Cardiovascular complications are the leading cause of morbidity and mortality in diabetic patients. Endothelial dysfunction with impaired endothelial nitric oxide (NO) synthase (eNOS) activity is a widely accepted cause of diabetic vasculopathy. The mechanisms of endothelial dysfunction in diabetes remain elusive, thus limiting effective therapeutic interventions. We report novel evidence demonstrating that the calcium-dependent protease calpain causes endothelial dysfunction and vascular inflammation in the microcirculation of the ZDF (Zucker diabetic fatty) rat, a genetic rat model of type 2 diabetes. We found evidence of increased calpain activity and leukocyte trafficking in the microcirculation of ZDF rats. Inhibition of calpain activity significantly attenuated leukocyte-endothelium interactions in the vasculature of ZDF rats. Expression of cell adhesion molecules in the vascular endothelium of ZDF rats was consistently increased, and it was suppressed by calpain inhibition. In vivo measurement of endothelial NO availability demonstrated a 60% decrease in NO levels in the microcirculation of diabetic rats, which was also prevented by calpain inhibition. Immunoprecipitation studies revealed calpain-dependent loss of association between eNOS and the regulatory protein heat shock protein 90. Collectively, these data provide evidence for a novel mechanism of endothelial dysfunction and vascular inflammation in diabetes. Calpains may represent a new molecular target for the prevention and treatment of diabetic vascular complications. *Diabetes* 54:1132–1140, 2005

The prevalence of type 2 diabetes is increasing dramatically in the U.S., with the number of patients diagnosed with type 2 diabetes escalating across all age-groups (1). Although vascular complications are the leading cause of morbidity and mortality in diabetic patients (1), the mechanisms responsible for diabetic vascular dysfunction remain poorly understood. Metabolic hallmarks of type 2 diabetes include hyperglycemia, obesity, and dyslipidemia, all of which are known risk factors for the development of vascular disease. All of these factors, especially hyperglycemia, have been associated with endothelial dysfunction (2). Overall, it is widely accepted that the endothelial dysfunction present in diabetic patients is a primary contributing factor to vascular complications.

Several studies in both animals (3,4) and humans (5,6) have demonstrated that hyperglycemia/diabetes causes endothelial dysfunction characterized by a loss of endothelium-derived nitric oxide (NO) and increased oxidative stress. Endothelial NO is an important regulator of vascular homeostasis. Loss of NO results in endothelial dysfunction, leading to increased vascular tone and abnormal endothelial adhesiveness, which increases platelet aggregation and leukocyte trafficking at the vessel wall (7). Thus, the diabetic vasculature experiences increased oxidative stress and abnormal inflammatory signals (8). Among other approaches, the mechanisms responsible for the loss of NO in the hyperglycemic vasculature are being intensely investigated with the aim to uncover novel therapeutic strategies for the treatment of diabetic vasculopathies.

The calpains are a family of calcium-dependent proteases that have previously been implicated in the pathophysiology of several inflammatory disorders of the cardiovascular system, including myocardial reperfusion injury (9), cerebral ischemia/reperfusion (10), and circulatory shock (11). In the current study, we have used the ZDF (Zucker diabetic fatty) rat, a genetic rat model of type 2 diabetes (12), to test the hypothesis of a role for calpains in the endothelial dysfunction of diabetes.

**RESEARCH DESIGN AND METHODS**

This study was performed in accordance with the National Institutes of Health and Thomas Jefferson University institutional animal care and use committee guidelines for the use of experimental animals. We used ZDF rats (Charles River Laboratories, Noblesville, IN) of 10–14 weeks of age, the age range at which they develop overt diabetes with hyperinsulinemia and permanent hyperglycemia (12). ZDF rats have a homozygous mutation in their leptin receptor gene (*fa/fa*) that causes them to develop a metabolic disorder similar to type 2 diabetes in humans (12). Zucker lean (ZL) rats are either homozygous wild-type or heterozygous at the leptin receptor gene (*fa/+/*), do not develop diabetes, and are used as control animals because of the common genetic background. Rats were divided into one of three groups: 1) control ZL rats injected with vehicle, 2) ZDF rats injected with vehicle, and 3) ZDF rats injected with 27 μg/kg i.p. calpain inhibitor benzoxycarbonyl-leucyl-leucinal (ZLLal) once a day for 5 consecutive days. The microcirculation of the mesentery was used for these studies because it is highly amenable to intravital microscopy and because it expresses a typical inflammatory phenotype in response to both hyperglycemia (13) and diabetes (14). In a preliminary study, administration of ZLLal 60 min before intravital microscopy, as well as 120 min local superfusion of ZLLal during intravital microscopy...
experiments, failed to inhibit leukocyte-endothelium interaction in the diabetic microcirculation (data not shown).

