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COMPARISON BETWEEN THE EFFECTS OF LISINOPRIL AND LOSARTAN ON THE COUGH REFLEX IN ANESTHETIZED AND AWAKE RABBITS

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The aim of the present study was to analyze differences in cough induction between losartan and lisinopril in both anaesthetized and awake rabbits, *i.e.*, under conditions in which the influences of higher brain areas on the cough reflex are strongly reduced or abolished. Losartan (500 μg/kg), lisinopril (100 μg/kg) and NaCl 0.9% saline solution (vehicle) were administered by intravenous injections. Animals were randomly assigned to the different experimental treatments. The cough reflex was induced by chemical (citric acid) and/or mechanical stimulation of the tracheobronchial tree. In anaesthetized rabbits, losartan and lisinopril caused similar hypotensive effects. Lisinopril, but not losartan, increased the cough response induced by both mechanical and chemical stimulation due to increases in the cough number, *i.e.* the number of coughs induced by each stimulation challenge. In awake animals, only lisinopril significantly increased the cough number. The results support the notion that cough potentiation induced by losartan, and possibly other sartans, is lower than that induced by most angiotensin-converting enzyme inhibitors despite the reduction or complete absence of higher brain functions. In this connection, the comparison between present results and our previous findings on ramipril and zofenopril shows that losartan and zofenopril display similar cough-inducing potency, much lower than that of lisinopril and ramipril.

Key words: *losartan, lisinopril, cough, hypertension, sartans, angiotensin II receptor inhibitors, angiotensin-converting enzyme inhibitors, prostaglandin E_2*

INTRODUCTION

Cough is one of the most important airway defensive reflexes (1-5) and the most common symptom for which patients consult a doctor. The cough reflex is purposeful under many circumstances ("appropriate cough"), but is without an apparent aim ("inappropriate cough") in cases of chronic cough (6) and, in particular, during treatment with angiotensin-converting enzyme (ACE) inhibitors. Dry cough is widely considered one of the major side effects of ACE inhibitors (7), a class of drugs widely used for hypertension, heart failure, and post-infarction treatment. ACE inhibitor-associated cough incidence has been estimated at 0.7 to 14.0% (8), with considerable variation between published reports, probably due to the method used for its assessment (9) and the pharmacological and kinetic differences between various ACE inhibitors (10). It is reversible when the drug is discontinued. The pathophysiological mechanism of this adverse effect is still unknown, but it has been suggested that local high concentrations of autacoids (such as substance P, bradykinin, and prostaglandins) resulting from ACE inhibition may play a role (11, 12). In a previous report (5), we have provided evidence that ramipril, but not zofenopril, increases the cough reflex in response to mechanical and chemical stimulation of the tracheobronchial tree in both anaesthetized and awake rabbits.

Losartan as well as other sartans are angiotensin II receptor blockers (ARBs) acting on subtype 1 (AT_1) receptors (13, 14). They have been associated with low cough incidence in humans and are employed to substitute ACE inhibitors in patients presenting as side effects cough or angioedema (15-21). Sartans-induced cough is due to not yet defined mechanisms probably different from those involved in the ACE inhibitor-associated cough. ARBs may involve not only the blockade of AT_1 receptors, but also the activation of the angiotensin II receptors, subtype 2 (AT_2) by increased levels of angiotensin II (12, 22) that may activate the bradykinin-prostaglandin-nitric oxide (NO) cascade (12, 23-26) and lead to increases in cough reflex sensitivity.

To our knowledge, a comparative study on the effects of ACE inhibitors and sartans on the cough reflex has not been yet performed in experimental animals either awake or under general anaesthesia. Such a study could confirm previous observations in humans (15-21), under conditions in which the influences of higher brain areas on the cough reflex are drastically reduced or completely removed. Such influences may include the volitional control as well as the influences of psychological and social factors known to regulate cough (27-30).

The present study is devoted to compare the effects of the ACE inhibitor lisinopril and the angiotensin II receptor antagonist losartan both in anaesthetized and unanaesthetized rabbits. Losartan is a long-lasting selective antagonist of AT_1 receptors (13, 14). Lisinopril is a carboxyl ACE inhibitor with long-lasting antihypertensive activity (31) differing for its functional group from other ACE inhibitors such as sulphydrylic

captopril and zofenopril or the phosphonylic fosinopril. The cough reflex was induced by chemical stimulation (citric acid inhalation) in awake animals and by both chemical and mechanical stimulation of the tracheobronchial tree in anaesthetized animals (5, 32-38).

MATERIALS AND METHODS

Preliminary remarks

All animal care and experimental procedures were conducted in accordance with the Italian legislation and the official regulations of the European Community Council on the use of laboratory animals (Directive 86/609/EEC). The experimental procedures were approved by the local Animal Care and Use Committee. Two different study protocols were used in two separate groups of experiments. A total number of 70 rabbits was used. Experimental procedures and details about the methods employed have previously been described (32-38), and particularly in our previous report (5) on the effects of ACE inhibitors on the cough reflex. However, a brief description of the methods employed has been reported.

Experiments on anaesthetized animals

Experiments were carried out on 28 male New Zealand white rabbits (3.0–3.8 kg) anaesthetized with 40 mg/kg intravenously (i.v.) sodium pentobarbitone, supplemented by 2–4 mg/kg every 30 min (Sigma-Aldrich, St. Louis, MO, USA). The adequacy of anaesthesia was assessed by the absence of reflex withdrawal of the hindlimb in response to noxious pinching of the hindpaw. Additional criteria were the absence of fluctuations in arterial blood pressure or phrenic nerve activity, spontaneously or in response to somatic nociceptive stimuli.

