Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 6506–6510, May 1999
Physiology

Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subtype-2 (AT_2) angiotensin receptor

(blood pressure/sodium/bradykinin)

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Communicated by Robert M. Berne, University of Virginia, Charlottesville, VA, April 1, 1999 (received for review December 1, 1998)

ABSTRACT **The vast majority of the known biological effects of the renin–angiotensin system are mediated by the type-1 (AT_1) receptor, and the functions of the type-2 (AT_2) receptor are largely unknown. We investigated the role of the AT_2 receptor in the vascular and renal responses to physiological increases in angiotensin II (ANG II) in mice with targeted deletion of the AT_2 receptor gene. Mice lacking the AT_2 receptor (AT_2-null mice) had slightly elevated systolic blood pressure (SBP) compared with that of wild-type (WT) control mice ($P < 0.0001$). In AT_2-null mice, infusion of ANG II (4 pmol/kg/min) for 7 days produced a marked and sustained increase in SBP [from 116 ± 0.5 to 208 ± 1 mmHg ($P < 0.0001$) (1 mmHg = 133 Pa)] and reduction in urinary sodium excretion ($U_{Na}V$) [from 0.6 ± 0.01 to 0.05 ± 0.002 mM/day ($P < 0.0001$)] whereas neither SBP nor $U_{Na}V$ changed in WT mice. AT_2-null mice had low basal levels of renal interstitial fluid bradykinin (BK), and cyclic guanosine 3′,5′-monophosphate, an index of nitric oxide production, compared with WT mice. In WT mice, dietary sodium restriction or ANG II infusion increased renal interstitial fluid BK, and cyclic guanosine 3′,5′-monophosphate by ≈4-fold ($P < 0.0001$) whereas no changes were observed in AT_2-null mice. These results demonstrate that the AT_2 receptor is necessary for normal physiological responses of BK and nitric oxide to ANG II. Absence of the AT_2 receptor leads to vascular and renal hypersensitivity to ANG II, including sustained antinatriuresis and hypertension. These results strongly suggest that the AT_2 receptor plays a counterregulatory protective role mediated via BK and nitric oxide against the antinatriuretic and pressor actions of ANG II.**

The renin–angiotensin system is a major hormonal cascade regulating body fluid volume, electrolyte balance, and arterial pressure (1, 2). Angiotensin II (ANG II), the principle effector peptide, acts by binding to its two isoform receptors, AT_1 and AT_2. The vast majority of the known vascular and renal actions of ANG II are thought to be mediated via the AT_1 receptor (1, 2). However, it has been demonstrated in the rat that ANG II acts at the renal AT_2 receptor to stimulate bradykinin (BK) and, via a nitric oxide mediated pathway, cyclic guanosine 3′, 5′-monophosphate (cGMP) production (3, 4, 5). Gohlke *et al.* (6) also recently showed in the rat that AT_2 receptor stimulation increased aortic cGMP by increasing BK and NO formation.

Disruption of the AT_2 receptor has been reported (7, 8). Mice lacking the AT_2 receptor develop normally but have reduced exploratory behavior, impaired drinking responses to water deprivation, normal or slightly increased baseline blood pressure, and an exaggerated acute pressor response to a pharmacological injection of ANG II. These results, together with previous studies, suggested that the AT_2 receptor may play a protective role in blood pressure regulation.

We conducted the present study to investigate the role of the AT_2 receptor in the acute and chronic vascular and renal responses to physiological increases in ANG II in conscious mice with targeted disruption of the AT_2 receptor gene. Our findings indicate that mice lacking the AT_2 receptor have slightly elevated baseline blood pressures and sustained hypersensitivity of blood pressure and sodium excretion to ANG II. Mice lacking the AT_2 receptor have low renal BK and nitric oxide production, as reflected by cGMP, and fail to increase BK or cGMP in response to dietary sodium restriction or exogenous ANG II. These studies strongly suggest a protective role of the AT_2 receptor in blood pressure regulation mediated by BK and nitric oxide.

