Angiotensin II and endothelin-1 augment the vascular complications of diabetes via JAK2 activation

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Banes-Berceli AK, Ketsawatsomkron P, Ogbi S, Patel B, Pollock DM, Marrero MB. Angiotensin II and endothelin-1 augment the vascular complications of diabetes via JAK2 activation. Am J Physiol Heart Circ Physiol 293: H1291–H1299, 2007. First published May 25, 2007; doi:10.1152/ajpheart.00181.2007.—The JAK/STAT pathway is activated in vitro by angiotensin II (ANG II) and endothelin-1 (ET-1), which are implicated in the development of diabetic complications. We hypothesized that ANG II and ET-1 activate the JAK/STAT pathway in vivo to participate in the development of diabetic vascular complications. Using male Sprague-Dawley rats, we performed a time course study [days 7, 14, and 28 after streptozotocin (STZ) injection] to determine changes in phosphorylation of JAK2, STAT1, and STAT3 in thoracic aorta using standard Western blot techniques. On day 7 there was no change in phosphorylation of JAK2, STAT1, and STAT3. Phosphorylation of JAK2, STAT1, and STAT3 was significantly increased on days 14 and 28 and was inhibited by treatment with candesartan (AT1 receptor antagonist, 10 mg·kg−1·day−1 orally in drinking water), atrasentan (ETα receptor antagonist, 10 mg·kg−1·day−1 orally in drinking water), and AG-490 (JAK2 inhibitor, 5 mg·kg−1·day−1 intraperitoneally). On day 28, treatment with all inhibitors prevented the significant increase in systolic blood pressure (SBP; tail cuff) of STZ-diabetic rats (SBP: 157 ± 9.0, 130 ± 3.3, 128 ± 6.8, and 131 ± 10.4 mmHg in STZ, STZ-candesartan, STZ-atrasentan, and STZ-AG-490 rats, respectively). In isolated tissue bath studies, diabetic rats displayed impaired endothelium-dependent relaxation in aorta (maximal relaxation: 95.3 ± 3.0, 92.6 ± 7.4, 76.9 ± 12.1, and 38.3 ± 13.1% in sham, sham + AG-490, STZ + AG-490, and STZ rats, respectively). Treatment of rats with AG-490 restored endothelium-dependent relaxation in aorta from diabetic rats at 14 and 28 days of treatment. These results demonstrate that JAK2 activation in vivo participates in the development of vascular complications associated with STZ-induced diabetes.

vascular smooth muscle cells; type 1 diabetes; angiotensin II; Janus-activated kinase 2; signal transducer and activator of transcription

Complications associated with type 1 and type 2 diabetes include both renal and vascular aspects. Vascular complications include an accelerated development of atherosclerosis and endothelial dysfunction (15). The loss of endothelial function has important implications for both the regulation of vascular tone and the unregulated growth of smooth muscle cells involved in the development of atherosclerosis.

One activator of the tyrosine kinase Janus kinase 2 (JAK2) pathway is angiotensin II (ANG II). ANG II is involved in the vascular dysfunction and renal complications associated with many diseases, including hypertension and diabetes (27, 18). In addition, our group (1, 26) has previously shown that JAK2 plays a critical role in the ANG II- and high glucose-induced growth in rat mesangial cells and ANG II-induced vascular smooth muscle cell (VSMC) proliferation. Another activator of the JAK2 pathway is endothelin-1 (ET-1). ET-1 also has been implicated as a causative factor in many diseases, including hypertension and diabetes (9, 10). ET-1 may be produced from multiple cell types in the vasculature, including the endothelium (11), VSMC (23), and adventitial fibroblasts (2).

Our group (7) recently reported that ET-1 can activate JAK2 in VSMC in vitro and that treatment of streptozotocin (STZ)-induced diabetic rats with the ETα receptor antagonist atrasentan for 14 days inhibits JAK2 phosphorylation in vivo in VSMC. Furthermore, a recent study by Sasser et al. (24) showed that blockade of the ETα receptor with the ETα receptor antagonist atrasentan resulted in a slowed progression of development of diabetic nephropathy in the STZ-induced model of diabetes through an anti-inflammatory mechanism. Several studies support the idea that ET-1 may mediate some of the vascular actions of ANG II (9, 7, 4, 17), perhaps via activation of JAK2.

