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The cough reflex is upregulated by lisinopril microinjected into the caudal nucleus tractus solitarii of the rabbit

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ABSTRACT

We have previously shown that cough potentiation induced by intravenous administration of the AT_1 receptor antagonist losartan is lower than that induced by the ACE inhibitor lisinopril in anesthetized and awake rabbits. Since losartan and lisinopril cross the blood–brain barrier, their central action on the cough reflex can be hypothesized. Mechanical stimulation of the tracheobronchial tree and citric acid inhalation were used to induce cough reflex responses in pentobarbital sodium-anesthetized, spontaneously breathing rabbits. Bilateral microinjections (30–50 nl) of losartan (5 mM), lisinopril (1 mM), bradykinin (0.05 mM), HOE-140 (0.2 mM, a bradykinin B_2 receptor antagonist) and CP-99,994 (1 mM, an NK_1 receptor antagonist) were performed into the caudal nucleus tractus solitarii, the predominant site of termination of cough-related afferents. Lisinopril, but not losartan increased the cough number. This effect was reverted by HOE-140 or CP-99,994. Cough potentiation was also induced by bradykinin. The results support for the first time a central protussive action of lisinopril mediated by an accumulation of bradykinin and substance P.

1. Introduction

Cough is a very important airway protective reflex, but also characterizes a debilitating disease under chronic conditions. Dry cough is largely considered one of the major side effects of angiotensin-converting enzyme (ACE) inhibitors (Berkin and Ball, 1988), a class of drugs widely used for hypertension, heart failure and post-infarction treatment (Paul et al., 2006). Angiotensin II is the main effector of the renin-angiotensin system (RAS) that controls cardiovascular hemodynamic and blood fluid homeostasis. In mammals there are two primary angiotensin II receptor subtypes, AT_1 and AT_2 (Paul et al., 2006; Wright and Harding, 2011). Losartan and other sartans block AT_1 receptors (McIntyre et al., 1997). They have been associated with low cough incidence in humans, so that they are usually employed to substitute ACE inhibitors in patients displaying as side effect persistent cough (e.g., Paster et al., 1998; Mutolo et al., 2013 also for further Refs.). The mechanism of ACE inhibitor-induced cough remains unclear, but likely involves mainly the protussive mediators bradykinin and substance P, agents that are degraded by ACE and therefore accumulated in the respiratory system when the enzyme is inhibited (e.g., Bali et al., 2014; Fox et al., 1996; Moreaux et al., 2001; Tomaki et al., 1996; Mutolo et al., 2010, 2013 also for further Refs.).

In a previous study (Mutolo et al., 2013), we have shown that cough upregulation induced by intravenous administration of losartan is lower than that induced by lisinopril both in awake and anesthetized rabbits. Since losartan and lisinopril cross the blood–brain barrier (Pediconi et al., 2005; Ranadive et al., 1992; Tan et al., 2005), a central action of these drugs on the cough reflex can be hypothesized especially at the level of the caudal nucleus tractus solitarii (NTS), the main central terminus of cough-related afferents (Kubin and Davies, 1995; Kubin et al., 2006; Mutolo et al., 2007).

Apart from their role in cardiovascular regulation, RAS components within the central nervous system have other functions mediated by AT_1 and/or AT_2 receptors (Paul et al., 2006; Premer et al., 2013; Wright and Harding, 2011). In particular, previous studies have demonstrated that several neuromodulators, including angiotensin II acting on AT_1 receptors, are involved in cardiovascular regulation within the NTS (Arnold et al., 2010; Cheng et al., 2010, 2012; Kasparov et al., 1998; Mosqueda-Garcia et al., 1990). Accordingly, high levels of ACE (Rogerson et al., 1995) and high densities of AT_1 receptors have been found at the level of the NTS in the rabbit (Aldred et al., 1993) as well as in many other mammals including humans (Paul et al., 2006; Premer et al., 2013; Wright and Harding, 2011).

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An attempt was made to ascertain a central action of losartan and lisinopril at the level of the caudal NTS and provide evidence supporting the hypothesis that upregulation of the cough reflex in the rabbit treated with lisinopril could be due, at least in part, to the same mechanisms already suggested to be active at the peripheral level. Thus, we carried out the present study on pentobarbital sodium-anesthetized, spontaneously breathing rabbits by evoking the cough reflex in response to both mechanical and chemical stimulation of the tracheobronchial tree. Changes in the cough reflex induced by microinjections of lisinopril, losartan, bradykinin, HOE-140 (a bradykinin B_2 receptor antagonist) and CP-99,994 (an NK_1 receptor antagonist) into the caudal NTS were investigated.

2. Materials and methods

2.1. Ethical approval

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 and 2010/63/UE). The study was approved by the Animal Care and Use Committee of the University of Florence. All efforts were made to minimize both the number of animals used and their suffering. Experimental procedures and details about the methods employed have previously been described (Cinelli et al., 2013; Mutolo et al., 2007, 2008, 2009, 2010, 2012, 2013, 2014).

