lisinopril + Bradykinin). The mean values of responses produced by each concentration of bradykinin used compared between Group II and Group III were found statistically significant *(P <0.0*5) (Table 1 and 2).

In comparison of Control Group I (Bradykinin) and Group III (Lisinopril +Bradykinin) The mean values of response produced by each concentration of bradykinin used compared between Group I and Group III were found statistically significant showing P values of 0.002, 0.002, 0.00, 0.00 and 0.001 *(P <0.05).* Comparison of concentration response curves of two drugs are shown in figure I. Figure I: Cumulative log concentration-response curves of bradykinin in the presence of fixed concentrations of captopril (10^{-5} M) and lisinopril (10^{-5} M).

In the second set of experiments, bradykinin in a fixed concentration of 66µg/dl was added in the organ bath and then concentration-response curve was obtained by increasing concentration of captopril. Same procedure was repeated with lisinopril. This was carried out to determine the concentration-dependent effects of these ACE inhibitors on bradykinin induced contraction. The concentration of bradykinin (66µg/dl) was chosen because it produced consistent and submaximal effects enabling us to observe potentiating or inhibition of contraction. Results were similar to first set of experiments in which lisinopril had produced less bradykinin-induced contraction than captopril. Enhancement of the contraction produced by lisinopril was near to the effect produced with captopril. Cumulative concentration-response curve with captopril has been taken as the control and curves with lisinopril was compared to that. The shift of the curve is statistically significant (P<0.05) (figure II).

Table 1: Comparison of responses to bradikinin between group 11 (captopril + bradykinin) and group 111 (lisinopril +bradykinin) with a drug dose of 22, 44 and 66 µg

Tissue 1-6	**Group 11**	**Group 111**	**Group 11**	**Group 111**	**Group 11**	**Group 111**
Dose of drug	22 µg	22 µg	44 µg	44 µg	66 µg	66 µg
Mean Response(mm)	13.00	12.83	26.5	23.00	35.00	29.50
SD	10.47	8.86	4.37	5.40	3.16	4.64
P value	0.03		0.01		0.02	

Table 2 : Comparison of responses to bradikinin between group 11 (captopril + bradykinin) and group 111 (lisinopril +bradykinin) with a drug dose of 88, 110 and 132 µg

Tissue 1-6	**Group 11**	**Group 111**	**Group 11**	**Group 111**	**Group 11**	**Group 111**
Dose of drug	88 µg	88 µg	110 µg	110 µg	132 µg	132 µg
Mean Response(mm)	41.67	35.0	46.5	36.67	51.33	38.17
SD	3.33	6.36	3.94	6.53	6.83	7.19
P value	0.00		0.038		0.009	

Fig. I:

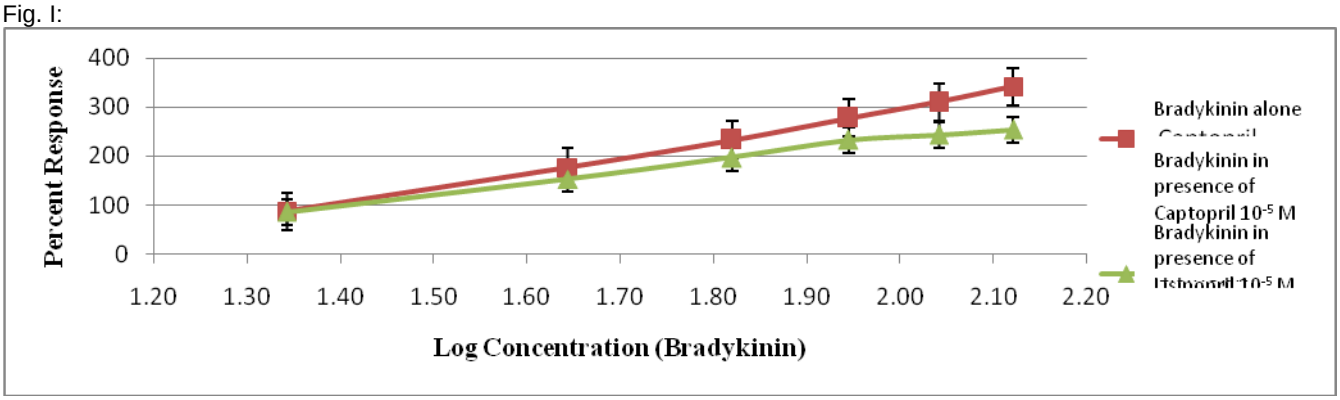
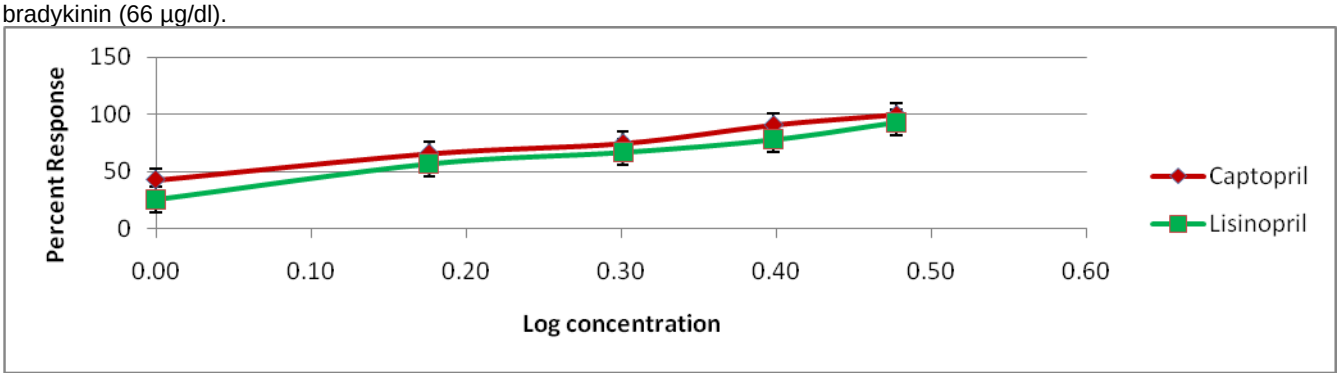


Fig. II: Cumulative log concentration-response curves of Captopril and lisinopril in the presence of fixed concentration of bradykinin (66 µg/dl).



DISCUSSION

ACE inhibitors are used for the treatment of hypertension and congestive heart disease may induce hyperreactivity of the airways with occurrence of a persistent dry cough, wheezing and dyspnoe in some of the patients. It is supposed that the hyperreactivity is associated with the accumulation of tachykinins, bradykinin and other inflammatory mediators present in the airways[19].

According to our study, captopril enhanced the amplitude of tracheal contraction from mean value of 7.7 mm to 35.6mm. Our study is in line with number of studies. One of the study reported that extreme contraction of tracheal smooth muscle is responsible for hyperresponsiveness of airway. This hyperresponsive airways show increased sensitivity to certain bronchoconstrictors, and the resulting airway obstruction is highly responsive to bronchodilators[20]. Another study stated that ACE inhibitors are often linked with an increased occurrence of bronchial responsiveness and cough that may cause more deterioration of patients with altered pulmonary function[21].

Present study has observed that lisinopril at 10^{-5} M concentration enhances the amplitude of tracheal contraction from mean value of 7.7mm to 29.1mm. A study found that lisinopril enhanced the cough response persuaded by both chemical and mechanical stimulation due to increase in the numeral of coughs induced by each stimulation[22]. The results of a study stated the central role of lisinopril mediated by an accumulation of substance P and bradykinin[23].

We also compared the response of bradykinin alone with the response produced by the combination of bradykinin with captopril. This shows an increased effect with a P value is <0.05. A study is experimentally proved that ACE inhibitors give the effects on the vascular system and increase the indirect effects of bradykinin on B2 receptors. ACE inhibitors directly activate B1 receptors via the zinc-binding present in B1 receptor[24]

According to a study bradykinin has been associated as a mediator of the acute pathophysiological and inflammatory outcomes of respiratory tract infections and may be responsible for chronic diseases of lung. Study experimentally proved that Bradykinin may stimulate cough and other conditions. It is found that these cough responses quickly desensitized, with desensitization of B2 receptor. Bradykinin-evoked cough was made effective by inhibition of both angiotensin-converting enzyme and neutral endopeptidase[25].