However, regulation of the activation of JAK2 in the vascular is not well understood under normal and diseased conditions. Seki et al. (25) have demonstrated that after vascular injury, ANG II, via the AT1 receptor, activates the JAK/STAT pathway in the rat carotid artery. Inhibition of the JAK2/STAT3 pathway activation resulted in reduced neointimal formation in the injured artery, suggesting a critical role for this pathway in vascular remodeling. In addition, in aortic endothelial cells our group (29) recently showed that high glucose-induced activation of JAK2 is detrimental to cell survival in vitro. However, there is currently no data in vivo to demonstrate that activation of JAK2 is detrimental to vascular function in diabetes. These data would suggest, however, that activation of the JAK/STAT pathway may be a critical component of vascular remodeling.

Furthermore, the mechanisms by which JAK2 phosphorylation is increased in vivo are not yet clearly elucidated. Previous in vitro studies have suggested that elevated levels of glucose alone increase JAK2 phosphorylation (1, 26). However, the time course of in vivo activation of JAK2 is as yet unknown. Our group (5) has previously demonstrated an increase in JAK2 phosphorylation in VSMC at 14 days of diabetes. Whether this activation is sustained is unknown. In addition, whether increased levels of JAK2 phosphorylation precede or
JAK2 inhibitor AG-490, sham and STZ rats treated with the ETA receptor antagonist atrasentan (ABT-627; 10 mg·kg⁻¹·day⁻¹) and the AT₁ receptor antagonist candesartan (10 mg·kg⁻¹·day⁻¹) were administered via intraperitoneal injection daily. The atrasentan, candesartan, and AG-490 treatments were all started on the day of STZ injection.

**Blood pressure measurements.** Standard tail-cuff methods (pneumatic transducer) were used on trained rats as previously described (20). Baseline measurements were taken before the administration of STZ. Data presented are averages of six measurements taken on each occasion. Final blood pressure measurements were taken 24 h before removal from the study.

**Metabolic measurements.** Animals were placed into metabolic cages for a 24-h equilibration period and then a 24-h measurement period. Fluid intake and food intake were measured, and urine was collected.

**Isolated tissue bath protocol.** Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and thoracic aortas were removed. Tissues were placed in physiological salt solution (PSS) consisting of (in mM) 118 NaCl, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄·7H₂O, 1.6 CaCl₂·2H₂O, 14.9 NaHCO₃, 5.5 dextrose, and 0.03 CaNa₂EDTA. The arteries were cleaned of loose connective tissue and cut into 3-mm rings. Tissues were mounted in tissue baths (50 ml) for isometric tension recordings and placed under optimum resting tension (3 g for aorta, determined previously). After 1 h of equilibration, tissues were challenged with KCl (64 mM) to determine viability. Tissues were then washed, and the status of the endothelium was examined by utilizing cumulative concentration-response curves to acetylcholine (ACh; 10⁻⁹ to 10⁻⁵ M) in preconstricted (KCl half-maximal concentration) vessels.

**Isolation of rat thoracic aorta.** Tissues were obtained from sham and STZ-induced diabetic rats with and without treatment with can-

are a result of the developing complications is also undetermined.

Currently, there is a lack of studies that have investigated the role of JAK2 activation in the diabetic vasculature and its contribution to the development of vascular complications. The aim of the present study was to determine the profile of changes in ANG II- and ET-1-dependent JAK2 phosphorylation in a model of type 1 diabetes. We hypothesized that inhibition of JAK2 would prevent endothelial cell dysfunction and the development of vascular complications.

