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Physiologic Activities of the Contact Activation System

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The plasma contact activation system has been recognized as a group of proteins [factor XII (FXII), prekallikrein (PK), high molecular weight kininogen(HK)] that influence surface-activated blood coagulation tests [the activated partial thromboplastin time, activated clotting time (ACT)] but are not associated with hemostasis, a process that leads to the cessation of bleeding. These proteins influence these assays because FXII and to a lesser extent PK have the ability to autoactivate into enzymes when incubated with some biologic and artificial surfaces. In vivo, contact activation occurs when there is an interaction of blood with artificial surfaces such as thrombus occurring on catheter tips, blood and platelet activation in cardiopulmonary bypass, and after excess kallikrein, activated FXII (FXIIa), or chondroitin sulfate adulterates intravenous preparations given to man [1-4]. In addition to the above man-made situations, several medical disorders such as sepsis from any etiology, hereditary angioedema due to C1 inhibitor deficiency or mutated FXII (Type III hereditary angioedema), adult respiratory distress syndrome, and allergic reactions, contact activation with plasma kallikrein formation and bradykinin liberation also is well-recognized to arise [5-9].

New interest in the contact activation system, also called the plasma kallikrein/kinin system, arose when it was discovered that FXII deficient mice (*F12*$^{-/-}$) are protected from surface activation-induced pulmonary embolism and after ferric chloride models of arterial thrombosis [10]. Furthermore, in an inferior vena cava venous stasis model, *F12*$^{-/-}$ mice have reduced thrombosis [11]. These findings contribute to a novel notion that the contact system is involved in thrombosis, but not hemostasis. Adding to the interest in the field, several laboratories have recognized a variety of previously unappreciated biologic substances such as exposed arterial vessel collagen, DNA and RNA, certain aggregated proteins, and long chain polyphosphates from any source that serve as platforms for FXII auto activation [12-15]. These substances support FXII auto activation to initiate thrombin formation and fibrin clots. To date, however, all the FXII activating substances and the disease states described above arise in pathologic states. Although these events occur *in vivo*, they become manifest in pathophysiology states. Are there physiologic conditions where the proteins of the contact activation or plasma kallikrein/kinin system participate in thrombosis risk regulation without some other insult occurring?

Several years ago our laboratory asked the question what are the constitutive physiologic activities of the plasma kallikrein/kinin system (KKS) and how does it contribute to vascular homeostasis? To address this question, we asked what other proteolytic systems interact with the KKS. After autoactivation of FXII, there is activation of PK with reciprocal activation of FXII in the presence of HK and dual amplification of plasma kallikrein and FXIIa formation (Figure 1). The formed enzymes concurrently cleave high molecular weight kininogen (HK) and liberate bradykinin (BK) (Figure 1). Formed BK is metabolized by at least two pathways: by its receptor binding and uptake and by proteolysis by the angiotensin converting enzyme (ACE). To date, three animals models of the KKS are associated with reduced BK delivery to tissues: the factor XII KO (*F12*$^{-/-}$), the kininogen I KO (*Kgn1*$^{-/-}$), and the BK B2 receptor KO (*Bdkrb2*$^{-/-}$) mice [10,16-18]. In each case, complete gene deletion is associated with thrombosis protection. With exception of the *Bdkrb2*$^{-/-}$ mice, the mechanism(s) for thrombosis protection has not as yet been fully elucidated in these animals' models.

Bdkrb2$^{-/-}$ mice are protected from thrombosis. This finding was not intuitive. For over 25 years, BK has been known to stimulate endothelial cell NO, prostacyclin (PGI_2) formation and tissue plasminogen activator (tPA) liberation, events that in most minds lead to thrombosis protection. Before embarking on studies on the *Bdkrb2*$^{-/-}$ mice, we postulated that these animals would be prothrombotic. To our surprise, the animals were protected from thrombosis [17,18]. The animals have elevated prekallikrein, BK and BK's ACE metabolite, bradykinin1-5, due to less uptake and metabolism by the absent receptor, the BK B2 receptor (B2R) (Figure 2). Bradykinin1-5 itself is a weak direct thrombin inhibitor directly binding to the active site of thrombin in a retrograde manner [19]. To explain why bradykinin1-5 was elevated we found increased ACE activity also yielding elevated angiotensin II (AngII). Usually, AngII stimulates the angiotensin receptor 1 (AT1R) to elevated blood pressure and increase thrombosis risk. However, if there are higher copy numbers of the angiotensin receptor 2 (AT2R) to AT1R, the AngII binds to more AT2R to increase NO and PGI_2. When examined, we found increased AT2R, NO, and PGI_2 in *Bdkrb2*$^{-/-}$ mice [17]. Treatment of *Bdkrb2*$^{-/-}$ with PD123314, an AT2R antagonist, L-NAME, an eNOS antagonist, or nimesulide, a COX2 inhibitor, corrected the

