ANG II enhances contractile responses via PI3-kinase p110β pathway in aortas from diabetic rats with systemic hyperinsulinemia

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Kobayashi, Tsuneo, Yuko Hayashi, Kumiko Taguchi, Takayuki Matsumoto, and Katsuo Kamata. ANG II enhances contractile responses via PI3-kinase p110β pathway in aortas from diabetic rats with systemic hyperinsulinemia. Am J Physiol Heart Circ Physiol 291: H846–H853, 2006. First published March 3, 2006; doi:10.1152/ajpheart.01349.2005.—We investigated the involvement of ANG II and phosphatidylinositol 3-kinase (PI3-K) in the enhanced aortic contractile responses induced by hyperinsulinemia in chronic insulin-treated Type 1 diabetic rats. Plasma ANG II levels were elevated in untreated compared with control diabetic rats and further increased in insulin-treated diabetic rats. Aortic contractile responses and systolic blood pressure were significantly enhanced in chronic insulin-treated diabetic rats compared with the other groups. These insulin-induced increases were largely prevented by cotreatment with losartan (an ANG II type 1 receptor antagonist) or enalapril (an angiotensin-converting enzyme inhibitor). LY-294002 (a PI3-K inhibitor) diminished the increases in contractile responses in ANG II-incubated aortas and aortas from diabetic rats compared with the other groups. The norepinephrine (NE)-stimulated levels of p110β-associated PI3-K activity were increased in aortic contractile responses and vascular contractility and blood pressure via a decrease in p110β-associated PI3-K activity.

Diabetes; phosphatidylinositol 3-kinase; contraction

Diabetes mellitus is an important risk factor for increased blood pressure and development of atherosclerosis (4–6, 11). Many of the vascular complications of Type 1 and Type 2 diabetes arise from a hyperglycemia that cannot be completely prevented by blood glucose control methods available today (23, 39, 33). Among these complications, diabetes-accelerated atherosclerosis and hypertension are most likely multifactorial in origin, with hyperinsulinemia being one of several possible causes (34, 37). Indeed, systemic hyperinsulinemia is inevitable during the insulin treatment of Type 1 diabetes mellitus, and it may play an important role in the progression of coronary artery disease (34, 37). One possibility raised by previous in vitro studies is that the hyperinsulinemia may increase the sensitivity of blood vessels to vasoconstrictors such as ANG II or catecholamines (9, 11, 19, 21). A few years ago, we demonstrated that, in aortas isolated from rats with streptozotocin (STZ)-induced diabetes, high-dose insulin treatment leading to systemic hyperinsulinemia can enhance norepinephrine (NE)-induced contractility (19). The above-mentioned effects may occur as a result of an enhancement of the insulin-like growth factor I system (21).

Class I phosphatidylinositol 3-kinase (PI3-K) is a multifunctional, heterodimer enzyme composed of a catalytic subunit (p85α, p85β, p55γ, and p101) and a regulatory subunit (p110α, p110β, p110δ, and p110γ) (8, 24, 27). Most attention has focused on the pathways involved in cell growth and migration; however, within the last few years, PI3-K activity has been implicated in vascular smooth muscle contractility and relaxation (3, 22, 26, 31, 32). Several groups have presented evidence that links PI3-K activity and phosphatidylinositol 1,4,5-trisphosphate (PIP3) production to the opening of voltage-gated and receptor-coupled Ca2+ channels (25, 26, 38). Furthermore, it has been suggested that NE and ANG II, as G protein-coupled receptor activators, exhibit cross talk with PI3-K activity in human vascular smooth muscle cells (10, 13). Northcott and co-workers (31, 32) suggested that, in aortas from deoxycorticosterone acetate-salt hypertensive rats, increases in PI3-K activity and PI3-K protein expression, specifically the p110β subunit, are associated with an increase in agonist-induced contractility.

ANG II is a vasoactive peptide that has been suggested to be involved in the pathogenesis of cardiovascular diseases. Indeed, an enhanced renin-angiotensin system has been reported in diabetic subjects with vascular complications, in diabetic rats, and in insulin-infused diabetic rats (15, 35). Clinical studies in Type 1 diabetic patients have revealed the cardiovascular protection afforded by blockade of the renin-angiotensin system (14). Furthermore, it has been suggested that the actions of insulin on blood vessels, especially its effects on cell growth, may be mediated through activation of the renin-angiotensin system (17). Thus we speculated that an increased ANG II level and an abnormality of the PI3-K pathway system might be related to the improvement in vascular contractility previously observed in insulin-treated Type 1 diabetic rats (see above).

