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Molecular mechanisms of bradykinin–induced angioedema

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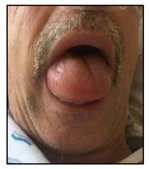
Introduction

Angioedema (AE) is a known adverse effect of renin-angiotensin-aldosterone system blockers. It is characterized by non-inflammatory swelling of the head and neck region, and increased plasma and tissue levels of bradykinin (BK) (Fig. 1). [1-2]



In-depth, elucidation of bradykinin-dependent mechanisms of microvascular destabilization are still needed to comprehend the molecular pathways causing angioedema and for rational design of anti-edematous therapy. Therefore, BK-induced changes in endothelial permeability and cellular architecture were investigated in a model of human dermis microvasculature.

Figure 1: Tongue swelling of BK-induced AE patient.

Materials and Methods

Human dermis endothelial cells (HDMECs) were obtained from a female Caucasian adult donor (PromoCell). All experiments were done between passages 4 and 6. Cells were plated on fibronectin coated plastic ware, Transwell® supports (0,4 µm membrane), or gold-film electrodes arrays and maintained in culture for 5 days. 24h before BK stimulation cells were deprived of growth factors. Macromolecular flux assays of FITC-conjugated dextran 70 kDa molecules were done to examine permeability. Impedance measurements were performed with xCELLigence® Real-Time Cell Analyzer (Acea). Modulation of cell junctional proteins expression was investigated by western blot (WB) and qRT-PCR (Roche). Reagents were obtained from Sigma-Aldrich.

Discussion

It was found that BK acute stimulation increases HDMECs permeability to macromolecules in a dose-dependent effect. It decreases the normalized cell index in a rapid and later effect, indicative of a transient barrier disruption. The observation of BK-induced changes in VE-cadherin phosphorylated pools further supports these findings. Increased phosphorylation of VE-cadherin Tyr658 is in agreement with published reports[3], being relevant for the selectively permeability regulation in microvascular beads. The observed dephosphorylation of Tyr731, shown to be relevant for leukocytes diapedesis[3], is a new finding that might be unique for BK in comparison to other mediators such as histamine. Furthermore, the down-regulation of tight junction protein Claudin-5 expression at protein and mRNA levels strongly suggests that BK stimulation leads to the opening of junctions. These effects were prevented by HOE 140, confirming BK mechanism through B2R. Finally, the up-regulation of vascular endothelial growth factor-C (vegfc) and angiopoietin-2 (ang-2) mRNA levels, barrier desestabilizing agents[4], might contribute to the loss of endothelial homeostasis in an autocrine manner that has to be further investigated.

Conclusion

BK-induced B2R signaling promotes microvascular homeostasis destabilization by transiently disrupting the endothelial barrier function through the opening of cellular junctions, and up-regulation of vegfc and ang-2 expression.

Literature

[1]Bas M, *et al.* (2007). Nonallergic angioedema: role of bradykinin. Allergy 62, 842-856.
[2]Leeb-Lundberg F, *et al.* (2005). Classification of the Kinin receptor Family: from Molecular Mechanisms to Pathophysiological Consequences. Pharm. Reviews 57, 27-77.
[3]Wessel F, *et al.* (2014). Leukocyte extravasation and vascular permeability are each controlled *in vivo* by different tyrosine residues of VE-cadherin. Nat Immunol 15, 223-230.
[4]Loffredo S et al. (2016) Hereditary angioedema patients show increased levels of VEGFs and Ang-2 in plasma. Allergy 71,989-996.

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Results

BK increases the permeability to macromolecules through B2R

Macromolecular permeability assay showed a concentration-dependent effect of BK in HDMECs permeability, with a maximal 2x increase at the highest concentration (Fig. 2A), that was effectively suppressed by B2R antagonist HOE140 (Fig. 2B).

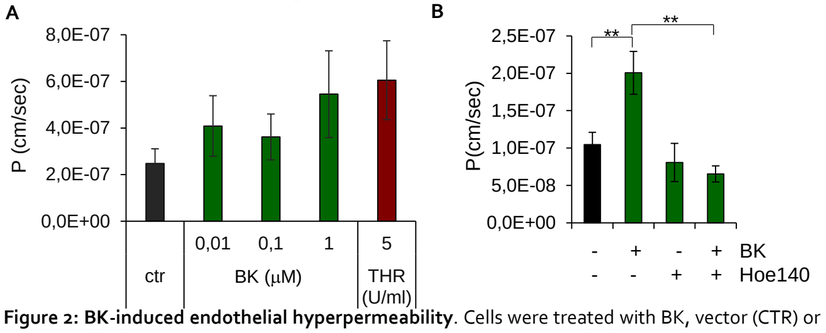


Figure 2: BK-induced endothelial hyperpermeability. Cells were treated with BK, vector (CTR) or thrombin (THR) as positive control for 4 h (A); or were treated with B2R inhibitor Hoe140 20 µM for 30 min and subsequently with BK 10 µM for 4 h. Permeability of FITC-dextran (70 kDa) across the cell monolayer was measured with a Transwell® system. Samples were taken from the lower chamber after 90 min (A) or 240 min (B) and fluorescence was measured. The results are depicted as absolute permeability. At least five independent experiments were performed (A) and one experiment in quadruplicate (B). Data are expressed as mean ± SD. ***P*≤ 0,01

BK-induced hyperpermeability was detected by impedance-based analysis

Two significant responses upon BK addition were observed.

