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Downregulation of the cough reflex by aclidinium and tiotropium in awake and anesthetized rabbits

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ABSTRACT

Long-acting muscarinic receptor antagonists (LAMAs) have been reported to attenuate cough in preclinical and clinical studies. The present study was performed on rabbits to compare aclidinium and tiotropium efficacy in the downregulation of the cough reflex. This reflex was evoked by citric acid inhalation in unanesthetized animals and by both citric acid inhalation and mechanical stimulation of the tracheobronchial tree in anesthetized animals 90 min following the inhalation of each drug (nebulizer output always at 1 mL/min). Aclidinium 4 mg/mL and tiotropium 200 μg/mL inhaled in 1 min proved to have similar protective effect on methacholine-induced bronchoconstriction in anesthetized animals. The total dosage employed for aclidinium and tiotropium was 4 mg and 200 μg, respectively. In awake animals, similar reductions in the cough number were observed following 10-min inhalation of each drug with a slight, not significant tendency to higher antitussive effects for aclidinium. In anesthetized animals, 1-min inhalation of each drug caused similar depressant effects on cough responses induced by both mechanical and chemical stimulation. A complete suppression of cough responses to mechanical stimuli was seen in some preparations. The results strongly suggest that the LAMA-induced downregulation of cough may be mediated not only by transient receptor potential vanilloid type 1 channels, as already reported, but also by acid-sensing ion channels and mechanoreceptors. The route of administration along with the more rapid hydrolysis of aclidinium into inactive metabolites minimize potential systemic side effects and give to this drug a very favorable safety profile.

1. Introduction

Cough is a very important airway defensive reflex [1–4] and is the most common symptom for which patients seek medical advice. Despite considerable efforts in the last decades to find appropriate therapies, a safe and effective cough remedy is still lacking [5–11].

Inhaled bronchodilator therapies with long-acting muscarinic receptor antagonists (LAMAs) are of crucial importance for chronic obstructive pulmonary disease (COPD) management and cause an improvement in symptoms including cough ([12–16] also for further Refs.). Tiotropium was the first LAMA, reaching the market in 2002 [17]. Dicpinigaitis et al. [18] reported that tiotropium (1 h after its inhalation) inhibits cough induced by capsaicin, a transient receptor potential vanilloid type 1 (TRPV1) agonist, in patients with upper respiratory tract infections. Furthermore, tiotropium inhalation has been shown to improve cough and other symptoms in patients with chronic pulmonary disease due to sulphur mustard lung injury [19]. More recently, it has been shown that inhaled tiotropium attenuates after 1 h cough induced by capsaicin in the guinea pig and that this effect is mediated by TRPV1 receptors through a mechanism unrelated to its anticholinergic activity [17]. However, Clay et al. [7] have reported that, in contrast to the guinea pig, the ozone-induced hypertussive responses to citric acid are not inhibited by tiotropium in the rabbit.

Aclidinium bromide is a LAMA that has recently been approved as a maintenance bronchodilator treatment for patients with COPD and asthma [14,20,21]. In clinical studies, aclidinium provides greater improvements in COPD symptoms, including cough, than tiotropium and is well tolerated, with a similar safety profile [12,13]. A recent study in the guinea pig chronically exposed to

Abbreviations: ASICs, acid-sensing ion channels; COPD, chronic obstructive pulmonary disease; DMSO, dimethyl sulfoxide; LAMAs, long-acting muscarinic receptor antagonists; MCh, methacholine; P_{ao}, alveolar pressure; P_{es}, esophageal pressure; P_{TP}, transpulmonary pressure; R_L, lung resistance; TRPV1, transient receptor potential vanilloid type 1.

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cigarette smoke (an experimental model of COPD) indicates that aclidinium, in addition to beneficial effects on lung structure and function, shows a trend toward fewer cough episodes [22]. A comparative study on the antitussive effects of aclidinium and tiotropium in animal models is lacking.

The present study was undertaken to compare aclidinium and tiotropium efficacy in the downregulation of the cough reflex in the rabbit. The two drugs were administered by inhalation, whilst the cough reflex was evoked by citric acid inhalation in awake animals and by both citric acid inhalation and mechanical stimulation of the tracheobronchial tree in anesthetized animals [8–11,23–28].

2. Materials and methods

2.1. Preliminary remarks

A total of 48 rabbits were enrolled in this study, including 2 rabbits used in preliminary trials and 6 rabbits employed to investigate the LAMA protective action on cholinergic-induced bronchoconstriction (see below). 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 [8–11,23–28].

2.2. Effects of aclidinium or tiotropium on methacholine-induced bronchoconstriction

The first step was to assess the concentrations of the two drugs effective in counteracting the bronchoconstriction induced by methacholine (MCh; Sigma-Aldrich, St. Louis, MO, USA). Experiments were performed in New Zealand white rabbits (3.1–3.6 kg) anesthetized with pentobarbital sodium (40 mg/kg i.v., supplemented by 2–4 mg/kg every 30 min; Sigma-Aldrich) and spontaneously breathing. The adequacy of anesthesia was assessed by the absence of reflex withdrawal of the hindlimb in response to noxious pinching of the hindpaw. The trachea was cannulated and polyethylene catheters were inserted into a femoral artery and vein for monitoring arterial blood pressure and for drug delivery, respectively. The animal was placed in a prone position and fixed by a stereotaxic head holder and vertebral clamps. Body temperature was maintained at 38.5–39 °C by a heating blanket controlled by a rectal thermistor probe.

