Physiol. Res. 54: 515-520, 2005

Enalapril and Diltiazem Co-Administration and Respiratory Side Effects of Enalapril

S. FRAŇOVÁ, G. NOSÁĽOVÁ, M. ANTOŠOVÁ, S. NOSÁĽ

Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Received February 9, 2004
Accepted November 30, 2004
On-line available January 10, 2005

Summary

A persistent, chronic dry cough is the most common adverse effect of angiotensin converting enzyme (ACE) inhibitors therapy. The mechanism of this respiratory adverse effect is related to the inhibition of ACE and the accumulation of bradykinin, substance P, prostanoids and other inflammatory neuropeptides in the airways. The aim of this study was to follow the relationship between 15-day administration of enalapril and the defense reflexes (cough and bronchoconstriction) of the airways in experimental animals, as well as the possibility of their pharmacological restriction with simultaneous diltiazem administration. Cough reflex was investigated by the method of mechanical irritation of laryngopharyngeal and tracheobronchial area in non-anesthetized cats. The reactivity of tracheal smooth muscles of the airways to bronchoconstrictor mediators (histamine 10 nM – 1 mM, acetylcholine 10 nM – 1 mM and KCl 1 mM – 100 mM) was evaluated by an *in vitro* method in guinea pigs. Enalapril 5 mg/kg/day and diltiazem 30 mg/kg/day were administered perorally for 15 days. The results showed that long-lasting administration of enalapril resulted in a significant increase of measured cough parameters and increased reactivity of tracheal smooth muscle to histamine and KCl. Simultaneous administration of enalapril together with diltiazem significantly decreased the enalapril induced cough, and decreased enalapril induced hyperreactivity of tracheal smooth muscles to KCl. The results showed a partially protective effect of diltiazem and enalapril co-administration on the respiratory adverse effects induced by enalapril therapy.

Key words
Enalapril • Cough • Bronchoconstriction • Diltiazem

Introduction

Angiotensin-converting enzyme (ACE) inhibitors are the drugs of choice in the treatment of hypertension and congestive heart failure. ACE-inhibitors lower the blood pressure without adverse effects on lipid and glucose metabolisms. However, it has been reported that in some patients ACE-inhibitors induce a dry non-productive cough with the incidence between 0.2-37 % (Israili and Hall 1992). Other airway reactions following ACE-inhibitor therapy such as dyspnoe and wheezing occur less frequently (Semple 1995).

The mechanism of respiratory adverse effects associated with ACE-inhibitors is related to the inhibition

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

of angiotensin convertase, which plays a pivotal role in the metabolism of bradykinin and substance P. Kinins (such as bradykinin), normally degraded by ACE, are accumulated in the airways as a result of ACE inhibition. The result of this effect is enhanced sensitivity of the cough reflex and the reactivity of airway smooth muscles (Trifilieff *et al.* 1993).

Bradykinin stimulates bronchial C-fibres and induces the release of substance P *via* axon reflexes (Sekizawa *et al.* 1996). Substance P is the second neuropeptide for the proteolytic action of ACE and may be involved in the stimulation of respiratory adverse effects of ACE-inhibitors (cough reflex and bronchoconstriction).

Another possible mechanism that can be involved here is that bradykinin and substance P may stimulate phospholipase A_2 activity that results in an increased formation of arachidonic derivates, mainly prostaglandins and tromboxane A_2 (Dendorfer *et al.* 1999). $PGF_{2\alpha}$ and PGE_1 belong to prostanoids stimulating the cough reflex (Ho *et al.* 2000).