METHODS

Targeted Disruption of the Mouse AT_2 Receptor Gene. This procedure was performed as described (8). In brief, the coding region of embryonic stem cells (E14-1) in the third exon of the AT_2 receptor gene on the X chromosome was disrupted with a construct containing a neomycin resistant expression cassette and a herpes simplex virus thymidine kinase expression cassette. Gene-disrupted cells were selected by G418 and gancyclovir followed by Southern blot analysis to validate the desired gene disruption. Four targeted clones were obtained from 800 double-resistant colonies, two of which were injected into blastocysts derived from C57BL6 mice, and the blastocysts were implanted into the uterus of pseudo-pregnant JCR mice. Of 34 chimeras, germ-line transmission occurred in 11 mice. Heterozygous female mice and wild-type (WT) male mice were mated to generate hemizygous male mice. Homozygous female mice were generated by mating heterozygous females with hemizygous males. The presence of a homozygous mutated AT_2 receptor gene was confirmed by Southern blot analysis (8).

***In Vivo* Microdialysis Technique.** For the determination of renal interstitial fluid (RIF) autacoids, we constructed a microdialysis probe by using a modification of a technique previously described (3, 4). Each end of a single 0.5-cm-long hollow fiber dialysis tubing (0.1-mm inner diameter; molecular mass cutoff, 5,000 Da; Hospal, Meyzieu, France) was inserted into a manually dilated end of two 30-cm-long (inflow and outflow) hollow polyethylene tubes (0.28-mm inner diameter, 0.61-mm outer diameter; Becton Dickinson). Substances with a molecular mass >10,000 Da cannot cross this membrane. This molecular mass cutoff allows free passage of BK, NO, and

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Abbreviations: ANG II, angiotensin II; BK, bradykinin; cGMP, cyclic guanosine 3′,5′-monophosphate; RIF, renal interstitial fluid; SBP, systolic blood pressure; WT, wild-type.

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cGMP. The distance between the ends of the polyethylene tubes was 3 mm (dialysis area), and the dialysis fiber was sealed in place within the polyethylene tubes with cyanoacrylic glue. The dead volume of the dialysis tubing and outflow tube was 3.6 μl. The microdialysis probe was sterilized by a gas sterilization method.

***In Vitro* Microdialysis.** *In vitro* best recoveries for renal autacoids were observed with a perfusion rate of 3 μl/min and were 75% for cGMP, 78% for BK, and 70% for nitrate and nitrite. It has been demonstrated that negligible amounts of these substances stick to the polyethylene tubes (3, 4).

Animal Preparation. Experiments were conducted in 20 homozygous and 20 WT mice ages 12–16 weeks. With mice under general anesthesia with 80 mg/kg ketamine i.m. (Fort Dodge Laboratories, Fort Dodge, IA) and 8 mg/kg xylazine i.m. (Bayer, Animal Health Division, Shawnee, KS), the right and left kidneys were exposed via a midline abdominal incision. Microdialysis probes were inserted into mouse kidneys. The renal capsule was penetrated with a 31-gauge needle that was tunneled into the outer renal cortex ≈1 mm deep from the outer renal surface and was threaded through the cortex for 0.5 cm before it exited by penetrating the capsule again. The tip of the needle was inserted into the end of the dialysis probe, and the needle was pulled through with the dialysis tube until the dialysis fiber was situated in the renal cortex. The inflow and outflow tubes of the dialysis probes were tunneled s.c. by using a bevel-tipped stainless steel guide tube and were exteriorized near the intrascapular region. The exterior ends of the tubes were secured in place by suturing them to the skin at the exit site. The exteriorized portions of the tubes were placed in a stainless steel spring (to prevent the mice from damaging them). To infuse ANG II or vehicle, an osmotic micro pump (Model 1007D, Alza) was implanted in the s.c. space in the interscapular area, and ANG II or vehicle were infused s.c.

Mice were housed under controlled conditions (temperature 21 ± 1°C, humidity 60 ± 10%, lighting 8–20 h). Experiments were initiated at the same time each day to avoid diurnal variation of the measured body weight, systolic blood pressure (SBP), or RIF mediators. For collection of RIF, the inflow tube was connected to a gas-tight syringe filled with lactated Ringer's solution and perfused at 3 μl/min. The effluent was collected from the outflow tube for 30-min sample collection periods in nonheparinized plastic tubes and was stored at −80°C until measured for BK and cGMP. The known BK and cGMP-generating and -degrading enzymes (molecular weights of 34,000 to 150,000) do not cross the dialysis membrane because of their size. A histological examination of the renal tissue 6 weeks after insertion of the dialysis probe did not show any fibrosis or scarring.

