Angiotensin II utilizes Janus kinase 2 in hypertension, but not in the physiological control of blood pressure, during low-salt intake

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JAK2 is activated in vascular smooth muscle cells and in vascular function by ANG II. The JAK2/STAT3 pathway could play an important role in the regulation of these responses by blocking JAK2 activation chronically with AG-490. In male Sprague-Dawley rats, after a control period, ANG II (10 ng·kg−1·min−1) was infused intravenously with or without AG-490 at 10 ng·kg−1·min−1 iv for 11 days. ANG II infusion (18 h/day) increased mean arterial pressure from 91 ± 3 to 168 ± 7 mmHg by day 11. That response was attenuated significantly in the ANG II + AG-490 group, with mean arterial pressure increasing only from 92 ± 5 to 127 ± 3 mmHg. ANG II infusion markedly decreased urinary sodium excretion, caused a rapid and sustained decrease in glomerular filtration rate to ∼60% of control, and increased renal JAK2 phosphorylation; all these responses were blocked by AG-490. However, chronic AG-490 treatment had no effect on the ability of a separate group of normal rats to maintain normal blood pressure when they were switched rapidly to a low-sodium diet, whereas blood pressure fell dramatically in losartan-treated rats on a low-sodium diet. These data suggest that activation of JAK2/STAT3 pathways is critical for the development of ANG II-induced hypertension by mediating its effects on renal sodium excretion, but the physiological control of blood pressure by ANG II with a low-salt diet does not require JAK2 activation.

Intracellular signaling; mean arterial pressure; AG-490; losartan; sodium-deficient diet

Janus kinase 2 (JAK2) has been implicated as a component of the angiotensin type 1 (AT1) receptor signaling cascades in many cell types in vitro, including vascular smooth muscle cells (11), kidney glomerular mesangial cells (1a), and endothelial cells (17). ANG II activates JAK2 and its target, the STAT3 pathway, to elicit its effects. In vitro and ex vivo studies have linked activation of JAK2 to vascular smooth muscle cell proliferation (11), endothelial cell apoptosis (17), and vascular contractility (7). This provides evidence that the JAK/STAT pathway could play an important role in the regulation of vascular function by ANG II.

Furthermore, in vivo in a rat model of type 1 diabetes, JAK2 is activated in vascular smooth muscle cells and in kidney glomerular mesangial cells (2, 3). In addition, in vivo inhibition of JAK with AG-490 protects against the development of endothelial dysfunction in diabetes and increased blood pressure (3) and decreases neointimal formation after balloon injury in the rat carotid artery (16). These data suggest that activation of JAK2 in vivo may contribute to the development of cardiovascular dysfunction and remodeling associated with disease states. While the exact role of JAK2 in cardiovascular disease is unknown, these studies suggest that chronic activation of JAK2 has an important role in cardiovascular pathophysiology.

Recently, in a mouse model of ANG II-induced hypertension, chronic treatment with AG-490 prevented the development of hypertension (7). However, the mechanism is still unclear. Our recent results in mice showed increased JAK2/STAT3 phosphorylation in kidneys during ANG II-induced hypertension (5). Therefore, the goal of this study was to determine whether the blood pressure-lowering actions of chronic JAK2 inhibition in ANG II-induced hypertension were accompanied by attenuation of the renal sodium-retaining and vasconstrictor actions of ANG II. This was tested by blocking JAK2 activation chronically with AG-490. In addition, we tested whether the role of ANG II in maintaining normal blood pressure in rats on a low-sodium regimen also was dependent on JAK2 activation, thereby possibly identifying the JAK2/STAT3 pathway as a mechanism that isolates the pathological from the physiological actions of chronic elevations in ANG II.

METHODS

Animal Instrumentation

All experiments were conducted in male Sprague-Dawley rats (350–375 g; Harlan Sprague Dawley, Madison, WI), and protocols were approved by the Institutional Animal Care and Use Committee. Rats were instrumented with chronic abdominal aortic and femoral vein catheters, as described previously (15). The catheters were routed subcutaneously to the scapular region and exteriorized through a Dacron-covered button sutured subcutaneously over the scapulae.