**Western blot analysis of calpain activity in the rat mesentery in vivo.** Mesenteric segments with dense microvascular networks were dissected under microscopy. Sections were snap-frozen in liquid nitrogen and homogenized as previously described (15). Calpain activity was studied by Western blot analysis using polyclonal antibodies against the NH₂-terminal domains (RP1 calpain-1 or RP2 calpain-2) or domain IV (RP3 calpain-1 and RP3 calpain-2; Triple Point Biologics, Portland, OR) of the large 80-kDa subunit of m- and μ-calpain, respectively. Once activated, m- and μ-calpains autolytically cleave their NH₂-terminal ends, respectively, resulting in the loss of NH₂-terminal antibody recognition, which can be used as a measure of calpain activation. Quantification of the stable domain IV was used to measure total m- and μ-calpain content in mesenteric extracts. Proteins were detected by chemiluminescence (Supersignal West Pico; Pierce, Rockford, IL) and quantified by laser densitometry (personal densitometer; Molecular Dynamics, Piscataway, NJ).

**Intravital microscopy.** Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and prepared for intravital microscopy as previously described (13). Leukocyte rolling, adhesion, and transmigration were studied in mesenteric postcapillary venules. Leukocyte rolling is defined as the number of leukocytes rolling past a fixed point per minute; leukocyte adherence is defined as leukocytes firmly adhered to a 100-μm length of endothelium for at least 30 s. The number of leukocytes extravasated within a 5-μm perivascular area were counted and normalized with respect to the area. Erythrocyte velocity was determined online using an optical Doppler velocimeter obtained from the Micrcirculation Research Institute (College Station, TX). Erythrocyte velocity \( V_{rbc} \) and venular diameter (D) were used to calculate the venular wall shear rate \( \gamma \) using the formula: \( \gamma = 8(V_{rbc}/D) \), where \( V_{rbc} = V_{v} - V_{r} \).

**Immunohistochemistry.** At the completion of intravital microscopy experiments, sections of mesentery and ileum were fixed in vivo, dehydrated using graded aceton washes, and embedded in plastic as previously described (13). Immunohistochemical localization of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) was accomplished using monoclonal antibodies (BD Transduction Laboratories, San Diego, CA) and the avidin/biotin immunoperoxidase technique. The percentage of positively staining venules was determined by examining 5–10 tissue sections per rat, with 50 venules analyzed per tissue section. NO release from rat mesenteric venules. NO levels in mesenteric postcapillary venules were measured during intravital microscopy experiments, using an ISO-NO microelectrode (100-nm diameter at the tip) connected to an ISO-NO Mark-II NO meter (World Precision Instruments, Sarasota, FL), according to a previously described technique (15,16). Briefly, the microelectrode was positioned directly over a mesenteric postcapillary venule at a distance of <5 μm from the venular wall. To calculate the amount of NO released by the vascular endothelium, background currents obtained at a distance of 0.5–1 cm from the venular wall were subtracted from currents measured at the venular wall. Endothelial NO values were normalized to the size of the blood vessel studied and reported as nanomolar NO/1,000 μm² vessel area.

**Association of endothelial NO synthase and heat shock protein 90.** The association of endothelial NO synthase (eNOS) and heat shock protein 90 (hsp90) in the mesentery of all experimental groups of rats was studied by immunoprecipitation and Western blotting according to a technique described previously (15). Briefly, mesentry extracts containing ~750 μg protein were incubated with anti-eNOS monoclonal antibody in immunoprecipitation buffer for 1 h followed by incubation with prewashsed protein G-agarose (Sigma) for 2 h. The resulting pellet was washed three times in PBS, boiled in SDS sample buffer, and resolved by SDS-PAGE. Immunoblot analysis was performed using primary antibodies against eNOS (BD Transduction) and hsp90 (Santa Cruz Biotechnology, Santa Cruz, Ca).