Mean dose of BK is needed to produce 100% increase in airway pressure. The dose-response

curve for the effect of BK was significantly shifted to the left by the captopril. Data suggest that ACE degrade BK in the airway lumen without the involvement of kininase I[26].

In comparison of Control Group I (Bradykinin) and Group III (Lisinopril +Bradykinin) The mean values of response produced by each concentration of bradykinin used compared between Group I and Group III were found statistically significant *(P <0.05).*

A study found that bradykinin (BK) causes sensitization of airway sensory neurons and an increase in the cough reflex of experimental animal. It is suggested that BK trigger the production of prostaglandin synthesis and increase the release of pro-inflammatory neuropeptides from neurons, a system that may be observed during inflammation, and this can be stopped by a bradykinin B2 receptor antagonist[27].

Bradykinin (BK) has many effects on airway function which may be applicable in obstructive airways disease in both animal and human. These effects are carried out via B2-receptors. Bradykinin is a effective bronchial vasodilator, increases microvascular leakage, triggers mucus secretion and epithelial cells to release bronchodilators and also triggers mucus secretion leading to response of bronchoconstriction, neurogenic inflammation and coughing via release of neuropeptides from sensory nerves[28].

REFERENCES

1. Jackson, Edwin K. "Chapter 30. Renin and Angiotensin". In Brunton, Laurence L.; Lazo, John S.; Parker, Keith. Goodman & Gilman's The Pharmacological Basis of Therapeutics 2006 (11th ed.). New York: McGraw-Hil
2. Ma TKW, Kam KKH, Yan BP, Lam YY. Renin–angiotensin–aldosterone system blockade for cardiovascular diseases: current status. Br J Pharmacol. 2010 Jul; 160(6): 1273–1292.
3. Dimopoulos NA, Salukhe TV, Coats AJ, Mayet J, Piepoli M, Francis DP. "Meta-analyses of mortality and morbidity effects of an angiotensin receptor blocker in patients with chronic heart failure already receiving an ACE inhibitor (alone or with a beta-blocker)". Int J Cardiol.2004; 92 (2): 105–111
4. Luno J, Praga M, de Vinuesa SG. "The reno-protective effect of the dual blockade of the renin angiotensin system (RAS)". Current pharmaceutical design. 2005; 11 (10): 1291–300.
5. Sidorenkov G, Navis G. "Safety of ACE inhibitor therapies in patients with chronic kidney disease". Expert Opinion on Drug Safety. 2014; 13 (10): 1383–1395
6. Fein A. "ACE inhibitors worsen inflammatory pain". Medical Hypotheses. 2009; 72 (6): 757.
7. Okumura H, Nishimura E, Kariya S, Ohtani M, Uchino K, Fukatsu Tet al. [No Relation between Angiotensin-Converting Enzyme (ACE) Inhibitor-Induced Cough and ACE Gene Polymorphism, Plasma Bradykinin, Substance P and ACE Inhibitor Concentration in Japanese Patients]. Yakugaku Zasshi (in Japanese). 2001;121 (3): 253–7.
8. Dicpinigaitis PV. Angiotensin-converting enzyme inhibitor-induced cough: ACCP evidence-based clinical practice guidelines. Chest. 2006 Jan;129(1 Suppl):169S-173S.
9. Inoue H, Koto H, Takata S, Aizawa H, Ikeda T. Excitatory role of axon reflex in bradykinin-induced contraction of guinea pig tracheal smooth muscle. Am Rev Respir Dis. 1992 Dec;146(6):1548-52.
