Diuretic response to acute hypertension is blunted during angiotensin II clamp

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Leong, Patrick K. K., Yibin Zhang, Li E. Yang, Niels Henrik Holstein-Rathlou, and Alicia A. McDonough. Diuretic response to acute hypertension is blunted during angiotensin II clamp. Am J Physiol Regul Integr Comp Physiol 283: R837–R842, 2002.—Acute hypertension inhibits proximal tubule (PT) fluid reabsorption. The resultant increase in end proximal flow rate provides the error signal to mediate tubuloglomerular feedback autoregulation of renal blood flow and glomerular filtration rate and suppresses renal renin secretion. To test whether the suppression of the renin-angiotensin system during acute hypertension affects the magnitude of the inhibition of PT fluid and sodium reabsorption, plasma ANG II levels were clamped by infusion of the angiotensin-converting enzyme (ACE) inhibitor captopril (12 μg/min) and ANG II after pretreatment with the bradykinin B2 receptor blocker HOE-140 (100 μg/kg bolus). Because ACE also degrades bradykinin, HOE-140 was included to block effect of accumulating vasodilatory bradykinins during captopril infusion. HOE-140 increased the sensitivity of arterial blood pressure to ANG II: after captopril infusion without HOE-140, 20 ng·kg⁻¹·min⁻¹ ANG II had no pressor effect, whereas with HOE-140, 20 ng·kg⁻¹·min⁻¹ ANG II increased blood pressure from 104 ± 4 to 140 ± 6 mmHg. ANG II infused at 2 ng·kg⁻¹·min⁻¹ had no pressor effect after captopril and HOE-140 infusion (“ANG II clamp”). When blood pressure was acutely increased 50–60 mmHg by arterial constriction without ANG II clamp, urine output and endogenous lithium clearance increased 4.0- and 6.7-fold, respectively. With ANG II clamp, the effects of acute hypertension were reduced 50%: urine output and endogenous lithium clearance increased two- and threefold, respectively. We conclude that HOE-140, an inhibitor of the B2 receptor, potentiates the sensitivity of arterial pressure to ANG II and that clamping systemic ANG II levels during acute hypertension blunts the magnitude of the pressure diuretic response.

captopril; HOE-140; bradykinin receptor; endogenous lithium clearance

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decreasing renal perfusion pressures to demonstrate that clamping systemic [ANG II] improves RBF autoregulation range but abolishes the ability to reset the range during prolonged depression in perfusion pressure. This ANG II clamp strategy was useful to establish the role of a responsive renin-angiotensin system for these hemodynamic responses.

In the present study, we investigate the hypothesis that diuretic and natriuretic responses to acute hypertension require a responsive systemic renin-angiotensin system with an “ANG II clamp” protocol. Plasma ANG II levels were clamped by inhibition of ACE (with captopril), infusion of a nonpressor level of ANG II, and pretreatment with the bradykinin B2 receptor blocker HOE-140 to eliminate any effect of the buildup of vasodilatory bradykinin during captopril infusion. The results demonstrate that the inhibition of the B2 receptor increases the sensitivity of arterial pressure to systemic ANG II and that clamping of systemic ANG II levels during acute hypertension blunts pressure diuresis.

METHODS

Animal and surgical protocols. All animal experiments were approved by the University of Southern California School of Medicine and conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (293 ± 22 g body wt) were kept under diurnal light conditions with free access to food and water. Before surgery, rats were anesthetized intramuscularly with ketamine (Fort Dodge Laboratories) and xylazine (Miles) (1:1 vol/vol) and placed on a thermostatically controlled operating table to maintain the body temperature at 37°C. Polyethylene catheters (PE-50) were placed into the jugular vein for infusion of drugs and 0.9% NaCl at 50 l/min. Ligatures were tightened acutely until mean arterial pressure increased by 50 mmHg. This ANG II clamp strategy was useful to establish the role of a responsive renin-angiotensin system with an ANG II clamp protocol. Plasma ANG II levels were clamped by inhibition of ACE (with captopril), infusion of a nonpressor level of ANG II, and pretreatment with the bradykinin B2 receptor blocker HOE-140 to eliminate any effect of the buildup of vasodilatory bradykinin during captopril infusion. The results demonstrate that the inhibition of the B2 receptor increases the sensitivity of arterial pressure to systemic ANG II and that clamping of systemic ANG II levels during acute hypertension blunts pressure diuresis.