After recovery, the rats were placed in individual metabolic cages with a 12:12-h light-dark cycle. The catheters were passed through a stainless steel spring that tethered the animals to a dual-channel hydraulic swivel (Instech) mounted above the cage. The venous catheter was connected, via the hydraulic swivel, to a syringe pump (Harvard Apparatus) that ran continuously throughout the study. All solutions were infused through a filter (0.22 μm; Cathivex, Millipore). The arterial catheter was filled with heparin solution (1,000 USP U/ml) and connected, also via the hydraulic swivel, to a pressure transducer for continuous measurement of arterial pressure. The amplified pulsatile arterial pressure signals (CB Sciences) were sam-
Sodium intake was clamped at ~3.1 mmol/day by continuous intravenous infusion of 18 ml of sterile saline (9 g/l NaCl) per day combined with sodium-deficient rat chow (0.006 mmol sodium/g; Teklad). A sodium-deficient diet ensured that the daily sodium intake could be controlled precisely by the infusion. This infusion was started immediately after placement of the rat in the metabolic cage. Animals were allowed ad libitum access to food and tap water. The sodium content of the tap water was measured and included in the daily sodium intake calculations.

Experimental Protocol

**Experiment 1: AG-490 in ANG II-induced hypertension.** Rats were divided randomly into five groups: control (n = 4), ANG II (n = 6), control + AG-490 (n = 5), ANG II + AG-490 (n = 10), and a subset of the ANG II + AG-490 group in which AG-490 was removed after 5 days (n = 5). After baseline measurements in animals on a normal-sodium regimen, high-salt intake (~12 mmol/day) was begun as follows: 18 ml of sterile 9 g/l NaCl solution was switched to 18 ml of sterile 36.4 g/l NaCl solution per day. All rats were allowed 4 days on the high-salt regimen before ANG II was added to the intravenous infusion for the respective groups at 10 ng·kg⁻¹·min⁻¹. In the rats receiving AG-490, ANG II at 10 ng·kg⁻¹·min⁻¹ was infused simultaneously with the ANG II infusion. The group in which AG-490 was removed, AG-490 was stopped after 5 days, but during continued ANG II infusion, to determine whether its effects were reversible.

The ANG II period (with or without AG-490) lasted 11 days. On day 3 of the baseline period and days 1 and 8 of the ANG II period, 1.0 ml of arterial blood was collected from the arterial catheter for measurement of glomerular filtration rate (GFR), hematocrit, and plasma protein and electrolyte concentrations. Samples were replaced with an equal volume of 9 g/l saline.

**Experiment 2: AG-490 during low-salt intake.** Rats were divided randomly into four groups: control low salt, control normal salt, low salt with losartan, and low salt with AG-490. AG-490 at 10 ng·kg⁻¹·min⁻¹ and losartan at 10 ng·kg⁻¹·min⁻¹ were administered intravenously starting on day 4 of the treatment protocol. The animals were switched from a normal-salt to a sodium-deficient infusion (18 ml of sterile water per day) on day 8 of the protocol and then euthanized on day 14.

**Experiment 3: ANG II in vivo dose response.** Rats were divided randomly into a control group and ANG II infusion groups as follows: intravenous infusion of ANG II at 1, 2.5, 5.0, and 10 ng·kg⁻¹·min⁻¹ starting on day 6 of the treatment protocol. The animals were maintained on a normal-salt diet and then euthanized on day 21.

**Analytic Methods**

GFR was measured after a 24-h intravenous infusion of [¹²³I]iothalamate (Glofil; ~20 μCi). Because steady state is achieved during the 24-h infusion, the isotope infusion rate was substituted for urinary isotope excretion rate to calculate clearance (4).

Urinary sodium and potassium concentrations were determined with atomic absorption spectrometry (model AA200, Perkin Elmer). Plasma electrolytes were measured by ion-sensitive electrodes (MEDICA Easy Electrolytes, Bedford, MA), plasma protein concentration was measured by refractometry, and hematocrit was expressed as percentage of red cell volume using microcapillary tubes. Fractional sodium excretion (FeNa) was calculated using standard formulas. Daily mean arterial pressure (MAP) was calculated by averaging 10-s periods from each minute during the 18 h of data collection.

