ENaC-mediated effects assessed by proton MRI in a rat model of hypertonic saline-induced hydration of airways

F-X. Blé[1,2], S. Zurbruegg[1], S. Collingwood[3], H. Danahay[3], and N. Beckmann[1]

[1]Global Imaging Group, Novartis Institutes for BioMedical Research, Basel, Switzerland, [2]Faculty of Pharmacy, University Louis Pasteur-Strasbourg-1, Illkirch, France, [3]Respiratory Diseases Department, Novartis Institutes for BioMedical Research, Horsham, United Kingdom

Introduction:

Cystic fibrosis (CF) is a common hereditary disease in Caucasians caused by mutations in the CF transmembrane conductance regulator (CFTR) chloride channel. Mortality in CF results primarily from chronic airway infection because of impaired mucus clearance, which leads to progressive deterioration of lung function. Recent studies have shown that nebulized hypertonic saline (HS) may improve the lung function in CF patients (1,2). By disturbing the osmotic equilibrium through the epithelium, HS increases the volume of airway surface liquid (ASL), thereby improving the mucociliary clearance in the airways. As the beneficial action of HS is of short duration, blockage of epithelial sodium channels (ENaC) previous to HS administration with the consequent prevention of ASL absorption is pursued as a strategy to improve the results obtained with saline (3). Using MRI, we examined the effects on the fluid status of the whole lung when compounds that interact directly or indirectly with ENaC were administered prior to HS or of physiological saline. Measurements were performed in intact and spontaneously breathing rats.

Materials and Methods:

Animals: Male Brown Norway (BN) rats weighing 270-300 g were supplied by IFFA CREDO (L'Arbresle, France).

Substance administration: For intra-tracheal (i.t.) substance administration, rats were anaesthetized (2% isoflurane; Abbott, Cham, Switzerland) and 0.2 ml of fluid was sprayed into the trachea.

Experimental protocols: (i) The ENaC blocker, amiloride (0.03, 0.3 or 3 mg/kg), was administered 20 min before HS (1.5 % NaCl); (ii) amiloride (3 mg/kg) 20 min before physiological saline (0.9 % NaCl) at 0; (iii) vehicle (dextrose 5%) 20 min before HS; (iv) the broad spectrum serine protease inhibitor, aprotinin (1 µg/kg) was administered 2 h before HS; (v) alpha-1-antitrypsin (15 nmol/kg), was administered 2 h before HS. MRI was performed at baseline (at least 3 h before substance application) and at time points 30 min, 1 h and 4 h after HS. All substances and the saline were sprayed i.t.

MRI: Rats were anaesthetized with isoflurane (1.5-2.0%) in a mixture of O_2/N_2O (1:2), administered via a face mask. Measurements were carried out with a Bruker Biospec 47/40 system. A gradient-echo sequence was used throughout the study for detecting fluid signals (TR = 5.6 ms; TE = 2.7 ms; FOV = 6x6 cm^2; matrix = 256x128; slice = 1.5 mm; 45 image averages with an interval of 530 ms between each image acquisition). Neither cardiac nor respiratory triggering was applied, and rats respired spontaneously.

Results and Discussion:

Figure 1a shows axial sections through the chest of two BN rats, acquired at various time points with respect to i.t. administration of HS as a spray (0.2 ml). The animals had been pretreated with vehicle (0.2 ml) or amiloride (3 mg/kg, 0.2 ml). Signals detected by MRI in the lung of the animal pretreated with amiloride were more prominent and of longer duration than those observed in the vehicle-pretreated rat. Remaining fluid signals were present even 4 h after HS in the amiloride-pretreated rat. The effects of amiloride were dose-dependent (fig. 1b). The fluid volumes detected for pre-treatment with amiloride (3 mg/kg) followed by physiological saline were similar to those for vehicle (dextrose) pre-treatment followed by HS. These results indicate that blockage of ENaC not only augments the magnitude but also prolongs the effect of the osmotic shock elicited by HS.

