Captopril augments acetylcholine-induced bronchial smooth muscle contractions in vitro via kinin-dependent mechanisms

Naman Agrawal, Aparna Akella & Shripad B Deshpande*
Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Received 10 June 2014; revised 05 February 2015

Angiotensin converting enzyme (ACE) inhibitors therapy is associated with bothersome dry cough as an adverse effect. The mechanisms underlying this adverse effect are not clear. Therefore, influence of captopril (an ACE inhibitor) on acetylcholine (ACh)-induced bronchial smooth muscle contractions was investigated. Further, the mechanisms underlying the captopril-induced changes were also explored. In vitro contractions of rat bronchial smooth muscle to cumulative concentrations of ACh were recorded before and after exposure to captopril. Further, the involvement of kinin and inositol triphosphate (IP$_3$) pathways for captopril-induced alterations were explored. ACh produced concentration-dependent (5-500 µM) increase in bronchial smooth muscle contractions. Pre-treatment with captopril augmented the ACh-induced contractions at each concentration significantly. Pre-treatment with aprotinin (kinin synthesis inhibitor) or heparin (inositol triphosphate, IP$_3$-inhibitor), blocked the captopril-induced augmentation of bronchial smooth muscle contractions evoked by ACh. Further, captopril-induced augmentation was absent in calcium-free medium. These results suggest that captopril sensitizes bronchial smooth muscles to ACh-induced contractions. This sensitization may be responsible for dry cough associated with captopril therapy.

Keywords: Angiotensin converting enzyme inhibitors, Aprotinin, Bronchospasm, Cholinergic contractions, Dry cough, IP$_3$, Kinin

Angiotensin converting enzyme inhibitors (ACEIs) are the class of drugs used extensively in the treatment of hypertension and heart failure. In spite of the therapeutic efficacy of ACEIs, dry cough is encountered in 5-35% patients receiving the treatment$^{1,4}$. Cough has emerged as a class effect occurring with all ACEIs$^{1,2}$. This symptom may occur at any point of time after institution of therapy. Replacement by another ACEI causes the cough to recur$^7$. Cough is usually resistant to treatment and the patients are subjected to unnecessary diagnostic tests and consultations. Treatment of cough with antitussive agents, bronchodilators, or antibiotics is not useful$^5$. Sometimes, the cough can be much troublesome to the patient, thereby leading to withdrawal of ACEI therapy and switching over to other drugs$^5$. The mechanism underlying dry cough associated with ACEI therapy is not known clearly$^4$.

Angiotensin converting enzyme (ACE) is a non-specific dipeptidyl carboxypeptidase that cleaves dipeptide units from substrates with diverse amino acid sequences$^{1,2}$. It cleaves angiotensin-I to form angiotensin-II. It also acts as kininase II to degrade kinins, thereby causing decreased endogenous kinin levels. Inhibition of ACE by ACEIs causes accumulation of kinins, angiotensin-I and many other downstream products in vivo$^{1,2}$. One or more of these products may be responsible for adverse effects associated with ACEI therapy. Angiotensin-I has a little physiological role in the body, apart from being a substrate for synthesis of angiotensin-II$^9$, and may not account for ACEI-associated dry cough. Dry cough can be produced by bronchoconstriction or bronchospasm, as seen with asthma. Kinins have been implicated to induce bronchoconstriction$^{1,3,6,7}$. Thus, it is hypothesized that ACEIs increase the kinin levels to produce bronchoconstriction and dry cough.

In order to understand the mechanisms underlying ACEI-associated dry cough, we investigated the effects of captopril (a prototype ACEI) on bronchial smooth muscle contractions in vitro. The predominant contractile innervation of airway smooth muscle is cholinergic in nature$^{7,8}$. Thus, bronchial smooth muscle contractions were evoked by the natural agonist, acetylcholine (ACh) and the effect of captopril pre-treatment on these contractions was examined. Further, the signalling pathways underlying the captopril-induced changes were investigated.

*Correspondence:
Phone: +91 542 2369069; Fax: +91 542 2367568
E-mail: desh48@yahoo.com
Materials and Methods

Animals

All animal experiments were approved by the Institutional Animal Ethical Clearance Committee. Experiments were performed on healthy Charles Foster strain male albino rats (200-300 g, n = 21), housed at 25±0.5°C, under 12:12 light:dark cycles. All animals received ad libitum diet (Hindustan Lever, Mumbai, India) and water. A day before experiment, the rats were fasted overnight and provided with water ad libitum.