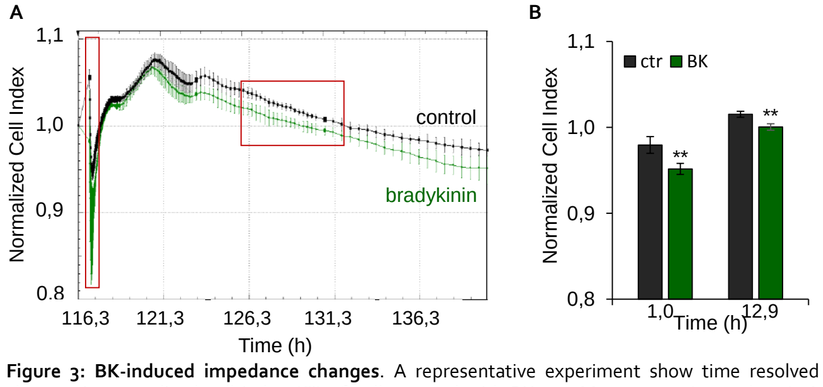
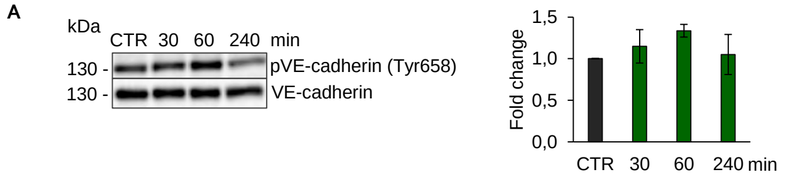


Figure 3: BK-induced impedance changes. A representative experiment show time resolved changes in normalized cell index (CI) of cells treated with BK 10 µM or vector (control, ctr). A decrease is observed within the first hour of BK addition, and after 9 h of stimulation lasting 6 h (red inserts) (A). The bar graph shows the normalized CI of two time points (B). Results correspond to mean ± SD of one experiment done in triplicate. ***P*≤ 0,01

BK changes the intracellular phosphorylated VE-cadherin pool



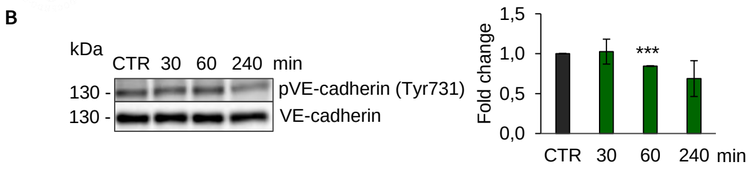


Figure 4: BK changes VE-cadherin phosphorylation. Representative image of WB analysis of total protein extracts prepared from cells treated with BK 1 µM, for the given time points. Status of pTyr658 (A) and pTyr731 (B) was analyzed. Bar graphs correspond to image density analysis of 3 independent experiments. Data are expressed as fold change mean of stimulated versus control samples ± SD. ****P*≤ 0,001

BK down-regulates the expression of Claudin-5 (Cldn-5) through B2R

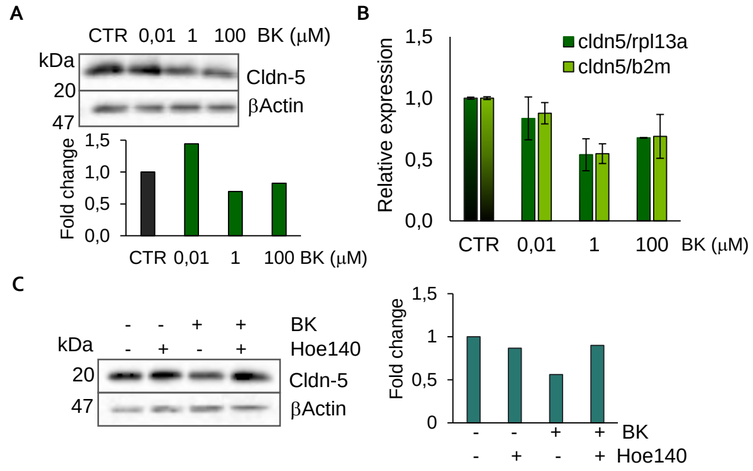


Figure 5: BK down regulates Cldn-5 expression. Cells were treated with BK or vector (CTR) for 4 h and Cldn-5 expression was determined by WB analysis of membrane enriched proteins extracts (A), and at mRNA levels by qRT-PCR. As housekeeping genes rpl13a and b2m were used. The results are depicted as relative expression of stimulated versus control. Data of one experiment are expressed as mean ±SD of 3 determinations (B). (C) WB analysis of membrane enriched proteins extracts of cells treated with B2R inhibitor Hoe140 1 µM for 30 min and subsequently with BK 1 µM for 4 h.

BK increases the expression of vegfc and ang2

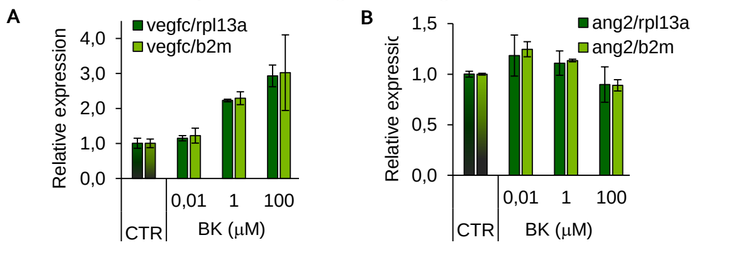


Figure 6: BK-induced expression of permeability target genes. Cells were treated with BK or vector (CTR) for 4 h. Expression levels of vegfc (A) and ang2 (B) were determined by qRT-PCR. Most stable housekeeping genes rpl13a and b2m were used for normalization. Results are depicted as relative expression of stimulated versus control. Data of one experiment are expressed as mean ± SD of 3 determinations.