RESULTS

Effects of ANG II clamp on blood pressure. ANG II level was clamped by inhibition of de novo ANG II synthesis with the ACE inhibitor captopril along with an infusion of a fixed level of ANG II. Because ANG II is a potent vasoconstrictor, it was necessary to first establish a systemic nonpressor level of ANG II to evaluate the primary effects of clamping ANG II levels on the renal responses to acute hypertension. To establish a nonpressor infusion level, ANG II was infused along with captopril at different rates in the presence or absence of a pretreatment bolus injection of HOE-140 to block the effect of accumulation of vasodilatory kinins. HOE-140 pretreatment per se did not affect blood pressure (Fig. 1A), whereas captopril infusion acutely (<5 min) lowered blood pressure whether the rats were pretreated with HOE-140 (P = 0.01; Fig. 1A) or not (P = 0.03; Fig. 1B). In the HOE-140-treated rats, blood pressure was restored to basal levels by subsequent infusion of ANG II at 2 ng·kg⁻¹·min⁻¹ and significantly increased from 104 ± 4 to 140 ± 6 mmHg (P = 0.001) with ANG II clamp at 20 ng·kg⁻¹·min⁻¹ (Fig. 1A). In contrast, without HOE-140 pretreatment, ANG II clamp at 20 ng·kg⁻¹·min⁻¹ was not hypertensive, but blood pressure did increase to ~120 mmHg when clamp level was raised to 50 ng·kg⁻¹·min⁻¹ (P = 0.01) or higher (Fig. 1B).

Effects of ANG II clamp on renal responses to acute hypertension. On the basis of the findings in Fig. 1A, ANG II clamp was defined as treatment with HOE-140 followed by confusion of captopril and 2 ng·kg⁻¹·min⁻¹ ANG II. This ANG II clamp protocol, which maintained the systemic [ANG II] at basal plasma levels (30), did not significantly change blood pressure or urine output, which was collected over 15-min intervals. Acutely raising blood pressure by arterial constriction caused a significant increase in urine output during ANG II clamp, demonstrating that a drop in the systemic level of this hormone is not requisite for a pressure diuretic response (Fig. 2).
Figure 3 compares the responses to acute hypertension in control vs. ANG II clamp rats. Arterial constriction induced similar elevations in blood pressure: from ~110 mmHg to 172 ± 8 (control) and 165 ± 6 mmHg (ANG II clamp), respectively (Fig. 3A). Acute hypertension induced a significant increase in urine output in control rats (P = 0.005; Fig. 3B). This diuretic response was significantly blunted in the ANG II clamp rats (17.5 ± 3.5 μg/min) compared with control rats (34.5 ± 4.3 μg/min; P = 0.03). Expressed as fold change, ANG II clamp reduced the pressure diuretic response from four- to twofold increase (Table 1). ANG II clamp conditions did not alter endogenous C\textsubscript{Li} (Fig. 3C). Acute hypertension induced a significant increase in C\textsubscript{Li} in control rats (P = 0.02). There was a significant blunting of the pressure-induced increase in C\textsubscript{Li} in ANG II clamp rats (0.09 ± 0.02 vs. 0.23 ± 0.05 ml/min in control rats; P = 0.04). Expressed as fold change, ANG II clamp reduced the pressure-induced increase in C\textsubscript{Li} from 6.7- to 3.0-fold (Table 1).

DISCUSSION

The present study aimed to test the hypothesis that a decrease in systemic ANG II levels provoked by acute hypertension contributes to the pressure diuretic response. When [ANG II] was clamped during acute hypertension, the pressure diuresis and pressure-induced increase in C\textsubscript{Li} were blunted ~50% (Fig. 3 and Table 1). These results suggest that the magnitude of the pressure diuretic/natriuretic responses during acute hypertension is dependent on a change in systemic ANG II levels, thus an intact renin-angiotensin system.