**Tissue Homogenization for Protein Work**

Tissues were obtained from rats, quick-frozen with liquid nitrogen, pulverized in a liquid nitrogen-cooled mortar and pestle, and solubilized in a 255 mM sucrose-10 mM Tris buffer (pH 7.4) with protease (0.5 mM PMSF, 2 mM EGTA, 10 μg/ml aproitin, and 10 μg/ml leupeptin) and tyrosine phosphatase (1 mM sodium orthovanadate) inhibitors. Homogenates were centrifuged (14,000 g for 10 min at 4°C), and supernatant total protein (Bio-Rad) was measured.

**Western Blot Analysis**

Tissue homogenate supernatant (diluted 4:1 in denaturing sample buffer and boiled for 5 min) was separated on SDS-polyacrylamide gels (7.5% SDS-PAGE) and transferred to Immobilon-P membrane. Membranes were blocked [3–4 h in Tris-buffered saline (TBS) + 0.1% Tween 20 containing 5% bovine serum albumin] and probed overnight (4°C) with primary antibody [1:1,000 dilution, phospho-specific JAK2 (Biosource), phospho-specific JAK1 (Cell Signaling), or phospho-specific STAT3 (Cell Signaling)]. Blots were washed three times (30 min, 5 min, and 5 min) with TBS + Tween 20 and once with TBS (5 min). An anti-rabbit horseradish peroxidase-linked secondary antibody (1:7,500 dilution; Amersham) was added for 1 h and incubated with the blots at 4°C. Blots were washed, and enhanced chemiluminescence (Super Signals Ultra, Pierce) was used to visualize labeled bands. Blots were stripped and reprobed with the total antibody [1:1,000 dilution, JAK2 and JAK1 (Biosource) and STAT3 (BD Transduction Labs)]. β-Actin was used to ensure equal total protein loading between lanes. Band density was quantified using the program NIH Image.

**Vascular Contractility**

A separate set of untreated male Sprague-Dawley rats were euthanized, the thoracic aorta was removed and cut into 4-mm rings, and each ring was mounted in a myograph for isometric force recording (Danish Myograph Technology, Aarhus, Denmark), as previously described (10). Tissues equilibrated for 45 min under a resting tension (30 mN), and functional integrity of the endothelial cells was evaluated by testing endothelium-dependent relaxation to acetylcholine (1 μM) in rings contracted with phenylephrine (10 nM). Vessels were incubated for 30 min with the vehicle (DMSO) or AG-490 (1 μM) prior to the addition of ANG II or 5-HT for the concentration-response curves. In separate tissues, the same preincubation with vehicle or AG-490 was performed, and a single concentration of 65 mM KCl was added to the baths to determine if there were global reductions in contraction in the presence of AG-490.

**Data Analysis and Statistics**

Values are means ± SE. Statistical analysis was carried out with the GraphPad Prism program (GraphPad Software, San Diego, CA) or with SPSS software. For comparison of two groups, the appropriate Student’s t-test was used. For comparison of three or more groups, one-way ANOVA followed by a Student-Newman-Keuls post hoc test was performed to determine significance, and two-factor repeated-measures ANOVA was used for the chronic blood pressure, sodium excretion, and GFR data. For all studies, P ≤ 0.05 was considered statistically significant.

**RESULTS**

**Experiment 1: AG-490 in ANG II-Induced Hypertension**

Figure 1 shows no difference in MAP between groups during the control or high-salt alone periods. ANG II caused a rapid increase in MAP that was statistically significant by day 2 of infusion and averaged 168 ± 7 mmHg by day 11. MAP
also increased significantly in the ANG II + AG-490 group, from 90 ± 5 to 127 ± 3 mmHg, but this response was significantly lower than that of the ANG II-alone group. When AG-490 was removed after 5 days from a subgroup of rats receiving ANG II and AG-490, there was an immediate increase in MAP, such that the final MAP on day 18 was not different from that of the group that received ANG II alone throughout the study (Fig. 1).