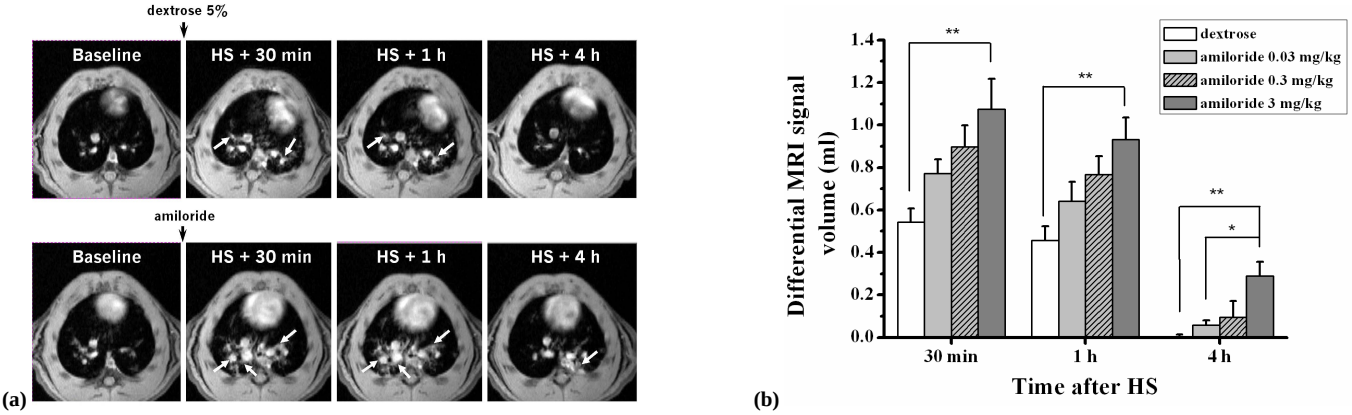

Fig. 1 – (a) *Axial MR images of two BN rats acquired before (baseline) and following i.t. instillation of HS (1.5% NaCl, 0.2 ml) as a spray. One animal (lower row) had been pretreated with amiloride (3 mg/kg i.t.) 20 min before HS administration, while the other rat (upper row) had received vehicle (dextrose 5%, 0.2 ml i.t.) at the same time point. Fluid signals (arrows) following HS were more prominent for the animal pre-treated with amiloride.* **(b)** *Volume (means±sem, n=6 animals per group) of fluid signals detected by MRI in the lungs of BN rats at different points after HS. Animals had been pretreated (-20 min) with either amiloride at the specified doses, or by its vehicle (dextrose 5%). Levels of significance * $0.01<p<0.05$ and ** $0.001<p<0.01$ correspond to Anova comparisons.*

Administration of aprotinin 2 h prior to HS led to similar fluid signal volumes as those observed following pre-treatment with amiloride (3 mg/kg) in the first hour after HS administration. However, the signals observed in the lungs of rats pre-treated with aprotinin were of longer duration, detectable even 6 h after HS, suggesting that aprotinin had a longer duration of action than amiloride. Application of alpha-1-antitrypsin 2 h before HS resulted in much smaller fluid responses following the osmotic shock, comparable to pre-treatment with the vehicle. These results are consistent with the view that ENaC is activated through cleavage by proteases such as prostasin, a membrane-anchored serine peptidase inhibited by e.g. protease nexin-1 (PN-1) or aprotinin (4).

In summary, proton MRI provides non-invasively information on the fluid dynamics in the lungs of spontaneously breathing rats. The technique is straightforward and gives quantitative and spatial information on the water content, thus having the potential to impact ENaC blocker research in the context of mucus clearance.

1. Donaldson SH et al. N Engl J Med 2006; 354:241–250.
2. Elkins MR et al. N Engl J Med 2006; 354:229–240.
3. Tarran R et al. J Gen Physiol 2006; 127:591-604.
4. Kleyman TR et al. Kidney Int 2006; 70:1391-1392.