Phrenic nerve activity was recorded by means of bipolar platinum electrodes from the central stump of cut and desheathed C_3 or C_5 phrenic roots. The electromyographic (EMG) activity of abdominal muscles was recorded using wire electrodes (Nichrome wires, insulated except for 1 mm at the tips; diameter 0.1 mm) inserted into the external or internal oblique abdominal muscles. Phrenic and abdominal activities were amplified, band pass filtered, full-wave rectified, and "integrated" (low-pass RC filter, time constant 100 ms). Arterial blood pressure was recorded by a strain-gauge manometer. End-tidal CO_2 partial pressure was measured by an infrared CO_2 analyzer (Datex, CD-102; Normocap, Helsinki, Finland). All recorded variables were fed into an eight-channel rectilinearly writing chart recorder (model 8K20; NEC San-ei, Tokyo, Japan). Cardiorespiratory variables were also acquired and analyzed using a personal computer, equipped with an analog-to-digital interface (Digidata 1200; Axon Instruments, Union City, CA, USA) and appropriate software (Axoscope, Axon Instruments).

Cough was induced both by mechanical and chemical stimulation of the tracheobronchial tree. Mechanical stimulation was performed by means of a 0.5-mm diameter nylon fiber inserted through a lateral port of the tracheal cannula until the tip was judged to be near the carina and main bronchi (32, 33, 35-38). Back and forth movements of the fiber aimed at touching repeatedly (~1× time every second) the carina or nearby airway walls were made over periods of 4–5 s. An interval of ~1 min was scheduled between cough stimulations. As a rule, three stimulation trials were performed in succession. Chemical stimulation was performed by means of citric acid inhalation. Citric acid (1 M, Sigma-Aldrich) was freshly dissolved in 0.9% NaCl solution and nebulized *via* an ultrasonic nebulizer (Projet, Artsana, Grandate, CO, Italy). The opening of the tracheal cannula, through which the rabbits were spontaneously breathing, was exposed to a steady stream of the nebulized citric acid solution for 3 s. All the animals coughed in response to such inhalation challenges. The interval between chemical challenges was at least 15 min (5, 34, 36).

The cough motor pattern in response to mechanical or chemical stimulation of the tracheobronchial tree is usually characterized by repeated coughs consisting of coordinated bursts of inspiratory and expiratory activity (32-38). Each cough usually consisted of a phrenic burst of variable amplitude (preparatory inspiration) immediately followed by a burst of expiratory abdominal activity. In some cases, the first obvious response following mechanical stimulation of the tracheobronchial tree was a small-amplitude expiratory effort without a preceding preparatory inspiration (32, 35, 37). This pattern could fit more appropriately the definition of expiration reflex that is typically evoked by mechanical stimulation of the vocal folds (1, 39), but that can be also produced by mechanical stimulation of the tracheobronchial tree (40, 41). For further details on this topic see our previous reports (33-35, 37, 38). In a previous study in the rabbit (35), we have observed by recording tracheal pressure during cough efforts that the expiration reflex clearly occurred after the beginning of the expiratory phase of a control breath (35, figure 2). From the scrutiny of tracheal pressure recordings we also found that in most cases the first expiratory effort following mechanical stimulation is immediately preceded by an inspiratory component of normal or even reduced amplitude. This expiratory effort was considered a real cough since it is preceded by an inspiration that is not aimed at preventing aspiration of foreign particles into the lungs (35). Thus, also in the present study expiratory efforts that were clearly found to be immediately preceded by an inspiration of normal or even reduced amplitude are considered real coughs. However, in our study an expiration reflex only occurred as the first motor event in a cough epoch, and its appearance was limited to a few occasions. Therefore, these expiratory responses were not considered for data analysis.

The animals were randomly assigned to one of the two experimental groups (14 for each group) and were treated i.v. with losartan (500 µg/kg; 1084 nmol/kg; Fluka- Sigma-Aldrich) or lisinopril (100 µg/kg; 226 nmol/kg; Sigma-Aldrich). Drugs were dissolved in 1 ml saline solution (NaCl 0.9%). The doses were based on the knowledge that the antihypertensive potency of zofenopril is similar to that of lisinopril (42), while the antihypertensive potency of lisinopril is ~5 times higher than that of losartan (31, 43-45). In a few preliminary experiments (n=4) devoted to other purposes, these doses were found to produce similar reductions in arterial blood pressure 30 min after their i.v. administration in anaesthetized rabbits. This finding was confirmed in the present results obtained in anaesthetized animals.

In each animal of the two groups, drug treatments were preceded by i.v. injections of saline solution (NaCl 0.9%). Cough was induced both by mechanical (3 trials) and chemical (1 trial) stimulation in each animal of the two groups before (control) and 30 min after the different types of treatment, *i.e.*, saline and losartan or saline and lisinopril. Thirty-min intervals after administration of both losartan and lisinopril were adequate for stabilization of blood pressure and blockade of the renin-angiotensin system (42, 46-50). Respiratory variables were measured for an average of 10 consecutive breaths during baseline respiration before and 30 min after each treatment. The inspiratory (T_I) and expiratory (T_E) times, as well as the total duration of the respiratory cycle (T_T) were measured on recordings of raw phrenic nerve activity. The respiratory frequency (breaths/min) was subsequently calculated. Peak

amplitude (arbitrary units) of the phrenic nerve activity and abdominal EMG activity were measured on integrated traces and were normalized by expressing them as a fraction of the highest amplitude obtained in each animal during coughing. Therefore, amplitudes were expressed in relative units (5, 36) (RU). During baseline respiration (10 consecutive breaths), systolic and diastolic blood pressures were measured at 2-s intervals both before and 30 min after each treatment. Mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure. Owing to the small variations in cardiorespiratory variables within each measurement period, average values were taken as single measurements for the purpose of statistical analysis. Under the different experimental conditions, cough-related variables were measured (an average of 3 trials for mechanical stimulation and a single trial for chemical stimulation) before and 30 min after the injections. These variables included cough-related T_I, T_E, and T_T, as well as peak phrenic amplitude, peak abdominal activity, and cough number, *i.e.* the number of coughs induced by each stimulation challenge.

