Angiotensin II hypertension is attenuated in interleukin-6 knockout mice

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Submitted 29 June 2005; accepted in final form 7 November 2005

Angiotensin II hypertension is attenuated in interleukin-6 knockout mice. Am J Physiol Heart Circ Physiol 290: H935–H940, 2006. First published November 11, 2005; doi:10.1152/ajpheart.00708.2005.—Plasma levels of IL-6 correlate with high blood pressure under many circumstances, and ANG II has been shown to stimulate IL-6 production from various cell types. This study tested the role of IL-6 in mediating the hypertension caused by high-dose ANG II and a high-salt diet. Male C57BL6 and IL-6 knockout (IL-6 KO) mice were implanted with biotelemetry devices and placed in metabolic cages to measure mean arterial pressure (MAP), heart rate (HR), sodium balance, and urinary albumin excretion. Baseline MAP during the control period averaged 114 ± 1 and 109 ± 1 mmHg for wild-type (WT) and IL-6 KO mice, respectively, and did not change significantly when the mice were placed on a high-salt diet (HS; 4% NaCl). ANG II (90 ng/min sc) caused a rapid increase in MAP in both groups, to 141 ± 9 and 141 ± 4 mmHg for WT and KO mice, respectively, on day 2. MAP plateaued at this level in KO mice (134 ± 2 mmHg on day 14 of ANG II) but began to increase further in WT mice by day 4, reaching an average of 160 ± 4 mmHg from days 10 to 14 of ANG II. Urinary albumin excretion on day 4 of ANG II was not different between groups (9.18 ± 4.34 and 8.53 ± 2.85 μg/day for WT and KO mice). By day 14, albumin excretion was nearly fourfold greater in WT mice, but MAP dropped rapidly back to control levels in both groups when the ANG II was stopped after 14 days. Thus the ~30 mmHg greater ANG II hypertension in the WT mice suggests that IL-6 contributes significantly to ANG II-salt hypertension. In addition, the early separation in MAP, the albumin excretion data, and the rapid, post-ANG II recovery of MAP suggest an IL-6-dependent mechanism that is independent of renal injury.

mean arterial pressure; high-salt diet

INTERLEUKIN-6 (IL-6) is a proinflammatory cytokine that is released from numerous cell types, including endothelial cells (12, 21), vascular smooth muscle cells (14, 16), and macrophages (32). The sympathetic nervous system (12, 20, 22) stimulates the release of IL-6 and other proinflammatory cytokines, and sympathetic nerves also are a source of IL-6. Previous results indicate that high IL-6 levels correlate with increased blood pressure and may be an independent risk factor for hypertension (5, 28). However, it has been difficult to establish a cause-and-effect link between IL-6 and chronic hypertension.

Short-term (5 day) infusion studies have begun to provide support for such a link. Alexander et al. (1) reported that a twofold elevation in the plasma levels of the closely related proinflammatory cytokine tumor necrosis factor α (TNF-α) caused a significant increase in arterial pressure in pregnant rats, and Orshal and Khalil (25) followed with similar findings infusing IL-6 in pregnant rats. However, although those studies demonstrated that sustained increases in IL-6 can cause hypertension, they did not test the role of IL-6 in mediating hypertension. Thus the question is whether the correlative relationship between IL-6 and blood pressure means that IL-6 is required for, or plays a role in, the hypertension, and that question must be addressed by blocking IL-6.

Our laboratory used mice with knockout of IL-6 to demonstrate recently that IL-6 is important in mediating the acute hypertensive response to psychosocial stress (18), and we also have shown that the hypertension in that model depends significantly on ANG II (19). ANG II stimulates the release of IL-6 (10, 14, 16, 29), and recent work has shown that an ANG II type 1 receptor antagonist lowers blood pressure, aortic mRNA expression of proinflammatory cytokines IL-1β, TNF-α, and IL-6, as well as plasma levels of IL-6 and IL-1β in spontaneously hypertensive rats (30). The goal of this study was to test more directly the role of IL-6 in long-term blood pressure control by testing the hypothesis that the hypertensive response to chronic (14 days) ANG II infusion would be attenuated significantly in IL-6 knockout (KO) mice compared with wild-type (WT; C57BL/6) mice. Because IL-6 has been linked to hypertensive glomerular injury (3, 17, 29) in addition to effects on vascular contractility (24, 25), we used a high dose of ANG II (90 ng/min) and a 4% high-salt diet to enhance our ability to detect a significant effect of IL-6 on ANG II hypertension.