Dissection and recording of bronchial smooth muscle contractions in vitro

The animals were sacrificed by cervical dislocation and exsanguination. The lungs along with primary bronchi were carefully dissected and transferred to a Sylgard plated petri-dish containing physiological solution bubbled with 100% O₂. Intrapulmonary bronchus of 3-5 mm length was dissected out carefully. One end of the bronchus was secured to a glass tissue holder. Then it was transferred to an organ bath containing physiological solution bubbled with 100% O₂. The other end was fastened firmly using a fine thread, to a force displacement transducer (ADInstruments, Australia) and an initial tension of 0.2 g was given. The preparation was allowed to stabilize for 30 min at 35°C. After stabilization the contractions evoked by ACh in presence or absence of antagonists were recorded on a computer-based chart recorder (PowerLab, ADInstruments, Australia).

Experimental design

The experiments were performed in three groups. In group I (n=8), the effect of captopril on ACh-induced contractions was assessed. Initially, contractions were recorded by exposing the tissue to cumulative concentrations of ACh (5-500 µM). The tissue was exposed to each concentration of ACh for 30 s before exposing it to next higher concentration. After exposing to 500 µM of ACh, the preparation was washed thrice with physiological solution for 5 min. Further, the preparation was exposed to captopril (70 µM). After 20 min, cumulative concentration-response to ACh was repeated as before. Peak amplitude of the contractile response from the baseline was measured.

In group II, the experiments were divided into two subgroups to evaluate the effects of kinin synthesis inhibitor (aprotinin) or inositol triphosphate (IP₃) antagonist (heparin) on the captopril-induced changes in bronchial smooth muscle contractions. After recording the initial responses to cumulative concentrations of ACh, the bronchial smooth muscle was exposed to aprotinin (1.53 µM; n=4)³,⁴,¹⁰/heparin (10 IU/mL, n=4)³,¹¹. After 10 min exposure to aprotinin/heparin, captopril (70 µM) was added to the organ bath. After 20 min, cumulative concentration-response to ACh was repeated as before.

In group III (n=5), the experiments were performed to evaluate the effect of extracellular calcium on captopril-induced changes in bronchial smooth muscle contractions evoked by ACh. After recording the initial responses to cumulative concentrations of ACh, the tissue was washed (thrice) with normal physiological solution and then exposed to calcium-free medium. After stabilization for 20 min, cumulative concentration-response of ACh was repeated. Then the tissue was washed with calcium-free physiological solution (as above) and then exposed to captopril (70 µM). After 20 min, cumulative concentration-response of ACh was repeated as before.

Data analysis and statistical procedures

The peak amplitude of contractions was recorded from the baseline. The amplitude of contraction evoked by the maximal concentration of ACh (500 µM) on naïve tissue was taken as 100% and other responses were normalized to it. The data were expressed as mean±SE. The concentration-response relationship was tested by one way ANOVA and the responses after treatment with captopril and aprotinin/heparin were analyzed by using two way ANOVA. Multiple comparisons were made by using Student Newman Keul test. A P <0.05 was considered significant.

Drugs and solutions

ACh and aprotinin were obtained from Sigma Chemical Company, St. Louis, MO, USA. Captopril was procured from Wockhardt Limited, Mumbai, India. Heparin was obtained from Biological Evans, Hyderabad, India. Stock solutions of the drugs were prepared in distilled water and final dilutions were made in physiological solution. The physiological solution had the following composition in mM: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; NaHCO₃, 5.0; KH₂PO₄, 1.2; glucose, 10 and pH of 7.4. Composition of calcium-free solution was similar to normal physiological solution but without CaCl₂. In addition, ethylene glycol tetra acetate (EGTA-0.2 mM) was added to it.
Results

Captopril augmented the ACh-induced bronchial smooth muscle contractions

In the preliminary experiments, responses to cumulative concentrations of ACh (5-500 µM) were found to reach the peak amplitude within 30 s. ACh (5-500 µM) produced concentration-dependent increase in bronchial smooth muscle contractions (P < 0.05, one way ANOVA) with maximal response at 500 µM. After exposure to captopril (70 µM) for 20 min, the contractile responses of bronchial tissue to ACh were significantly greater at all concentrations (P < 0.05, Two way ANOVA; Fig. 1 A and B).