Experiments on unanaesthetized animals

Experiments were performed on 42 unanaesthetized male New Zealand white rabbits (3.2–3.9 kg). In each cough induction test, the rabbits were placed individually into a transparent Perspex inhalation chamber (approximately 0.050 m^3) and exposed for 3 min to 1 M nebulized citric acid (5).

The number of coughs during the 3-min citric acid challenge was evaluated by two trained observers unaware of the treatment used. Coughs were recognized mainly from the characteristic behavior of the animal and the sound produced, and readily distinguished from sneezing. Cough sounds were recorded by a microphone placed in the box and monitored both visually and acoustically, making use of a personal computer equipped with an analog-to-digital interface (Digidata 1200, Axon Instruments) and appropriate software (Axoscope, Axon Instruments). An example of a microphonic cough recording has been shown in a previous report (5). Control trials (first citric acid challenge) were performed on all the animals. Rabbits that did not cough in response to citric acid inhalation were not included in the study.

All studies were carried out at the same time of day. The animals were randomly assigned to three groups (14 for each group) to undergo one of the following treatments administered i.v. in 1 ml saline solution (NaCl 0.9%): 1) vehicle; 2) losartan (500 μg/kg; 1084 nmol/kg); 3) lisinopril (100 μg/kg; 226 nmol/kg). During each treatment, the animals were exposed to citric acid (second citric acid challenge) 30 min after the injection and the number of coughs was counted again. The second citric acid challenge was performed 5–7 days after the first challenge to avoid tachyphylaxis (5).

Statistical analysis

In each group of anaesthetized animals, the effects of treatment on the respiratory pattern, arterial blood pressure, and cough-related variables were compared by means of one-way repeated-measures analysis of variance followed by Student-Newman-Keuls test. Comparisons between groups of the variables that were significant were performed by means of unpaired *t*-tests.

In unanaesthetized animals, the number of coughs for each 3-min citric acid challenge was counted for each animal and the mean value for each group was calculated. Changes in the number of coughs for each treatment were evaluated by Student's paired *t*-tests. The changes in the number of coughs due to the three treatments were compared by one-way analysis of variance followed by Student-Newman-Keuls test. All reported values represent mean ±S.E. (standard error of the mean). $P<0.05$ was taken as significant.

RESULTS

Anaesthetized animals

Losartan (500 μg/kg) and lisinopril (100 μg/kg) caused similar ($P>0.05$) changes in arterial blood pressure 30 min after i.v. injection (mean arterial blood pressure in controls 102.1±3.7 mmHg versus losartan 93.5±3.5 mmHg, $P<0.05$; in controls 105.7±3.5 mmHg versus lisinopril 96.5±3.9 mmHg, $P<0.05$). Decreases in arterial blood pressure ranged for losartan from 8 to 10 mmHg (8.6±0.2 mmHg; n=14) and for lisinopril from 7 to 12 mmHg (9.1±0.6 mmHg; n=14). As expected, NaCl 0.9% saline solution did not cause any significant changes in cardiorespiratory variables (data not shown). Among the studied cough-related variables, only the cough number significantly increased in all investigated rabbits in response to both mechanical stimulation (*Table 1*) and chemical stimulation (*Table 2*) of the tracheobronchial tree following i.v. lisinopril administration. Nevertheless, it should be mentioned that losartan increased the cough number in 3 animals during cough induced by mechanical stimulation and in 1 animal during cough induced by chemical stimulation. The effects of the 3 treatments on the cough number are illustrated in *Fig. 1*. *Figs. 2* and *3* report examples of original recordings of drug-induced changes in cough responses induced by mechanical and chemical

Table 1. Effects of losartan (*n*=14) and lisinopril (*n*=14) on cough responses induced by mechanical stimulation of the tracheobronchial tree in anaesthetized rabbits.

	Control	Losartan 500 g/kg	Control	Lisinopril 100 g/kg
T_T (s)	0.52 ± 0.03	0.52 ± 0.03	0.57 ± 0.03	0.56 ± 0.02
T_I (s)	0.33 ± 0.02	0.33 ± 0.02	0.36 ± 0.01	0.34 ± 0.01
T_E (s)	0.18 ± 0.02	0.19 ± 0.01	0.21 ± 0.02	0.23 ± 0.02
PPA (RU)	0.62 ± 0.02	0.66 ± 0.02	0.64 ± 0.02	0.64 ± 0.02
PAA (RU)	0.61 ± 0.02	0.54 ± 0.03	0.59 ± 0.02	0.56 ± 0.03
CN	3.26 ± 0.13	3.19 ± 0.19	2.81 ± 0.14	3.85 ± 0.15*

Values are means ±S.E.M.; *n*, number of animals; T_T, cycle duration; T_I, inspiratory time; T_E, expiratory time; PPA, peak phrenic activity in relative units (RU); PAA, peak abdominal activity; CN, cough number. *$P<0.001$ compared with control.

