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ADRENOMEDULLIN, A NEWLY DISCOVERED HYPOTENSIVE PEPTIDE, IS A POTENT BRONCHODILATOR

Hiroshi Kanazawa, Naotsugu Kurihara, Kazuto Hirata, Shinzoh Kudoh, Takashi Kawaguchi, and Tadanao Takeda

The First Department of Internal Medicine
Osaka City University Medical School
1-5-7, Asahi-machi, Abenoku, Osaka 545, Japan

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The present study was designed to examine the effect of adrenomedullin (AM), a novel vasorelaxant peptide originally isolated from human pheochromocytoma, on histamine-or acetylcholine-induced bronchoconstriction in anesthetized guinea pigs in vivo. AM significantly inhibited acetylcholine-induced bronchoconstriction in a dose-dependent fashion. AM also significantly inhibited histamine-induced bronchoconstriction, but 10^{-10} M AM had no significant inhibitory effect on this response. We also found that AM induced a long-lasting bronchodilator response, while isoproterenol induced transient bronchodilation. These preliminary findings suggest that AM may play important roles in airway function.

Adrenomedullin (AM) is a hypotensive peptide recently discovered in human pheochromocytoma. The peptide, consisting of 52 amino acids, has one intramolecular disulfide bond, and shows slight homology with calcitonin gene - related peptide[1]. A cDNA clone encoding the porcine AM precursor was isolated and sequenced from rat and human tissue. Using RNA blot analysis, porcine AM mRNA was shown to be highly expressed in several porcine tissues including lung and kidney as well as adrenal medulla[2]. In studies of the regional distribution of immunoreactive AM in human tissue, AM was found to be abundant in normal adrenal medulla as well as in pheochromocytoma tissue, but only relatively small amounts of AM were detected in the lung[3]. These findings suggest the possibility that AM synthesized in lung tissue is rapidly released into the peripheral blood and airway lumen. AM is an important circulating hormone which participates in the regulation of blood pressure, and is found in peripheral blood in a considerable concentrations. Since endothelins, which are especially important regulators of the

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cardiovascular system function, elicit bronchoconstrictor responses in human and guinea pig airway[4], AM might also play roles in airway function. However, the airway functions of AM have not yet been studied. In this study, the bronchodilating effects of AM were studied in vivo in anesthetized guinea pigs.

Materials and Methods

Pulmonary resistance (R_L) measurement : Hartley male guinea pigs (400-500g) were used in this study. They were anesthetized using sodium pentobarbital (50 mg/kg i.p. ; Abbott Laboratories) and then ventilated artificially with a tracheal cannula using a constant-volume ventilator (Model 680; Harvard Apparatus Co.) at a frequency of 60 breaths / min. The tidal volume was set at 6 ml/kg. Airflow was monitored continuously using a pneumotachograph(TV-241T, Nihon Koden Co.) connected to a differential pressure transducer (TP-602T, Nihon Koden Co.). The tidal volume was determined by electrical integration of airflow. A fluid-filled polyethylene catheter was introduced into the esophagus to measure esophageal pressure as an approximation of pleural pressure. Intratracheal pressure was measured using a polyethylene catheter inserted into a short tube connecting the tracheal cannula to the pneumotachograph. The transpulmonary pressure (defined as the pressure difference between the intratracheal and esophageal pressures) was measured with a differential pressure transducer. R_L was calculated as previously described[5]. Aerosols of test agents were generated with an ultrasonic nebulizer and delivered to the airways by the ventilator .

Effect of AM on acetylcholine - induced bronchoconstriction : AM (10^{-8} ~ 10^{-6}M, 30 breaths at each concentration) and acetylcholine (10^{-2}M, 60 breaths) were administered continuously by an ultrasonic nebulizer. Concentration curves for the response to 10^{-8} to 10^{-6}M AM were recorded.

Effect of AM or isoproterenol on histamine-induced bronchoconstriction: Histamine(10^{-3}M, 60 breaths) was administered using an ultrasonic nebulizer 5min before AM administration. Concentration curves for the response to 10^{-10} to 10^{-6}M AM (30 breaths at each concentration) were recorded. Similarly, we recorded the concentration curves for the response to 10^{-8} to 10^{-6}M isoproterenol (30 breaths at each concentration) 5 min after histamine inhalation.

Drugs : Histamine, acetylcholine and isoproterenol were obtained from Sigma Chemical Company (St.Louis, Mo). Human AM was purchased from the Peptide Institute, Inc.(Japan).

Statistical Analysis: Values are expressed as mean ± SEM. Comparisons between groups were made using Students' t-test. Statistical significance was claimed at $p<0.05$.

