

MEETING ABSTRACT

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Natriuretic peptides, via GC-A/cGMP, moderate hypoxia-induced VEGF release from astrocytes and thereby pathological neovascularization in the retina

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Background

Our previous studies demonstrated that natriuretic peptides, i.e. BNP produced by activated satellite cells within ischemic skeletal muscle, stimulate the regeneration of neighboring endothelia via endothelial GC-A/cGMP signaling [1]. This paracrine communication might be critically involved in coordinating postischemic muscle regeneration and angiogenesis. In the retina, angiogenesis occurs as a part of normal development as well as in proliferative vascular diseases, such as diabetic retinopathy (DR) or retinopathy of prematurity (ROP) [2]. Retinal vascular development is controlled by interactions between ganglion cells, astrocytes and endothelial cells. In particular, reciprocal feedback between endothelial cells and astrocytes is crucial for proper vascular patterning. Hypoxia-induced vascular endothelial growth factor (VEGF) expression in astrocytes plays a key role in (patho)physiological retinal endothelial growth. Notably, immunohistochemistry on postnatal (P7) retinal whole-mounts revealed the expression of immunoreactive BNP in glial fibrillary acidic protein (GFAP)-expressing astrocytes. Therefore we postulated that BNP participates in this astrocyte-endothelial communication during physiological vascularization and/or pathological revascularization of the retina.

Materials and methods

We compared physiological postnatal retinal vascularization in mice with conditional, either endothelial (EC

GC-A KO [1]) or astrocyte-restricted deletion of GC-A (astrocyte GC-A KO) and respective control littermates (GC-A^{fl/fl}). The latter mouse model was generated by crossing GC-A^{fl/fl} mice with GFAP-Cre^{TG} mice. In addition, we studied pathological neoangiogenesis in oxygen-induced retinopathy (OIR), a disease model for DR and ROP [3]. These *in vivo* studies were complemented with electrophysiological and molecular studies (intracellular cGMP, VEGF secretion) in primary cultured murine brain astrocytes.

Results

Firstly we studied whether crossing GC-A^{fl/fl} mice with GFAP-Cre^{TG} mice resulted in efficient and selective inactivation of GC-A in astrocytes. Indeed, arterial blood pressure and GC-A expression levels in peripheral tissues of GC-A^{fl/fl};GFAP-Cre^{+/-} mice were unaltered. ANP and BNP enhanced cGMP levels in cultured control astrocytes and these effects were significantly attenuated in astrocytes prepared from GC-A^{fl/fl};GFAP-Cre^{+/-} mice. Since these cultures contained ~10% contaminating cells such as microglia, we also performed single-astrocyte patch-clamp recordings. ANP and BNP provoked membrane depolarizations in control astrocytes by ΔV_m 1.58 ± 0.46 mV (at 10 nM NPs) and 2.08 ± 0.51 mV (100 nM NPs). In GC-A^{fl/fl};GFAP-Cre^{+/-} astrocytes, NP-induced depolarizations were almost abolished: ΔV_m 0.59 ± 0.35 and 0.25 ± 0.4 mV at 10 and 100 nM of the NPs.

Retinal whole-mount stainings with FITC-isolectin demonstrated that physiological vasculogenesis 5 and 7 days after birth was unaltered both in EC GC-A KO and astrocyte GC-A KO mice (compared with respective control littermates). In the OIR model, pathological

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vascular regression (at P12) was also unaltered in both genotypes. However, unexpectedly, hypoxic neovessel formation (at P17) was unchanged in EC GC-A KO but enhanced in astrocyte GC-A KO mice. GFAP stainings revealed unaltered astrocyte density within and around the neovascular zones of astrocyte GC-A KO retinas. Hence, since astrocyte GC-A disruption apparently does not change the vitality/proliferation of these cells but still enhances the pathological proliferation of adjacent endothelia, we hypothesized that NP/GC-A signaling modulates astrocyte VEGF release.

To follow this hypothesis lastly we studied the effects of ANP and BNP on VEGF secretion by cultured astrocytes. Hypoxia (1% O₂ during 6 or 24h) increased VEGF secretion by 21 fold. ANP and BNP did not significantly modulate basal VEGF secretion but markedly and concentration-dependently attenuated the stimulation by hypoxia. These inhibitory effects were almost fully abolished in GC-A-deficient astrocytes.

Conclusions

Our observations indicate that BNP and/or ANP, via GC-A/cGMP signaling, participate in a local, autocrine or paracrine feedback which inhibits hypoxia-induced VEGF release from retinal astrocytes and thereby moderates pathological neoangiogenesis. The modulation of VEGF-mediated communication between astrocytes and endothelial cells by natriuretic peptides may have a key role during processes of pathological neoangiogenesis in the brain and retina.

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