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New Insights of μ-Calpain in the Pathogenesis of Diabetic Vascular Injury

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The prevalence of diabetes, the most common noncommunicable health condition, is increasing at an alarming rate. It is estimated that over the next 20 years, this condition will affect 400 million people, or ~4.4% of the world's population (1,2). Diabetes leads to a number of well-described long-term consequences involving multiple organ systems. Of these, cardiovascular disorders, end-stage renal disease, and blindness are of particular concern (3). Both type 1 and type 2 diabetes may be accompanied with circulatory anomalies that account for up to 80% of premature mortality and a heavy health care burden (3). Macrovascular circulatory abnormalities include angiopathy, atherosclerosis, increased vascular tone, arterial hypertension, and calcification in both medium and large arteries. Microvascular complications include retinopathy, nephropathy, and peripheral neuropathy (3,4). Regardless of the affected site, vascular injury shares some common histopathological features of endothelial and smooth muscle dysfunction. A plethora of theories have been postulated for diabetic vascular injury. They include increased flux through polyol and hexosamine pathways, activation of protein kinase C (PKC), oxidative stress, and accumulation of advanced glycation end products that lead to abnormal blood flow, apoptosis, hyperpermeability, and accumulation of extracellular matrix (3,5). Therapeutic strategies to address diabetic vascular injury include removing risk factors, such as hyperinsulinemia, hypertension, obesity, and hyperglycemia; blocking injurious mechanisms; and promoting protective processes, such as insulin-regulated genes, antioxidant or anti-inflammatory factors, or cell survival factors (5). Recent evidence supports an independent role for hyperhomocysteinemia (HHcy), or elevated levels of plasma homocysteine (Hcy), in the pathogenesis of vascular and neurodegenerative diseases, autoimmune disorders, birth defects, diabetes, renal disease, osteoporosis, neuropsychiatric disorders, and cancer (6). Despite this growing evidence, whether elevated HHcy and diabetes—a combination that is commonly observed in humans—affect the development of diabetic cardiovascular complications is poorly understood. Improved knowledge of the interplay between HHcy and hyperglycemia may help tailor clinical management of diabetes complications in patients with concurrent HHcy.

In this issue of *Diabetes*, Cheng et al. (7) report that concurrent HHcy and hyperglycemia induced endothelial dysfunction, an observation that was ablated by the endothelial nitric oxide synthase (eNOS) inhibitor L-N^G-nitro-L-arginine methyl ester (L-NAME). More importantly, the new report suggests an essential role for μ-calpain, a cleavage activator for PKC, in HHcy-aggravated endothelial dysfunction in diabetes. The study shows that calpain inhibitors or small interfering RNA (siRNA) rescued against HHcy- and HHcy/hyperglycemia−induced endothelial dysfunction (7). The new research suggests that oxidative stress is largely responsible for calpain activation. HHcy aggravated high glucose−increased phosphorylation of eNOS at threonine 497/495 and resulted in loss of nitric oxide (NO) production. This was reversed by μ-calpain siRNA and dominant negative PKCβ_2 (7). These results provide new insight for μ-calpain in HHcy-aggravated diabetic vascular injury in which μ-calpain mediates PKCβ_2 activation and eNOS inactivation (Fig. 1). Together, these findings suggest the potential for μ-calpain as a therapeutic target for the prevention of diabetes complications. The investigators tested three different pharmacological inhibitors and a μ-calpain siRNA, all of which reversed endothelial dysfunction and eNOS inactivation in diabetic and HHcy vasculature. Given that vascular dysfunction is an early event in the development of cardiovascular disease prior to any visible

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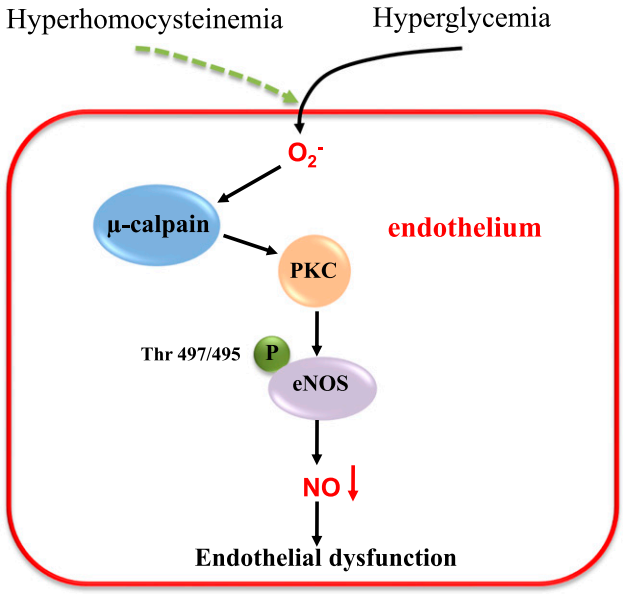

Figure 1—Schematic diagram depicting possible mechanism(s) of calpain in the interplay between HHcy and hyperglycemia in endothelial function. HHcy aggravates hyperglycemia-induced endothelial dysfunction through a μ-calpain–mediated PKC/eNOS/NO pathway.

morphological changes in the endothelium, this study supports the notion that calpain might be an ideal target for the treatment and prevention of cardiovascular disease in HHcy, diabetes, and other metabolic disorders.