Results and Discussion

Acetylcholine inhalation induced significant bronchoconstriction, which was maximal 1 min after inhalation. AM (10^{-8} ~ 10^{-6}M) significantly inhibited acetylcholine-induced bronchoconstriction in a dose-dependent fashion (Fig. 1). AM(10^{-9}~10^{-6}M) also significantly inhibited histamine-induced bronchoconstriction in a dose - dependent fashion, but 10^{-10}M AM had no significant inhibitory effect on this response (Fig. 2). Isoproterenol, a β-adrenergic agonist, inhibited histamine-induced bronchoconstriction to the same extent as AM (Fig. 3). We also found

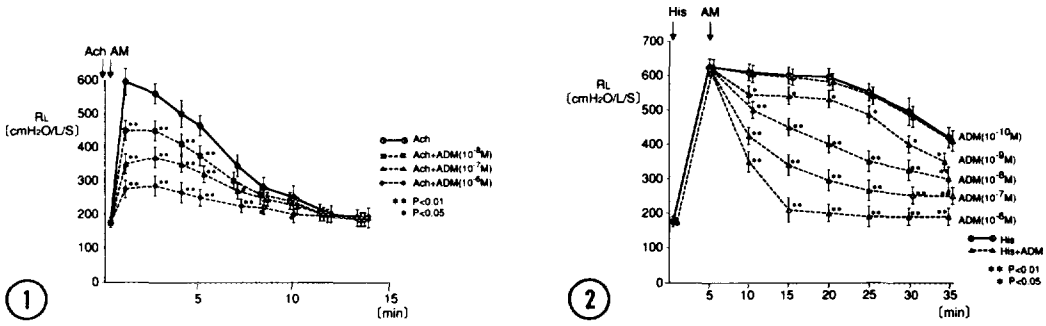

Fig. 1. Effect of adrenomedullin on acetylcholine-induced bronchoconstriction.
Ach = acetylcholine, AM = adrenomedullin
Each point represents the mean ± SEM for six animals. Significant differences from values for acetylcholine inhalation are indicated by by * $p<0.05$, ** $p<0.01$.

Fig. 2. Effect of adrenomedullin on histamine-induced bronchoconstriction.
His = histamine, AM = adrenomedullin
Each point represents the mean ± SEM for six animals. Significant differences from values for histamine inhalation are indicated by * $p<0.05$, ** $p<0.01$.

that AM induced a long-lasting bronchodilator response, while isoproterenol induced transient bronchodilation. Although these findings are preliminary, we have shown for the first time that AM has a bronchodilative effect in anesthetized guinea pigs in vivo. However the machanism of AM-induced bronchodilatation is not clearly known. In perfused mesenteric vascular bed, perfusion of AM induced a concentration-dependent decrease in perfusion pressure due to vasodilation[6]. In addition, Ishiyama et al reported that intravenous administration of AM induced a rapid and marked reduction in mean blood

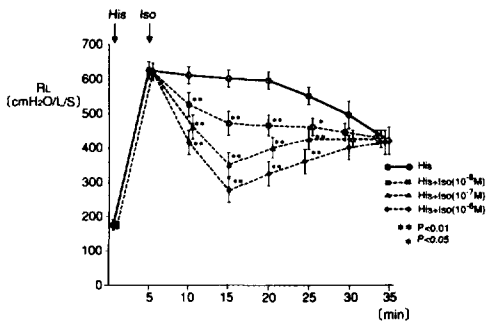

Fig. 3. Effect of isoproterenol on histamine-induced bronchoconstriction.
His = histamine, Iso = isoproterenol
Each point represents the mean ± SEM for six animals. Significant differences from values for histamine inhalation are indicated by * $p<0.05$, ** $p<0.01$.

pressure in anesthetized rats[7]. AM induces vasodilation by increasing cyclic adenosine monophosphate (cAMP) levels in vascular smooth muscle cells, which possess AM receptors functionally coupled to adenylate cyclase[8]. cAMP is thought to play an important role as an intracellular mediator in the regulation of airway functions. Our findings suggest the possibility that binding sites for AM are present on guinea pig smooth muscle, and that their stimulation by AM may result in bronchodilation. β-adrenergic agonists and phosphodiesterase inhibitors are used clinically for treatment of bronchial asthma. Both of these drugs may also stimulate the production of cAMP in airway smooth muscle cells directly, and may consequently participate in the bronchodilation. However, we have noted unfavorable effects with the use of these drugs including tachycardia. Interestingly, in this study, inhaled isoproterenol induced tachycardia, but AM did not . This findings agrees with that of a recent study by Ishiyama et al, that intravenous administration of AM did not induce tachycardia despite a marked reduction in mean blood pressure[7]. It is therefore possible that inhaled AM will be useful clinically for the management of bronchial asthma. However, further studies will be required to clarify the physiological role of AM in the regulation of airway functions.

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