Alpha-Human Atrial Natriuretic Peptide (α-hANP) Prevents Pulmonary Edema Induced by Arachidonic Acid Treatment in Isolated Perfused Lung from Guinea Pig

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Abstract—Protective effect of α-human atrial natriuretic peptide (α-hANP) on pulmonary edema was investigated using an isolated perfused lung model. Infusion of α-hANP (1.7 to 22 ng/ml or 0.56 to 7.3 nM) prevented the edema induced in isolated lung from guinea pig by repeated treatment of 50 μg of arachidonic acid at 30 min intervals via the pulmonary artery. The antiedematous action of α-hANP was considered to be receptor mediated because the effective concentration was close to the Kd value of the binding of the ANP receptors in the lung homogenate.

It has been established that atrial natriuretic peptide (ANP) plays an important role in regulating salt and water balance and blood pressure (1–3). Autoradiographic studies on localization of a radioactive iodinated ANP (125I-ANP) demonstrated that receptors specific for ANP are distributed to various organs which include the brain, posterior pituitary gland, kidney, adrenal gland, vascular system, lung, liver, ciliary body of the eye and probably the small intestine (4). Receptors in the brain, posterior pituitary gland kidney, adrenal gland and vascular system have been understood in relation to the integrated regulation of salt and water balance and blood pressure by ANP (1). However, the effect of ANP in such organs as liver, lung and small intestine has not been known, although the lung has been suggested to be a possible target organ for ANP based on the observation that a considerable amount of α-rat atrial natriuretic peptide (α-rANP)-like immunoreactivity was determined in the rat lung (5). As to a possible action of ANP in the lung, we suspected that ANP might prevent pulmonary edema which is encountered in congestive heart failure, renal disease, shock, infections within the lung, because it has been understood that the function of ANP is to integrally regulate body fluid volume and electrolyte balance in concert with other cardiovascular and hemodynamic or hemostatic systems (1–3). We examined this hypothesis by using an isolated perfused lung model.

The lungs were isolated from male Hartley guinea pigs weighing 500 to 600 g under pentobarbital anesthesia with minor modification of the techniques previously described by Bassett et al. (6), and hung on force transducers to monitor lung wet weight. Pulmonary arterial perfusion pressure was monitored by a pressure transducer. The lungs were perfused in an atelectatic condition with Krebs-Ringer bicarbonate buffer (pH 7.4) via the cannulated pulmonary artery at a flow rate of 3.5 ml/min. The buffer was equilibrated with a gas mixture of 95% O2/5% CO2 and maintained at 37°C throughout the experiment. To induce pulmonary edema, 50 μg of arachidonic acid was given repeatedly at 30 min intervals through the pulmonary artery cannula by bolus. Arachidonic acid was chosen as a candidate substance which may cause...
edema in the lung because arachidonic acid has been known to be metabolized in the lung by cyclooxygenase and lipoxygenase to give substances which are capable of causing vasoconstriction and enhancement in capillary permeability (7). This idea was confirmed by the following observations: increases in wet lung weight and pulmonary perfusion pressure following bolus administration of arachidonic acid were suppressed when the lung was infused with 0.4 mM of indomethacin (data not shown).

When arachidonic acid was administered, wet lung weight, an indicator of pulmonary edema in this system, increased irreversibly after the 6th to 8th applications, with transient increase in pulmonary arterial perfusion pressure (Fig. 1A). In contrast, when chemically synthesized α-hANP (8) was infused at a concentration of 22 ng/ml (7.3 nM), edema was not produced, while the edema was produced after the infusion was discontinued (Fig. 1B). The protective effect of α-hANP against edema formation was also observed at a concentration of 1.7 ng/ml (data not shown).

