Vascular receptors as new substrates for matrix metalloproteinases in hypertension and other inflammatory states

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The matrix metalloproteinase (MMP) family consists of a large group of zinc- and calcium-requiring enzymes that include interstitial collagensases, gelatinases, elastases, stromelysins, and secreted as well as membrane-type MMPs (2, 13). These hydrolytic enzymes act synergistically to degrade a broad array of extracellular matrix (ECM) components and, as such, are involved in a variety of physiological and pathological processes including embryonic development, tissue remodeling, angiogenesis, invasive cell behavior, wound healing, fibrosis, and inflammation. While ECM components have long been viewed as the major target for hydrolytic digestion by the MMPs, a growing body of evidence indicates that members of this family of proteinases can target a spectrum of non-ECM substrates for processing such as cytokines, bioactive peptides, and growth factors (2). Once secreted, intracellular proteins may also be severed by MMPs, allowing them to perform different functions in the extracellular space (2). MMPs have also been reported to translocate into cells, where they can exert proteolytic and novel nonconventional actions such as the participation in transcription factor activities (2).

The study described by Rodrigues and coworkers (16) in this issue of American Journal of Physiology-Heart and Circulatory Physiology adds to the growing list of surface receptors on vascular cells that are substrates for MMP-mediated cleavage such as ICAM-1, vascular endothelial growth factor receptor 2 (VEGFR2), and insulin receptor-α (2, 6, 22) by demonstrating that MMP-mediated severing of extracellular domains of the β2-adrenergic receptor (β2-AR) occurs in conduit arteries (aorta) and coronary microvessels of spontaneously hypertensive rats (SHRs). As a consequence, arterial vessels in SHRs exhibit an MMP-dependent increase in arteriolar tone compared with their normotensive counterparts (6, 22). Thus, these studies not only provide new insight regarding the mechanisms contributing to high blood pressure in SHRs but also provide a novel explanation for the reductions in vascular responsiveness to β2-AR agonists and antagonists that occur in hypertension (19).

Elevated levels of several MMPs were noted in plasma obtained from hypertensive patients in an earlier study from Schmid-Schönbein’s group (8), as were the effects of long-term treatment with an MMP inhibitor to reduce protease activity in plasma and microvessels and to return arterial blood pressure toward normal levels in SHRs (6, 22). Moreover, exposing arterioles in normotensive animals to exogenous MMP-7 and MMP-9 produced vasoconstriction that was inhibited by a broad-spectrum MMP inhibitor (16), although the MMP concentrations used were three orders of magnitude higher than measured in SHRs (8). While the data reported by Rodrigues et al. (16) were interpreted with caution, it should be noted that broad-spectrum MMP inhibitors also inhibit closely related metalloproteinases such as a disintegrin and metalloproteinases (ADAMs) and ADAMs with thrombospondin domains, which could potentially play a role in receptor cleavage. Indeed, other proteases have been implicated by this group in shear-induced activation and cleavage of CD18 from neutrophils (9, 18).

A surprising finding reported by Rodrigues et al. (16) was that β2-AR labeling density in aortas obtained from normotensive controls was reduced by an exposure to plasma obtained from SHRs, an effect that was attenuated by the addition of an MMP inhibitor to the SHR plasma. Although these studies suggest that the plasma of SHRs contains significant protease activity, plasma also contains abundant levels of tissue inhibitor of metalloproteinases and α2-macroglobulin, both of which should effectively inhibit the activity of MMPs (2). Although it is possible that the expression of tissue inhibitor of metalloproteinases and/or α2-macroglobulin is reduced in hypertensive states, which would diminish MMPs in SHR plasma, this postulate was not tested in these studies.

Important unanswered questions that arise from the studies of Rodrigues et al. (16) relate to the identification of the cellular sources and mechanisms for the activation of the MMPs that cleave β2-AR receptors in SHRs. Given the large number of potential cellular source(s), which include leukocytes, vascular smooth muscle and endothelial cells, macrophages, and platelets, as well as fibroblasts, mast cells, and other cells in the tissue parenchyma, this question may be difficult to address. Perhaps the use of bone marrow transplant approaches or cell-specific MMP knockout would be informative. With regard to mechanisms for MMP activation in SHRs, this group has demonstrated that elevations in MMP levels occur in large arteries and at all levels of the microcirculation in hypertensive animals, even in those regions (capillaries and venules) that do not experience elevations in intraluminal pressure relative to normotensive controls (16). Thus it would appear that elevated MMP levels and their consequences are not entirely caused by the pressure elevation. This suggests that a genetically derived mechanism may contribute to these responses. Alternatively, the oxidant stress that accompanies hypertension and occurs throughout the vascular tree may activate MMPs as well as increase MMP transcription (23). It is also well established that active MMPs (and other proteases) can activate other MMPs (2, 13). This may be an important contributing factor, given that patients with essential hypertension exhibit elevated plasma levels of multiple MMPs (8). Indeed, proteases typically do not act alone in that many of their substrates are...
zymogens and inhibitors, such that cleavage can modulate the net proteolytic activity in a "protease web" (2).