10. Comini L, Bachetti T, Cargnoni A, Bastianon D, Gitti GL, Ceconi C and Ferrari R. Therapeutic modulation of nitric oxide: all ace inhibitors are not equivalent. Pharmacological Research 2007; 56; 42-48.
11. Acharya KR, Sturrock ED, Riordan J and Ehlers, MRW. ACE revisited: a new target for structure-based drug design. Nat Rev Drug Discov 2003; 2: 891-902.
12. Ramanathan N, Sylva LU, Schwager, Edward D, Sturrock and K Ravi Acharya. Crystal structure of human angiotensin converting enzyme-lisinopril complex Nature 2003;421, 551.
13. Agrawal N, Akella A, Deshpande SB. Captopril augments acetylcholine-induced bronchial smooth muscle contractions in vitro via kinin-dependent mechanisms. Indian J Exp Biol. 2016 Jun;54(6):365-9.
14. Mohammadi-Karakani A, Ghazi-Khansari M, Sotoudeh M. Lisinopril ameliorates paraquat-induced lung fibrosis. Clin Chim Acta. 2006 May;367(1-2):170-4.
15. Song JC, White CM. Clinical pharmacokinetics and selective pharmacodynamics of new angiotensin converting enzyme inhibitors: an update. Clin Pharmacokinet. 2002;41(3):207-24.
16. Rappelli A. Controlling hypertension: lisinopril-hydrochlorothiazide vs captopril-hydrochlorothiazide. An Italian multicentre study. J Hum Hypertens. 1991 Dec;5 Suppl 2:55-7
17. Graham RD. Treating mild-to-moderate hypertension: a comparison of lisinopril-hydrochlorothiazide fixed combination with captopril and hydrochlorothiazide free combination. J Hum Hypertens. 1991 Dec;5 Suppl 2:59-60
18. Chang CH, Lin JW, Caffrey JL, Wu LC, Lai MS. Different Angiotensin-converting enzyme inhibitors and the associations with overall and cause-specific mortalities in patients with hypertension. Am J Hypertens. 2015 Jun;28(6):823-30.
19. Franová S, Nosál'ová G.ACE-inhibitors and defence reflexes of the airways. Acta Physiol Hung. 1997-1998;85(4):359-66.
20. Govindaraju V, Michoud MC, Ferraro P, Arkinson J, Safka K, Valderrama-Carvajal H, et al. The effects of interleukin-8 on airway smooth muscle contraction in cystic fibrosis. Respir. Res. 2008;9:76
21. Packard KA, Wurdeman RL, Arouni AJ. ACE inhibitor-induced bronchial reactivity in patients with respiratory dysfunction. Ann Pharmacother. 2002 Jun;36(6):1058-67.
22. Mutolo D, Cinelli E, Bongianni F, Evangelista S, Pantaleo T. Comparison between the effects of lisinopril and losartan on the cougn reflex in anesthetized and awake rabbits. J Physiol Pharmacol. 2013 Apr;64(2):201-10.
23. Cinelli E, Bongianni F, Pantaleo T, Mutolo D. The cough reflex is upregulated by lisinopril microinjected into the caudal nucleus tractus solitarii of the rabbit. Respir Physiol Neurobiol. 2015 Dec;219:9-17
24. Kugaevskaia EV, Eliseeva IuE. [ACE inhibitors--activators of kinin receptors]. Biomed Khim. 2011 May-Jun;57(3):282-99.
25. Hewitt MM[1], Adams G Jr, Mazzone SB, Mori N, Yu L, Canning BJ Pharmacology of Bradykinin-Evoked Coughing in Guinea Pigs. J Pharmacol Exp Ther. 2016 Jun;357(3):620-8.
26. Ichinose M, Barnes PJ. The effect of peptidase inhibitors on bradykinin-induced bronchoconstriction in guinea-pigs in vivo. Br J Pharmacol. 1990 Sep;101(1):77-80.
27. Schuligoi R[1], Peskar BA, Donnerer J, Amann R. Bradykinin-evoked sensitization of neuropeptide release from afferent neurons in the guinea-pig lung. Br J Pharmacol. 1998 Sep;125(2):388-92
28. Barnes PJ. Effect of bradykinin on airway function. Agents Actions Suppl. 1992;38 (Pt 3):432-8.

.