**Isolation of mesenteric microvascular endothelial cells and quantification of calpain in vitro.** Densely vascularized segments of mesentery were isolated. Mesenteric microvascular endothelial cells (MMECs) were then isolated using magnetic microbeads (Miltenyi Biotech, Auburn, CA) ligated with anti-rat platelet endothelial cell adhesion molecule-1 (PECAM-1) antibody (17). MMECs were phenotyped by fluorescence-activated cell sorting analysis of PECAM-1 and by functional uptake of acetylated LDL. For fluorescence-activated cell sorting studies, MMECs were incubated with monoclonal CD31 antibody conjugated with phycoerythrin (BD Pharmingen) for 30 min on ice and analyzed using a FacsCalibur (Becton Dickinson, Franklin Lakes, NJ).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mmol/l)</th>
<th>Mean arterial blood pressure (mmHg)</th>
<th>Venular shear rate ( (s^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ZL rat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5.5 ± 0.2</td>
<td>126.6 ± 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>581 ± 60.1</td>
</tr>
<tr>
<td><strong>ZDF rat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>17.2 ± 1.4*</td>
<td>125.8 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>335 ± 26.7†</td>
</tr>
<tr>
<td><strong>ZDF rat + 27</strong></td>
<td></td>
<td>22.0 ± 3.7*</td>
<td>128.5 ± 2.1</td>
</tr>
<tr>
<td>μg/kg ZLal</td>
<td>5</td>
<td></td>
<td>461 ± 88.2</td>
</tr>
</tbody>
</table>

*P < 0.01, †P < 0.05 vs. ZL rat.

**RESULTS**

**Blood glucose levels and hemodynamics in ZDF rats.** Compared with nondiabetic ZL rats, diabetic ZDF rats displayed fasting hyperglycemia that was not lowered by calpain treatment (Table 1). Mean arterial blood pressures were not significantly different among the three groups of rats studied (Table 1). However, venular shear rate values in postcapillary venules of ZDF rat mesenteries were significantly decreased, despite remaining within physiologic range (Table 1). This finding is in agreement with previous studies demonstrating decreased shear rates (14) and increased vascular permeability (20) in the microcirculation of diabetic rats. Thus, 10- to 14-week-old ZDF rats develop overt hyperglycemia and hemodynamic perturbations in the microcirculation that are highly relevant to diabetic vascular disease.

**Assessment of calpain activity in ZDF rats.** To investigate the effect of diabetes on calpain activity in vivo, we measured autophosphorylation of m- and μ-calpain, a well-established indicator of calpain proteolytic activity in biological systems (21). On activation, calpains undergo autophosphorylation with removal of the NH₂,9–14 amino acids from the large 80-kDa subunit (22,23). Accordingly, evaluation of m- and μ-calpain autophosphorylation in freshly isolated vascular segments of mesenteric tissue from ZL and ZDF rats was assessed by immunoblot analysis, using primary antibodies to the NH₂-terminal domains of the m-calpain.
and μ-calpain large subunits (Fig. 1A). We found increased proteolytic activity of μ-calpain in ZDF rats, as demonstrated by 75% loss in NH2 terminus antibody binding (Fig. 1). Administration of 27 μg/kg i.p. of the calpain inhibitor ZLLal to ZDF rats for 5 consecutive days completely blocked μ-calpain autoproteolysis in the rat mesentery (Fig. 1). Total μ-calpain content was quantified using a primary antibody against domain IV of the large subunit, which recognizes both unautolyzed and autolyzed μ-calpain. No significant differences in total μ-calpain protein expression were found in ZDF rats (Fig. 1A). In contrast, the proteolytic activity of m-calpain was not increased in ZDF rat mesenteries, as demonstrated by a lack of changes in the m-calpain NH2 binding domain expression level (Fig. 1). No significant changes were observed in total m-calpain expression, as detected using a primary antibody against m-calpain domain IV (data not shown). These findings demonstrate that type 2 diabetes selectively increases the proteolytic activity of μ-calpain in densely vascularized tissues.