Acute hypertension provokes a pressure diuretic response within 5–10 min in rats (6, 37). In order for systemic ANG II to be an important signaling component of this pressure diuretic response, it is necessary that plasma concentration of ANG II is rapidly responsive. Indeed, the in vivo half-life for ANG II has been reported to be <1 min in rats and other mammals (7, 16, 34). In agreement with this finding, we observed that captopril infusion induced an immediate (~3 min) and significant drop in blood pressure leading us to two conclusions: endogenous ANG II has a short half-life, and baseline physiological levels of ANG II contribute significantly to baseline blood pressure. A longer half-life for endogenous ANG II would have delayed or even
Because captopril not only decreases ANG II production but also decreases degradation of kinins that may have vasodilatory and natriuretic actions (5), rats in this study were pretreated with the bradykinin B₂ receptor blocker HOE-140 to avoid the complication of kinin buildup. This design leads to the question as to whether the hypotensive effect of captopril should be attributed to a decrease in ANG II, as proposed, or to accumulation of vasodilatory kinins (20). The answer depends on whether the dose of HOE-140 used was adequate to block the kinin receptors. The dose was, indeed, effective as it increased the sensitivity of mean arterial pressure to infused ANG II. In this study, we identified and used a nonpressor clamp level of ANG II to avoid complicating the interpretation of the study of acute hypertension on renal function. Without HOE-140 pretreatment, the nonpressor dose of ANG II was 20 ng·kg⁻¹·min⁻¹, as reported by other investigators (21), and when ANG II clamp was raised to 200 ng·kg⁻¹·min⁻¹ ANG II had a pressor effect, increasing blood pressure to 140 ± 6 mmHg (higher than that seen with 200 ng·kg⁻¹·min⁻¹ ANG II), and the nonpressor clamp level for ANG II was reduced to 2 ng·kg⁻¹·min⁻¹. This suggests that in the absence of HOE-140, the accumulation of vasodilatory kinins antagonizes or counters the pressor effect of 20 ng·kg⁻¹·min⁻¹ ANG II. The fact that baseline blood pressure was restored with 10-fold less ANG II after HOE-140 pretreatment indicates that the vasodilatory effects of kinins were blunted or prevented by the HOE-140 treatment. This finding illustrates the physiological role of kinins in opposing the actions of ANG II and presents a simple acute model to induce an upward shift of the ANG II/pressor dose-response curve.

Another question that arises from the use of the bradykinin B₂ receptor blocker is whether the blunting of the diuretic response to acute hypertension is attributed to preventing a drop in ANG II, as proposed, or secondary to the effective blocking of the B₂ receptor. Because HOE-140 pretreatment alone had no effect on blood pressure, it is unlikely that baseline levels of kinins contribute to baseline blood pressure. We conducted a single experiment (not shown) that demonstrated that pretreatment with HOE-140 followed by acute hypertension did not alter the magnitude of the hypotensive effect of captopril. The rapid and steady hypotensive effect of captopril also provides evidence that the captopril infusion protocol was sufficient and effective in inhibiting systemic ACE activity. Furthermore, it has been shown in Sprague-Dawley rats that captopril infusion at 3 mg·kg⁻¹·h⁻¹ (current study: 2.4 mg captopril·kg⁻¹·h⁻¹) completely abolishes the systemic pressor response to ANG I injection in 45 min (11).

![Fig. 3. Effect of ANG II clamp on physiological responses to acute hypertension (↑ BP). Measurements were made before (basal) and after (↑ BP) blood pressure was increased by arterial constriction (over 10 min periods in control and over 15 min in ANG II clamp). A: arterial pressure recorded from the carotid artery. B: urine output rate measured gravimetrically. C: endogenous lithium clearance calculated as [Li⁺] in urine × V/[Li⁺] in a plasma sample obtained at the end of the experiment. Values are means ± SE, n = 3 for control, n = 4 for ANG II clamp. *P < 0.05 vs. basal values. †P < 0.05 vs. control BP group.](image-url)

Table 1. Relative increase in urine output and endogenous lithium clearance during acute hypertension

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<tr>
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<th>Control</th>
<th>ANG II Clamp</th>
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<tr>
<td></td>
<td>Basal</td>
<td>↑ BP</td>
</tr>
<tr>
<td>Urine output</td>
<td>1.00 ± 0.20(3)</td>
<td>3.96 ± 0.37(3)*</td>
</tr>
<tr>
<td>Lithium clearance</td>
<td>1.00 ± 0.25(3)</td>
<td>6.74 ± 2.57(3)#</td>
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Values are means ± SE expressed as value after induction of acute hypertension/basal value. (n) Represents number of animals. Urine output was collected and endogenous lithium clearance was measured for 10 min before (Basal) and after induced acute hypertension (↑ BP) in control rats and for 15 min before (Basal) and after induced acute hypertension (↑ BP) in ANG II clamp rats. The basal value for each rat was normalized to a standard defined as 1 with its SE value proportionately calculated. *P < 0.05 compared with corresponding basal values; †P < 0.05 compared with corresponding acute hypertension values from control rats.