GFR averaged 3.6 ± 0.3, 4.4 ± 0.7, 3.4 ± 0.2, 3.6 ± 0.3, and 3.0 ± 0.5 ml/min at baseline in the control, ANG II, control + AG-490, ANG II + AG-490, and AG-490 removal groups, respectively, and there were no statistically significant differences between groups. At 24 h after the start of ANG II infusion, there was a significant decrease in GFR in the ANG II treatment group that was sustained throughout the study (Fig. 2A), but there was no change in GFR in any of the other groups. Removal of AG-490 caused GFR to decrease in the AG-490 removal group and approach levels in the ANG II-treated rats that did not receive any AG-490 (Fig. 2A).

Urinary sodium excretion tracked sodium intake, and there were no significant differences between groups during the baseline and high-salt-alone periods (Fig. 2B). However, ANG II infusion decreased sodium excretion significantly for the first 2 days in the ANG II group, while there was no evidence of sodium retention in either of the ANG II groups that also received AG-490 (Fig. 2B). It is not clear why a sodium excretion “overshoot” was not observed in the ANG II group, as they returned to sodium balance, but there was no significant difference or change in FE$_{\text{Na}}$ during control) between or within control, control + AG-490, ANG II, and ANG II + AG-490 groups, respectively. FE$_{\text{Na}}$ averaged 0.70 ± 0.09, 0.69 ± 0.13, 0.7 ± 0.14, and 0.61 ± 0.20% in the control, control + AG-490, ANG II, and ANG II + AG-490 groups, respectively, on day 4. There was no significant difference or change in FE$_{\text{Na}}$ between or within any group, except for a significant increase to 1.21 ± 0.16% in the ANG II group on day 15.

A preliminary AG-490 dose-response curve consisting of 1, 10, and 100 ng·kg$^{-1}$·min$^{-1}$ showed that 10 ng·kg$^{-1}$·min$^{-1}$ was as effective as 100 ng·kg$^{-1}$·min$^{-1}$ at inhibiting ANG II-induced JAK/STAT activation, so this was the dose we utilized (data not shown). Figure 3A shows that chronic intravenous AG-490 treatment had no effect on the basal levels of JAK2 activation, as measured by the phosphorylation levels of the protein, in kidney cortex homogenates. However, ANG II significantly increased JAK2 activation, and that effect was prevented by AG-490 treatment. These responses were paralleled by STAT3 phosphorylation (Fig. 3B), and there were similar changes in JAK2 and STAT3 phosphorylation in aortic homogenates (data not shown).

To address whether this dose of AG-490 was targeting JAK2 selectively, we measured the phosphorylated levels of JAK1. Figure 4 shows that ANG II stimulated phosphorylation of JAK1 in kidney cortex homogenates and that the response was not affected by AG-490.

Figure 5A shows that AG-490 significantly inhibited the ANG II-induced contraction in the thoracic aorta (Fig. 5A). Similar results were obtained in the superior mesenteric artery as well (data not shown). To demonstrate that AG-490 did not globally inhibit the ability of the smooth muscle to contract, we used KCl and observed that AG-490 did not affect the ability of KCl to maximally stimulate contraction (Fig. 5B).

The involvement of JAK2 in contraction mediated by vasoconstrictors utilizing G protein-coupled receptors is unknown. 5-HT is an established vasoconstrictor that utilizes the 5-HT$_{2A}$ receptor, a G protein-coupled receptor, to mediate contraction in vascular smooth muscle cells. However, the involvement of JAK2 in the contractile mechanisms appears to be selective for ANG II, as 5-HT-induced contraction of the thoracic aorta was unaffected by the presence of AG-490 (Fig. 6).

**Experiment 2: AG-490 During Low-Salt Intake**

There were no differences in baseline MAP between groups, and similar to the response in experiment 1, AG-490 alone did not change blood pressure from baseline (Fig. 7A). This was the same AG-490 dose administered in the same way as in experiment 1, but it also did not affect blood pressure during the switch to low-sodium intake. However, chronic losartan infusion did decrease baseline MAP and caused significantly lower MAP during low-sodium intake. The lack of effect of AG-490 on blood pressure during low-sodium intake is consistent with no increase in JAK2 activation in rats on the low-sodium regimen (Fig. 7B).