thrombosis protection in these animals. Since the bleeding times are prolonged in *Bdkrb2*$^{-/-}$ mice we postulated both an effect on vessel wall and platelets as the basis for thrombosis protection in these mice.

However, there was a confounding experiment that needed explanation. When *Bdkrb2*$^{-/-}$ mice were treated with losartan, an AT1R antagonist, AngII levels fell, but the whole animal is still protected from thrombosis. We found that AT1R receptor antagonists like losartan lowers ACE. Yet, how is thrombosis protection maintained? Regardless of the ACE level, there was a constant level of elevated angiotensin-[1-7] [Ang-[1-7] in *Bdkrb2*$^{-/-}$ mice (Figure 2). Ang-[1-7] is the prolylcarboxypeptidase (PRCP) or angiotensin converting enzyme 2 (ACE2) breakdown product of AngII [20]. Further, the Ang-[1-7] receptor, Mas, was increased as well. Stimulation of the Mas receptor which has been associated with thrombosis risk modification also increases NO and PGI_2 [21,22]. The Mas antagonist A-779 corrects the prolonged thrombosis time back to normal. Hence, in *Bdkrb2*$^{-/-}$ mice, thrombosis protection is produced by a double increase in AngII and Ang-[1-7] working on increased receptors AT2R and Mas [17,18]. Thus, physiologically, plasma BK levels regulate arterial thrombosis risk indirectly through its influence on peptides and receptors of the RAS.

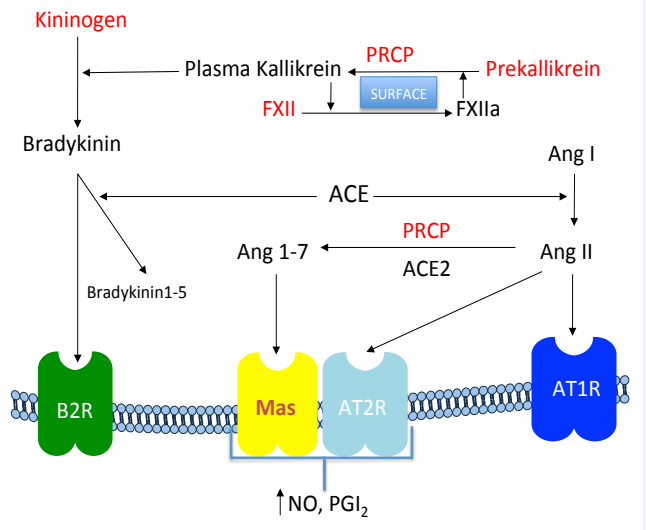

Figure 1 Physiologic interactions of the plasma kallikrein/kinin system with the renin-angiotensin system. In contact activation, factor XII (FXII) autoactivates to factor XIIa (FXIIa) to activate prekallikrein to plasma kallikrein on many different artificial and biologic surfaces. A serine protease prolylcarboxypeptidase (PRCP) also activates prekallikrein to plasma kallikrein independent of FXIIa. Formed plasma kallikrein as well as FXIIa cleaves high molecular weight kininogen (kininogen) to liberate bradykinin (BK). In vivo BK is metabolized after binding to its receptor, the bradykinin B2 receptor (B2R) or it is degraded by angiotensin converting enzyme (ACE) to bradykinin1-5. ACE also converts angiotensin I (AngI) to angiotensin II (AngII). AngII normally binds to the angiotensin receptor 1 (AT1R). However, it also binds the angiotensin receptor 2 (AT2R) and, if there are more copies than the AT1R, becomes the dominant systemic pehnotype. AngII is metabolized to angiotensin-[1-7]. [Ang-[1-7]. by PRCP or angiotensin converting enzyme 2 (ACE2). Simulation of both the AT2R with AngII or Mas with Ang-[1-7]. increases NO and prostacyclin (PGI2). Both NO and PGI2 contribute to thrombosis protection through several mechanisms.