Blood Pressure Measurements. SBP was measured in the tail artery in homozygous mice and WT mice under restraint by using an automated sphygmomanometer (Model 679, IITC/Life Sciences Instruments). Blood pressures were recorded at 10-min intervals for 30 min each morning during the study period (Model 179 Apollo Recorder, Life Sciences Instruments), and values were averaged each day.

Analytical Methods. Urinary sodium concentrations were measured with a Nova analyzer (Nova Biochem). RIF BK levels were measured by ELISA assay (Sigma). The sensitivity of this assay is 1 pg/ml. This assay is 100% specific for BK and does not react with any other peptide. cGMP levels in the dialysate samples were measured by enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI). Sensitivity was 0.11 pmol/ml, and specificity was 100% for cGMP. The intra- and interassay cross reactivity was <0.01% with other cyclic nucleotides.

Effects of Dietary Sodium Restriction on Blood Pressure, Sodium Excretion, and RIF Mediators. Homozygous ($n = 10$) and WT ($n = 10$) mice were placed in metabolic cages. Baseline SBP and heart rate were measured, a baseline 24-h urine collection was obtained for calculation of urine flow rate (V) and sodium excretion ($U_{Na}V$), and RIF samples were obtained for BK and cGMP (experimental day 1) while mice were consuming a normal sodium diet (0.28% NaCl, Bioserv, Frenchtown, NJ). After experimental day 1, mice were placed on a low sodium diet (0.05% NaCl) for 7 days. On the 7th day of low sodium intake, the study was repeated as outlined above.

Effects of Acute ANG II Infusion on Blood Pressure. Homozygous and WT (both $n = 10$) mice were studied during normal sodium intake. Blood pressure was monitored during a control period [vehicle, 5% dextrose in water (D_5W)] was infused into the right jugular vein at 20 μl/min for 30 min and during a treatment period during which ANG II 30 ng/kg/min was infused for 60 min.

Effects of Chronic ANG II Infusion on Blood Pressure, Sodium Excretion, and RIF Autacoids. Homozygous and WT (both $n = 10$) mice were placed in metabolic cages on normal sodium intake for 10 days. On experimental day 0, baseline SBP and 24-h urine collection were obtained for calculation of V and $U_{Na}V$ and RIF samples for BK and cGMP. At 8 a.m. on experimental day 1, a s.c. infusion of a subpressor dose of ANG II at 4 pmol/kg/min (4, 5) or vehicle was initiated and continued for 7 days (experimental days 1–7) via the microosmotic pump. SBP, heart rate, V, and $U_{Na}V$ were monitored daily. On experimental day 7, RIF samples for autacoids were obtained again. At 8 a.m. on experimental day 8, the infusion of ANG II was discontinued, and a vehicle infusion was substituted for 7 additional days (experimental days 8–14) while SBP, HR, V, and $U_{Na}V$ measurements were continued.

Histological Analysis. At the conclusion of these experiments, AT_2-null and WT mice were deeply anesthetized, and their kidneys were collected, sectioned, and fixed with 2% paraformaldehyde and were cryoprotected with 30% sucrose. The frozen sections were examined microscopically. No histological differences between kidneys of AT_2-null and WT mice were found.

Statistical Analysis. Comparisons between normal and low sodium intake and between ANG II and vehicle were estimated by ANOVA, including a repeated measures term, by using the General Linear Models procedure of the Statistical Analysis System (Virginia Polytechnic and State University, Blacksburg, VA). Multiple comparisons of individual pairs of effect means were conducted by the use of values of least-square means pooled variance. Data are expressed as mean ± standard error. Statistical significance was identified at $P < 0.05$.

RESULTS

Blood Pressure, Urine Volume, and Urinary Sodium Excretion Responses to Dietary Sodium Restriction (Fig. 1). Baseline SBP was slightly but significantly higher in the AT_2-null mice than in WT mice (Fig. 1*A*; $P < 0.0001$). Dietary sodium restriction did not significantly alter SBP in either the AT_2-null or WT mice. Heart rate was not significantly different among normal sodium or sodium-restricted AT_2-null or WT mice (data not shown). Sodium excretion (Fig. 1*B*) was similar in AT_2-null and WT mice (P was not significant) on normal sodium diet. AT_2-null mice were able to conserve sodium normally in response to dietary sodium restriction (Fig. 1*B*). Urinary volume was not significantly different between AT_2-null and WT mice and was unchanged by sodium restriction (Fig. 1*C*).