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diuresis seen in response to acute hypertension alone. In addition, ongoing experiments examining the effects of ANG II clamp without HOE-140 pretreatment demonstrate a similar blunting to that seen when animals were pretreated with the bradykinin receptor blocker. These results lead to the conclusion that the blunting of the diuretic response to acute hypertension is secondary to a change in systemic ANG II levels.

The change in volume flow out of the PT during acute hypertension, estimated from endogenous C_{Li}, increased during acute hypertension: 6.7-fold in controls and only threefold with ANG II clamp. C_{Li} is a noninvasive measure of volume flow out of the PT determined by both the GFR and proximal reabsorption rate. Therefore, the blunting of the increase in C_{Li} with ANG II clamp may be due to either a blunting of inhibition of proximal Na^+ reabsorption or to a blunting of an increase in GFR during acute hypertension. In the original study that presented the in vivo rat model of pressure diuresis adopted in the present study, Roman and Cowley (27) reported a transient elevation (15%) of GFR in the absence of changes in RBF during the first 5 min of induced acute hypertension. Because of the lack of changes in RBF, Roman and Cowley (27) attributed the transient increase to the washout of preexisting urine in the kidney rather than a genuine elevation in GFR. We did not measure GFR in this study, but in parallel ongoing studies, we found that GFR increased transiently during acute hypertension (5–15 min) and that clamping of systemic ANG II minimized or prevented this transient change as has been reported by others (21). It is therefore likely that the blunting of the increase in C_{Li} with ANG II clamp during acute hypertension observed in this study was mostly, if not exclusively, due to a blunting of pressure-induced inhibition of proximal Na^+ reabsorption per se and not to changes in GFR.

Previous work reported a blunting effect of chronic infusion of ANG II (60 ng/min for 13 days) on pressure diuresis and natriuresis (36). In a related study, the water immersion-induced natriuresis and increase in C_{Li} in humans can be attenuated by low-level ANG II infusion (29a). Multiple mechanisms could be responsible for the blunting of pressure diuresis and natriuresis by ANG II infusion. In chronic ANG II infusion rat models, direct stimulation of tubular reabsorption by ANG II and decrease in GFR have been suggested to be the mechanisms (33, 36). It is well documented that ANG II contributes significantly to the maintenance of PT fluid reabsorption (13), and our C_{Li} data also suggest that a blunting of the pressure-induced inhibition of PT fluid reabsorption may contribute to the blunting of the pressure diuretic response in our ANG II clamp model. Facilitation of distal tubular reabsorption by ANG II may also contribute to the blunting effect but was not investigated in the present study.

The blunting of the inhibition of pressure-induced increase in C_{Li} and the pressure diuretic response by ANG II clamp demonstrated here are likely a systemic effect, because the brief ANG II infusion time (<1 h) we adopted was not likely to have any effects on the intrarenal ANG II levels (35, 39). With the use of subcutaneous osmotic minipump, Zhou and co-workers (39) showed that intrarenal ANG II levels are not significantly elevated until 10 days of continuous ANG II infusion at 40 ng/min in rats. Furthermore, increase in intrarenal [ANG II] resulting from direct intrarenal ANG II infusion (1–1.5 ng·kg^{-1}·min^{-1}) produced a sustained increase in arterial pressure (25) and a decrease in urine volume (10), but neither responses were observed in the current ANG II clamp study (25). It has also been shown that acute ACE inhibition has no significant effects on intraluminal (3) and renal interstitial fluid (24) concentrations of ANG II. It is, however, possible that captopril and HOE-140 have an ANG II-independent intrarenal effect that contributed to the blunting effect of the ANG II clamp.

In summary, the present study demonstrates that inhibition of the bradykinin B2 receptor increases the sensitivity of arterial pressure to systemic ANG II and that ANG II clamp reduces the magnitude of both the increase in volume flow out of the PT and the diuresis during acute hypertension by 50%. This finding implicates a rapid drop in systemic [ANG II] as a significant signaling component for the pressure diuretic response in rats affecting tubular Na^+ reabsorption rate.

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