Table 2. Effects of losartan (*n*=14) and lisinopril (*n*=14) on cough responses induced by citric acid inhalation in anaesthetized rabbits.

	Control	Losartan 500 g/kg	Control	Lisinopril 100 g/kg
T_T (s)	0.51 ± 0.02	0.49 ± 0.03	0.54 ± 0.02	0.55 ± 0.03
T_I (s)	0.33 ± 0.01	0.33 ± 0.02	0.36 ± 0.01	0.35 ± 0.02
T_E (s)	0.18 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
PPA (RU)	0.59 ± 0.02	0.56 ± 0.03	0.59 ± 0.03	0.58 ± 0.02
PAA (RU)	0.48 ± 0.02	0.51 ± 0.03	0.53 ± 0.02	0.50 ± 0.03
CN	3.92 ± 0.29	3.81 ± 0.29	3.71 ± 0.32	4.83 ± 0.31*

Values are means ±S.E.M.; *n*, number of animals; T_T, cycle duration; T_I, inspiratory time; T_E, expiratory time; PPA, peak phrenic activity in relative units (RU); PAA, peak abdominal activity; CN, cough number. *P<0.001 compared with control.

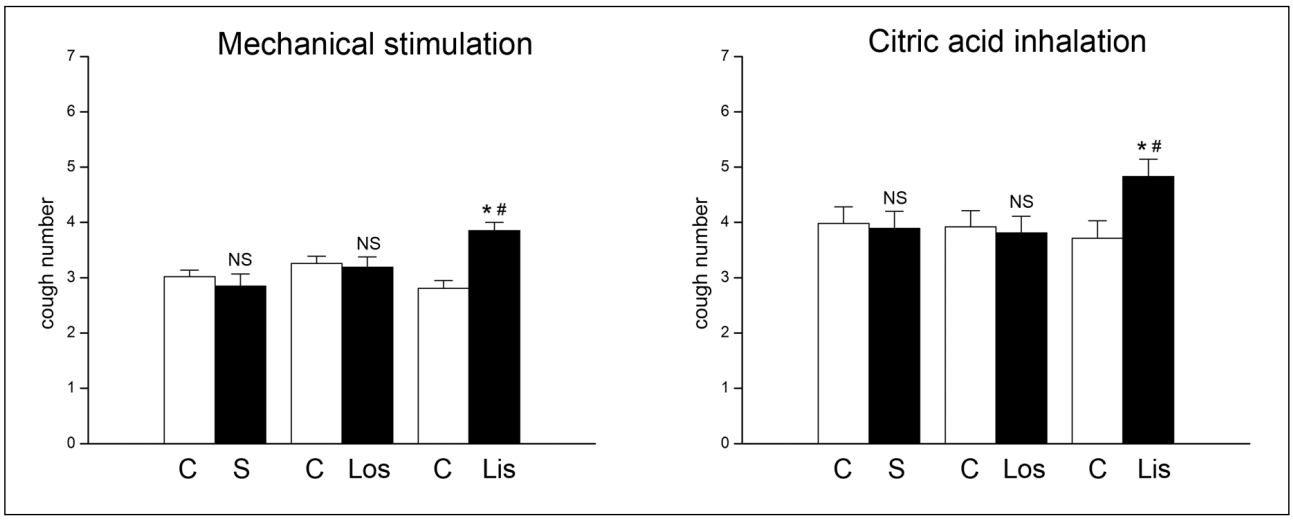

Fig. 1. Histograms showing the number of coughs induced by mechanical or chemical stimulation of the tracheobronchial tree before (control conditions, C) and 30 min following intravenous injection of saline (S), losartan (Los), and lisinopril (Lis) in anaesthetized rabbits. NS, not significant; * P<0.001 compared with controls; # P<0.001 compared with losartan or saline solution.

Table 3. Cough number induced by citric acid inhalation under control conditions and 30 min after each treatment in unanaesthetized rabbits.

Treatment	Control	30 min after treatment
Saline (*n*=14)	21.79 ± 5.17	21.64 ± 5.50
Losartan (*n*=14) 500 g/kg	25.57 ± 6.90	24.71 ± 6.20
Lisinopril (*n*=14) 100 g/kg	18.79 ± 3.10	29.36 ± 5.22*#

Values are means ±S.E.M.; *n*, number of animals; * *P*<0.05 compared with control. # *P*<0.05 compared with losartan and saline.

stimulation, respectively. No treatment caused changes in the ongoing pattern of breathing.

Unanaesthetized animals

Table 3 and *Fig. 4A* show that the saline solution and losartan did not induce any significant changes in the cough number compared with their respective control values, whereas lisinopril significantly increased the number of coughs. Changes in the cough number following the three treatments are also illustrated (*Fig. 4B*). However, it is worth noting that in 5 animals losartan caused increases in the cough number.