RIF, BK, and cGMP Responses to Sodium Restriction (Fig. 2). BK levels (Fig. 2*A*) were significantly reduced in AT_2-null mice as compared with WT mice on normal sodium intake. In WT mice, sodium restriction increased BK by ≈4-fold ($P < 0.0001$). In marked contrast, AT_2-null mice had no BK response to dietary sodium restriction. cGMP levels (Fig. 2*B*) responded in parallel with BK. Cyclic GMP was lower in AT_2-null mice than in WT mice. In WT mice, cGMP increased

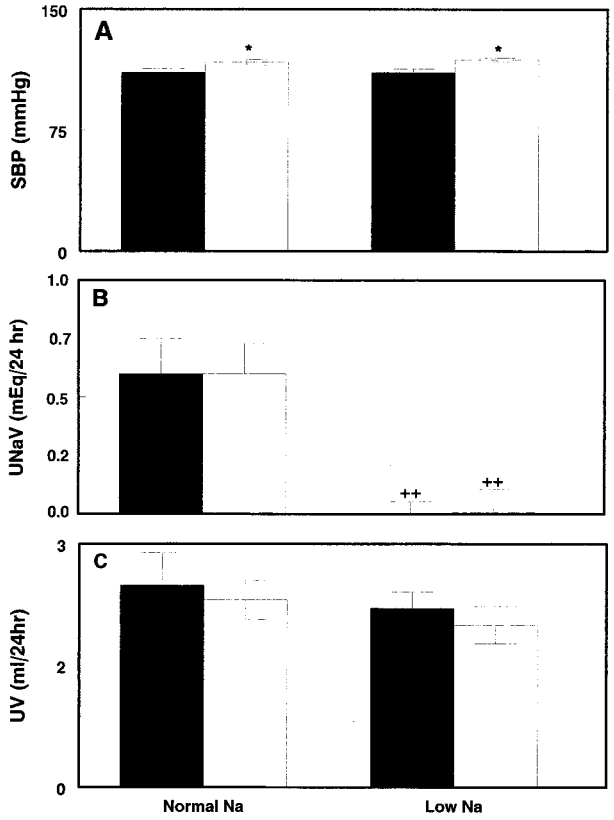

FIG. 1. SBP (*A*), 24-h urine sodium excretion ($U_{Na}V$) (*B*), and 24-h urine volume (V) (*C*) of mice ($n = 10$) lacking the subtype-2 (AT_2) angiotensin receptor (open bars) and WT mice ($n = 10$; closed bars) on normal or low sodium intake. *, $P < 0.01$ from WT mice; +, $P < 0.0001$ from normal sodium intake.

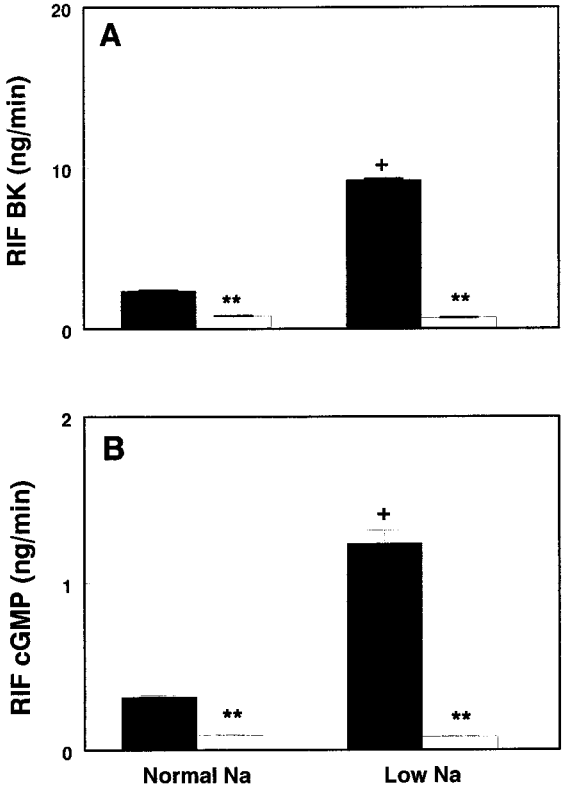

FIG. 2. RIF, BK (*A*), and cGMP (*B*) in mice ($n = 10$) lacking the subtype-2 (AT_2) angiotensin receptor (open bars) and WT mice ($n = 10$; closed bars) on normal or low sodium intake. **, $P < 0.0001$ from WT mice; +, $P < 0.0001$ from normal sodium intake.

by ≈4-fold ($P < 0.0001$) in response to sodium restriction but did not increase in AT_2-null mice.