METHODS

Procedures involving animals were approved by the Animal Care and Use Committee of the Medical College of Georgia. Blood pressure transmitters (Data Science, PA-C20) were implanted in IL-6 KO (KO) mice (n = 9; Jackson Laboratories B6.129S6-IL6tm1Kopf), and their WT controls (n = 12; Jackson Laboratories C57BL/6J 000664) as previously described (18). Mice were allowed to recover for 7 days and then were placed in individual metabolic cages (Lab Products) that prevented food and fecal contamination of urine samples. Two-day measurement periods were used to collect urine and monitor food and water intake because the increased volumes minimized variability. Food and water were available ad libitum.

Mean arterial pressure (MAP) and heart rate were measured over a continuous 19-h period every 2nd day, and studies were begun only after normal circadian rhythm was reestablished (~4–7 days postsurgery). The mice were monitored for 4 days on standard laboratory chow before being switched to a high-salt diet (4% NaCl diet, Teklad)
for the remainder of the study. After 4 days on the high-salt diet, the ANG II mice (KO-ANG II, n = 6; WT-ANG II, n = 9) were placed under isoflurane anesthesia and implanted with an osmotic minipump (Alzet, Durect, Cupertino, CA) to deliver ANG II subcutaneously for 2 wk at a rate of 90 ng/min. This corresponds to ~3,600 ng·kg⁻¹·min⁻¹, which is approximately fourfold higher than the range of doses typically employed for chronic subcutaneous ANG II infusion in mice. The time control mice (KO n = 3, WT n = 3) were maintained on the high-salt diet on the same timeline as the other mice but were not infused with ANG II.

ANG II recovery study. Separate groups of three WT and three KO mice were instrumented similarly and placed on high-salt diet, but the mice were housed singly in standard polycarbonate cages rather than metabolic cages. MAP was measured every 2nd day, and after 3 days of control measurements, ANG II minipumps were implanted as described above. After 14 days of ANG II infusion, the pumps were removed under isoflurane anesthesia to enable rapid and coordinated termination of the ANG II infusion for measurement of recovery-period MAP.

Analytical methods. MAP data were collected at 500 Hz for 5 s each minute, from 3 PM until 10 AM (i.e., 19 h) every 2nd day. The 3 PM to 6 PM period and the 6 AM to 10 AM period together (7 h) were analyzed as “day” MAP, and the 6 PM to 6 AM period (12 h) was “night.” Sodium balance was measured as sodium intake – urinary sodium excretion – insensitive sodium loss, where sodium intake = (food weight × food [Na]) + (water intake × water [Na]), sodium excretion = urine volume × urine [Na], and insensitive sodium loss = average intake minus average excretion during the pre-ANG II high-salt period. Albumin concentration was measured from a 2-day timed urine collection on control day 2 and ANG II days 4 and 14 in the KO-ANG II and WT-ANG II mice, using an Albuwell murine microalbuminuria ELISA (Exocell). Plasma IL-6 levels were measured by enzyme immunoassay (R&D Systems) from blood samples obtained on day 6 of ANG II infusion by cardiac puncture under isoflurane anesthesia in separate groups of identically treated mice. Data were analyzed with a two-factor, repeated-measures ANOVA. Significant F-tests from the ANOVA at P < 0.05 were followed by post hoc comparisons using the Newman-Keuls multiple range test.

RESULTS

Blood pressure. The day- and nighttime averages for MAP during control conditions were 113 ± 4 and 118 ± 4 mmHg, respectively, in the WT-ANG II mice and 106 ± 2 and 113 ± 1 mmHg, respectively, in the KO-ANG II mice. These values were not different from average baseline values in the WT and KO control groups (Fig. 1). The high-salt diet did not change MAP in any group. ANG II infusion, however, had a rapid hypertensive effect in both treated groups that was not different between groups, averaging 141 ± 9 and 141 ± 4 mmHg on day 2 for WT-ANG II and KO-ANG II mice, respectively. A difference in MAP between WT-ANG II and KO-ANG II mice was evident on day 4 and was statistically significant by day 6, with daytime MAP on day 6 averaging 153 ± 8 and 141 ± 3 mmHg in WT-ANG II and KO-ANG II mice, respectively. The difference between WT-ANG II and KO-ANG II mice continued to increase, with daytime MAP on day 14 of ANG II averaging a respective 159 ± 7 and 132 ± 3 mmHg. The average daytime MAP differed between the two groups by 30 ± 2 mmHg over days 8–14 of ANG II. WT-ANG II and KO-ANG II groups were significantly higher (P < 0.05) than WT-control and KO-control groups during the entire period of ANG II infusion. Figure 2 shows that there was an ~10-fold increase in plasma IL-6 on day 6 caused by the ANG II infusion in the WT mice, and it is noteworthy that these data coincide with the time at which MAP began to differ significantly between groups.

