Carmen Sjulammiet Rozendal
Dr. med.

Effects of endothelin and hypoxia on lung alveolar fluid clearance

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Doktorvater: Prof. Dr. H. Mairbäurl

Rationale: Endothelin-1 (ET-1) is a potent vasoconstrictor. Together with hypoxia it is thought to play an important role in the regulation of pulmonary vascular tone and alveolar fluid balance in health, but also in ARDS and hypoxic pulmonary edema. Hypoxia inhibits transepithelial Na^+- and fluid reabsorption in the lung. However it is not known whether and how ET-1 affects alveolar fluid reabsorption.
Objective: It was the goal of this study to test whether ET-1 inhibits alveolar Na^+-transport and fluid reabsorption in the lung and to clarify involved mechanisms contributing to edema formation.
Methods: In a first set of experiments the effects of hypoxia **(**8% O_2, 24 hrs) and ET-1 (10^{-7} M) on alveolar fluid reabsorption were investigated in the fluid instilled lung of anaesthetized rats. Amiloride (100µM) was added to inhibit Na^+ transport. In a second set of experiments it was tested whether changes in absorption could be prevented by ET-1 receptor antagonist. Effects of both the non-specific receptor antagonist Bosentan and selective ET-A or ET-B receptor antagonists on alveolar fluid reabsorption were tested. The direct effects of ET-1 on transepithelial Na^+ transport was tested by measuring Isc in primary rat alveolar epithelial cells monolayers. PCR was used to detect ET-1, ET-A and ET-B mRNA expression.
Results: Alveolar fluid clearance was about 18% per 30 min in normoxic control rats. It was inhibited by hypoxia by ~70% ($P<0.05$). Bosentan attenuated this effect ($P=0.032$). In normoxia ET-1 inhibited reabsorption to the same level as hypoxia (P=0,561). There was no difference in the rate of reabsorption in presence of amiloride between the different experimental states (P=0,359). In presence of the ET-A antagonist BQ123, ET-1 still inhibited alveolar fluid clearance ($P=<0.05$) In contrast, addition of the ET-B antagonist BQ788 prevented the decrease in reabsorption by ET-1. Ussing chamber measurements on primary AT-II monolayers show no effect of ET-1 on Isc, although expression of ET-1, ET-A and ET-B receptors was found by PCR.
Conclusions: We show that ET-1 and hypoxia inhibit alveolar fluid clearance of anesthetized rats by an amiloride-sensitive pathway, probably involving ENaC. Furthermore this effect is caused by activation of the ET-B receptor, most likely involving the endothelium, as there was no direct effect of ET-1 on transepithelial transport of primary AT-II cells. Since the non-selective ET-1 antagonist Bosentan prevents hypoxic inhibition of reabsorption, it appears likely that this effect is caused by a hypoxic induced release of ET-1. Since ET-1 levels are also elevated in patients with

ARDS and hypoxic pulmonary edema such as HAPE, it seems likely that the formation of pulmonary edema in these diseases is promoted by the ET-1 induced inhibition of alveolar fluid clearance. Thus, ET-1 might favor edema formation by two mechanisms; increased filtration and inhibition of alveolar reabsorption, both of which are mediated by ET-1 action on pulmonary vascular endothelium. Based on this, ET-1 receptor antagonists offer a promising pharmacological tool in the treatment of hypoxic pulmonary edema (such as HAPE) and ARDS.