**METHODS**

**Streptozotocin-induced diabetes.** All studies were conducted with the approval of the Medical College of Georgia Institutional Animal Use Care Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (225–250 g) from Harlan Laboratories (Indianapolis, IN) were rendered diabetic by a single intraperitoneal injection of STZ (60 mg/kg) made up in fresh 0.1 M citrate buffer, pH 4.5, as previously described (5). Age-matched control rats received buffer only. Forty-eight hours later the diabetic state was confirmed by measurement of tail blood glucose (BG) level, obtained using the Accu-chek glucometer. All rats given STZ had a blood glucose concentration exceeding 15 mM and thus were considered diabetic. Diabetic rats were treated with 2 units of insulin per day (via insulin pellets subcutaneously; LinShin, Ontario, Canada) to prevent ketoadisosis and maintain blood glucose levels in the 300–500 mg/dl range. In some experiments we also included a group of STZ-treated rats given higher doses of insulin to normalize blood glucose and test for non-specific effects of STZ. Animals were fed standard Purina rat chow (Ralston Purina, Richmond, IN), had free access to tap water ad libitum, and were kept on a 12:12-h light-dark cycle. The ETA receptor antagonist atrasentan (ABT-627; 10 mg·kg⁻¹·day⁻¹) and the AT₁ receptor antagonist candesartan (10 mg·kg⁻¹·day⁻¹) were administered via drinking water for 7, 14, or 28 days. Animals were monitored daily for fluid intake to ensure adequate dosing. The JAK2 inhibitor AG-490 (5 mg·kg⁻¹·day⁻¹) was administered via intraperitoneal injection daily. The atrasentan, candesartan, and AG-490 treatments were all started on the day of STZ injection.
desartan, atrasentan, and AG-490. Tissues were cleaned of debris, endothelium denuded, quick frozen with liquid nitrogen, pulverized in a liquid nitrogen-cooled mortar and pestle, and solubilized in a lysis buffer (0.5 M Tris·HCl, pH 6.8, 10% SDS, and 10% glycerol) with protease inhibitors (0.5 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) and a tyrosine phosphatase inhibitor (1 mM sodium orthovanadate). Homogenates were sonicated (1 min in a Polytron at setting 7) and then centrifuged (11,000 g for 10 min at 4°C), and the protein concentration for each sample was assessed using a modification of Bradford’s method. We found no difference in the phosphorylation levels examined in aortas with and without endothelium (data not shown).

**Immunoblotting protocol.** Aortic lysates were resolved by 7.5% SDS-PAGE gel electrophoresis, transferred to nitrocellulose membranes, and blocked (60 min, 22°C) in T-TBS (Tris-buffered saline with 0.05% Tween 20, pH 7.4) with 5% skimmed milk powder. Membranes were incubated overnight (4°C) with affinity-purified anti-phosphotyrosine-specific and total JAK (JAK2) and STAT antibodies (STAT1 and STAT3). Membranes were also probed with phosphospecific and total Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) and SHP-2 antibodies (Abcam, Cambridge, MA). Membranes were washed with T-TBS and then incubated with the appropriate secondary antibodies (for 60 min at 22°C). After washing with T-TBS, the bands were visualized using Pierce Supersignal substrate chemiluminescence and Kodak Biomax film. Membranes were also incubated with smooth muscle α-actin antibody (Calbiochem, La Jolla, CA) and the appropriate secondary antibody to ensure equal loading of total protein. Molecular weight markers assessed specificity of the bands.

**Data analysis and statistics.** Quantitation of band density was performed using NIH Image (Scion USA). Band density is reported in arbitrary densitometry units. Pharmacological parameters were calculated for contractility studies with the use of GraphPad Prism software (GraphPad Software, San Diego, CA). Data are means ± SE for the number of samples indicated, and *P* ≤ 0.05 was considered statistically significant. One-way ANOVA with a Newman-Keuls post hoc test and ANOVA with repeated measures tests were used to analyze the data.

**Chemicals.** Molecular weight standards, acrylamide, SDS, N,N′-methylene-bisacrylamide, N,N,N′,N′-tetramethylenediamine, protein assay reagents, and nitrocellulose membranes were purchased from Bio-Rad Laboratories (Hercules, CA). STZ was purchased from Sigma Chemical (St. Louis, MO). The anti-phosphotyrosine and total antibodies for JAK2 were obtained from BioSource International (Carlsbad, CA). The anti-phosphotyrosine and total STAT-1 and STAT-3 antibodies were obtained from Upstate (Charlottesville, VA).

![Fig. 3. Densitometric analysis and representative Western blots of the levels of JAK2 phosphorylation (p) in sham nondiabetic rats, STZ-induced diabetic rats, sham and STZ rats treated with the AT1 receptor antagonist candesartan, sham and STZ rats treated with the JAK2 inhibitor AG-490, sham and STZ rats treated with the ETA receptor antagonist atrasentan, and STZ diabetic rats treated with high insulin on days 7 (A), 14 (B), and 28 (C). Values are means ± SE (n = 6–7). *P* ≤ 0.05 compared with sham counterpart.](http://ajpheart.physiology.org/10.1152/ajpheart.00632.2006)
Total and phosphospecific SHP-1 and SHP-2 antibodies were purchased from Abcam. The Pierce Supersignal substrate chemiluminescence detection kit was obtained from Pierce (Rockford, IL). Goat anti-mouse IgG and anti-rabbit IgG were acquired from Amersham BioScience (Princeton, NJ). Tween 20 and all other chemicals were purchased from Sigma Chemical.