The aim of the present study was to investigate the effect of ACE-inhibitor enalapril administration on the mechanically stimulated cough and reactivity of the airway smooth muscle in experimental animals. ACE-inhibitors are frequently used in clinical practice for the treatment of hypertension in combination with the group of calcium channel blockers**.** Ca^{2+} channel blockers exert their therapeutic effects by reversibly blocking the L-type voltage-dependent Ca^{2+} channels (Striessnig *et al.* 1998). The block of transmembrane calcium ion flux in the respiratory tract through these channels causes inhibition of bronchoconstriction and modulation of the cough reflex (Undem *et al.* 2002). The second phase of the study was to examine the possibility of lowering respiratory adverse effects of enalapril by means of simultaneous administration of Ca^{2+} channel blocker diltiazem.

Methods

Material

Enalapril, diltiazem, histamine hydrochloride, acetylcholine were purchased from Sigma-Aldrich. All other chemicals and solvents used were purchased from commercial sources.

Mechanically induced cough by in vivo method

A method of mechanical stimulation of the laryngopharyngeal and tracheobronchial area of the airways in non-anesthetized cats of both sexes weighing 1500-2500 g was used in the experiment (Korpáš and Nosáľová 1991). After several days of quarantine, a tracheal cannula was surgically implanted into the animals. The tracheal cannula served for five consecutive mechanical irritation of the laryngopharyngeal (LPh) and tracheobronchial (TB) mucous area of the airways with nylon fiber 0.35 mm in diameter. The single cough parameter (changes in lateral tracheal pressure different from the normal breathing pattern) was reviewed from registered pressure changes by Mingograph Elema device. The number of cough efforts (NE) obtained before drug administration represented control values. Enalapril was administered perorally as a saline solution in the dose 5 mg/kg b.w. to the first group of experimental animals for 15 days. The second group of animals was treated for 15 days with enalapril (dose 5 mg/kg b.w. perorally) and diltiazem (dose 30 mg/kg b.w. perorally). The effect of enalapril and simultaneous administration of enalapril with diltiazem on the cough parameters was monitored in the intervals 3, 5, 8, 10, 12 and 15 days.

Reactivity of smooth muscles of the airways by in vitro method

The reactivity of tracheal smooth muscles was estimated *in vitro*, after 15 days administration of enalapril (5 mg/kg/day) and after 15 days combined administration of enalapril (5 mg/kg/day) with diltiazem (30 mg/kg/day).

TRIK guinea pigs (250-350 g) of either sex were used in the experiment. The guinea pig tracheal strips were placed in 20-ml organ chamber containing Krebs-Henseleit buffer of the following composition (mM): NaCl, 110.0; KCl, 4.8; $CaCl_2$, 2.35; $MgSO_4$, 1.20; $KHPO_4$, 1.20; $NaHCO_3$, 25.0; in glass-distilled water. Organ chambers were maintained at 36.5±0.5 ^oC and were aerated continuously with the mixture 95 % O_2 and 5 % CO_2, to maintain pH 7.5±0.1. The tissue strips were initially set to 4 g of tension (30 min loading phase). After this period, the tension in each tissue segment was readjusted to a baseline of 2 g (30 min adaptation phase). During these periods the tissue was washed at 15 min intervals. The amplitude of isometric contraction (mN) of the tracheal smooth muscle to the cumulative doses of histamine (10 nM – 1 mM), acetyl-choline (10 nM – 1 mM) and KCl (1 mM – 100 mM) were used for the reactivity evaluation (Urdzik *et al.* 2003).

Statistical analysis

The results of the experiments, estimated the cough response, were evaluated by the Wilcoxon-Wilcox statistical method. In *in vitro* experiments the reactivity of the tracheal smooth muscle was evaluated by Student´s t-test for unpaired data.

Results

During 15-days peroral administration of enalapril (5 mg/kg b.w.) the sensitivity of the cough reflex was investigated by the method of mechanical stimulation of the airways in non-anesthetized cats. In comparison with control values, a statistically significant increase in the number of cough efforts (Fig. 1) was observed in day 3, 5, 8, 10, 12, 15 after enalapril administration. The measured cough parameter was increased from both parts of the airways. The 15 days simultaneous administration of enalapril (5 mg/kg b.w.) together with diltiazem (30 mg/kg b.w.) revealed a decline in the number of cough efforts from laryngopharyngeal and tracheobronchial part of the airways (Fig. 2).