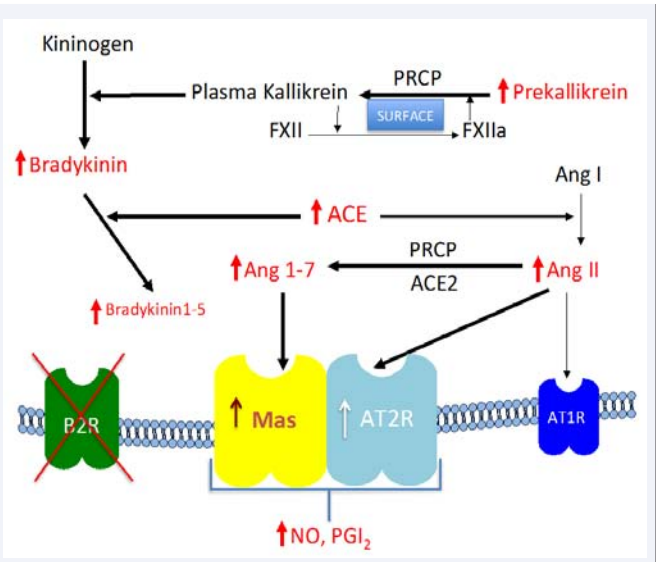

Figure 2 Changes in the plasma KKS and RAS in bradykinin B2 receptor deleted (*Bdkrb2*$^{-/-}$) mice that influence thrombosis risk. In *Bdkrb2*$^{-/-}$ mice there is increased plasma prekallikrein. Further there is elevation of BK, bradykinin 1-5, ACE activity, AngII, and Ang-[1-7]. The elevated AngII and Ang-[1-7]. stimulate elevated receptors AT2R and Mas to produced increased NO and PGI2. These latter entities inhibit platelet activation and influence vasculature to create an animal that is protected from arterial thrombosis [17,18]. All abbreviations in this figure are identical to those in Figure 1.

How do the elevated NO and PGI_2 precisely influence thrombosis risk? Since *Bdkrb2*$^{-/-}$ mice have long bleeding times, we examined their platelet function. We found that PGI_2, not NO, is mostly responsible for the platelet defect [18]. *Bdkrb2*$^{-/-}$ mice have 3-fold PGI_2 elevation, increased cGMP and cAMP, but normal thrombin- and ADP-induced $\propto_{2b}\beta_3$ integrin complex formation and P-selectin secretion and fibrinogen binding, respectively. *Bdkrb2*$^{-/-}$ mice also have an integrin-mediated spreading defect on fibrinogen and collagen and a GPVI activation defect to convulxin, CRP, and collagen [18]. Furthermore, it is host dependent, not intrinsic to their platelets because after bone marrow transplantation of normal bone marrow into *Bdkrb2*$^{-/-}$ mice, the formed platelets acquire the same platelet function defect as host [18]. Thus in *Bdkrb2*$^{-/-}$ mice, BK levels influence platelet function and arterial thrombosis risk through modulation of the RAS. We believe these pathways are a physiologic means to influence thrombosis independent of hemostasis. To date, we have not examined the influence of NO and PGI_2 on vascular function in *Bdkrb2*$^{-/-}$ mice, but postulate that there may be additional mechanisms for arterial thrombosis protection as well.

What about the other mice of the plasma KKS that have been shown to have reduced BK delivery? *F12*$^{-/-}$ mice have been shown to have increased resistance to contact activation-induced thrombosis models. Using collagen-epinephrine and polyphosphates, *F12*$^{-/-}$ mice have increased survival on contact activation-induced pulmonary embolism model [10,23]. Furthermore, these animals are protected from venous thrombosis associated with ketosis of citrullinated DNA. Presumably, this result is due to reduced contact activation, but that point as of yet has not been shown precisely [11]. No other efforts have been made to ascertain what influence low BK levels

in these animals have on thrombosis risk [24]. Last, *Kgn1*$^{-/-}$ mice are deficient in BK because kininogen is the parent protein for BK [16]. Although shown to be protected from thrombosis on the Rose Bengal assay for carotid artery thrombosis, there are no mechanistic data on these animals to date.