DISCUSSION

The results of this study on rabbits show for the first time in experimental animals that the cough potentiation induced by the AT_1 receptor blocker losartan is lower than that of the ACE inhibitor lisinopril. They are in agreement with previous findings in humans (15-21) and consistent with the results of a recent study showing that the ACE inhibitor enalapril, but not the newly developed angiotensin II type 1 receptor antagonist KD3-671 enhances the cough reflex in awake sensitized guinea pigs (51). Noticeably, present results were obtained both in awake and anaesthetized preparations, in which the influences on the cough reflex from higher brain areas, including not only volitional and general psychological factors, but also placebo/nocebo effects (27-30), are greatly attenuated or completely removed. This is, in our opinion, of particular interest in that it can be relevant to the cardiovascular therapy and in particular to the discontinuation of ACE inhibitors. In this context, it should be also mentioned that, according to a recent study (52), the incidence of cough associated with several ACE inhibitors and the withdrawal rate due to cough is significantly greater in the literature than reported in the Physicians' Desk Reference/drug label. As in our previous report (5), the cough number was the only variable affected in both types of preparation. The interpretation of this finding is obscure. We can hypothesize that the effects caused in a few instances by the AT_1 receptor blocker and those consistently provoked by the ACE inhibitor are mainly mediated at the peripheral level, *i.e.*, at the

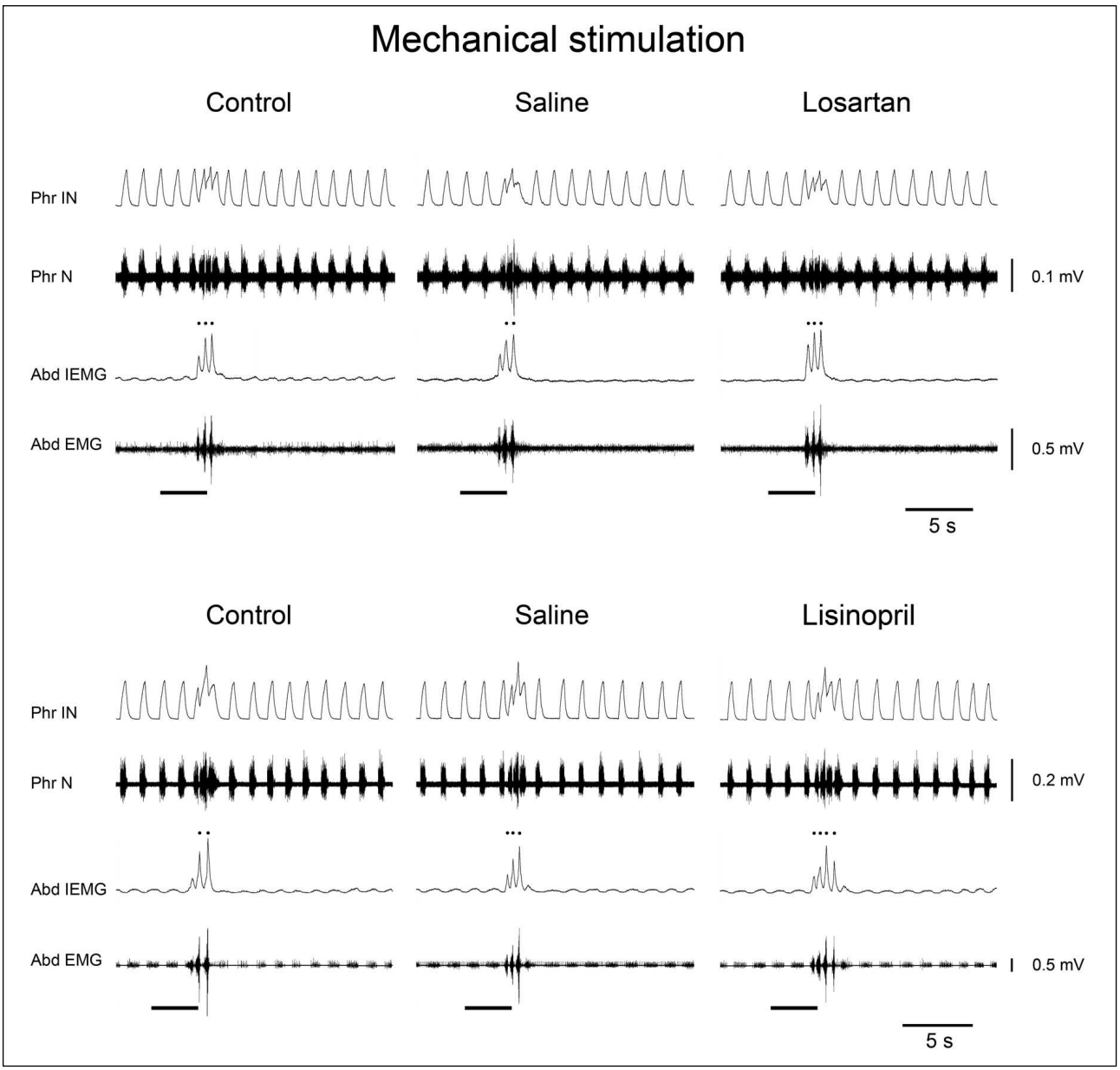

Fig. 2. Examples of cough responses induced in anaesthetized rabbits by mechanical stimulation of the tracheobronchial tree under control conditions and 30 min after intravenous injection of saline, losartan, and lisinopril. Dots indicate the expiratory component of each cough. Expiration reflexes are not indicated. The greater effects of lisinopril on the number of evoked coughs can be seen in the third panel.
Traces are: Phr IN, phrenic integrated neurogram; Phr N, phrenic neurogram; Abd IEMG, abdominal integrated electromyographic activity; Abd EMG, abdominal electromyographic activity. Stimulation periods (~4 s) marked by filled bars.

level of cough-related receptors that trigger stereotyped and fixed cough motor patterns by increasing the cough-related afferent input (increases in the number of recruited receptors and their discharge). In fact, it has been suggested the existence of a central gating mechanism that does not participate in breathing pattern control, but specifically regulates the cough number as a function of the intensity of cough-related afferent input (53-55). However, since both ACE inhibitors and sartans (56-59) cross the blood-brain barrier, a central action at the level of the cough-gating mechanism can be suggested. By contrast, in previous studies not only the cough number but also other cough related variables were altered by the antitussive or protussive drugs applied through different routes of administration (33, 35, 37, 38, 54). The reasons of these discrepancies are not known. In agreement with our previous suggestions (33, 35), we propose that several neural substrates are responsive to administered drugs and that the effects on cough-related variables may be different according to the responsive neural structures involved. In other words, the drug-induced effects may depend upon the route of administration, the actual concentration reached at the neuronal level and the involvement of single or multiple responsive sites including neural substrates subserving not only the cough reflex, but also the control of breathing.