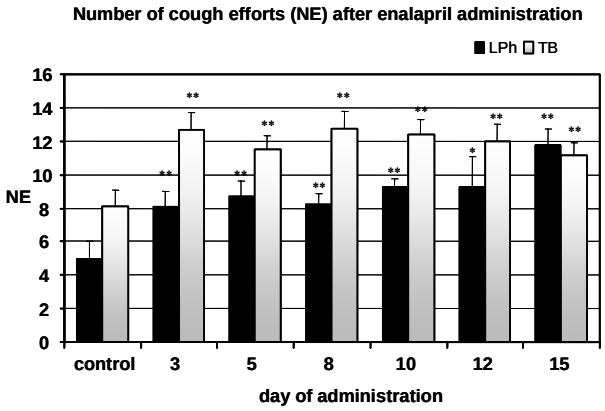

Fig. 1. Changes in the number of cough efforts (NE) from laryngopharyngeal (LPh) and tracheobronchial (TB) area of the airways of the non-anesthetized cats during 15 days of enalapril administration The control represent the number of the cough efforts before drug administration. Data represent mean ± S.E.M., n=12, *p<0.05, ** p<0.01.

After 15 days of drug administration the reactivity of the tracheal smooth muscle was investigated *in vitro*. The 15 days of treatment with enalapril (5 mg/kg/day) resulted in a significant increase of the reactivity of tracheal smooth muscle to cumulative doses of histamine. This increase in bronchoconstrictor activity compared to the control values was significant at the concentration range of histamine 10 nM – 1 mM. However, 15-day combination treatment with enalapril and diltiazem did not cause the lowering of the contraction of tracheal smooth muscle to histamine (Fig. 3).

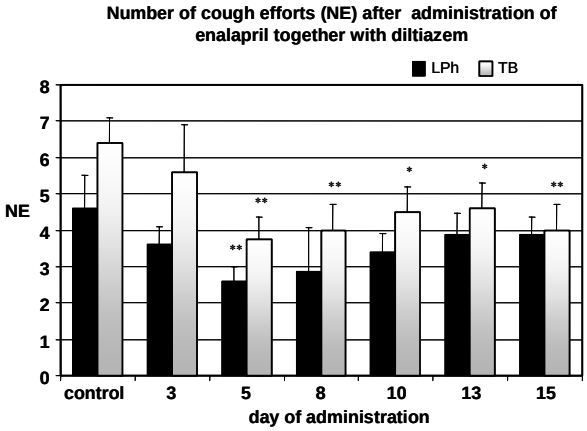

Fig. 2. Changes in the number of cough efforts (NE) from laryngopharyngeal (LPh) and tracheobronchial (TB) area of the airways of the non-anesthetized cats during 15 days of administration of enalapril with diltiazem. The control represent the number of the cough efforts before drug administration. Data represent mean ± S.E.M., n=12, *p<0.05, ** p<0.01.