**Intravital microscopy.** Nondiabetic ZL rats exhibited a low baseline leukocyte rolling value of 24.1 ± 2.4 cells/min in mesenteric postcapillary venules (Fig. 2A). In contrast, ZDF rats had a threefold increase in leukocyte rolling (P < 0.05 vs. ZL). Injection of 27 μg/kg i.p. ZLLal once a day for 5 consecutive days to ZDF rats attenuated leukocyte rolling to 47.8 ± 10.0 cells/min, a value that did not reach statistical significance.

There were a low number of leukocytes firmly adhering to the vascular endothelium of mesenteric postcapillary venules in ZL rats (Fig. 2B). Leukocyte adherence was increased fivefold in ZDF rats (P < 0.01 vs. ZL rats). Administration of 27 μg/kg i.p. ZLLal once a day for 5 days significantly attenuated the number of adherent leukocytes in mesenteric postcapillary venules of ZDF rats (Fig. 2B).

The number of leukocytes that had transmigrated into the perivascular area was also studied. Few leukocytes were found in the perivascular area of postcapillary venules in nondiabetic ZL rats (Fig. 2C). In contrast, increased numbers of transmigrated leukocytes were observed within 5 μm of the postcapillary venular wall in ZDF rats (P < 0.01 vs. ZL rats). Inhibition of calpain activity with ZLLal greatly reduced the number of leukocytes extravasated in the mesentery of ZDF rats (Fig. 2C).

These data demonstrate that the diabetic microcirculation of ZDF rats experiences chronic inflammatory signals with increased leukocyte trafficking and that inhibition of calpain activity acutely attenuates vascular inflammation in the face of chronic hyperglycemia.

**Immunohistochemistry.** To investigate the molecular mechanisms of increased leukocyte trafficking interactions in the diabetic microcirculation, we studied endothe-
lial cell surface expression of ICAM-1 and VCAM-1 (Fig. 3),
two cell adhesion molecules relevant to vascular inflam-
mation and atherogenesis. Compared with nondiabetic ZL
rats, expression of ICAM-1 increased 2.5-fold in the vascul-
lar endothelium of ZDF rats ($P < 0.01$), and it was
attenuated to control values by ZLLal treatment ($P < 0.01$
vs. untreated ZDF rats). Similarly, we found evidence of a
2.8-fold increase in VCAM-1 expression in the vascular
endothelium of ZDF rats. Treatment of ZDF rats with the
calpain inhibitor ZLLal attenuated VCAM-1 expression
levels to control values ($P < 0.01$ vs. untreated ZDF rats).
These data demonstrate that increased calpain activity
plays a role in the upregulation of endothelial cell adhe-
sion molecules in diabetes.

**Basal release of endothelial NO in postcapillary
venules of ZDF rats.** Endothelial NO suppresses VCAM-1
expression at the molecular level (24). Accordingly, we
measured levels of endothelial NO in mesenteric postcap-
illary venules to further study the molecular mechanisms
of vascular dysfunction in diabetes. We found that levels
of NO decreased by 2.4-fold in the inflamed postcapillary
venules of ZDF rats (Fig. 6). Inhibition of calpain activity
with ZLLal significantly restored endothelial NO levels in
mesenteric venules of ZDF rats ($P < 0.05$ vs. untreated
ZDF rats) (Fig. 4). Thus, loss of endothelial NO in the
diabetic vasculature is at least in part calpain dependent.

**Association of eNOS and hsp90.** Previous studies have
indicated that increased calpain activity impairs NO pro-
duction by decreasing the association of the regulatory
protein hsp90 with the eNOS complex (25). Accordingly,
we measured total hsp90 content in the mesentery of
ZL and ZDF rats using Western blot analyses. We also
performed immunoprecipitation studies to determine
the extent of eNOS/hsp90 association in vascular seg-
ments of the ZDF rat mesentery. Densitometric analysis
revealed a $32 \pm 1.7\%$ reduction in hsp90 expression levels
in ZDF rat mesenteries, compared with nondiabetic ZL
rats ($P < 0.05$). Furthermore, less hsp90 was associated
with eNOS in the vasculature of ZDF rats as compared
with ZL rats (Fig. 5). Inhibition of calpain activity signifi-
cantly attenuated the loss of hsp90 from the eNOS complex (Fig. 5). These data demonstrate that increased calpain activity impairs the posttranslational assembly of eNOS in the diabetic vasculature, and they also correlate with the evidence of calpain-dependent loss of NO illustrated in Fig. 4.