A discussion on the possible receptors involved in cough production has already been provided in our previous report (5). In brief, we believe that the cough reflex in anaesthetized animals is mainly due to the activation of rapidly adapting receptors, while it depends upon both rapidly adapting and C-

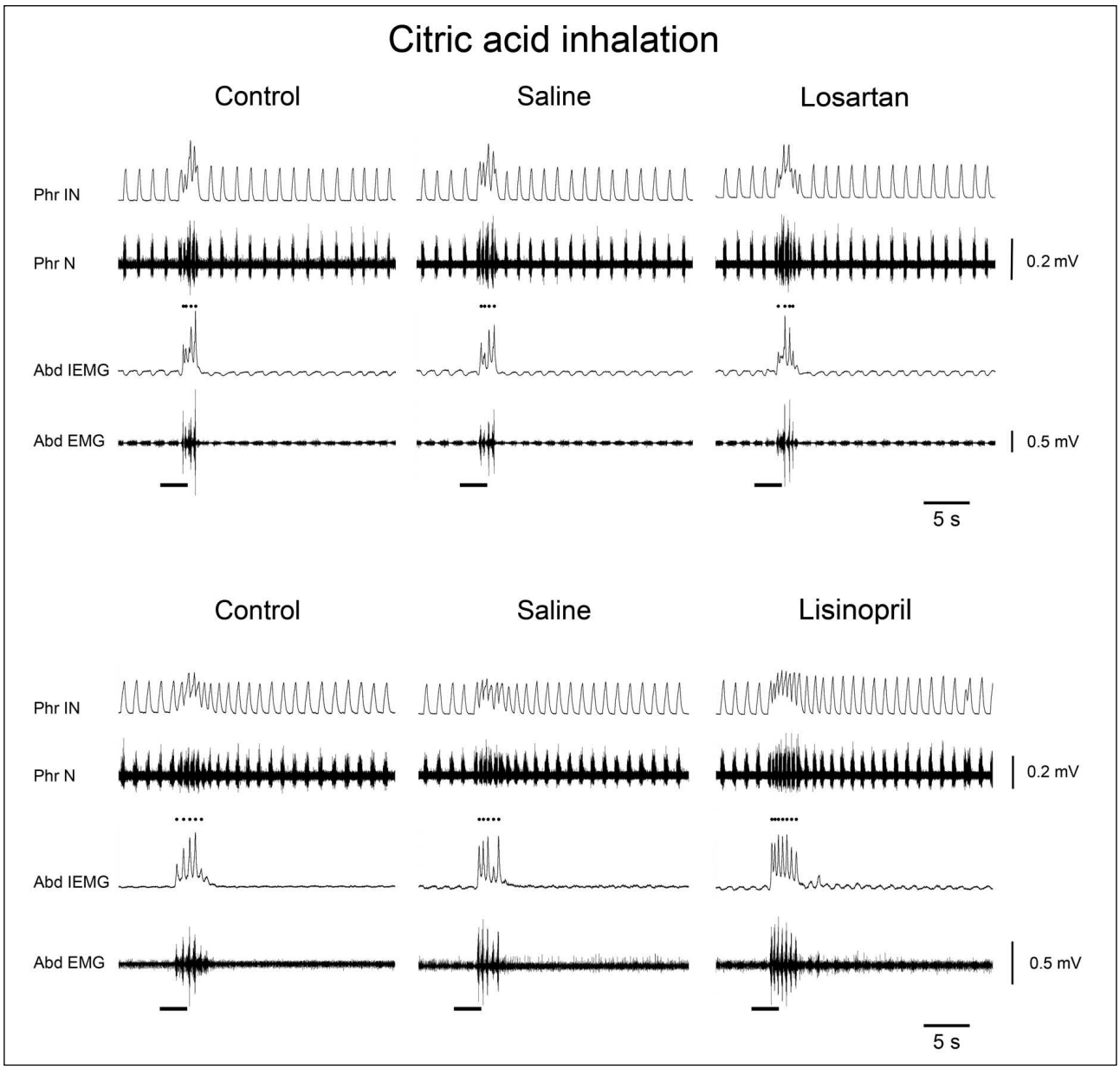

Fig. 3. Examples of cough responses induced in anaesthetized rabbits by citric acid inhalation under control conditions and 30 min after intravenous injection of saline, losartan, and lisinopril. Dots indicate the expiratory component of each cough. The greater effects of lisinopril on the number of evoked coughs are very clear (third panel).
Traces are: Phr IN, phrenic integrated neurogram; Phr N, phrenic neurogram; Abd IEMG, abdominal integrated electromyographic activity; Abd EMG, abdominal electromyographic activity. Stimulation periods marked by filled bars.