RESULTS

The STZ model of diabetes is characterized by a loss of or inability to gain body weight in the diabetic animals. The lack of gain in body weight in the diabetic rats was unaffected by treatment with the AT1 receptor antagonist candesartan, the ETA receptor antagonist atrasentan, and the JAK2 inhibitor AG-490 (Fig. 1A). There was no effect on body weight by any of the inhibitors in any of the control animals (Fig. 1A). STZ alone had no effect on these parameters, since treatment with high insulin (STZ-HI) to normalize blood glucose levels maintained the animals at control levels.

Blood glucose levels. At days 7, 14, and 28, all of the diabetic animals exhibited a significantly increased blood glucose level compared with their control counterparts (Fig. 1B). In the STZ-induced diabetic rats receiving candesartan, there was a small but significant decrease in the blood glucose level (Fig. 1B). However, it was still significantly higher than in the control rats (Fig. 1B). Treatment with AG-490 and atrasentan had no effect on the blood glucose levels of the diabetic and control rats (Fig. 1B). The STZ-HI group was maintained at the same level as the control group.

Systolic blood pressure. To determine whether there were changes in the systolic blood pressure and whether those...
changes were causally related to changes in the activation of the JAK/STAT pathway, we measured the blood pressure of the animals using standard tail-cuff techniques. We found no significant increase in the systolic blood pressure on days 7 and 14 in any group (Fig. 2). However, on day 28 in the untreated STZ-induced diabetic rats, there was a significant increase in the systolic blood pressure, and this was prevented by treatment with candesartan, atrasentan, and AG-490 (Fig. 2). At all time points, the STZ-HI group was no different from the control group.

**JAK/STAT pathway.** To understand the potential role of the JAK/STAT pathway in the development of these complications, we examined the activation of JAK2, STAT1, and STAT3 in endothelium-denuded rat thoracic aorta. We observed no difference in the levels of JAK2 phosphorylation in any of the treatment groups on day 7 (Fig. 3A). There also were no differences in the levels of STAT1 and STAT3 phosphorylation on day 7 (data not shown). On day 14 there was a significant increase in the levels of JAK2 phosphorylation only in the untreated diabetic rats (Fig. 3B). This increase was prevented by treatment with candesartan, atrasentan, and AG-490 (Fig. 3B). We observed a similar increase in the phosphorylation levels of STAT1 and STAT3 that also was inhibited by treatment with candesartan, atrasentan, and AG-490 (data not shown). At day 28 the levels of JAK2 phosphorylation were still significantly elevated only in the untreated diabetic rats compared with the rest of the treatment groups (Fig. 3C). This increase was again prevented by treatment with candesartan, atrasentan, and AG-490 (Fig. 3C). We observed similar results with the levels of STAT1 and STAT3 phosphorylation (data not shown). At no time point were the phosphorylation levels of JAK2, STAT1, or STAT3 altered from control in the STZ-HI treatment group. These data suggest that the JAK/STAT pathway is activated in the vasculature of diabetic rats and precedes the increase in blood pressure.

Fig. 5. Densitometric analysis and representative Western blots of the levels of SHP-1 (A) and SHP-2 (B) phosphorylation in sham non-diabetic rats, STZ-induced diabetic rats, sham and STZ rats treated with the AT1 receptor antagonist candesartan, sham and STZ rats treated with the ETA receptor antagonist atrasentan, and STZ diabetic rats treated with high insulin on day 14. Values are means ± SE (n = 6–7). *P ≤ 0.05 compared with sham counterpart.
Tyrosine phosphatases. Since the in vivo regulation of the JAK/STAT pathway is not clearly defined, we investigated two known regulators, the tyrosine phosphatases SHP-1 and SHP-2. In vitro, SHP-1 has been shown to dephosphorylate JAK2, whereas SHP-2 has been proposed to act as a facilitator of JAK2 activation (16). We investigated two tyrosine phosphorylation sites on each protein, since tyrosine phosphorylation of SHP-1 and SHP-2 has been shown to increase activity of the proteins (21). An increase in SHP-1 activation would result in a decrease in JAK2 phosphorylation levels, whereas an increase in SHP-2 would result in an increase in JAK2 phosphorylation levels. On day 7 we observed that the untreated diabetic rats had significantly less SHP-1 phosphorylation on both site 11 and site 15 (Fig. 4A). There was no phosphorylation of site 580 on SHP-2 on day 7 in any group. However, there was significant phosphorylation of site 542 in the sham rats treated with AG-490 and atrasentan and the untreated STZ-induced diabetic rats (Fig. 4B). These data suggest that activation of JAK2 requires both a decrease in SHP-1 activation and full activation of SHP-2 by dual phosphorylation on sites 542 and 580. The STZ-HI rats were no different from control rats (data not shown).