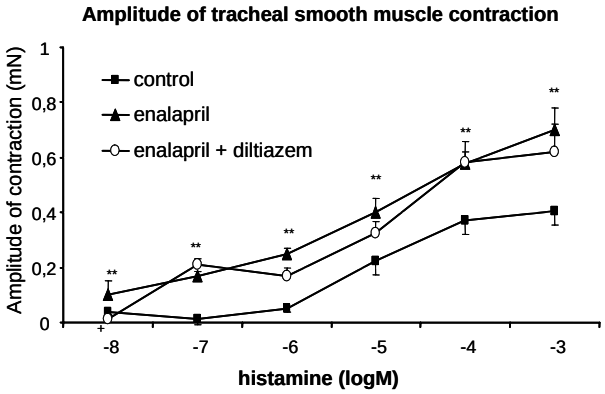

Fig. 3. Effect of enalapril and enalapril in combination with diltiazem on the amplitude of tracheal smooth muscle contraction to histamine (10^{-8} - 10^{-3} mol/l) in guinea pigs after 15 days of treatment. -♦- control group (15 days physiological solution); -▲- enalapril (15 days 5 mg/kg p.o.); -O- enalapril with diltiazem (15 days enalapril with diltiazem 30 mg/kg p.o.); Data are expressed as mean ± S.E.M.; n=12 for each group; Statistically significant difference * p<0.05 and ** p<0.01 control group vs. enalapril; + p<0.05 and ++ p<0.01 enalapril vs. enalapril with diltiazem.

The reactivity of tracheal smooth muscles to acetylcholine (10 nM – 1 mM) after enalapril treatment showed a significant increase of contraction amplitude even to low doses of acetylcholine. Simultaneous administration of enalapril with diltiazem did not influence the tracheal reactivity to acetylcholine (Fig. 4).

An unambiguous lowering in the reactivity of tracheal smooth muscle to other bronchoconstrictor mediator KCl (1 mM – 100 mM) was observed after 15-day combined therapy with enalapril and diltiazem, in comparison to enalapril monotherapy (Fig. 5).

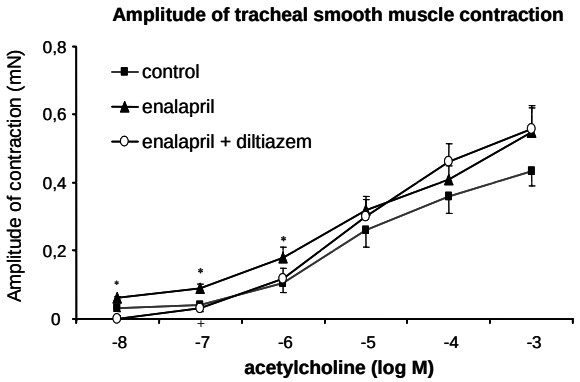

Fig. 4. The effect of enalapril and enalapril in combination with diltiazem on the amplitude of tracheal smooth muscle contraction to acetylcholine (10^{-8} - 10^{-3} mol/l) in guinea pigs after 15 days of treatment. -♦- control group (15 days physiological solution); -▲- enalapril (15 days 5 mg/kg p.o.); -O- enalapril with diltiazem (15 days enalapril with diltiazem 30 mg/kg p.o.); Data are expressed as mean ± S.E.M.; n=12 for each group; Statistically significant difference * $p<0.05$ and ** $p<0.01$ control group vs. enalapril; + $p<0.05$ and ++ $p<0.01$ enalapril vs. enalapril with diltiazem.

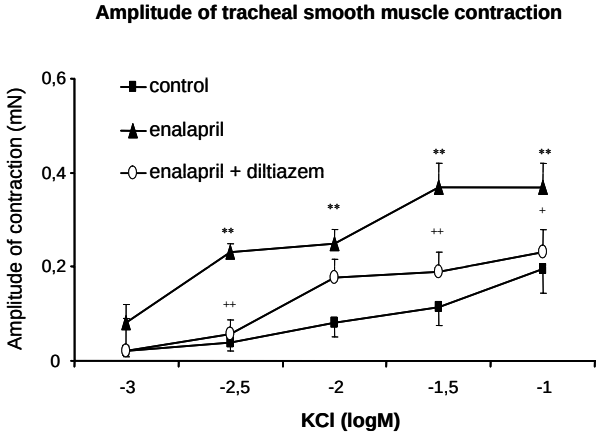

Fig. 5. The effect of enalapril and enalapril in combination with diltiazem on the amplitude of tracheal smooth muscle contraction to KCl (10^{-3} - 10^{-1} mol/l) in guinea pigs after 15 days of treatment. -♦- control group (15 days physiological solution); -▲- enalapril (15 days 5 mg/kg p.o.); -O- enalapril with diltiazem (15 days enalapril with diltiazem 30 mg/kg p.o.); Data are expressed as mean ± S.E.M.; n=12 for each group; Statistically significant difference * $p<0.05$ and ** $p<0.01$ control group vs. enalapril; + $p<0.05$ and ++ $p<0.01$ enalapril vs. enalapril with diltiazem.

