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Rapidly adapting receptor activity during oxidative stress induced airway hyperresponsiveness

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ABSTRACT

The responses of airway rapidly adapting receptors (RARs) to ovalbumin challenge and histamine were investigated in guinea pigs which were sensitized with ovalbumin. Sensitization alone increased the basal RAR activity. Antigen challenge stimulated them. Histamine doses which caused a 50% increase in airway resistance (ED_{50}) were reduced immediately and 24 h after antigen challenge indicating respectively early and late onset airway hyperresponsiveness. At these doses, there was a greater stimulation of the RARs compared to controls. An increase in lipid peroxidation and a decrease in glutathione peroxidase were observed also. With oral intake of vitamins C and E, attenuations in the basal RAR activity, the responses of RARs to antigen challenge and the oxidative stress were observed. With an increase in ED_{50}, the RAR response to histamine became similar as in control. It is concluded that by decreasing the RAR responses to allergen and histamine, antioxidants may reduce reflex bronchoconstriction occurring in asthmatics.

1. Introduction

Asthma is an inflammatory disease of the airways in which there is airway obstruction and increased bronchial responsiveness to a variety of stimuli (Wood et al., 2003). These responses could be due to several factors including an increase in the production of reactive oxygen species (ROS) as excessive ROS also promote bronchoconstriction, airway hypersecretion and microvascular leakage (Rhoden and Barnes, 1989; Adler et al., 1990; Barnes, 1990; Yukawa et al., 1990; Sadeghi-Hashjin et al., 1996; Lei et al., 1996). It is known that following the administration of agents such as histamine which cause bronchoconstriction (Barnes et al., 1998), inhalation of irritant gases such as cigarette smoke which increase airway secretion (Takeyama et al., 2001) and pulmonary edema where there is microvascular leakage (Bhagat et al., 2011), there is stimulation of the airway rapidly adapting receptors (RARs) (Sellick and Widdicombe, 1971; Vidruk et al., 1977; Ravi et al., 1989, 1994; Kou and Lee, 1990; Bhagat et al., 2011). However, there is negligible information on the effects of *in vivo* generation of ROS on RARs in animals with hyperresponsive airways.

Even though it was reported several years ago that during anaphylaxis, there was an increase in RAR activity (Mills et al., 1969), there are not many subsequent studies which have examined the behavior of RARs in an animal model with hyperresponsive airways. In an attempt made in guinea pigs which were sensitized with ovalbumin, it was reported that there was an increase in the sensitivity of RARs as evidenced by an augmentation in their responses to capsaicin (Bergren, 2001). This study did not investigate the responses of RARs to an antigen challenge or lung autacoids. Additionally, it did not explore whether ROS contributed to the observed responses.

Oxidative stress was reported in guinea pigs which had been previously sensitized and challenged with ovalbumin (Jain et al., 2004). There was airway hyperresponsiveness also as evidenced by an increase in the airway reactivity to histamine (Jain et al., 2005). In such a model, first we hypothesized that following an antigen challenge, there would be an increase in the activity of RARs and in this background, there would be an increase in their sensitivity to bronchoactive agents such as histamine. The RAR responses were investigated immediately and 24 h after the antigen challenge in order to assess whether the RARs contribute to the respiratory symptoms associated with early and late onset airway responses. Then, to determine the role of ROS, we repeated the study after oral administration of the antioxidants, vitamin C and vitamin E. Various oxidative stress parameters and plasma concentrations of vitamin C and vitamin E were determined also. To assess the effect of *in vivo* generation of ROS *per se* on RARs, the effects of xanthine–xanthine oxidase inhalation (Katsumata et al., 1990) were studied as a subsidiary investigation.

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2. Materials and methods

Adult guinea pigs ($n = 36$), weighing 400–800 g were used as experimental animals. They were fed on commercially available feed and were given food and water *ad libitum*. All the experimental procedures mentioned in the protocols were approved by the Institutional Animal Ethics Committee and conforms to the National guidelines on the use and care of experimental animals.

2.1. Surgery

Animals of all the groups were anesthetized with urethane (1 g/kg, i.p., Merck, Germany) (Mazzone et al., 2005). Anesthesia was maintained by periodic injections of urethane (0.25 g/kg, i.p.), as and when required. The adequacy of anesthesia was examined by pinching the paw and checking the corneal reflex. Polyethylene cannulae were introduced into the right external jugular vein and common carotid artery. The venous cannula was utilized for giving infusions and drugs. The carotid arterial cannula was utilized for measurement of blood pressure and for periodic withdrawal of arterial blood samples for the determination of arterial blood gases (Nova Biomedical, USA). The temperature of the animal was constantly monitored by a thermistor. It was maintained between 37 and 38 °C using heating pads.

