Editorial

Kallikrein–Kinin System in Neovascularization

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The formation of new blood vessels is viewed completely differently by cardiologists than by oncologists and ophthalmologists. Whereas the former try to stimulate this process, the latter put all efforts into blocking it. In any case, factors involved in neovascularization are of highest therapeutic relevance. The article by Stone et al in this issue of *ATVB*[1] corroborates that the kallikrein–kinin system (KKS) is one very important but yet underestimated player in this process. This peptide hormone system acts via kinins which are generated from precursors, called kininogens, by enzymes called kallikreins, two of which exist, plasma (PK) and tissue kallikrein (TK). The most important kinin is the nonapeptide bradykinin, which activates the G protein–coupled receptor B2. When kininase I (carboxypeptidase M or N) truncates the peptide by 1 amino acid at the C terminus, the resulting des-Arg^{9}bradykinin binds the B1 receptor. Interestingly, this receptor is 1 of the rare G protein–coupled receptors, which is inducible by inflammatory mediators, in contrast to the B2 receptor, which is constitutively expressed in multiple cell types.[2] Kininase II degrades kinins further to inactive fragments and is identical to angiotensin-converting enzyme (ACE). Consequently ACE inhibitors, one of the most popular classes of cardiovascular drugs, not only inhibit angiotensin generation but also stabilize kinins with important consequences in particular in their effects on vessel formation.

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The first evidence for a role of the KKS in neovascularization was published already more than 15 years ago. Hu and Fan[3] showed that bradykinin increases angiogenesis in a sponge implantation model through the B1 receptor. This explained earlier puzzling findings showing that angiotensin II as well as ACE inhibitors increase vessel density in several animal models, which seemed paradoxical at first sight.[4,5] Obviously in this case, ACE inhibitors act via the stabilization of kinins and not by the inhibition of angiotensin II generation.[6] Numerous studies have confirmed these findings. Brown-Norway Katholiek rats, which lack secreted kininogen, showed a reduced capacity for new vessel formation in a sponge implantation and in tumor models.[7] When kininogen binding to endothelial cells, the prerequisite for efficient kinin generation by plasma kallikrein, was inhibited either by fragments of the protein or by a specific antibody, new vessel formation was impaired in experimental models[8] and in tumors.[9] Reduced neovascularization was also observed in knockout mice for the B1 receptor using a hindlimb ischemia model[10] and in B2-deficient animals when endothelial cell sprouting was analyzed.[11] The study by Stone et al[1] in this issue adds TK knockout mice to the list of animals with defective neovascularization again using the hindlimb ischemia model. Accordingly, the local overproduction of TK was shown to increase angiogenesis in various situations.[1,12–14]

The mechanisms of action of kinins in the formation of new vessels seem to include both kinin receptors on endothelial and other cells. The B1 receptor induces the expression of vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR2),[7,15] whereas the B2 receptor transactivates VEGFR2 by phosphorylation of its cytoplasmic domain.[16] However, there is also recent evidence that the B2 receptor increases the expression of VEGF and VEGFR2 via the PI3 kinase/Akt/GSK3beta signaling pathway.[17] All 3 receptors, B1, B2, and VEGFR2, can activate endothelial NO synthase (eNOS) by increasing intracellular concentrations of Ca^{2+} and changing the phosphorylation state of the enzyme mediated by PI3 kinase, Akt, and calcineurin.[18] Moreover, the B2 receptor increases the expression of eNOS.[6] On the other hand, the B1 receptor–induced NO synthesis was shown to increase the expression of fibroblast growth factor 2 (FGF2), which is itself proangiogenic via its receptor FGFR1.[19] Interactions between the 2 kinin receptors seem to be essential for some of the actions of kinins, because B1 agonists are ineffective in B2-deficient mice.[11] The detailed nature of these interactions is unclear, but an increasing amount of publications show such interdependencies of B1 and B2 receptor activities.[2]

The actions of the KKS (summarized in the Figure) finally enhance the concentration and signaling of the well-known mediators of endothelial cell proliferation and migration, NO, VEGF, and FGF2, and thereby stimulate angiogenesis. Furthermore, it was recently shown that bradykinin via the B2 receptor enhances the homing of circulating endothelial progenitor cells, which is another important source for the formation of new vessels.[20]

The study of Stone et al in this issue[1] confirms and extends the evidence about the KKS in the development and adaptation of the vascular system using various animal models. In TK-deficient mice the authors confirm the importance of kinins for neovascularization after hindlimb ischemia. In normal rat mesentery, they provide evidence for an increased vasculogenesis after TK overexpression dependent on B2 receptor activation. In the zebrafish embryo, TK overexpression also caused a disturbance of vascular development. Interestingly, no vascular abnormalities have been observed in all known knockout models for the KKS, such as the B1, B1/B2 double, kininogen I, and tissue kallikrein knockout