The implications of the study by Rodrigues et al. (16) are far reaching and may provide a mechanistic insight with respect to other pathological consequences of hypertension. Although the current study (16) focused on MMP-mediated β2-AR cleavage, it is likely that other cell surface receptors and perhaps vasodilator peptides are disrupted by MMPs and contribute to alterations in vascular tone in hypertensive subjects. Indeed, earlier work from Schmid-Schönbein’s group (6, 22) showed that MMPs cleaved the extracellular domains of insulin receptor-α and VEGFR2 in SHRs. A long-term treatment of these hypertensive rats with broad-spectrum MMP inhibitors prevented cleavage of these receptors, reduced protease activity in plasma and microvessels, attenuated oxidant production in the microcirculation, limited endothelial apoptosis and restored capillary density, and normalized blood pressure and blood glucose levels (6, 22). These earlier results not only support the findings of the present study but also demonstrate that MMPs play an important role in the development of insulin resistance and capillary rarefaction that occurs in SHRs by a MMP-mediated, receptor cleavage-dependent mechanism.

It seems likely that receptor severing may also contribute to the pathology of other inflammatory conditions characterized by enhanced MMP (or other proteases) activation. For example, endothelium-dependent, nitric oxide (NO)-mediated vasodilator dysfunction not only is an early hallmark event of the hypertensive state but also occurs in ischemia-reperfusion, hemorrhagic shock, sepsis, hypercholesterolemia, obesity, and diabetes (21). Although it is thought to be related to enhanced superoxide production (which quenches NO) and endothelial NO synthase (eNOS) uncoupling (where eNOS produces superoxide), the possibility that receptors for acetylcholine and other endothelium-dependent vasodilators are cleaved by MMPs in these models warrants attention. Indeed, oxidants and MMPs may be mechanistically linked in such disease states since peroxynitrite not only induces eNOS uncoupling but also activates MMPs (23). Support for this possibility is provided by the observations that MMP-9 knockout mice exhibit increased acetylcholine-induced vasodilatory responses (20), whereas sepsis-induced decreases in responsiveness to vasoconstrictor agonists is prevented by the inhibition of MMP activity (3). Both observations may be explained by receptor sparing associated with MMP deficiency or inhibition.

As another potential implication, the findings of Rodrigues et al. (16) suggest a possible mechanism to explain the failure of ischemic preconditioning (IPC) to prevent ischemia-reperfusion-induced myocardial and vascular dysfunction in some hypertensive models as well as with other comorbidities and aging (1, 5, 7). This notion is related to the fact that IPC is triggered by the activation of β-adrenergic, adenosine, CGRP, and other receptors (1, 5, 7). Hypertension-induced, MMP-mediated severing of such receptors may thus prevent the activation of the downstream signaling events that mediate the development of the protected phenotype induced by IPC. The MMP-dependent receptor cleavage hypothesis may also have implications for the therapeutic mechanisms underlying the efficacy of angiotensin-converting enzyme (ACE) inhibitors as anti-hypertensive agents. While their effects on the renin-angiotension system are well known, many ACE inhibitors also inhibit MMPs. Thus an evaluation of the effect of ACE inhibitors on receptor cleavage may provide new insight regarding the mechanisms underlying the antihypertensive effects of these agents.

In addition to receptor cleavage, the enzymatic activity of MMPs also exposes cryptic functions in basement membrane and ECM molecules that modify vascular myogenic tone and responsiveness to vasoactive agents, provoke vascular cell motility and proliferation, and induce vascular cells to adopt either a pro- or anti-inflammatory phenotype, depending on the nature of the exposed cryptic binding sequences (matricryptic sites) in, and/or fragments (matricryptins) released from, the disrupted ECM (4, 12, 15). The role that an MMP-induced exposure of matricryptic sites and a release of matricryptins may play in the elevation of blood pressure in SHRs and other models of hypertension is not clear but should be investigated in light of the findings of Rodrigues et al. (16).

The contribution of inflammation in hypertension is now well appreciated, and whereas much of the work in this area has focused on the role of reactive oxygen and/or nitrogen species, kidneys, and autonomic nervous system (10), more recent efforts have revealed an important role for the adaptive immune system (10, 11). The study reported by Rodrigues et al. (16), when coupled with earlier work from this group (6, 8, 17, 22), now establishes that the enzymatic activity of MMPs contributes to elevated blood pressure, capillary rarefaction, and insulin resistance in hypertension by cleaving extracellular domains of β2-AR, VEGFR2, and insulin receptor-α. This novel discovery has implications that are far reaching, perhaps extending not only to other manifestations of cardiovascular disease but also to a wide range of pathological conditions that are characterized by the inflammation and activation of MMPs. Rodrigues, Schmid-Schönbein, and coworkers (6, 8, 16, 17, 22) have thus set the stage for future inquiries regarding the role of MMP (and other proteases)-dependent receptor cleavage in the pathophysiology of inflammatory states.

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**DISCLOSURES**

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