**Experiment 3: ANG II In Vivo Dose Response**

There were no differences in baseline MAP in any of the treatment groups (Fig. 8A). There were also no differences in
MAP in the control group and the group treated with ANG II at 1 ng·kg\(^{-1}\)·min\(^{-1}\). MAP increased at the fastest rate in the group treated with ANG II at 10 ng·kg\(^{-1}\)·min\(^{-1}\).

Infusion of ANG II at 2.5, 5, and 10 ng·kg\(^{-1}\)·min\(^{-1}\) resulted in significant elevations of blood pressure by day 21. MAP increased at the fastest rate in the group treated with ANG II at 10 ng·kg\(^{-1}\)·min\(^{-1}\) (Fig. 8A). In addition, there was no difference between the control group and the group treated with ANG II at 1 ng·kg\(^{-1}\)·min\(^{-1}\) with or without AG-490 at 10 ng·kg\(^{-1}\)·min\(^{-1}\) and a subset of the ANG II + AG-490 group in which AG-490 was removed on day 13. *P ≤ 0.05 compared with ANG II + AG-490.

**DISCUSSION**

The most important finding from this study is that the effect of chronic JAK2 inhibition to attenuate ANG II hypertension was accompanied by blockade of the GFR-lowering and sodium-retaining actions of ANG II. Chronic administration of AG-490 to inhibit JAK2 had no effects on the basal phosphorylation levels of JAK2 or blood pressure and kidney function in the normotensive state. However, in the ANG II-induced hypertensive state, we observed significantly elevated levels of JAK2 phosphorylation and significant decreases in sodium excretion and GFR, and all those changes were prevented and the hypertension was markedly ameliorated by chronic inhibition of JAK2 with AG-490. On the other hand, the low-sodium diet caused blood pressure to be highly ANG II-dependent, but it did not activate JAK2 or cause blood pressure to be JAK2-dependent. These results confirm the role of JAK2 in ANG II-induced hypertension, reveal a role for renal JAK2 activation in mediating the renal actions of ANG II, and demonstrate that JAK2 is not required for the physiological action of ANG II to support normal blood pressure during low-salt intake.

Previous studies (2, 3) demonstrated that, in a model of type 1 diabetes, JAK2 activation occurred in parallel with the development of proteinuria and was linked to increased activity of ANG II. Those events preceded the development of hypertension (3). However, that model was characterized by the presence of hyperglycemia, which has its own effect on JAK2 activation and glomerular injury independent of ANG II (1a).
control/H11001 at 10 ng·kg

hypertension was not known. The recent report by Guilluy et al. (7) was a major advance, because it linked JAK2 activation directly to ANG II-mediated hypertension and showed that blocking activation therefore, because it linked JAK2 activation directly to ANG II-induced hypertension and showed that blocking activation with AG-490 also attenuated the hypertension. However, the mechanism through which JAK2 mediated ANG II-induced hypertension remains poorly understood. We found that acute ANG II-induced constriction of superior mesenteric artery and thoracic aorta was also blocked by JAK2 inhibition with AG-490. Therefore, our GFR and blood vessel data suggest that ANG II-mediated renal vasoconstriction requires JAK/STAT pathway activation, but renal blood flow measurements are required for more definitive conclusions.

The technique through which JAK2 could mediate vasoconstriction by ANG II is not known, but there are several possibilities. Endothelin-1 in rabbit basilar arteries utilized JAK2 to cause contraction via activation of the mitogen-activated protein kinase (p42/44 MAPK) pathway (20). The mechanism utilized by ANG II may be similar, as ANG II and endothelin-1 share many signaling pathways. Additionally, ANG II has many molecular targets with which it may interact and may use JAK2 to modulate these intracellular signaling pathways. For example, transactivation of epidermal growth factor receptors by ANG II is an established mechanism in vitro (6, 19). In DOCA-salt hypertension, activation of the JAK1. *P < 0.05 compared with control.