The trachea was cannulated and an uncuffed endotracheal tube was introduced into it. The animals were artificially ventilated with a ventilator (Model 683, Harvard apparatus, USA) at the rate of 38 breaths/min and a tidal volume of approximately 0.75 ml/100 mg body wt. The animals were paralyzed by gallamine triethiodide (1 mg/kg i.v., Sigma). The adequacy of anesthesia was checked first before giving gallamine. Gallamine triethiodide injections were repeated every hour after checking the depth of anesthesia and administered after giving the anesthetic first. The inspired air was supplemented with 40% oxygen. The arterial blood gases were monitored and maintained in the normal range: PO_2 ~100 mm Hg, PCO_2 ~40 mm Hg and pH ~7.4. The PCO_2 and pH were kept at the normal range by adjusting the tidal volume and by infusing sodium bicarbonate (8.5%, w/v). A polyethylene cannula (i.d. 0.86 mm) connected to a differential pressure transducer (MPX, Harvard apparatus, USA) was introduced through the tracheal cannula for the measurement of airway pressure. A pneumotachograph (DP 45-14, Harvard apparatus, USA) was introduced in between the ventilator and the tracheal cannula and it was used for measuring airflow. From the pressure and flow measurements airway resistance was obtained electronically using a pulmonary mechanics analyzing system (Pulmodyn, Harvard apparatus, USA).

2.2. Recording of airway RAR activity

The right cervical vagus nerve was separated from the carotid sheath and prepared for recording afferent activity originating from RARs using conventional techniques (Paintal, 1955). The neural signals were amplified by a pre-amplifier (Tektronix TM 503, USA) and fed into a thermal array recorder (WindoGraf, Gould, USA) and audio amplifier (Ahuja CA15, India) connected to loud speaker. The location of the receptor was ascertained at the end of each experiment after a midline thoracotomy by gently probing of the lungs and airways externally using a blunt glass rod of 3 mm diameter. A positive end-expiratory pressure of 1 cmH_2O was applied immediately after opening the chest.

The RARs were identified by their irregular resting discharge and rapid adaptation to a maintained hyperinflation (3 times of the tidal volume) of the lungs (Kappagoda et al., 1987) as shown in Fig. 1.

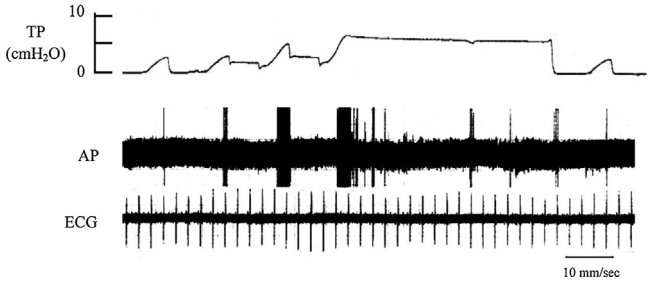

Fig. 1. Identification of rapidly adapting receptor (RAR). After a normal ventilatory cycle, the expiratory line of the ventilator was occluded and the lungs were inflated for 3 cycles. At the 3rd cycle, the ventilator was switched off and the inflation was maintained. Note the high frequency discharge during each inflation adapting rapidly. TP – tracheal pressure, AP – action potentials and ECG – electrocardiogram.

2.3. Airway sensitization followed by inhalation challenge

Guinea pigs were sensitized as per standard procedures (Santing et al., 1994). For sensitization, 100 mg aluminum hydroxide (Sigma) mixed with 100 μg ovalbumin (Sigma) per ml of normal saline was used. 0.5 ml of the antigen-adjuvant solution was injected intraperitoneally and 0.5 ml was injected subcutaneously, with the dose divided equally and injected into 7 different sites near the lymph nodes. After 4 weeks, for observing the early asthmatic response, the sensitized animal was anesthetized and then challenged with 0.2% ovalbumin for one min using an ultrasonic nebulizer (Hico-Ultrasonat, 806 EH, Germany), connected to the ventilator. In another set of animals, after sensitization with ovalbumin for 4 weeks, to observe the late asthmatic response, they were placed in the guinea pig body box and challenged with 0.2% ovalbumin for 1 min in the conscious state. They were kept under observation and utilized for the receptor study 24 h later.

2.4. In vivo generation of ROS

Xanthine (MP Biomedicals, USA) and xanthine oxidase (Sigma) were dissolved in phosphate buffered saline, and each was made up to a volume of 10 ml (Katsumata et al., 1990). Successive inhalations of xanthine (0.1%) for 1 min and xanthine oxidase (1 U/ml) for 1 min were given using the ultrasonic nebulizer through the ventilator.

2.5. Administration of histamine

Histamine (Sigma) inhalation was given successively in increasing concentrations using the ultrasonic nebulizer connected to the ventilator. Starting with a dose of 0.04 mg/ml histamine in phosphate buffered saline, the histamine concentration was doubled every time and continued until it produced an increase in airway resistance by 50% or more. This dose of histamine was termed as ED_{50} (the effective dose required to produce at least 50% increase in airway resistance). The percentage increase in airway resistance was calculated from the baseline value just before histamine inhalation for each dose. An interval of 15 min was given between successive injections to avoid tachyphylaxis.

2.6. Antioxidant supplementation

Vitamin E was obtained in the form of capsules (Evion-400 IU, Merck). The oil was removed from the capsule using a syringe, and emulsified by sonification in presence of gelatin as stabilizing agent. The final concentration was adjusted to 1 ml in water. Each animal was given 1 ml of the suspension everyday orally. Vitamin C was obtained in the form of tablets (Celin-500 mg, Glaxo Smithkline). It