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ANP changes microvascular endothelial barrier function *in vivo*

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Background

Atrial natriuretic peptide (ANP) regulates blood pressure and volume (for review see [1]). Its receptor, the guanylyl cyclase-A (GC-A) is expressed in vascular endothelium and mediates increases in intracellular cyclic GMP levels, but the functional relevance is controversial. Notably, mice with endothelial-restricted GC-A deletion (EC *GC-A* KO mice) exhibit significant hypervolemia and hypertension, suggesting that the ANP/GC-A system regulates transvascular fluid balance via increases in endothelial permeability [2].

The aim of our study was to elucidate the effect of ANP on endothelial cells, using intravital microscopy models.

Methods

An indirect method to monitor acute changes in intravascular fluid volume is the estimation of the hematocrit (Hct). We therefore examined the effect of synthetic ANP (500 ng/kg BW/min, 60 min, intravenous infusion) on the Hct of wildtype (GC-A+/+) and GC-A knockout (GC-A-/-) mice.

To elucidate whether ANP modulates the permeability of the microcirculation to macromolecules such as albumin (BSA), a dorsal skinfold chamber was implanted [3] or the cremaster muscle was prepared for intravital microscopy studies, as described [4]. FITC-labelled BSA was injected via a tail vein. In the dorsal skinfold chamber model synthetic ANP (final concentration 10^{-7} M) or vehicle were superfused locally. In the cremaster model, ANP was applied systemically at dose of 500 ng/kg BW/min. The changes in microvascular permeability were measured by the extravasation of FITC-BSA into the interstitium.

Results

In both, GC-A+/+ as well as in the GC-A-/- mice, the Hct upon ANP infusion changed. While in GC-A+/+ mice the Hct increased (before infusion: 43.3 ± 1.1%, after infusion: 49.4 ± 0.9%; n = 7), the Hct in GC-A-/- mice decreased (42.9 ± 0.4% and 35.0 ± 1.1%; n = 4).

When we locally superfused the subcutaneous tissue in the dorsal skinfold chamber with ANP, an 1.6 fold increase in interstitial FITC-fluorescence was observed in GC-A+/+ mice. In contrast, in GC-A-/- mice ANP led to a 0.9 fold reduction in interstitial fluorescence as compared to the initial fluorescence intensity.

In the cremaster model, the interstitial fluorescence in the GC-A+/+ mice increased to 1.12 fold of the initial fluorescence intensity, while the interstitial fluorescence in GC-A-/- mice did not change in response to ANP.

Discussion

Taken together, our vital microscopy studies in wild-type (GC-A+/+) mice show that ANP, via GC-A, increases the permeability of the microvasculature to macromolecules such as albumin. The changes in Hct indicate that this is accompanied by an acute contraction of intravascular vol-

ume. Intriguingly, in GC-A-/- mice the effects of ANP on the extravasation of FITC-BSA or on Hct are not only abolished, but even reversed. One possible explanation is that in mice lacking GC-A ANP binds to a higher extend to NPR-C, the natriuretic peptide clearance receptor. Remarkably, published studies showed that the hematocrit of NPR-C knockout mice was significantly higher as compared to wildtype littermates [5]. This increase in hematocrit could be due to an increase in endothelial permeability leading to a shift of macromolecules and water to the extravascular space. Our future studies will be directed to explore whether GC-A and NPR-C indeed mediate opposite (increasing vs decreasing) effects of ANP on microvascular endothelial permeability.

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