Fig. 3. A: JAK2 phosphorylation in kidney cortex homogenates from chronically catheterized rats treated with vehicle (control) or ANG II at 10 ng·kg

−1·min

−1 with or without AG-490 at 10 ng·kg

−1·min

−1, control (n = 4), control + AG-490 (n = 5), ANG II (n = 6), and ANG II + AG-490 (n = 5). pJAK2, phosphorylated JAK2. *P < 0.05 compared with ANG II + AG-490. B: STAT3 phosphorylation in kidney cortex from chronically catheterized rats treated with vehicle or ANG II at 10 ng·kg

−1·min

−1 with or without AG-490 at 10 ng·kg

−1·min

−1: control (n = 4), control + AG-490 (n = 5), ANG II (n = 6), and ANG II + AG-490 (n = 5). pSTAT3, phosphorylated STAT3. *P < 0.05 compared with ANG II + AG-490.

The recent report by Guilluy et al. (7) was a major advance, therefore, because it linked JAK2 activation directly to ANG II-induced hypertension and showed that blocking activation with AG-490 also attenuated the hypertension. However, the mechanism through which JAK2 mediated ANG II-induced hypertension was not known.

Infusion of ANG II at 10 ng·kg

−1·min

−1 in the present study caused a progressive increase in MAP that was statistically significant on day 2. A significant decrease in urinary sodium excretion was measured over the first 2 days of ANG II infusion and was accompanied by a significant decrease in GFR. It is not possible to determine precisely the extent to which direct tubular actions of ANG II may have contributed to the decrease in sodium excretion, but the marked decrease in GFR would be expected to decrease sodium excretion significantly. The role of GFR is supported by the lack of a significant decrease in FE_{Na} on day 8, when GFR and sodium excretion were decreased significantly. The increase in FE_{Na} at day 15 in the ANG II group is consistent with a decrease in tubular reabsorption that would be required to maintain sodium balance in the face of a sustained reduction in GFR. These GFR and sodium excretion responses were virtually eliminated by the addition of AG-490. Although we did not measure renal blood flow and cannot ascribe the ANG II and AG-490 effects on GFR directly to renal vasoconstriction, we recently reported dose-dependent decreases in renal blood flow by ANG II sustained for 7 days in mice chronically instrumented with renal artery flow probes (5). A role for JAK2 in ANG II-mediated renal vasoconstriction would be consistent with in vitro and in vivo evidence linking ANG II to stimulation of the JAK/STAT pathway in vascular smooth muscle cells (3, 7, 11).

We found that acute ANG II-induced constriction of superior mesenteric artery and thoracic aorta also was blocked by JAK2 inhibition with AG-490. Therefore, our GFR and blood vessel data suggest that ANG II-mediated renal vasoconstriction requires JAK/STAT pathway activation, but renal blood flow measurements are required for more definitive conclusions.

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Fig. 4. JAK1 phosphorylation in kidney cortex from chronically catheterized rats treated with vehicle (control) or ANG II at 10 ng·kg

−1·min

−1 with or without AG-490 at 10 ng·kg

−1·min

−1: control (n = 4), control + AG-490 (n = 5), ANG II (n = 6), and ANG II + AG-490 (n = 5). pJAK1, phosphorylated JAK1. *P < 0.05 compared with control.

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epidermal growth factor receptor has been linked to enhanced activation of phosphoinositide 3-kinase, which is coupled to enhanced vascular contraction and the development of spontaneous tone (12, 13, 18). JAK2 in vitro has been shown to interact with phosphoinositide 3-kinase directly via the docking protein GAB2 (14). Another pathway with which JAK2 has been shown to interact, i.e., the p42/p44 MAPK pathway, is also activated by ANG II (9). Furthermore, recent work has shown that activation of JAK2 in vitro is a critical step in ANG II’s molecular mechanisms used to activate the Rho kinase pathway (7). This is an important avenue of future research, and these studies provide the rationale for pursuing it.

The substantial reduction in ANG II-induced hypertension in the AG-490-treated rats implies that the JAK/STAT pathway has a role in the overall response to chronic increases in ANG II, and the association with the renal responses supports the hypothesis that JAK/STAT-mediated renal actions of ANG II underlie the blood pressure effect. Endothelin (3) and norepinephrine (1) have been shown to activate JAK2, and diabetic proteinuria and hypertension are blocked by AG-490. Activation of JAK2 in diabetes occurred before the onset of hypertension, suggesting that hypertension per se does not underlie JAK2 activation, but those reports indicate that JAK/STAT does not exclusively mediate ANG II-induced hypertension. In addition, our present results show a JAK/STAT-independent hypertension component, because MAP still increased by \(10.220.33.1\) mmHg in the ANG II + AG-490 groups, despite nearly complete elimination of the increase in JAK2 activation. Also, we did not measure any sodium retention in those groups. However, the increase in MAP in the two ANG II + AG-490 groups was delayed compared with the ANG II-
compared with control.