Effect of antagonists on captopril-induced augmentation of bronchial contractions

In this group, initial responses to cumulative concentrations of ACh were similar to the previous group. Pre-treatment with kinin synthesis inhibitor, aprotinin (1.53 µΜ) blocked the captopril-induced augmentation of bronchial contractions evoked by ACh (n=4, P < 0.05, two way ANOVA, Fig. 1C as compared to ‘After’ response in Fig. 1B).

Pre-treatment with IP<sub>3</sub> antagonist, heparin (10 IU/mL) also blocked the captopril-induced augmentation of bronchial contractions evoked by ACh (n=4, P < 0.05, two way ANOVA, Fig. 1D as compared to ‘After’ response in Fig. 1B). Instead, there was a significant attenuation of ACh-evoked contractile response after exposure to heparin (P <0.05, two way ANOVA).

Fig. 1.—Captopril augmented the acetylcholine induced bronchial smooth muscle contractions. The augmentation was blocked by pretreatment with aprotinin or heparin. (A): original tracings from an experiment showing the response of bronchial smooth muscles to cumulative concentrations of ACh, before and after captopril (70 µM). Arrowheads indicate the points of administration of ACh; (B) concentration-response of ACh before and after captopril; (C) the responses to cumulative concentrations of ACh before and after ‘aprotinin (1.53 µΜ) + captopril’; (D) the responses to cumulative concentrations of ACh before and after ‘heparin (10 IU/mL) + captopril’. [Values represent mean ± SE from 4-8 different experiments. In B, an asterisk (*) indicates significant increase in the contractile response after captopril administration (P <0.05, two way ANOVA). In D, (#) indicates significant attenuation of the contractile responses after ‘heparin + captopril’ administration (P <0.05, two way ANOVA)]

Discussion

The present observations show that captopril (ACE inhibitor) augmented the bronchial smooth muscle contractions induced by ACh. The augmentation was blocked by kinin synthesis inhibitor (aprotinin) and IP<sub>3</sub>-antagonist (heparin). Further, augmentation of ACh-induced contractions by captopril was absent in Ca<sup>2+</sup>-free medium.
ACE is an endogenous dipeptidyl carboxypeptidase that cleaves angiotensin-I to form angiotensin-II. In addition, ACE also cleaves kinins owing to its kininase-II activity, thereby reducing endogenous kinin levels. Thus, by inhibiting ACE, captopril increases endogenous kinins and angiotensin-I levels. It has been reported that angiotensin-I appears to function only as the precursor of angiotensin-II without having any significant physiologic action. On the other hand, kinins have diverse biological actions including the actions on smooth muscles. Involvement of kinins has been reported for augmentation of cough induced by other ACEIs like zofenopril, ramipril and TAME-esterase. Present observations showing the blockade of captopril-induced augmentation of bronchial smooth muscle contractions by kinin synthesis inhibitor (aprotinin) supports the involvement of kinins.

Kinins are endogenous peptides released in response to tissue damage, allergic reactions or inflammatory events. Kinins act via B1 and B2 receptors, which mediate the actions through G protein coupled receptors (GPCRs). Binding of agonist (kinin) with the GPCR activates downstream signaling through phospholipase C (PLC)-IP3-Ca2+ pathway. Present results demonstrate that IP3 antagonist blocked the captopril-induced augmentation of bronchial smooth muscle contractions, supporting the involvement of IP3 pathway in mediating the action of kinins on bronchial smooth muscles. These findings are further supported by the fact that bradykinin-induced activation of Ca2+ current was blocked by IP3 antagonist (heparin) in human airway smooth muscle.

Bronchial smooth muscles are innervated by vagus (parasympathetic) nerves. Further, it has been shown that the vagal inputs mediate both contractile (cholinergic) and relaxatory (non-cholinergic) responses. The non-cholinergic inhibitory responses are shown to balance the cholinergic excitatory activity. Hence, bronchiolar spasm can result from the dysbalance of excitatory and inhibitory inputs. Further, it is shown that non-cholinergic mechanisms are mediated through the prejunctional muscarinic receptors.