fiber receptors in conscious animals (2-4, 33, 34, 36 ,37 ,60). The subgroup of rapidly adapting receptors called "cough receptors", that has been described in the larynx and trachea of guinea pigs, but that is possibly present in other animals, may also have had a role in both anaesthetized and unanaesthetized preparations since these receptors are responsive to punctate mechanical stimuli and acid solutions (61-63). The differences in cough potentiation between the two drugs employed cannot be likely ascribed to differences in their antihypertensive activity. The two drugs proved to produce similar arterial blood pressure-lowering effects in line with available data of the literature (31, 42-45). However, Poliacek *et al*. (64) reported that blood pressure changes could alter tracheobronchial cough in cats. Thus, we cannot exclude that the ACE inhibitor-induced sensitizing effect on the cough reflex combined with the cough-potentiating effect of blood pressure changes could underlie the observed increases in cough number. Nevertheless, it should be also recalled that changes in arterial blood pressure induced by lisinopril and losartan were fairly small as compared with those induced by Poliacek *et al*. (64). In this context, it should be also mentioned that the cough reflex is subject to several peripheral and central regulatory influences (55, 64). Interestingly, the cough reflex induced by mechanical stimulation of the tracheobronchial region is enhanced by intranasal capsaicin challenges (65) and suppressed by spasmodic inspirations of aspiration reflexes induced by mechanical stimulation of the nasopharyngeal mucosa (66).

The in-deep pathogenesis of the ACE inhibitors-induced cough is not completely known, but cough is thought to be related to a cascade of effects that begins with the accumulation

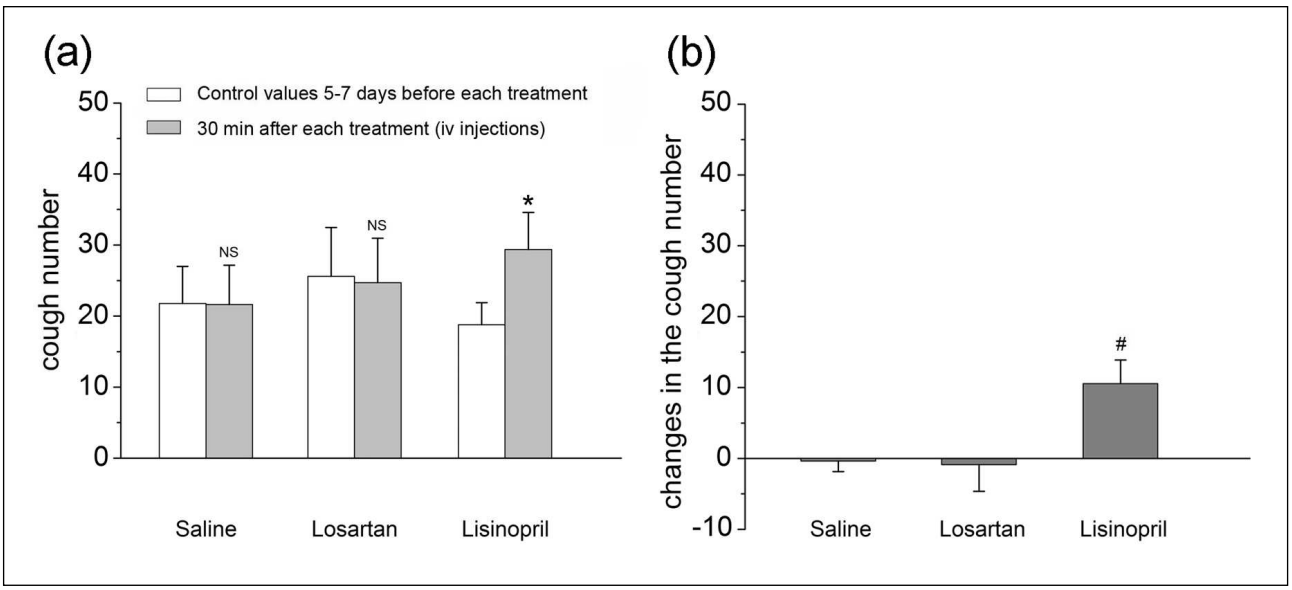

Fig. 4. Effects of saline, losartan, and lisinopril on the cough number in unanaesthetized rabbits.
(a), cough number before and after each treatment. NS, not significant; * $P<0.05$ compared with controls.
(b), changes in the cough number induced by each treatment. # $P<0.05$ compared with the other treatments.

of kinins, and then involves the production of arachidonic acid metabolites and NO (12). ACE inhibition is known to block the metabolism of bradykinin and leads to the accumulation of this bronchoconstrictor autacoid in the airways. The indirect demonstration that ACE inhibitor-induced cough is linked to the accumulation of kinins is proved by the fact that ACE is able to degrade kinins and that ACE inhibitor protussive effect is reduced by kinin antagonists (67). Bradykinin has many local effects, including the release of histamine from mast cells, and also interferes with locally produced neurotransmitters, such as substance P and neuropeptide Y (68). Both bradykinin and substance P stimulate vagal receptors that subserve the cough reflex, particularly the endings of unmyelinated C fibers (3, 69). Local effects on prostaglandin synthesis have also been suggested to play a role since prostaglandins act locally as inflammatory agents. Besides directly interacting with C fibers, bradykinin may also cause bronchoconstriction indirectly by the release of arachidonic acid derivatives, such as leukotrienes and prostaglandin E_2 (PGE_2) and I_2 (68). Similarly to bradykinin, PGE_2 stimulates the endings of unmyelinated bronchopulmonary C fibers resulting in cough. A recent study in guinea-pigs has shown that ramipril-induced cough was associated with increases (32%) in bradykinin levels and with marked increases (232%) in PGEM, a metabolite of PGE_2 in the bronchoalveolar lavage fluid (70). Noticeably, in the same experimental conditions, zofenopril did not enhance bradykinin and PGEM levels in the bronchoalveolar lavage fluid. In addition, it has been reported that captopril increases PGE_2 production in hypertensive patients and that the treatment with a cycloxygenase inhibitor may alleviate cough in affected patients (71). Actually, it has been reported that in guinea pigs cough and bronchoconstriction induced by citric acid inhalation are mediated by bradykinin release (72, 73). Although cough-potentiating effects were observed during both chemical and mechanical stimulation following lisinopril administration, the additional release of bradykinin during citric acid challenges may have contributed to the observed effects.