Abnormal endothelium-dependent vasodilation is well established in diabetes (3,5). It has been suggested that activation of endothelial PKC leads to endothelium-dependent vasodilatory defects through inhibition of vasodilation mediated by NO, endothelium-derived hyperpolarizing factor, and prostacyclin in diabetes. Meanwhile, PKC activation promotes endothelium-dependent vasoconstriction triggered by endothelin 1, prostaglandin E_2, and thromboxane A_2 (5,6). A role for PKC in diabetes complications, and in particular the PKCβ isoform, was demonstrated by the beneficial effect of PKC inhibitors against hyperglycemia-induced endothelium-dependent vasodilatory defects (5,6).

As a family of ubiquitous nonlysosomal Ca^{2+}-dependent cysteine proteases expressed in a variety of cell types, including endothelial cells, calpains are implicated in the pathogenesis of atherosclerosis, circulatory shock, diabetes, cerebral/ischemia injury, and myocardial reperfusion injury (8,9). Elevated calpain expression or activity is found in humans (10) and experimental animals (11,12) with diabetes, and calpain inhibition rescues hyperglycemia-mediated vascular injury, inflammation, and endothelial dysfunction (11,12). Although the precise role for calpain is still unknown in the pathogenesis of diabetic vascular injury, calpains are believed to alter glucose metabolism as well as insulin secretion and action (9). In addition, there is a suggestion of interactions between PKC and calpain. While some studies suggest calpain is an upstream regulator of PKC activation, it can also serve as a downstream target of PKC signaling. Although calpain activity is reported to be stimulated by Hcy in mouse primary hepatocytes and rat heart microvascular endothelial cells (13,14), the precise role of calpain activation in HHcy-induced endothelial dysfunction remains largely unknown. The new report by Cheng et al. focused on the impact of HHcy on hyperglycemia-induced endothelial dysfunction in mouse aorta. They induced mild (22 μmol/L) and moderate HHcy (88 μmol/L) in cystathionine β-synthase wild-type ($Cbs^{+/+}$) and heterozygous-deficient ($Cbs^{-/+}$) mice using a high methionine diet. A synergy was identified between HHcy and hyperglycemia in NO-mediated vasodilation, the effect of which was rescued by calpain inhibitors or siRNA. These experiments also revealed that HHcy/hyperglycemia–induced loss of NO, eNOS inactivation, and eNOS phosphorylation at threonine 497/495 were rescued by the calpain inhibitor MDL28170 and μ-calpain siRNA. Despite these findings, regulation of calpain activity in the setting of HHcy/hyperglycemia remains poorly understood (15). One puzzling issue that remains is that intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) required for calpain activation (μ-calpain: 3–50 μmol/L and m-calpain: 400–800 μmol/L) seem to be much higher in cells than in vivo physiological conditions (50–300 nmol/L) (15). The most likely explanation for this observation is that other factors affect calpain sensitivity to $[Ca^{2+}]_i$. For example, reactive oxygen species trigger calpain activation, possibly through cysteine oxidation of Ca^{2+}-ATPase (16) and activation of L-type voltage-sensitive Ca^{2+} channels, leading to intracellular Ca^{2+} overloading (17). Cheng et al. (7) reported that antioxidant polyethylene glycol superoxide dismutase prevented HHcy/hyperglycemia–induced calpain activation. Future research on HHcy/hyperglycemia–induced calpain activation might help to clarify the underlying mechanisms and provide fundamental insights that could lead to the development of new therapeutic strategies.

One intriguing finding from the current study is the doubling of plasma Hcy levels in diabetic mice in response to a high methionine diet (7). This seems to be consistent with the fact that in high-risk patients with diabetes, secondary prevention, such as diet modification, improves clinical outcomes including HHcy (18). A number of factors could explain the higher plasma Hcy level in diabetes, including age and renal function (19), cystathionine β-synthase activity, and circulating insulin levels (20). Cheng et al. (7) observed that the combination of HHcy and hyperglycemia induced glomerular sclerosis, a finding that suggests a role for renal damage in elevated plasma Hcy levels. Nonetheless, other mechanisms remain to be explored concerning the precise interplay between HHcy and hyperglycemia. Given the promise of calpain as a target for early treatment and prevention of cardiovascular disease, future work should focus on identifying the cellular mechanism of calpain in the regulation of cell survival. This work could provide a rationale for extending

calpain inhibitors into clinical testing for appropriate indications.

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