To examine whether this action was receptor mediated, saturable binding kinetics of ANP to the rat lung homogenate was studied by using radiolabeled α-rANP ($^{125}$I-α-rANP). The monoiodinated radioactive ligand was prepared by the lactoperoxidase method (9) and purified by a C18 reversed phase HPLC (Comisol 5C18-P, 4.6 x 100 mm) with a linear gradient of acetonitrile (10 to 60%) in 0.1% trifluoroacetic acid solution. The specific radioactivity was approximately 0.6 mCi/μg. Nonradioactive monoiodinated peptide was prepared, and the equipotency of the material to α-rANP was confirmed by the chick rectum assay (10). The lung was dissected from Sprague-Dawley male rats, and homogenized with polytron to give a 15% homogenate in a cold buffer containing 10 mM Tris-HCl, pH 7.4, 0.25 M sucrose, 1 mM EDTA, 1 mM PMSF (phenylmethylsulfonyl fluoride) and 1 μM captopril. A 100 μl aliquot of the supernatant after centrifugation of the homogenate at 1,700 rpm for 10 min was added into a test tube which contained 150 μl of a buffer consisting of 10 mM Tris-HCl, pH 7.4, and 0.2% bovine serum albumin. Binding was allowed to proceed for 15 min to equilibrium. Bound $^{125}$I-α-rANP was separated from free ligand by rapid centrifugation at 11,000 rpm for 5 min. The supernatant was removed and the pellet was assayed for radioactivity. The specific binding of $^{125}$I-α-rANP was defined as the difference between total counts determined in the pellet in the absence and in

![Fig. 1. Antiedematic action of α-hANP in isolated perfused guinea pig lungs. Changes of lung wet weight (ΔWt) and pulmonary arterial perfusion pressure (ΔP) were recorded as indicators of pulmonary edema. A: ΔWt and ΔP induced by repeated treatment of 50 μg of arachidonic acid when the lung was perfused with Krebs-Ringer bicarbonate buffer alone. B: ΔWt and ΔP recorded when the lung was co-perfused with α-hANP (22 ng/ml) and the Krebs-Ringer bicarbonate buffer. Arrows indicate bolus injections of arachidonic acid to the lung via pulmonary artery cannula.](image-url)
the presence of 1 nM of unlabeled α-rANP. The dissociation rate constant (Kd) was obtained by Scatchard analysis.

The binding of 125I-α-rANP to the homogenate competed with unlabeled α-hANP as well as α-rANP but not with angiotensin II (1.2 nM) (data not shown). The apparent Kd value of the binding was estimated to be 1.75 nM (Fig. 2A, B). Thus it is considered that the antiedematous action of ANP is probably receptor mediated because the effective concentration of α-hANP (0.56 to 7.3 nM) is close to the Kd value (1.75 nM) of the specific binding of 125I-α-rANP to the lung homogenate.

Very recently it has been reported that ANP is capable of ameliorating pulmonary edema manifested by congestive heart failure (11) and ascites due to cirrhosis of the liver (12). However, it is not clear whether these antiedematous actions of ANP are solely derived from its natriuretic action and/or improved cardiac function. In the present study, we could demonstrate that ANP has a direct antiedematous action in an isolated perfused lung model.

It has also been reported that patients suffering from congestive heart failure, renal disease, and cirrhosis of the liver are associated with elevated concentration of ANP in plasma (13–15), and the elevated ANP levels are understood as a consequence of enhanced secretion of ANP from the atrium in response to elevated atrial pressure and/or increased vascular volume (16, 17). Based on the observation that an intravenous infusion of ANP increases the hematocrit value even in nephrectomized rats (18), one might suspect that the elevated ANP may induce edema in the lung in consonance with pulmonary hypertension encountered in congestive heart failure. However, our observation reported here suggests to us that the elevated ANP may counter water movement across blood vessels to the interstitial space of the lung, and consequently, normal pulmonary function can be maintained.

In this study, the binding profile of ANP was examined using rat lung homogenate, and the protective effect of ANP was demonstrated in the lung from guinea pigs. Circulating major ANPs in humans, and rats have been identified as α-hANP and α-rANP, respectively (19), while the ANP in guinea pigs has not been identified yet. Since the biological profiles of α-hANP are almost identical with α-rANP which differs from α-hANP by an isoleucine substitution (20), the results obtained here are thought to be adoptable for α-rANP. The significance and the mechanism of the phenomenon is currently under investigation.

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