Discussion

Clinical trials and experimental studies dealing with ACE-inhibitor treatment have been currently aimed at management of the cough induced by administration of the above mentioned group of substances. The basic condition for the cough to be eliminated by means of the pharmacological intervention, consists of maintaining the primary pharmacological efficacy of ACE-inhibitors, thanks to which they are so widely used in clinical practice.

ACE-inhibitors and calcium channel blockers are in combination widely used in the treatment of cardiovascular diseases. This co-administration exhibits synergic hemodynamic, antiproliferative, antithrombotic and antiatherogenic effects (Ruschitzka *et al.* 1998). In our experimental conditions, the animals treated for 15 days with enalapril showed a statistically significant increase of the cough response to mechanical stimuli. Simultaneous administration of enalapril with diltiazem decreased the number of cough efforts in comparison to enalapril monotherapy. A significant decline was found mainly in the tracheobronchial region.

The neural pathway responsible for the cough regulation may undergo disease-related changes (plasticity), which cause that the protective aspects of the cough reflex are replaced by exaggerated and inappropriate coughing in response to stimuli that are otherwise only slightly irritating (Mazzone and Canning 2002). Increased incidence of the cough after enalapril treatment is linked with ACE-inhibition and accumulation of bradykinin, substance P, prostaglandins and other pro-inflammatory mediators in the airways (Gajdoš *et al.* 2000). These accumulated substances may sensitize airway afferent nerve endings, thereby lowering their chemical and mechanical threshold for activation. From the point of view of afferent nerve endings, the cough reflex is induced by stimulation of rapidly adapting airway mechanoreceptors (RARs) (Hargreaves *et al.* 1992), bronchopulmonary C-fibres (Fox 1996) and Aδ nociceptors (Undem *et al.* 2002). While all these three types of receptors are activated differently by tussigenic agents, RARs cause a cough directly, C-fibre receptors by local release of tachykinins that stimulate RARs. The reflex role of Aδ nociceptors is not known (Widdicombe 2001). Peripheral afferent nerve sensitization may lead to increased input to the nucleus tractus solitarius (nTS) in the brainstem and contribute to the cough plasticity (Mazzone and Canning 2002).

The mechanism of diltiazem action in suppression of the cough induced by enalapril administration is unknown. Modulation of the cough reflex with diltiazem could involve the peripheral and central level. The antitussive effect of diltiazem can be

the result of its ability to inhibit the activity of peripheral nerve endings regulating the cough reflex. The modulation of the central transmission of the cough reflex through inhibition of calcium-dependent glutamate release in nucleus tractus solitarius (nTS) may be the second location where the calcium channel blockers might act (Korpáš and Nosáľová 1991).

Apart from the cough, bronchospasm is another typical reflex response occurring secondarily to RAR stimulation. On the other hand, increased reactivity of airway smooth muscles can enhance the cough reflex (Canning *et al.* 2001). Our experimental results confirmed the increase of tracheal smooth muscle activity after enalapril treatment.

The increased reactivity of the tracheal smooth muscle after enalapril treatment could be caused by a release of kinins, tachykinins and other proinflammatory mediators. During the treatment with ACE-inhibitors, bradykinin and substance P could contribute to the enhanced reactivity of airways smooth muscles directly by inducing smooth muscle contraction and indirectly by local edema. Furthermore, by stimulating phospholipase A_2, bradykinin could augment the formation of other bronchoconstrictor mediators from the group of prostaglandins and tromboxane A_2. Bradykinin and substance P can also release histamine from mast cells (Israili and Hall 1992).