We should mention that especially in unanaesthetized rabbits losartan-induced cough responses showed a relatively high degree of variability and were also slightly potentiated by losartan in a few cases (see Results). On the possible mechanism of losartan-induced cough potentiation there is at present no available explanation (74). Since AT_1 receptor blockers, such as losartan, do not have an effect on agents involved in cough mediation, such as bradykinin and substance P (37, 63, 75-77), theoretically they should not be associated with cough (5, 18, 20, 68, 69). However, it should be also mentioned that recently it has been reported that losartan causes PGE_2 increases both in plasma (78) and bronchoalveolar lavage fluid (79).

Losartan involves the blockade of AT_1 receptors, but also the activation of AT_2 receptors due to the blockade-induced increase in plasma levels of angiotensin II (12, 22). In turn, angiotensin II may activate bradykinin-prostaglandin-NO cascade (12, 23-26) and produce increases in cough reflex sensitivity and therefore in the cough number. In this context, it should be also recalled that the activation of AT_2 receptors may induce a suppression of ERK 1/2 activity in adult cardiac myocytes (80). Interestingly, we are provided evidence that inhibition of ERK 1/2 activation within the caudal nucleus tractus solitarii (NTS) causes suppressant effects on the cough reflex in the anaesthetized rabbit (36). Since evidence has been provided that AT_2 receptors are present in the NTS (81-83), including its caudal portions (82), we can hypothesize that a suppression of ERK 1/2 activity due to the activation of caudal NTS AT_2 receptors could explain the low occurrence of cough potentiation following losartan administration in our preparations. Finally, we cannot exclude that genetic factors may underlie differences in the susceptibility to blockers of the renin-angiotensin system (84, 85).

Previous studies in humans have shown that the prevalence of cough is lower with several ARBs than with ACE inhibitors (15, 17-20) and is similar to that of placebo (16, 18) or the diuretic hydrochlorothiazide (17, 21). In a study carried out on 100 hypertensive patients with a history of ACE inhibitor-induced cough (18), it was found that the incidence, severity, and frequency of dry cough were significantly lower in those treated with losartan than in those treated with lisinopril and are similar to the incidence, severity, and frequency of dry cough in those receiving placebo. Ramsay *et al.* (21) compared the prevalence of cough associated with the AT_1 receptor blocker losartan, the ACE inhibitor lisinopril, and the diuretic hydrochlorothiazide in

135 hypertensive patients with a history of ACE inhibitor-induced cough. They observed a significantly lower prevalence of cough associated with losartan compared with lisinopril and similar to that of hydrochlorothiazide. Cough frequency was also significantly lower for losartan compared with lisinopril and similar to that of hydrochlorothiazide.

In a preceding report (5), we analyzed the effects of the two ACE inhibitors zofenopril and ramipril on the cough reflex. The results of the present study can be compared with those of this previous investigation. The comparison seems appropriate since the employed experimental procedures were exactly the same in both studies and, in particular, decreases in arterial blood pressure induced by zofenopril, ramipril, losartan and lisinopril in anaesthetized rabbits (one-way analysis of variance followed by Student-Newman-Keuls test, $P>0.05$) were quite comparable. We made use of the same statistical analysis to compare the changes induced on the cough number by each treatment (zofenopril, ramipril, losartan and lisinopril) both in awake and anaesthetized preparations. The statistical analysis showed that saline solution, losartan and zofenopril had similar effects and caused no changes in the cough reflex (P always >0.05). At variance, ramipril and lisinopril increased the cough number to a similar extent ($P>0.05$) and displayed obvious differences (P always <0.05) with the other drugs. These results are consistent with previous findings by Cialdai *et al*. (70) in guinea pigs and with the conclusions of previous reports on the lower incidence of dry cough in patients treated with losartan (15-18, 20, 21, 86). In short, the following conclusions of our studies on cough potentiation can be drawn: saline=losartan=zofenopril<<ramipril=lisinopril.

In conclusion, this study supports the notion that losartan and possibly other sartans are less effective than most ACE inhibitors in inducing cough also when influences arising from higher brain structures are reduced or completely removed. In this connection, we have also shown that losartan and zofenopril display a similar cough-inducing potency, much lower than that lisinopril and ramipril. The finding that losartan could potentiate cough in some experimental animals along with the recent case report by Dashti-Khavidaki *et al*. (74) and the fact that sartans, and in particular losartan, can induce dry cough in a low percentage of treated patients (15-18, 20, 21, 86) may reflect biological variability including genetic factors (84, 85), but could also raise the problem of the presence of different mechanisms underlying the incidence of dry cough induced by the treatment with different antihypertensive drugs.

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