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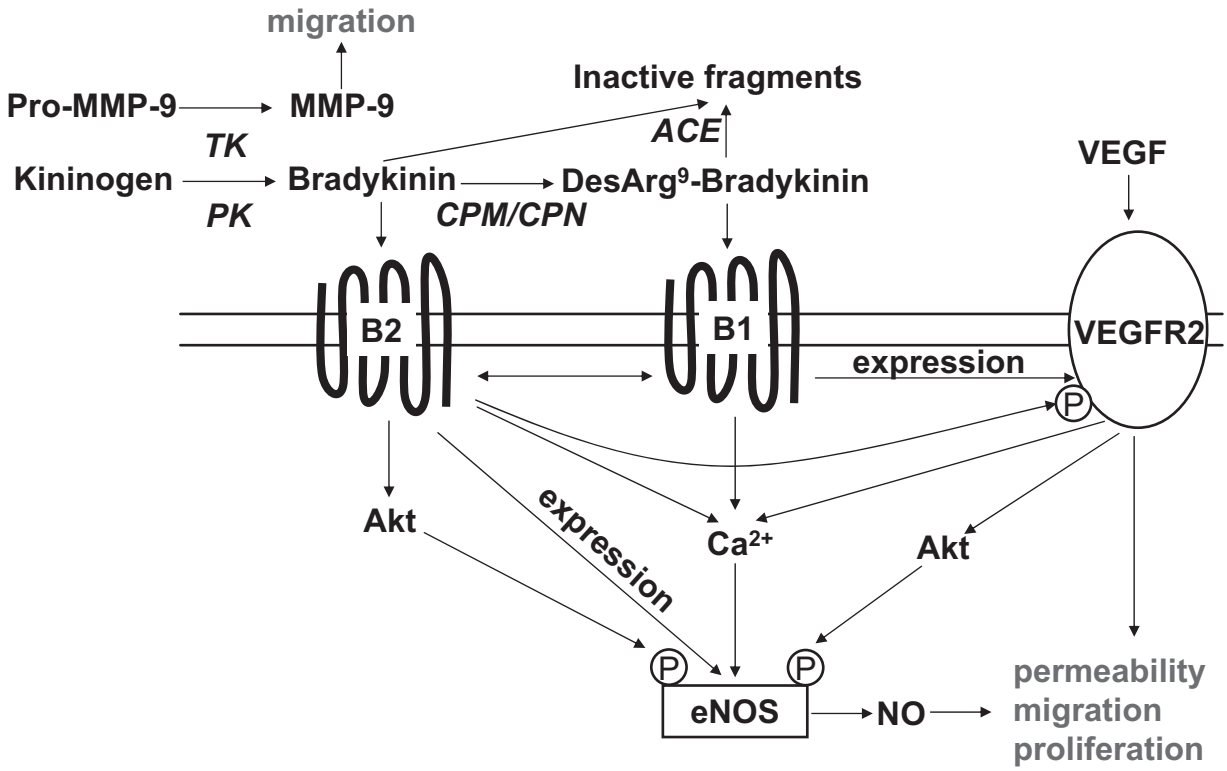

Figure. Actions of the kallikrein-kinin system on endothelial cells. Plasma (PK) and tissue (TK) kallikrein form bradykinin interacting with the B2 receptor. Further metabolism by carboxypeptidases M (CPM) or N (CPN) generates the B1 agonist des-Arg^9 bradykinin. Angiotensin-converting enzyme (ACE) degrades these active kinin peptides. Signaling of the 2 kinin receptors directly and by enhancing the VEGF/VEGFR2 pathway activates eNOS, and the resulting increase in NO is a strong proangiogenic stimulus inducing permeability, migration, and proliferation of endothelial cells. Furthermore, TK activates pro-MMP-9 which in turn also facilitates migration (more details in text).

mice. The only exception is the observation of a mild capillary rarefaction in the heart of B2 knockout mice,[21] which was not described in another study.[22] This indicates that the KKS is not essential for the embryonic development of the vascular system but centrally involved in its adaptation to ischemic stress.

Stone et al[1] also provide evidence for a second activity of TK besides its kinin generating capacity, the activation of matrix metalloproteases (MMP), which was discovered already 20 years ago by in vitro studies.[23] They show that TK overexpression increases MMP-9 activity in tissues and that mice lacking MMP-9 do not respond to TK treatment after hindlimb ischemia. Because MMP-9 is a prominent mediator of angiogenesis by facilitating migration of endothelial cells, its activation contributes to the actions of TK in the formation of new vessels.

Neovascularization is particularly important in ischemic diseases such as limb ischemia and myocardial infarction. For both cases, evidence is available in animal models for the beneficial effects of ACE inhibitors,[4,5,14] probably acting via the stabilization of kinins, which in turn stimulate the proliferation of resident endothelial cells and the homing of circulating precursors. Thus, it is conceivable that ACE inhibitors should be very suitable drugs in myocardial infarction and limb ischemia in patients. Indeed, large clinical trials have proven the extraordinary efficiency of these drugs after myocardial infarction.

Because tumors can only grow when they organize their blood supply by inducing angiogenesis, inhibition of the KKS may be of high therapeutic relevance in oncology. The suitability of this approach has already been shown in experimental models,[7,9,24] but clinical evidence is still lacking.

Diabetic retinopathy is a disease of the microvessels in the eye characterized by increased permeability and proliferation of endothelial cells. Recent findings show a pivotal role of the KKS in this disease initiated by the activation of PK attributable to a decrease in intraocular pH, and treatments with B2 antagonists and novel kallikrein inhibitors were shown to be effective at least in mice.[25] Because angiogenesis is also a major pathogenetic factor in age-related macular degeneration, as evidenced by the clinical effectiveness of anti-VEGF antibodies, also this disease of growing significance may be a target of KKS-inhibiting drugs. Moreover, the formation of new vessels is considered pathophysiologically relevant in other common diseases such as rheumatoid arthritis and asthma, further widening the therapeutic applicability for such drugs.

In conclusion, the KKS is an important system in the generation of novel vessels in ischemia and in other pathological situations but possibly not in normal development. Whereas cardiovascular physicians will aim to support angiogenesis in ischemic diseases by increasing local kinin generation or signaling, eg, by ACE inhibitors, oncologists intend to promote tumor ischemia and ophtalmologists want to stop the uncontrolled vascular growth in certain retinopathies and both may in the future consider to inhibit the local

KKS, eg, by receptor antagonists, such as icatibant for B2, or kallikrein inhibitors.

Disclosures

None.

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