Although it is known that changes in the p85 regulatory subunit of PI3-K occur in insulin-resistance models, suggesting that the expression level of this regulatory subunit might modulate insulin sensitivity in vivo (1, 29), the mechanisms underlying the changes in the PI3-K pathway that are associated with macrovascular disease in diabetes are not understood. To address this issue, we decided to examine the rat aorta, which is dependent on PI3-K for its contraction. The aim of the...
present study was to investigate the relation between the PI3-K system and ANG II in the enhancement of aortic contraction in insulin-treated diabetic rats. We also examined whether chronic ANG II type 1 receptor blockade might blunt the enhancements of vascular contractility and blood pressure and any increases in PI3-K activities/proteins that occur in association with systemic hyperinsulinemia.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats (8 wk old, 180–250 g body wt) were treated with a single injection of STZ (65 mg/kg dissolved in a citrate buffer) via the tail vein (19, 20, 28). Food and water were given ad libitum. The experimental design was approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals,” published by the National Institutes of Health and adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (accredited by the Ministry of Education, Culture, Sports, Science and Technology, Japan).

**Chronic administrations.** The STZ-induced diabetic rats (8 wk after STZ injection) and the control rats were treated with a gradually increasing daily dose of insulin (human crystalline insulin zinc, 5–30 U·kg⁻¹·day⁻¹; Novo Nordisk) for 2 wk (19–21). For chronic insulin + losartan or insulin + enalapril treatment, losartan (25 mg·kg⁻¹·day⁻¹; Nulotan, Banyu, Tsukuba, Japan) or enalapril (20 mg·kg⁻¹·day⁻¹, Renivase, Banyu) was administered concomitantly with the above-described insulin treatment for 2 wk (starting 8 wk after STZ injection).

**Measurement of plasma glucose, insulin, ANG II, and blood pressure.** Plasma glucose and insulin were measured as described previously (19–21). Plasma ANG II was eluted with methanol using C-18 reverse-phase HPLC columns (Cayman Chemical) and measured using a commercially available ANG II enzyme immunoassay kit (SPI-BIO, Massy Cedex, France) according to the manufacturer’s instructions. Systolic blood pressure was measured using a standard tail-cuff procedure.

**Measurement of isometric force.** Rats were anesthetized with diethyl ether and killed by decapitation. A section of the thoracic aorta from the region between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution. The aorta (cut into helical strips) was placed in a bath containing 95% O₂ (95 mol/mol)/l) in the presence of LY-294002 (2 × 10⁻⁵ mol/l), or vehicle for 5 min at 37°C, and PI3-K activity was estimated using a commercially available PI3-K ELISA kit (Echelon Biosciences, Salt Lake City, UT) according to the manufacturer’s instructions. Briefly, the tissues were pulverized in a liquid nitrogen-cooled mortar and then solubilized in PI3-K lysis buffer, as previously described (18). The supernatant was mixed with p110α antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and incubated for 2 h. Then protein A-agarose beads were added to equal amounts of total protein, and the samples were rocked (4°C) for 1 h. The immunoprecipitated p110α was incubated with phosphatidylinositol 4,5-bisphosphate substrate and reaction buffer in the absence or presence of LY-294002 (2 × 10⁻⁵ mol/l) for 2 h. The amount of PIP₃ formed from phosphatidylinositol-4,5-bisphosphate by PI3-K activity was detected using a competitive ELISA.

**Measurement of protein expressions of p85 and p110 subunits of PI3-K by Western blotting.** Aortas (100 μg of total protein) were homogenized in ice-cold lysis buffer, and Western blotting was performed as previously reported (21, 22). The membrane was incubated with anti-p85α subunit antibody (1:2,000 dilution; BD Bioscience, San Jose, CA), anti-p110α subunit antibody (1:300 dilution; Santa Cruz Biotechnology), or anti-p110β subunit antibody (1:500 dilution; Santa Cruz Biotechnology), or β-actin antibody (1:5,000 dilution; Sigma, St. Louis, MO) in blocking solution. To normalize the data, we used β-actin as a housekeeping protein.

**Statistical analysis.** The contractile force developed by aortic strips is expressed in milligrams of tension per milligram of tissue. Values are means ± SE. When appropriate, statistical differences were assessed by Dunnett’s test for multiple comparisons after a one- or two-way ANOVA; *P < 0.05 was regarded as significant. Statistical comparisons between concentration-response curves were made using a one-way ANOVA, with post hoc correction for multiple comparisons by Bonferroni’s test; P < 0.05 was considered significant.