Esophageal pressure (P_{es}) was measured with a thin-walled latex balloon (5-cm length) sealed over a polyethylene catheter (100-cm length, 1.7 mm ID) with several holes in the section covered by the balloon, positioned in the midesophagus and connected to a pressure transducer. This corresponds to the transpulmonary pressure (P_{TP}) under static conditions, i.e. $P_{TP} = P_{es} - P_{ao}$, where P_{ao} is the alveolar pressure at end inspiration or expiration, equal to the atmospheric pressure (see e.g. Ref. [29]). The flow signal was recorded by means of a pneumotachograph (Fleish no. 00) and a differential pressure transducer. The quotient of the maximum inspiratory value of P_{es} and the maximum inspiratory value of tidal flow (Fig. 1A) was considered a reliable index of lung resistance (R_L; see e.g. Refs. [30,31]). All recorded 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). Obviously, the pneumotachograph was disconnected during drug inhalation and connected again for R_L assessment.

MCh, dissolved in 0.9% NaCl solution, was delivered by an ultrasonic nebulizer (Projet, Artsana, Grandate, CO, Italy). The diameter of the droplets ranged from 0.5 to 8 μm and the nebulizer output was always set at 1 mL/min. The opening of the tracheal cannula, through which the rabbits were spontaneously breathing, was exposed to a steady stream of the nebulized MCh solution for ~30 s. In preliminary trials (two rabbits), MCh was delivered at increasing concentrations (8, 16 and 32 mg/mL) as previously reported [30] to select the dose able to induce a submaximal increase of R_L. The selected dose was 16 mg/mL. MCh challenges and induced bronchoconstriction were evaluated before and after aclidinium (1, 2 and 4 mg/mL for 1 min) and tiotropium (50, 100 and 200 μg/mL for 1 min) to counteract bronchoconstriction and cause a fall in $R_L > 90\%$. R_L was measured under basal conditions and after aerosolized MCh at 16 mg/mL. A maximum bronchoconstriction occurred within about 1 min and was maintained during the following 2–3 min. A rapid recovery followed. Nevertheless, a time interval of at least 30 min was allowed before 1-min inhalation of aclidinium or tiotropium. Ninety min after drug inhalation, the MCh challenge was repeated and R_L re-evaluated. The interval of 90 min was chosen to ensure LAMA maximum effects; both aclidinium and tiotropium (as other LAMAs) have been proved to fully express their protective action on cholinergic-induced bronchoconstriction at least 1 h post-administration [32]. As illustrated in Fig. 1, we found that the adequate concentration of each drug to produce similar decreases in R_L (range 90–95%) was 4 mg/mL of aclidinium and 200 μg/mL of tiotropium. Since these muscarinic receptor antagonists are long-acting [33], it was necessary to use one animal for each drug concentration, i.e. three rabbits for each drug.

Aclidinium bromide (ShanHai Biochempartner Co., Ltd, China) was initially dissolved in a 0.1 N hydrochloric acid/DMSO (10:90, v/v) mixture and then diluted in 0.9% NaCl solution at the desired concentrations [34]. In the final solution, the concentration of DMSO was less than 5%. Tiotropium bromide monohydrate (Sigma-Aldrich) was dissolved in 0.9% NaCl solution at the desired concentrations [7,33]. All drugs were freshly prepared on the day of administration. Vehicle solutions administered in the same preparations proved to be without effects on MCh-induced bronchoconstriction.

We roughly estimate that over the 1-min challenge, the lungs will be exposed to 160–200 μg of aclidinium. This was based on a normal breathing rate of 40–50 breaths/min and a tidal volume of ~30 mL as well as a deposition rate of 10% with the aerosolized 4 mg/mL solution, which is “diluted” ~3000-fold with room air from the nebulizer system (airflow of the nebulizer set at ~3 l/min). Taking into consideration an aerosolized 200 μg/mL solution of tiotropium, similar calculations led to the estimation that over the 1 min of challenge the lungs will be exposed to 8.4–10.5 μg of tiotropium. For the total dose calculation see also Birrell et al. [17]. The same total dosage (aclidinium 4 mg and tiotropium 200 μg) was employed to investigate drug effects on the cough reflex in both unanesthetized and anesthetized animals. In particular, for an evaluation of total lung exposition to aclidinium and tiotropium in awake animals a similar calculation was performed, taking into account in addition the 10-min duration of the inhalation period in the Perspex chamber and accordingly reducing the concentration of drug solutions to be aerosolized (see below).

2.3. Experiments on unanesthetized animals

Experiments were performed on 20 unanesthetized male New Zealand white rabbits (3.0–3.6 kg). In each cough induction test [23,24], the rabbits were placed individually into a transparent Perspex inhalation chamber (approximately 0.050 m^3) and exposed