In our experiments, 15 days' enalapril treatment increased the amplitude of contractions to cumulative doses of histamine under the *in vitro* conditions. This finding partially supports the results of Bucknall *et al.* (1988), who demonstrated increased bronchial reactivity to histamine in subjects who cough after taking an ACE-inhibitor. However, simultaneous administration of enalapril with diltiazem did not decrease the reactivity of tracheal smooth muscle to histamine. The contractile response of airway smooth muscle to histamine depends upon stimulation of phospholipase C-dependent pathway, and release of calcium from intracellular stores. The classical voltage-dependent calcium channel antagonists, which inhibit calcium entrance from extracellular sources, are not able to block histamine-induced tracheal smooth muscle contraction (Hall 2000).

Another contractile agonist used in our experimental conditions for evaluation of tracheal smooth muscle reactivity in guinea pigs was acetylcholine. However, after 15 days of enalapril administration acetylcholine added to the organ bath caused a contraction of tracheal smooth muscle strips only in low concentrations and this action was not influenced by combination of enalapril with diltiazem. This result cannot be explained exactly on the basis of our experiments. Long-lasting administration of ACE-inhibitors causes an accumulation of bradykinin, substance P in the airways. These neuropeptides increase the tracheal smooth muscle contraction induced by acetylcholine (Lundberg *et al.* 1983). Besides the contractile effect, substance P plays a role in the regulation of airway smooth muscle tone through sensory nerve inhibitory system, which modulates cholinergic contraction. According to some experiments the contractile response of tracheal smooth muscle induced by acetylcholine was inhibited by substance P (Szarek *et al.* 1996).

Acetylcholine induces the tracheal smooth muscle contraction through the release of calcium from intracellular stores. For this reason, diltiazem in combination with enalapril is not able to influence the contraction of tracheal smooth muscles induced by acetylcholine.

The airway smooth muscle displays a concentration-related contraction by the administration of KCl in terms of a depolarization mechanism. It has been demonstrated that Ca^{2+} antagonists cause relaxation of airway smooth muscles precontracted with KCl by blocking the transmembrane Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Koga *et al.* 1989). In our experiments, cumulative administration of KCl induced the concentration-dependent increase in tracheal smooth muscle reactivity after enalapril treatment. The enalapril-induced release of bronchoconstrictor mediators might potentiate the potassium-induced contraction of tracheal smooth muscle. The combination of enalapril and diltiazem caused the significant decline in the amplitude of tracheal smooth muscle contraction induced by KCl. Moreover, the bronchodilatory effect of diltiazem could enhance its cough-suppressing activity in the cough induced enalapril administration.

In conclusion, the present study demonstrates the protective effect of diltiazem administration on the incidence of the cough and partially on the occurrence of bronchoconstriction during enalapril treatment. The combination of ACE-inhibitors with calcium channel blockers is useful in the treatment of cardiovascular diseases, but it is also beneficial in the management of respiratory side effects of ACE-inhibitors.

References

BUCKNALL CE, NEILLY JB, CARTER R, STEVENSON RD, SEMPLE PF: Bronchial hyperreactivity in patients who cough after receiving angiotensin converting enzyme inhibitors. *Br Med J* **269**: 86-88, 1988.

CANNING BJ, REYNOLDS SM, MAZZONE SB: Multiple mechanisms of reflex bronchospasm in guinea pigs. *J Appl Physiol* **91**: 2642-2653, 2001.

DENDORFER A, WOLFRUM S, DOMINIAK P: Pharmacology and cardiovascular implications of the kinin-kallikrein system. *Jpn J Pharmacol* **79**: 403-426, 1999.

FOX AJ: Modulation of the cough and airways sensory fibres. *Pulm Pharmacol* **9**: 335-342, 1996.