Looking at the other side of the coin, can alterations in the interaction between the RAS and KKS lead to a prothrombotic state? We have examined the thrombosis phenotype of the enzyme propylcarboxypeptidase (PRCP) and have found that gene trap mice with ~25% PRCP antigen in tissues are prothrombotic on two reactive oxygen species inducing models for carotid artery thrombosis, the Rose Bengal and ferric chloride assays [25]. PRCP is a membrane serine protease that degrades biologically active peptides with C-terminus Pro-X bonds and activates prekallikrein [20,26,27]. Its physiologic activities are $\propto MSH_{1-13}$ metabolism regulating central anorexia and AngII metabolism leading to the production of Ang-[1-7]. In kidney, PRCP is as an important producer of Ang-[1-7] as ACE2 [20]. PRCP$^{gt/gt}$ mice are lean, hypertensive, and prothrombotic. In PRCP deficiency, there is increased plasma PK and reduced FXII [25]. Further, there is reduced Ang-[1-7]. production in the kidney [20] (Figure 3). How this information translates in vivo is not fully known. PRCP$^{gt/gt}$ mice have increased vessel ROS with reduced eNOS, uncoupled eNOS, reduced protein C activation due to reduced thrombomodulin expression, increased vascular tissue factor and plasminogen activator inhibitor [25]. These results follow from reduced Ang-[1-7]. and ROS. Further, these animals have reduced cell growth, angiogenesis, wound injury repair, ischemia/reperfusion repair, and increased arterial neointima/media growth after endothelial cell denudation [28]. PRCP and its influence of the KKS and RAS have profound effects on vascular well-being and should be considered a vasculoprotective gene.

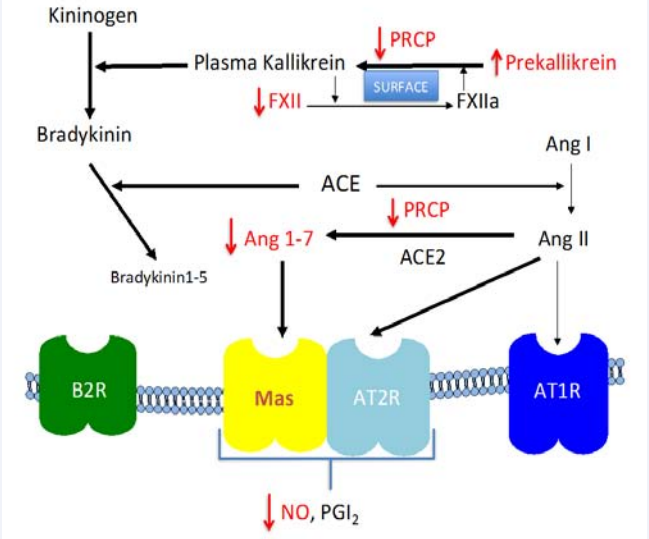

Figure 3 Changes in the plasma KKS and RAS in prolylcarboxypeptidase (PRCP) gene trap mice (*PRCP*$^{gt/gt}$) mice that influence thrombosis risk. In *PRCP*$^{gt/gt}$, there is reduced prekallikrein activation and reduced Ang-[1-7]. formation. These animals have hypertension and arterial thrombosis. Their vasculature shows inflammation with increased vascular reactive oxygen species associated with reduced and uncoupled eNOS, reduced and dysfunctional thrombomodulin, increased tissue factor, and increased plasminogen activator inhibitor [25,28].

Importantly, it has no influence on contact activation.

In sum, we propose that the proteins of the plasma kallikrein/kinin system have profound effects on thrombosis independent of contact activation. These proteins, PRCP, bradykinin B2 receptor, and BK through peptides and receptors of the renin angiotensin system regulate an individual's risk for thrombosis. The regulation of thrombosis risk is not directly through reduced thrombin formation from contact activation, but rather through its interaction with the renin-angiotensin system and, perhaps, other modulating pathways. As we become more knowledgeable in the mechanism(s) by which each of the proteins of the plasma KKS influence thrombosis risk in vivo, the contributions of RAS and other pathways should become more evident.

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