Metabolic data. Figure 3 shows that sodium and water intakes and urine volume were not different between groups during the control period and increased similarly in all groups during the control period and increased similarly in all groups.
implantation of saline-filled pumps in identically prepared and treated WT (n = 4) and KO (n = 4) mice. MAP averaged 101 ± 2 and 105 ± 4 mmHg in WT and KO mice, respectively, before implant, and 103 ± 1 and 103 ± 2 mmHg in those respective mice after implant, providing assurance that the ANG II hypertension was caused by ANG II and was not an artifact of the minipump procedure.

Even though there was an initial decrease in food intake, Fig. 3 shows that normal sodium (and food) intake was restored in the ANG II-infused groups. Over the last 4 days of the 2-wk ANG II infusion period, however, there was a tendency for sodium intake to decrease slightly in the WT-ANG II mice. On day 14 of ANG II, sodium intake averaged 5.4 ± 0.2, 5.3 ± 0.1, and 5.8 ± 0.3 meq sodium/2 days in the WT-control, KO-control, and KO-ANG II groups, respectively, but averaged 4.8 ± 0.5 meq sodium/2 days in the WT-ANG II mice. The explanation is not known, but the decrease was not statistically significant and was accompanied by slight drops in water intake and urine volume as well (Fig. 3). The response is interesting because it shows that sodium intake was not greater in the WT-ANG II mice vs. the KO-ANG II mice. It is also important to note that both groups were in sodium balance during the ANG II infusion period (Fig. 4). Thus neither sodium retention nor greater sodium and water intake appear to explain the greater MAP in the WT mice than the KO mice during ANG II infusion.

**Urinary albumin excretion.** Urinary albumin excretion was measured on control day 2 and on days 4 and 14 of ANG II to provide an index of renal injury (Fig. 5). Albumin excretion increased in both groups on day 4 of ANG II; importantly, however, there was no difference between the WT-ANG II and KO-ANG II mice. Albumin excretion was further, and significantly, increased by day 14 in both groups, but there was a markedly greater excretion of albumin in the WT-ANG II mice, averaging 155 ± 142 µg/2 days compared with 45 ± 13 µg/2 days in KO-ANG II mice. Although these data suggest renal injury may be greater in the WT-ANG II than the KO-ANG II mice by day 14, it is possible that the greater excretion was secondary to the greater blood pressure. In addition, the blood pressure data from our recovery study suggest that the injury was not enough to have explained...
completely the different blood pressure responses to ANG II over the 2-wk period.

ANG II recovery study. MAP increased rapidly in the WT and KO mice in the recovery study after implanting the ANG II pumps (Fig. 6), similar to the response shown in Fig. 1 for the main experimental groups. The same basic difference in MAP between the WT-ANG II and KO-ANG II mice also was measured. Interestingly, however, removal of the ANG II minipumps caused immediate return of MAP to control levels in both groups, suggesting that any renal injury that was present in either group after 14 days of ANG II was not sufficient to have an effect on blood pressure.

DISCUSSION

The major finding from this study is that the hypertension caused by a high dose of ANG II and a high-salt diet depends significantly on the presence of IL-6. The mice with KO of IL-6 had significantly lower MAP than WT mice during 2 wk of ANG II infusion, and sodium intake between the groups was not different. This suggests an important role for IL-6 in mediating the chronic hypertensive response to ANG II. Furthermore, because there was no post-ANG II hypertension, and because the between-group differences in blood pressure became apparent by day 4 and significant by day 6 of ANG II, which was before there was a difference in urinary albumin excretion, this suggests the mechanisms that underlie the direct hypertensive actions of ANG II may be dependent significantly on IL-6.

Since the report by Ridker et al. (28), there has been considerable interest in the potential role for inflammatory cytokines in blood pressure control. However, despite the strong correlational links, there has not been consistent evidence for a direct hypertensive effect. Acutely, IL-1β and TNF-α have been shown to increase endothelin-mediated vasoconstriction (35), and IL-1β and IL-6 have been shown to cause thromboxane-dependent vasoconstriction (33). However, acute infusion of IL-6 has been reported not to increase blood pressure in dogs (26), and the increased IL-6 that occurs with endotoxin shock, cirrhosis, or septic shock is associated with decreased blood pressure (9, 11, 23, 27). However, our laboratory has reported recently that the acute increase in blood pressure caused by psychosocial stress is dependent significantly on IL-6 (18). Thus there is evidence from acute studies to support the hypothesis that inflammatory cytokines may play a role in hypertension, but the data are far from conclusive.