Fig. 8. A: MAP in chronically catheterized rats treated with vehicle (control) and ANG II at 1.0, 2.5, 5.0, and 10 ng·kg⁻¹·min⁻¹. *P ≤ 0.05 compared with control. B: JAK2 phosphorylation in kidney cortex homogenates from chronically catheterized rats treated vehicle (control, n = 6) or ANG II at 1.0, 2.5, 5.0, and 10 ng·kg⁻¹·min⁻¹ (n = 6 each). *P ≤ 0.05 compared with control.

only group, so whether the renal and blood pressure responses in those groups implicate a role for nonrenal mechanisms, or more modest, slowly developing renal tubular mechanisms that we could not detect, cannot be determined. However, since many transporters are regulated by phosphorylation, this is a potential avenue of future research. Nevertheless, these results suggest that ANG II-induced hypertension is not completely JAK2-dependent, but given that ANG II is known to interact with NAD(P)H and increase reactive oxygen species (8), this is not unexpected.

The JAK2 response to low-salt intake was particularly important in this regard, because that model steps back further and examines the nonhypertensive blood pressure actions of ANG II. Because of the focus on pathological consequences that accompany chronic ANG II-induced hypertension, it is easy to overlook the fact that a major physiological function of ANG II is protection against low blood pressure during salt and volume depletion. The marked hypotensive response to losartan in this study reveals the importance of ANG II in maintaining baseline blood pressure and preserving normal blood pressure during low-salt intake. Interestingly, we did not measure increased JAK2 activation during low-salt intake, nor did blockade of JAK2 activation with AG-490 affect blood pressure. Given that the losartan data revealed a ~30-mmHg effect of ANG II on blood pressure during low-salt intake, the lack of any evidence for a role for JAK2 is all the more striking. Additionally, the dose-response data suggest that only doses of ANG II that increase blood pressure activate JAK2. A systematic study has not been done in rats to compare plasma ANG II during low-salt intake with plasma levels measured during a range of ANG II infusion doses, but Olsen et al. (14a) compared rat, dog, and human data to show that intravenous ANG II infusions at 3–10 ng·kg⁻¹·min⁻¹ yield plasma concentrations comparable to those during sodium depletion. This of course is the infusion range used to cause hypertension in the present study, which suggests that the activation of JAK2 and its role in ANG II-dependent blood pressure control may not be due to a simple ANG II-dose relationship. Rather, JAK2 activation may be uniquely linked to mechanisms mediating the pathological, hypertensive actions of ANG II that occur when ANG II levels are inappropriate for a given level of sodium intake.

In summary, these results show that the effect of chronic ANG II infusion to cause hypertension, sodium retention, decreased GFR, vasoconstriction, and JAK2 activation in the kidney and vasculature was blocked by chronic intravenous infusion of the specific JAK2 inhibitor AG-490. This suggests that the chronic renal and hypertensive actions of ANG II require JAK2 activation for their full manifestation. Furthermore, these data also show that ANG II is not utilizing JAK2 to physiologically control blood pressure in response to sodium depletion. The relationship between plasma ANG II and salt intake will be an important future direction for understanding how the ANG II-JAK2 mechanism differentiates between physiological and hypertensive blood pressure actions of ANG II.

Significance and Perspectives

These data clearly demonstrate that ANG II does not use JAK2 activation to regulate physiological blood pressure mechanisms in response to sodium depletion. However, our data also show that JAK2 mediates the powerful renal actions of ANG II that contribute to its pathological hypertensive action. This is a clinically relevant end point, and future studies also may reveal a role for JAK2 activation in ANG II-mediated end-organ injury. The apparent specificity of JAK2 for pathological actions of ANG II has exciting potential for therapeutic targeting of JAK2 activation as a way to block ANG II-dependent disease processes without affecting the physiological actions of ANG II.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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