The M2 and M3 muscarinic acetylcholine receptors are expressed on airway smooth muscles. The M2 receptors are located on parasympathetic ganglionic site as well as in the prejunctional sites (autoreceptors) and M3 receptors are located on bronchial smooth muscle at postjunctional sites. The M2 receptors are shown to modulate the ganglionic and prejunctional transmitter release. On the other hand, M3 receptors mediate the excitatory contractile response of the bronchial smooth muscle. A number of inflammatory mediators (tachykinins, bradykinin, histamine, prostaglandins, interleukins, thrombaxane, etc.) are known to facilitate the ganglionic transmitter release, while allergens, ozone, major basic protein, etc., inhibit the prejunctional M2 receptors thus enhance the transmitter (ACh) release at the junctional site. Thus, these mechanisms result in increased smooth muscle contraction/hyperresponsiveness of bronchial smooth muscles during inflammation or asthma.

Our results show that captopril augmented the ACh response even at the concentration that produced maximal response (Fig. 1). These observations indicate the existence of additional mechanism operating for the increased contractility of bronchial smooth muscles in the presence of kinins (captopril treated group). Kinins and ACh mediate the actions via GPCR and these agonists results in the activation of PLC-IP3-Ca2+ pathway to increase intracellular Ca2+ and contraction. Thus, it is likely that the intracellular Ca2+ release induced by kinins is added to the ACh-induced Ca2+ release thereby bringing greater contraction in bronchial smooth muscles. Such synergism of bradykinin receptor and M2 receptors-induced responses has been reported, thus supports our findings for an additional mechanism.

Cytosolic Ca2+ plays key role in smooth muscle contractions. Ca2+ influx into the smooth muscle cells release Ca2+ from smooth endoplasmic reticulum (SER) to increase the cytosolic Ca2+ which triggers series of changes leading to smooth muscle contraction. IP3 releases the Ca2+ from SER which is shown to trigger the opening of store-operated Ca2+ channels (SOCs), thereby causing Ca2+-influx into the cell. Our experiments with Ca2+-free medium show the absence of augmentation of ACh-induced contractions of bronchial smooth muscles in captopril treated group. This observation supports that captopril-induced augmentation is dependent on Ca2+ influx from extracellular compartment.

Based on the above observations, the following mechanism for captopril-induced augmentation of bronchial smooth muscle contractions is proposed. Captopril prevents the degradation of kinins, thereby increasing endogenous kinins. Increased kinins in vivo activate kinin receptors on bronchial smooth muscles, causing increased intracellular Ca2+ levels via IP3 pathway. In addition, kinins increase the ganglionic and prejunctional ACh release involving M2 receptors.
thus released, activates the M3 receptors located on the postjunctional sites to increase the intracellular Ca\(^{2+}\) level via GPCR-PLC-PI dependent mechanisms. Further, synergistic elevation of the intracellular Ca\(^{2+}\) resulting from kinins and ACh causes formation of more actin-myosin cross links leading to the augmentation of bronchial smooth muscle contractions to ACh in captopril treated group. The enhanced bronchial smooth muscle contractile response (bronchial spasm) to ACh after captopril may be responsible for the dry cough associated with captopril therapy.

Dry cough similar to the one encountered with ACEI therapy is reported with stimulation of J receptors\(^{20}\). J-reflex has a protective physiological role in exercise by causing hypotension and bradycardia, thereby reducing pulmonary capillary pressure\(^{21}\). J-reflex augmentation by scorpion venom has been shown to be mediated by kinins\(^5\)^\(^{22,23}\). Thus, kinin accumulation causing J-reflex activation can be another mechanism underlying ACEI-associated dry cough.

To conclude, captopril sensitizes the bronchial smooth muscles to the action of ACh via kinin-dependent mechanisms. Further, the kinin-dependent sensitization of ACh response is mediated via IP\(_3\)-Ca\(^{2+}\)-dependent pathways. Thus, captopril-induced enhanced bronchial contractility may lead to bronchial spasm and dry cough seen in patients receiving captopril therapy.

Acknowledgement

Financial assistance from ICMR (Indian Council for Medical Research), New Delhi is acknowledged. NA is a recipient of KVPY (Kishore Vaigyanik Protsahan Yojana; New Delhi) fellowship.

References