ANG II has been shown in many reports to increase IL-6 levels (10, 14, 16), and our data in Fig. 2 confirm that effect. That response, plus the effects of IL-6 on vascular contractile mechanisms (2, 8, 24, 25), lends support to the hypothesis that IL-6 could play a role in the hypertensive actions of ANG II. Thus we chose the ANG II-salt model of chronic hypertension to test the long-term blood pressure actions of IL-6. Our results demonstrated that loss of IL-6 significantly ameliorated, by ~30 mmHg, the ability of ANG II and high salt intake to cause hypertension. Similar to the findings of Alexander et al. (1) with TNF-α, the loss of IL-6 had no effect on blood pressure under control conditions, nor was blood pressure during high salt intake alone affected by loss of IL-6, thus pointing toward a direct interaction between IL-6 and ANG II.

The albumin excretion data are intriguing because they provide evidence that the influence of IL-6 on the hypertensive response to ANG II was not due to a significant difference in the onset or progression of renal injury, because there was no difference in albumin excretion between groups on day 4 of
ANG II. Moreover, even though our high dose of ANG II and 4% sodium intake were employed to capitalize on the potential role of IL-6 to affect blood pressure through renal injury mechanisms (3, 17, 29), and there was a difference in urinary albumin excretion between groups at day 14, the blood pressure data from the ANG II recovery mice suggest that there still was not sufficient renal injury to explain the blood pressure differences. We do not have renal histology data, however, so even though the recovery data show that there was not enough renal injury to affect baseline blood pressure or its sensitivity to high salt intake, we cannot rule out the possibility that there was enough injury developing near day 14 of ANG II (commensurate with the albumin excretion differences) to enhance the ANG II sensitivity of blood pressure. Thus, although not ruling out an effect of IL-6 to affect blood pressure by influencing the progression of hypertensive renal injury, whether due to ANG II or renal perfusion pressure per se (3, 13, 17, 30), our data suggest that some of the vascular and/or renal tubular mechanisms that underlie the direct hypertensive actions of ANG II may be dependent significantly on IL-6.

The precise identity of those mechanisms, of course, must be the focus of future studies, but it is interesting to note that there virtually was no difference in the initial, rapid increase in MAP between the two groups on starting ANG II. It was unfortunate that the effect of the ANG II minipump surgery to suppress appetite prevented us from observing any early, transient effects of ANG II on renal sodium handling, so it is difficult to guess at the relative importance of renal vs. systemic vasoconstrictor mechanisms in mediating the initial blood pressure responses in the WT-ANG II vs. KO-ANG II mice. The sodium balance data, on the other hand, particularly when food intake recovered after the first 2 days of ANG II, provide support for the hypothesis that the effects of IL-6 on renal sodium excretory capability may have contributed to the blood pressure response. This is because both groups were in sodium balance and had stable arterial pressure, but MAP was significantly lower in the IL-6 KO-ANG II mice compared with the WT-ANG II mice. This can be interpreted as indicating that the kidneys of the WT mice required a greater arterial pressure to maintain sodium balance during ANG II infusion, or, in other words, that the kidneys of the IL-6 KO mice were more capable of eliminating a salt load during ANG II infusion than were those in the WT mice (4, 6, 7). These data suggest there is a powerful effect of IL-6 in mediating the rightward shift in the renal pressure-natriuresis relationship caused by ANG II and high salt intake and provide evidence for an important long-term blood pressure effect of IL-6, but additional studies will be required to determine the role of direct renal mechanisms.

These results in this study demonstrate that the hypertensive shift in the renal pressure-natriuresis relationship caused by high levels of ANG II is dependent significantly on IL-6 and suggest that mechanisms independent of renal injury may contribute to that effect. A subtle point also is that the plasma IL-6 levels may not tell the complete story regarding dependence of ANG II hypertension on IL-6. Knockout of IL-6 showed that IL-6 is required for full development of ANG II hypertension, and that relationship would hold even if plasma levels did not increase. However, the increase in circulating IL-6 levels shows that ANG II indeed stimulated IL-6 release, but additional studies will be needed to determine tissue-specific IL-6 production and its relationship to the blood pressure response to ANG II. In addition, it will be important in future studies to identify the systemic and renal ANG II targets at which IL-6 could be acting, as well as the potential role of IL-6 in the link between ANG II, superoxide, and hypertension (15, 31, 34). How these relationships hold during low-dose ANG II infusion or when ANG II is increased by very low sodium intake also needs to be established.

ACKNOWLEDGMENTS

We thank Amy Phillips for excellent technical assistance with the plasma IL-6 measurements.

GRANTS

This research was supported by National Heart, Lung, and Blood Institute Grants HL-74167, HL-56259, HL-75625, and T32-HL-66993.

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