Increased calpain activity in MMECs isolated from diabetic ZDF rats. Measuring of calpain activity in mesenteric tissue homogenates does not provide absolute evidence of increased calpain activity in the vascular endothelium. We isolated MMECs from ZL and ZDF rats to further confirm that diabetes does increase calpain activity

FIG. 3. Representative photomicrographs illustrating the effect of calpain inhibition on ICAM-1 (left panels) and VCAM-1 (right panels) expression in rat ileal venules. Brown immunoperoxidase reaction product (arrows) indicates positive staining. ICAM-1 and VCAM-1 were significantly upregulated in the vascular endothelium of ZDF rats (middle panels). Calpain inhibition attenuates endothelial cell surface expression of ICAM-1 and VCAM-1 in diabetic rats (lower panels). V, venule.

FIG. 4. Measurement of NO in mesenteric venules from ZL (upper left panel) and ZDF (lower left panel) rats by an NO polarographic microelectrode. The NO electrode was placed parallel to the long axes of the venule and advanced to a 5-μm distance from the vessel wall under direct microscopy observation. Large black dots are the optical projection of the optical Doppler velocimeter used to measure blood flow velocity and venular shear rates during intravital microscopy. Arrows indicate rolling and adhering leukocytes. All values are means ± SE. Basal NO release is expressed as nanomolar NO/1,000 μm². Numbers at the base of the columns indicate the number of rats studied in each group.

CALPAINS AND DIABETIC VASCULOPATHY

1136 DIABETES, VOL. 54, APRIL 2005
in the endothelium of the microcirculation. Microvascular endothelial cells were phenotyped by uptake of acetylated LDL and flow cytometry analyses of PECAM-1 (Fig. 6). Calpain activity in normal and diabetic MMECs was measured with the use of the fluorescent probe t-BOC (Fig. 7 upper panel). We detected a 70% increase in basal calpain activity in MMECs isolated from ZDF rats, compared with MMECs from nondiabetic ZL rats (Fig. 7, lower panel). In additional control experiments, a comparable degree of calpain activation in the range of 1.6 ± 0.19-fold change was detected in nondiabetic MMECs incubated with 20 mmol/l D-glucose for 48 h (P < 0.05 vs. nondiabetic MMECs cultured under normal glucose conditions). This result points to hyperglycemia as the leading cause of increased calpain activity in the diabetic vasculature.

Treatment of diabetic MMECs with 10 µmol/l antisense oligonucleotides to µ-calpain for 48 h attenuated calpain activity to values found in nondiabetic control MMECs (Fig. 7). A comparable degree of calpain activity attenuation was also obtained after treatment of diabetic MMECs with ZLLal for 24 h (Fig. 7), a result that strengthens the significance of our in vivo data. Taken together with the biochemical results reported in Fig. 1, these data provide strong evidence that hyperglycemia and diabetes increase calpain activity in the vascular endothelium.

DISCUSSION

The present study demonstrates a novel role for the calcium-dependent protease calpain in the pathophysiology of diabetic vascular disease. We provide novel evidence implicating µ-calpain in the endothelial dysfunction and vascular inflammation of the ZDF rat, a genetically occurring animal model of type 2 diabetes.

Oxidative stress, upregulation of endothelial cell adhesion molecules, and leukocyte trafficking has been linked with damage to blood vessels and organ tissue in diabetes (8,26,27). In vivo studies have demonstrated that accumulation of adherent leukocytes is increased in the aortic endothelium of alloxan-induced diabetic rabbits (28) and in the retinal microcirculation of diabetic rats (27), where capillary occlusions by leukocytes appears to precede destruction of the retinal capillary bed (29). The relationship between diabetes and cell surface expression of adhesion molecules has been and continues to be the subject of several in vivo and in vitro studies. Upregulation of cell adhesion molecules occurs in spontaneously hyperglycemic mice (30). Circulating levels of soluble ICAM-1 and VCAM-1 are increased in diabetic patients (31) and in response to transient hyperglycemia (6). Monocytes isolated from diabetic patients are more adhesive to cultured human endothelium (32), and they are in a proinflammatory state (33). Overall, the proinflammatory effects of diabetes on endothelial cell adhesion molecules in humans is associated with glucose-driven oxidative stress (34) and reduced release of NO (2,8).