On day 14 there was still a significant decrease in SHP-1 phosphorylation at both sites in the untreated STZ-induced diabetic rats (Fig. 5A). However, on site 11 the diabetic rats treated with candesartan and atrasentan showed significantly elevated levels of phosphorylation (Fig. 5A). The phosphorylation of site 542 of SHP-2 was elevated only in the STZ diabetic rats (Fig. 5B). On day 14 there was also an elevated level of phosphorylation on site 580 in the sham rats treated with AG-490 and atrasentan as well as the STZ-induced diabetic rats (Fig. 5B). The STZ-HI rats were no different from control rats (data not shown).

On day 28 we did not observe any increase levels of phosphorylation on site 11 of SHP-1 in any group (Fig. 6A). Site 15 of SHP-1, however, had significantly increased levels of phosphorylation in the diabetic rats treated with candesartan and AG-490 (Fig. 6A). There were no significant differences in the phosphorylation of site 542 of SHP-2 on day 28 (Fig. 6B). There were significant increases in the levels of phosphorylation on site 580 in the sham rats treated with AG-490 and atrasentan as well as the STZ-induced diabetic rats (Fig. 6B). The STZ-HI rats were no different from control rats (data not shown).

Fig. 6. Densitometric analysis and representative Western blots of the levels of SHP-1 (A) and SHP-2 (B) phosphorylation in sham non-diabetic rats, STZ-induced diabetic rats, sham and STZ rats treated with the AT1 receptor antagonist candesartan, sham and STZ rats treated with the JAK2 inhibitor AG-490, and sham and STZ rats treated with the ETA receptor antagonist atrasentan on day 28. Values are means ± SE (n = 6–7). *P ≤ 0.05 compared with sham counterpart.
The STZ-HI rats were no different from control rats at all time points and parameters investigated (data not shown).


diabetic rats (Fig. 7). This was not observed in the rats treated with AG-490, suggesting that preceding an increase in blood pressure, AG-490 inhibition is beneficial. AG-490 treatment had no significant effect on the endothelium-dependent relaxation in the sham rats or the contraction to KCl in either sham or STZ-treated rats (Fig. 7A). In contrast, on day 28 there was a significant loss of endothelium-dependent relaxation in the diabetic rats, and this was significantly inhibited by treatment with AG-490 (maximal relaxation obtained: 95.3 ± 3.0, 92.6 ± 7.4, 76.9 ± 12.1, and 38.3 ± 13.1 in sham, sham + AG-490, STZ + AG-490, and STZ rats, respectively) (Fig. 7B). Similarly to day 14, AG-490 treatment did not affect the KCl-induced contraction on day 28 in the aorta from either the sham or diabetic animals. These data demonstrate that inhibition of the JAK/STAT pathway in vivo does attenuate the loss of endothelial cell function and is consistent with a role for this pathway in mediating endothelial dysfunction associated with diabetes.

**DISCUSSION**

These findings are the first to show the time line for activation of the JAK/STAT pathway in vivo in type 1 diabetes in VSMC. Furthermore, these data suggest that the increased activation of the JAK/STAT pathway precedes any changes in blood pressure observed in the diabetic rats. Activation of JAK2 appears to be dependent on ET-1 and ANG II, since their inhibition prevented the increased activation of JAK2. These are the only studies to examine the site-specific phosphorylation and resultant activation of SHP-1 and SHP-2 in VSMC in type 1 diabetes as well. In addition, these novel findings are the first to suggest that inhibition of JAK2 in vivo prevents loss of endothelial function as measured by Ach-induced relaxation. These results support the hypothesis that inhibition of JAK2 would prevent endothelial cell dysfunction and the development of vascular complications.

Inhibition of JAK2 does not appear to have any adverse metabolic effects in vivo. This is the only study to our knowledge to administer chronic (28 days) AG-490 to animals, and the control animals showed no adverse reaction as measured by their body weight and blood pressure. Furthermore, inhibition of JAK2 and the ETA receptor did not improve the blood glucose levels of the diabetic rats. However, treatment with candesartan did result in an improvement in the blood glucose levels in the diabetic animals. These data are consistent with the literature. Other investigators have reported similar findings with some AT1 receptor antagonists, such as telmisartan, exhibiting partial peroxisome proliferator-activated receptor (PPAR)-γ receptor agonist activity (19, 30).