**RESULTS**

**Plasma glucose, insulin, ANG II, and systolic blood pressure.** Nonfasting plasma glucose levels were significantly elevated after STZ treatment, whereas 2 wk of treatment of our diabetic rats with insulin (5–30 U·kg⁻¹·day⁻¹) or insulin + losartan resulted in a plasma glucose concentration that was not different from that of the controls (Table 1). Plasma insulin levels were significantly lower in the diabetic rats than in their controls and significantly higher in the insulin-treated diabetic group than in the non-insulin-treated control group. It is known that ANG II antagonism (e.g., by losartan) can improve insulin sensitivity in insulin-resistance models, such as fructose-induced and insulin-infused hyperinsulinemic rats (6, 30). Among our various insulin-treated diabetic rats, plasma insulin levels tended to be lower (but not significantly) in animals treated with insulin + losartan than in those treated with insulin alone (insulin-treated diabetic vs. insulin + losartan-treated, P = 0.11). Plasma ANG II levels were significantly elevated in diabetic rats compared with controls and further

**Table 1. Glucose, insulin, ANG II, and systolic blood pressure in control and diabetic rats**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Insulin-Treated Control</th>
<th>Insulin-Treated Diabetic</th>
<th>Insulin + Losartan-Treated Diabetic</th>
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<tr>
<td>Glucose, mg/dl</td>
<td>156 ± 6</td>
<td>541 ± 26*</td>
<td>136 ± 22</td>
<td>191 ± 61†</td>
<td>195 ± 77†</td>
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<tr>
<td>Insulin, pg/ml</td>
<td>881 ± 128</td>
<td>ND</td>
<td>2,882 ± 461*</td>
<td>3,355 ± 261*</td>
<td>2,610 ± 314*</td>
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<tr>
<td>ANG II, pg/ml</td>
<td>20.2 ± 2.3</td>
<td>30.8 ± 5.0*</td>
<td>35.5 ± 4.3*</td>
<td>91.8 ± 11.3†</td>
<td>80.4 ± 7.8†</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>103 ± 3</td>
<td>100 ± 2</td>
<td>108 ± 4</td>
<td>118 ± 2†</td>
<td>95 ± 2§</td>
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*Values are means ± SE (n = 8). ND, beneath lower limit of detection. *P < 0.05 vs. control. †P < 0.05 vs. diabetic. ‡P < 0.05 vs. insulin-treated control. §P < 0.05 vs. insulin-treated diabetic.
elevated in insulin-treated diabetic rats, and they were significantly higher in insulin-treated diabetic rats than in insulin-treated controls. Among the various control rats, plasma ANG II levels were slightly but significantly higher in insulin-treated than in untreated controls. The significantly higher systolic blood pressure in insulin- than in non-insulin-treated diabetic rats was significantly reduced by treatment with losartan.

**Vascular reactivity in aorta.** Insulin treatment had no significant effect on the contraction induced by NE or isotonic high K⁺ in control rats (Fig. 1, A and B). There were no significant differences, in terms of maximum contractile force or sensitivity, between control and diabetic rats (data not shown). Insulin treatment of our diabetic rats enhanced the NE- or isotonic high K⁺-induced aortic contractility to above that of the untreated diabetic rats (Fig. 1, C and D). These enhancement effects induced by chronic insulin treatment were largely prevented by chronic cotreatment with losartan, an ANG II type 1 receptor antagonist, or enalapril, an angiotensin-converting enzyme (ACE) inhibitor (Fig. 1, C and D). There was no significant difference in the above-described contractile responses between insulin + losartan-treated and untreated diabetic rats or between insulin + enalapril-treated and untreated diabetic rats. In control (vs. untreated control) or diabetic (vs. untreated diabetic) rats, losartan had no significant effects on the NE-induced contraction (data not shown).

When LY-294002 (2 × 10⁻⁵ mol/l) was added to aortic strips, the NE sensitivity tended to be reduced, whether the strips were from control (data not shown) or diabetic (Fig. 2A)

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Fig. 1. Concentration-response curves for norepinephrine (NE)-induced (A and C) and isotonic K⁺-induced (B and D) contractions in endothelium-denuded strips from age-matched control (A and B) and untreated diabetic (C and D) rat aortas. Aortic strips were treated with chronic insulin (insulin), insulin + losartan, or insulin + enalapril. Increase in tension was measured at peak of response. Values are means ± SE from 8–10 experiments; SE is shown only when it is larger than the symbol. *P < 0.05, insulin-treated vs. untreated diabetic. †P < 0.05 vs. insulin-treated diabetic.