It is now well appreciated that inflammation of the microcirculation can increase the risk of developing cardiovascular disease and atherosclerosis (35). Thus, inflammatory microangiopathy is considered an additional contributing factor to accelerated atherogenesis in diabetes. In fact, infiltration of leukocytes through the micro
large calcium fluxes, such as during apoptosis, may be more important in the setting of cell signaling under physiological and pathological conditions (21). Further studies addressing the signaling cascade(s) responsible for calpain activation in states of insulin resistance and type 2 diabetes cause vascular inflammation with endothelial dysfunction (8), loss of endothelial NO (2), and activation of circulating leukocytes (27,33). Activated leukocytes adhere to the vascular endothelium, where they quench NO, increase oxidative stress, and release proinflammatory cytokines. Given the important role played by oxidative stress in the cardiovascular complications of obesity and diabetes (33,34), further studies are needed to better understand the impact of calpain inhibition on the production of oxidant species and inflammatory cytokines in the vascular wall during states of hyperglycemia and insulin resistance.

There is a general consensus in the literature that endothelial NO exerts potent anti-inflammatory effects in the cardiovascular system. Accordingly, endothelial dysfunction with impaired release of NO has been demonstrated in experimental animal models of hyperglycemia (4,13) and diabetes (14,30), as well as in diabetic humans (2,5). Notable to diabetes, insulin exerts important anti-inflammatory effects during acute experimental hyperglycemia of the microcirculation in the rat (13). In human endothelial cells, insulin increases release of NO (38), induces eNOS expression (39), and suppresses levels of ICAM-1 (40). Interestingly, the NO-potentiating effect of insulin is inhibited by tumor necrosis factor-α, a leukocyte-derived proinflammatory cytokine elevated in states of insulin resistance (41). Thus, it is likely that recurrent transient inflammatory events of the vascular wall initiated by primary loss of NO may exacerbate insulin resistance and cardiovascular disease. Interestingly, statins have been recently proven to exert cardiovascular protective actions, which are independent of their cholesterol-lowering activity because of direct anti-inflammatory and NO-potentiating effects (42). Recent studies have demonstrated reduced expression of eNOS in cultured endothelial cells exposed to elevated ambient glucose (43) or dysfunctional eNOS under hyperglycemic conditions (44). The anti-inflammatory action of NO is largely based on its inhibitory effect on leukocyte-endothelium interactions. At the molecular level, NO suppresses leukocyte-endothelium interactions by modulating the activity of nuclear factor-κB, a nuclear transcription factor that increases transcription of proinflammatory cell adhesion molecules such as VCAM-1 (24). Loss of basal release of NO in postcapillary venules induces inflammatory responses in the microcirculation characterized by increased leukocyte-endothelium interactions and upregulation of endothelial cell adhesion molecules (42). Thus, it is likely that after calpain inhibition, the attenuation in VCAM-1 expression levels observed in the present study were a consequence of increased availability of NO in the vascular endothelium.

The production of NO by eNOS is dependent, among other factors and regulatory proteins (45), on the molecular chaperone hsp90 (46). There is compelling evidence...
that hsp90 plays a key role in activating eNOS, as suggested by a recent study showing that the interaction of hsp90 and eNOS permits hsp90 to serve as a docking site for Akt-dependent phosphorylation of eNOS (47). Thus, decreased association of hsp90 with the eNOS complex decreases eNOS activity and endothelial NO release (46). Interestingly, it has been shown that the neutral cysteine protease calpain degrades hsp90 in several cell systems (48,49). Recently, a study by Su and Block (25) demonstrated that calpain-mediated degradation of hsp90 decreases NO production in pulmonary artery endothelial cells. These observations agree with our finding of reduced availability of endothelial NO and decreased hsp90/eNOS association in the microcirculation of diabetic rats. Indeed, inhibition of calpain activity in ZDF rats restored the association of eNOS with hsp90 along with increased NO release and attenuation of leukocyte trafficking in the microcirculation.

In conclusion, we have demonstrated that the activity of the calcium-dependent protease μ-calpain is increased in the diabetic vasculature and that inhibition of calpain activity attenuates the vascular dysfunction associated with chronic diabetes. These findings uncover a role for the calcium-dependent protease calpain in the pathophysiology of diabetic vascular disease.

ACKNOWLEDGMENTS
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CALPAINS AND DIABETIC VASCULOPATHY