2.2. Animal preparation

Experiments were performed on 43 male New Zealand white rabbits (2.8–3.5 kg) anesthetized with pentobarbital sodium (40 mg/kg i.v., supplemented by 2–4 mg/kg every 30 min; Sigma–Aldrich, St. Louis, MO). Atropine (0.15 mg/kg i.m.) was administered to reduce mucosal secretion in the airways. The adequacy of anesthesia was continuously assessed during the experiment as in our previous studies. The trachea was cannulated and polyethylene catheters were inserted into a femoral artery and vein for monitoring arterial blood pressure and drug delivery, respectively. The C_3 or C_5 phrenic root on one side was prepared for recordings. The animal was placed in a prone position and fixed by a stereotaxic head holder and vertebral clamps. The head was ventroflexed for optimal exposure of the dorsal surface of the medulla by occipital craniotomy. Body temperature was maintained at 38.5–39 °C.

2.3. Recording procedures

Bipolar platinum electrodes were used to record efferent phrenic nerve activity from the central stump of one cut and desheathed phrenic root. Wire electrodes were used to record abdominal muscle electromyographic (EMG) activity. Phrenic and abdominal activities were amplified, full-wave rectified, and "integrated" (low-pass RC filter, time constant 100 ms). Arterial blood pressure and end-tidal CO_2 partial pressure were recorded. Cardiorespiratory variables were acquired and analyzed using a personal computer, equipped with an analog-to-digital interface (Digidata 1440, Molecular Devices, Sunnyvale, CA, USA) and appropriate software (Axoscope, Molecular Devices).

2.4. Microinjection procedures

Bilateral microinjections were performed at two different sites along the rostrocaudal extent of the caudal NTS, and particularly into the lateral commissural NTS. The first was at the level of the caudal-most end of the area postrema, 0.6–0.8 mm lateral to the midline and 0.7–0.8 mm below the dorsal medullary surface. The second was 0.5 mm more caudal, 0.4–0.5 mm lateral to the midline and 0.7–0.8 mm below the dorsal medullary surface. The stereotaxic coordinates were selected according to the atlas of Meessen and Olszewski (1949).

Microinjections (30–50 nl) of the following drugs were performed: losartan (5 mM; Fluka–Sigma–Aldrich), lisinopril (1 mM; Sigma–Aldrich), bradykinin (0.05 mM; Tocris Bioscience, Bristol, UK), HOE-140 (0.2 mM; Tocris Bioscience), a potent and selective bradykinin B_2 receptor antagonist, CP-99,994 (1 mM; gift from Pfizer, Groton, CT, USA), an NK_1 receptor antagonist and D,L-homocysteic acid (DLH, 20 mM; Sigma–Aldrich), a broad-spectrum excitatory amino acid agonist. Only one of these drugs was tested in each preparation unless otherwise stated. Drug concentrations were in the same range as those previously used in in vivo preparations (Arnold et al., 2010; Caligiorne et al., 1996; Kasparov et al., 1998; Mutolo et al., 2008). In particular, the concentrations of lisinopril and losartan were based on the knowledge that the antihypertensive potency of lisinopril is ~5 times higher than that of losartan (see e.g., Mutolo et al., 2013). All drugs were dissolved in 0.9% NaCl solution. Control injections of equal volumes of the vehicle solution at the responsive sites were also performed. The localization of injection sites is illustrated in Fig. 1. Green fluorescent latex microspheres (LumaFluor, New City, NY, USA) were injected for *post hoc* confirmation of injection sites within the caudal NTS of some preparations (3 for losartan and 4 for lisinopril).

2.5. Stimulation procedures

Both mechanical and chemical stimulation of the tracheobronchial tree were employed to induce cough. Mechanical stimulation was delivered by a custom-built device recently described and validated (Mutolo et al., 2014) using a 0.5 mm diameter nylon fiber with a smoothed tip inserted through a lateral port of the tracheal cannula. The device allowed to set the number of forth and back movements or cycles (1–3 cycles), shaft velocity (10–20 mm/s), and shaft displacement (10–20 mm). Mechanical stimulation was adjusted to the following parameters: 1 cycle, 15 mm/s velocity, and 15 mm displacement. These parameters proved to produce a bout of 2–4 coughs. The stimulation protocol comprised three stimulation trials performed in succession (at ~1 min interval) before drug administration, repeated ~5 min after the completion of all the microinjections and at appropriate intervals (at least 5 min) until complete recovery was observed.

Chemical stimulation of the tracheobronchial tree was performed by means of citric acid inhalation (for details see Mutolo et al., 2009). Citric acid (1 M, Sigma–Aldrich) was freshly dissolved in 0.9% NaCl solution and nebulized. 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. This short period as well as time intervals between chemical challenges >10 min proved to be adequate to avoid tachyphylaxis. Chemical stimulation was always applied 2–3 min after mechanically-induced cough and caused a bout of several coughs usually immediately followed by a tachypneic response. As a rule, chemical stimulation was performed both before and ~15 min after the completion of the injections and repeated at appropriate intervals to follow the time course of the recovery process. All stimulation procedures were performed at least 5–6 min after each supplemental dose of pentobarbital to avoid its possible immediate influence on the recorded variables.

2.6. Histology

The histological control of pipette tracks and injection sites was performed as previously described (for details, see Mutolo et al., 2007, 2012). Medullary sections in which injection sites