Although these data support the idea that activation of the JAK/STAT pathway is involved in the development of diabetic complications, the exact mechanisms of regulation of activation of the JAK/STAT pathway in vivo are not yet clear. Previous work has demonstrated an enhancement of JAK2 activation in VSMC after 24 h of exposure to high glucose in vitro (7). However, increased levels of JAK2 phosphorylation were not observed in vivo until 14 days of diabetes even though the diabetic animals had elevated levels of blood glucose 48 h post-STZ injection. These data suggest that the regulatory mechanisms of the JAK/STAT pathway in vivo may be different from those observed in vitro. Therefore, further investigation into the potential regulators of activation, particularly SHP-1 and SHP-2 but also the suppressors of cytokine signaling (SOCS), in disease states is an important avenue of future research.

JAK2 signaling is normally controlled by a balance between activation and deactivation by phosphatases. In vitro, SHP-1 dephosphorylates JAK2, whereas SHP-2 acts as a facilitator of JAK2 activation (16). Previous studies in rat glomeruli have shown that SHP-1 and SHP-2 phosphorylation is altered in diabetes (5). After 14 days of STZ-induced diabetes, the...
tyrosine phosphorylation of SHP-1 was significantly reduced, and this was unaffected by treatment with the AT1 receptor antagonist candesartan (5). The tyrosine phosphorylation of SHP-2 was significantly increased on day 14 of diabetes and was significantly reduced by treatment with candesartan (5). These data suggest that changes in the activation of these phosphatases may contribute to the altered levels of JAK2 activation and that there are ANG II-dependent and -independent effects.

Furthermore, SHP-1 activity has been shown to be sensitive to levels of reactive oxygen species (ROS) and is inhibited by high levels of ROS (14). Levels of ROS have been shown to be elevated in diabetes (8, 22). Therefore, the role of ROS in diabetes and its effect on phosphatases, for SHP-1, SHP-2, protein tyrosine phosphatase (PTP)-1B, T-cell PTP, and others is an area that requires further research. Future research into the regulation of SHP-1 in diabetes might involve examination of the serine 591 site. This site has been suggested by Jones et al. (12) to be a negative regulator of SHP-1 activation and a substrate of PKC in platelets. However, there is very little information available about the regulation of SHP-1 in VSMC. Studies have previously demonstrated that high glucose alone can activate PKC (3), and alterations in PKC levels have been reported previously in vivo under diabetic conditions (13, 28). Therefore, the elevated levels of ROS and PKC activity may inhibit SHP-1 and remove its negative regulation of JAK2, thereby contributing to the development of diabetic complications. These data also suggest that activation of SHP-2 and the resultant activation of JAK2 in vivo are dependent on both sites of SHP-2 being phosphorylated.

Further understanding of the role of JAK2 and its regulation in the various cell types is of obvious importance to the vasculature. Others have demonstrated that after vascular injury, ANG II activates the JAK/STAT pathway in the rat carotid artery and inhibition of the JAK2/STAT3 pathway resulted in reduced neointimal formation in the injured artery (25). These data suggest a critical role for this pathway in vascular remodeling, which is as yet poorly understood. Previous work by our group (29) in vitro demonstrated that activation of JAK2 in the endothelial cells was detrimental to survival; however, activation of JAK2 in VSMC results in increased DNA synthesis and growth (6). These findings are further supported by our in vivo findings, which suggest that activation of JAK2 is detrimental to endothelial cell function and may result in loss of vascular function and uncontrolled VSMC growth that contributes to the development of atherosclerosis and vascular disease common to diabetes. At this time it is not possible to separate the effects of lowered blood pressure on the protection of endothelial function and inhibition of JAK2, ANG II, and ET-1. However, the data obtained on day 14 preceding the increase in blood pressure support the idea that JAK2 activation is detrimental to endothelial cell function.

Conclusions. These data are the first to show alterations in the regulators SHP-1 and SHP-2 in VSMC in type 1 diabetics. These novel data also demonstrate for the first time the importance of the JAK/STAT pathway in the development of endothelial dysfunction in vivo. These data support the hypothesis that both ANG II and ET-1 can mediate the activation of JAK2 in the vasculature of the STZ-induced diabetic rat and that JAK2 phosphorylation is an important step in the development of diabetic vascular complications. Inhibition of JAK2 may provide a novel therapeutic approach in the treatment of diabetic complications.

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