Fig. 2. Concentration-response curves for NE-induced (A) and isotonic K⁺-induced (B) contractions of aortic strips from diabetic and chronic insulin-treated diabetic (chronic insulin) rats, together with effects of LY-294002 (2 × 10⁻⁵ mol/l). Increase in tension was measured at peak of response. Values are means ± SE from 8–10 experiments; SE is shown only when it is larger than the symbol. *P < 0.05, insulin-treated vs. untreated diabetic. †P < 0.05, LY-294002-incubated insulin-treated diabetic vs. insulin-treated diabetic.
r artists, but the differences were not significant. In aortic strips from insulin-treated diabetic rats, NE-induced (Fig. 2A) and K⁺-induced (Fig. 2B) contractions were enhanced after pretreatment with LY-294002. In the presence of LY-294002, there was no significant difference in the maximum contractile response between the groups. The isotonic high-K⁺-induced contractions were not changed by pretreatment with LY-294002 in control (data not shown) or diabetic rats (Fig. 2).

In rat aortas, the contraction induced by ANG II (10⁻⁹–10⁻⁶ mol/l) was markedly weaker, in terms of maximal tension, than that induced by NE or isotonic K⁺, as previously described (36), and there was no significant difference in the ANG II-induced contractile response between untreated control and untreated diabetic rats. LY-294002 inhibited ANG II-induced contraction in control and diabetic rats, with significantly weaker contraction in treated strips from diabetic than from control rats (Fig. 3A). Incubation of control aortas with a low concentration (3 × 10⁻⁸ mol/l) of ANG II (to induce ~10% of the maximum contraction), a physiological dose (10⁻⁷, 10⁻⁶ mol/l) of ANG II (to induce 10–40% of maximum contraction), or low-dose ANG II + LY-294002 had no significant effect on NE-induced (Fig. 3B) or isotonic K⁺-induced (data not shown) contraction. In diabetic aortas, the NE-induced (Fig. 3C) and isotonic K⁺-induced (data not shown) contractions were enhanced by incubation with ANG II (3 × 10⁻⁸ mol/l). After aortas from diabetic rats were incubated with a physiological dose of ANG II (10⁻⁷ or 10⁻⁶ mol/l), the NE- and isotonic K⁺-induced contractile responses were increased (data not shown). Incubation with ANG II + LY-294002 prevented the enhancement of contraction observed after incubation with ANG II alone (Fig. 3C).

**p110α-Associated PI3-K activity.** Having previously established the existence of a functional change in p110α-associated PI3-K in aortas from diabetic rats, we set out to measure PI3-K activity stimulated by NE (10⁻⁸ mol/l) and NE (10⁻⁸ mol/l) + ANG II (10⁻⁸ mol/l). Incubation with NE or ANG II increased p110α-associated PI3-K activity in aortas from control and diabetic rats (vs. basal). NE-stimulated PI3-K activity was not significantly different between control and diabetic groups. However, the basal and ANG II-stimulated PI3-K activity levels were significantly elevated in diabetic compared with control rats (Fig. 4). Stimulation with NE + ANG II increased PI3-K activity in aortas from diabetic compared with control rats; in the diabetic group, however, PI3-K activity was significantly higher in ANG II + NE- than in NE-stimulated aortas (Fig. 4).

Chronic treatment of control rats with insulin did not significantly change the NE-stimulated PI3-K activity: 13.3 ± 1.0 vs. 12.5 ± 1.0 mol·mg protein⁻¹·h⁻¹ for untreated controls (P = 0.52). Among diabetic rats, NE-stimulated PI3-K activity was significantly higher (~29%) in aortas from insulin-treated than from untreated animals: 21.1 ± 1.7 vs. 16.4 ± 1.2 mol·mg protein⁻¹·h⁻¹ (P = 0.039). Insulin + losartan greatly attenuated (P < 0.001) the increase in NE-stimulated p110α-associated PI3-K activity (9.9 ± 0.8 mol·mg protein⁻¹·h⁻¹) compared with that observed in aortas from diabetic rats treated with insulin alone (Fig. 5). LY-294002 was tested for its ability to inhibit PI3-K activity at 2 × 10⁻⁵ mol/l, which is the concentration used in the contractile studies. LY-294002 completely abolished (to below the lower limit of detection) enzymatic activity (basal and ANG II-stimulated PI3-K activity) in aortas from control and diabetic rats. No significant change in NE-stimulated PI3-K activity (vs. the untreated groups) was observed after chronic losartan treatment in control (9.9 ± 1.2 mol·mg protein⁻¹·h⁻¹, P = 0.14) or diabetic (13.3 ± 1.4 mol·mg protein⁻¹·h⁻¹, P = 0.23) rats.