GAJDOŠ M, KRIVOŠÍKOVÁ Z, ŠEBEKOVÁ K, LAJDOVÁ I, SPUSTOVÁ V, DZÚRIK R: Enalapril inhibits growth and proliferation of various tissue in rat normotensive four-sixths kidney ablation nephropathy. *Kidney Blood Press Res* **23**: 106-112, 2000.

HALL IP: Second messengers, ion channels and pharmacology of airway smooth muscle. *Eur Respir J* **15**: 1120-1127, 2000.

HARGREAVES MR, RAVI K, SENARATNE MP, KAPPAGODA, CT: Responses of airway rapidly adapting receptors to bradykinin before and after administration of enalapril in rabbits. *Clin Sci* **83**: 399-407, 1992.

HO CY, GU Q, HONG JL, LEE LY: Prostaglandin E_2 enhances chemical and mechanical sensitivities of pulmonary C fibres in the rat. *Am J Respir Crit Care Med* **162**: 528-533, 2000.

ISRAILI ZH, HALL D: Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. *Ann Intern Med* **117**: 234-242, 1992.

KOGA Y, SODEYAMA N, SATOH S, TESHIGAWARA T, YANAGISAWA T, TAIRA N, ENDOH M: Relaxation of airway smooth muscle induced by potassium in the presence of Ca-antagonists. *Jpn J Pharmacol* **50**: 387-396, 1989.

KORPÁŠ J, NOSÁĽOVÁ G: *Pharmacotherapy of the Cough*. Martin, Osveta, 1991.

LUNDBERG JM, SARIA A, BRODIN E, RUSSELL S, FOLKERS K: A substance P antagonist inhibits vagally-induced increase in vascular permeability and bronchial smooth muscle contraction in guinea pigs. *Proc Natl Acad Sci USA* **80**: 1120-1124, 1983.

MAZZONE SB, CANNING BJ: Plasticity of the cough reflex. *Eur Respir Rev* **85**: 236-242, 2002.

RUSCHITZKA FT, NOLL G, LÜSCHER TF: Combination of ACE inhibitors and calcium antagonists: a logical approach. *J Cardiovasc Pharmacol* **31** (Suppl 2): S5-S16, 1998.

SEKIZAWA K, JIA YX, EBIHARA T, HIROSE Y, HIRAYAMA Y, SASAKI H: Role of substance P in cough. *Pulm Pharmacol* **9**: 323-328, 1996.

SEMPLE PF: Putative mechanism of cough after treatment with angiotensin converting enzyme inhibitors. *J Hypertens* **13** (Suppl 3): S17-S21, 1995.

STRIESSNIG J, GRABNER M, METTEDORFER J, HERING S, SINNEGGER M J, GLOSSMANN H: Structural basis of drug binding to L Ca^{2+} channels. *TIPS* **19**: 108-115, 1998.

SZAREK JL, SPURLOCK B: Antagonism of cholinergic nerve-mediated contraction by sensory nerve inhibitory system in rat bronchi. *J Appl Physiol* **81**: 260-265, 1996.

TRIFILIEFF A, DA SILVA A, GIES JP: Kinins and respiratory tract diseases. *Eur Respir J* **6**: 576 – 587, 1993.

UNDEM BJ, CARR MJ, KOLLARIK M: Physiology and plasticity of putative cough fibres in guinea pig. *Pulm Pharmacol Ther* **15**: 193-198, 2002.

URDZIK J, JAKUBESOVA M, HUDEC M, MOKRÝ J, ŠVIHRA J: In vitro reactivity of urinary bladder smooth muscle influenced by oxybutinin, propranolol, and indomethacin in guinea pigs. *Acta Med Mart* **4/3***:* 23-29, 2003.

WIDDICOMBE JG: Airway receptors. *Respir Physiol* **125**: 3-15, 2001.

Reprint requests

S. Fraňová, Department of Pharmacology JMF UC, Sklabinská 26, 037 53 Martin, Slovakia. E-mail: franova@jfmed.uniba.sk