Expression of proteins for p85 and p110 subunits of PI3-K. Western blotting was performed on rat aortas. Use of anti-p85, anti-p110α, and anti-p110β subunit antibodies allowed detection of 85- or 110-kDa immunoreactive proteins (Fig. 6A). Expression of PI3-K p85 was significantly increased in aortas from diabetic compared with control rats (P = 0.045). Each insulin-treated group showed a greatly attenuated p85 expres-
mersion [control vs. insulin-treated control (P = 0.045) and diabetic vs. insulin-treated diabetic (P = 0.004)], and insulin + losartan treatment did not alter this decreased expression (Fig. 6B). Expression of p110γ was not significantly different among the various groups (data not shown). In contrast, expression of p110 was significantly elevated in aortas from diabetic compared with control rats (P < 0.001) and further elevated in aortas from insulin-treated diabetic rats (diabetic vs. insulin-treated diabetic, P = 0.02). Insulin treatment of control rats raised their plasma insulin concentration to a level not different from that of the insulin-treated controls. Such insulin admin-

**DISCUSSION**

The main conclusion to be drawn from the present study is that the mechanism underlying the enhancement of aortic contraction in insulin-treated Type 1 diabetic rats with hyperinsulinemia may involve increased activity in the PI3-K pathway. Furthermore, our data suggest that chronic ANG II type 1 receptor blockade blunts the increase in blood pressure and aortic contractility in insulin-treated diabetic rats via decreases in p110 subunit-associated PI3-K activity and p110 protein expression.

Administration of insulin to our established diabetic rats raised their plasma insulin concentration to a level not different from that of the insulin-treated controls. Such insulin admin-
istration to diabetic rats increased aortic contractility and systolic blood pressure to values above those seen in the insulin-treated controls. Thus insulin alone is not sufficient to increase aortic contraction in the rat aorta; instead, we suspect that a high insulin level and a diabetic state are needed to enhance contractility. In this study, the plasma ANG II level was considerably higher in the insulin-treated diabetic rats than in the insulin-treated controls, suggesting that the insulin-induced enhancement of aortic contractility in diabetic rats may be due to an increase in plasma ANG II concentration.

Systemic hyperinsulinemia is inevitable during insulin treatment of Type 1 diabetes mellitus, and it may play an important role in the progression of coronary artery disease (34, 37). In accordance with previous results, the hyperinsulinemia resulting from our insulin treatment of diabetic rats enhanced the NE- and isotonics high-K⁺-induced aortic contractility to levels above those seen in the other groups (19, 21). Recently, PI3-K and PIP₃ formation were found to activate voltage-gated Ca²⁺ channels and Ca²⁺ influx in vascular myocytes, and PI3-K and PIP₃ were shown to be involved in contractile hyperactivity in vascular smooth muscle (25, 26, 32). Here, we found that a PI3-K inhibitor, LY-294002, had a normalizing effect on the enhanced NE- and K⁺-induced contractions in aortas from insulin-treated diabetic rats. Because this PI3-K inhibitor had no effects on NE- or K⁺-induced contractile responses in aortas from control, untreated diabetic, or insulin-treated control rats, any abnormalities in the PI3-K system were presumably specific to aortas from insulin-treated diabetic rats. In addition, the NE-stimulated p110α-associated PI3-K activity (PIP₃ production assay) and p110α protein expression were greatly increased in aortas from insulin-treated diabetic rats. Thus our data suggest that the increase in vascular contractility in the aorta of the insulin-treated diabetic rat may be due to increased p110α-associated PI3-K activity.

ANG II and insulin are multifunctional peptide hormones that exert major regulatory influences over the cardiovascular system. Clinical studies in Type 1 diabetic patients have demonstrated the benefits of blockade of the renin-angiotensin system in terms of cardiovascular protection (14). In our hyperinsulinemia model, the plasma ANG II level was greatly elevated in insulin-treated diabetic rats. Chronic ANG II type 1 receptor blockade or ACE inhibition (by treatment with losartan or enalapril, respectively) blunted the increases in aortic contractility and blood pressure and in p110α subunit-associated PI3-K activity and p110α protein expression in the insulin-treated diabetic group. These observations suggest that the increased plasma ANG II level enhanced aortic contractility through the p110α subunit-associated PI3-K pathway.

We wondered whether, physiologically, ANG II increases PI3-K activity and whether a noncontractile concentration of ANG II might augment NE- or K⁺-induced contractility in the diabetic aorta via the PI3-K pathway. In our study, 1) the ANG II-induced contractile response was unchanged between control and diabetic rats, and 2) treatment with a PI3-K inhibitor attenuated ANG II-induced contraction in the control and diabetic groups, with weaker contraction in treated strips from diabetic than from control rats. These results suggest that the ANG II-induced contractile response is enhanced by PI3-K activity in the diabetic aorta. ANG II stimulated p110α-associated PI3-K activity in aortas from diabetic rats. However, our study and other studies have found that in the rat aorta the ANG II-induced contraction is markedly weaker (in terms of maximal tension) than NE- or isotonics K⁺-induced contraction (36). Furthermore, the main substances in the plasma that influence vascular tone are NE (the agent used in the present study) and epinephrine (40). We found that preincubating aortas with a low concentration of ANG II augmented NE-induced contractility and p110α-associated PI3-K activity in the diabetic group. Moreover, a PI3-K inhibitor prevented these ANG II-induced enhancements of NE- and K⁺-induced contractions. Thus the NE-induced contractile response in diabetic aorta may be enhanced by ANG II via increases in PI3-K activity and protein expression. This conclusion is consistent with the finding that the contractile response to NE was greater in aortas from insulin-treated diabetic than from untreated diabetic rats, whereas control aortas displayed no such effect after the same insulin treatment. These results strongly suggest that in diabetic rats with systemic hyperinsulinemia the NE-induced contractile response is enhanced by ANG II via an increase in p110α-associated PI3-K activity.

Interestingly, PI3-K p85 expression was slightly increased in aortas from diabetic rats. However, each insulin-treated group showed a greatly attenuated p85 expression, and ANG II receptor blockade did not alter this decreased expression. Thus changing the ANG II levels in vivo may not necessarily have beneficial effects in terms of p85 expression. During glucose metabolism, the activation of PI3-K by insulin is mainly mediated by binding of the p85 regulatory subunit to tyrosine-phosphorylated insulin receptor substrate protein (24). A recent study demonstrated that expression of the p85 subunit in skeletal muscle can be regulated by growth hormone (2). Therefore, it is possible that a number of growth factors and hormones could play important roles in the development of diabetic complications and insulin resistance. In our hyperinsulinemic (STZ diabetes) or systemic hyperinsulinemic (insulin-treated) diabetic models, increases in p85 expression might be initiated by changes in the level of insulin or any of several hormones in plasma.

PI3-K activity is complex, because PI3-K comprises, e.g., a p85 monomer, p85-p85 dimers, and p85-p110 heterodimers, which are regulated in ways that together lead to an alteration in PI3-K activity in response to an insulin signal (24, 27). We found that an ANG II receptor blocker prevented the insulin-induced increase in PI3-K activity in the diabetic aorta without changing the decreased expression of p85 subunit protein. It is unclear, however, whether complex alterations in the p85/p110 subunits contribute to the increased PI3-K activity and increased vascular contractility in insulin-treated diabetic rats. In the last few years, evidence has accumulated to suggest that an increase in the p85 monomer and a decrease in the p85-p110 heterodimer may play roles in negative PI3-K regulation as part of the insulin response (1, 29). Furthermore, cross-talk regulation of PI3-K activity by ANG II (although, admittedly, the biological actions of ANG II on PI3-K activities remain controversial) could play an important role in the development of insulin resistance (12, 16). Because PI3-K-associated hyperactivity in the aorta might be due to alterations in the p85-p110 complex, further studies focusing on the vascular hyperactivity seen in association with systemic hyperinsulinemia are needed to examine the effects of the p85-p110 complex in insulin-treated diabetic rats.
Taken together, our findings indicate that, in insulin-treated Type 1 diabetic rats with systemic hyperinsulinemia, treatment with an ANG II type I receptor blocker or an ACE inhibitor can almost totally prevent development of the hypertension and aortic vascular hyperactivity that are associated with an increase in the p110β subunit of PI3-K. It is suggested that, in diabetic patients with hyperinsulinemia, an increase in plasma ANG II might contribute to an augmentation of vascular contractile function via the PI3-K pathway and, hence, might accelerate the progression of hypertension.

REFERENCES