Blood Pressure Response to Acute Bolus Injection of ANG II. In response to an i.v. bolus injection of 30 pmol/kg, SBP increased by 97 mmHg (1 mmHg = 133 Pa), from 122 ± 0.6 to 219 ± 1.6 mmHg ($P < 0.0001$) in the AT_2-null mice, and by 16 mmHg, from 103 ± 1.6 to 119 ± 1.1, in the WT mice ($P < 0.05$). The increase in SBP in response to ANG II was statistically greater in AT_2-null mice than in WT mice ($P < 0.0001$).

Blood Pressure and Sodium Excretory Responses to Chronic ANG II Infusion (Fig. 3). Fig. 3*A* compares SBP in AT_2-null and WT mice. SBP in WT mice was stable throughout the 14-day infusion period and did not increase when ANG II was infused at a rate of 4 pmol/kg/min on days 1–7. SBP in AT_2-null mice was slightly higher than that of WT mice ($P < 0.05$) during the control period before ANG II infusion. In response to infusion of ANG II at 4 pmol/kg/min, SBP increased each day. On day 7, SBP reached values of >200 mmHg, approximately twice the control values. After cessation of the ANG II infusion, SBP dropped rapidly in the first day followed by a steady decrease to control values before the infusion. When vehicle was infused in AT_2-null mice during the entire 14-day period, there was no change in SBP from baseline control values (data not shown).

Fig. 3*B* illustrates urinary sodium excretion in AT_2-null and WT mice infused with vehicle or ANG II 4 pmol/kg/min. In the WT mice, sodium excretion was steady during the 14-day period and, in particular, did not change in response to ANG II (experimental days 1–7). Baseline urinary sodium excretion of AT_2-null animals was similar to that of WT mice. In marked contrast to the lack of response to ANG II in WT mice, in AT_2-null mice, ANG II caused a significant and progressive antinatriuresis that was maximum at day 7 of infusion. After cessation of ANG II, sodium excretion increased in stepwise fashion through day 14 (the 7th day of vehicle infusion), representing a highly significant rebound natriuresis. Infusion of vehicle for the entire 14 days in AT_2-null mice resulted in no change in $U_{Na}V$ from baseline control levels (data not shown).

In Fig. 3*C*, it can be observed that the reduction in sodium excretion caused by ANG II was accompanied by a similar reduction in urine flow rate. There was no difference in water intake or body weight in either AT_2-null or WT mice in response to either vehicle or ANG II (data not shown). Body weights were 27.4 ± 0.8 and 28.5 ± 0.5 g (*P* was not significant) in WT and AT_2-null mice, respectively, on day 0 before ANG II infusion. At the end of the ANG II infusion on day 7, body weights were unchanged, at 27.7 ± 0.3 and 28.9 ± 0.3 g (*P* was not significant), for WT and AT_2-null mice, respectively. On day 14, body weights also were unchanged, at 27.8 ± 0.4 and 28.2 ± 0.4 g, respectively.

RIF, BK, and cGMP in Response to Chronic ANG II Infusion (Fig. 4). On the control day before ANG II infusion, BK levels were significantly lower in AT_2-null mice than in WT mice. In WT mice, ANG II infusion significantly increased BK levels, but no such increase occurred in the AT_2-null mice (Fig. 4*A*). cGMP levels were lower in AT_2-null mice than in WT mice (Fig. 4*B*) during vehicle infusion on experimental day 0. In WT mice, ANG II infusion resulted in an ≈4-fold increase in cGMP. In AT_2-null mice, cGMP levels were unchanged after ANG II infusion.

DISCUSSION

This study demonstrates a pronounced and sustained pressor and antidiuretic/antinatriuretic hypersensitivity to ANG II in mice lacking the subtype-2 (AT_2) angiotensin receptor. The study further elucidates the renal mechanisms for these find-

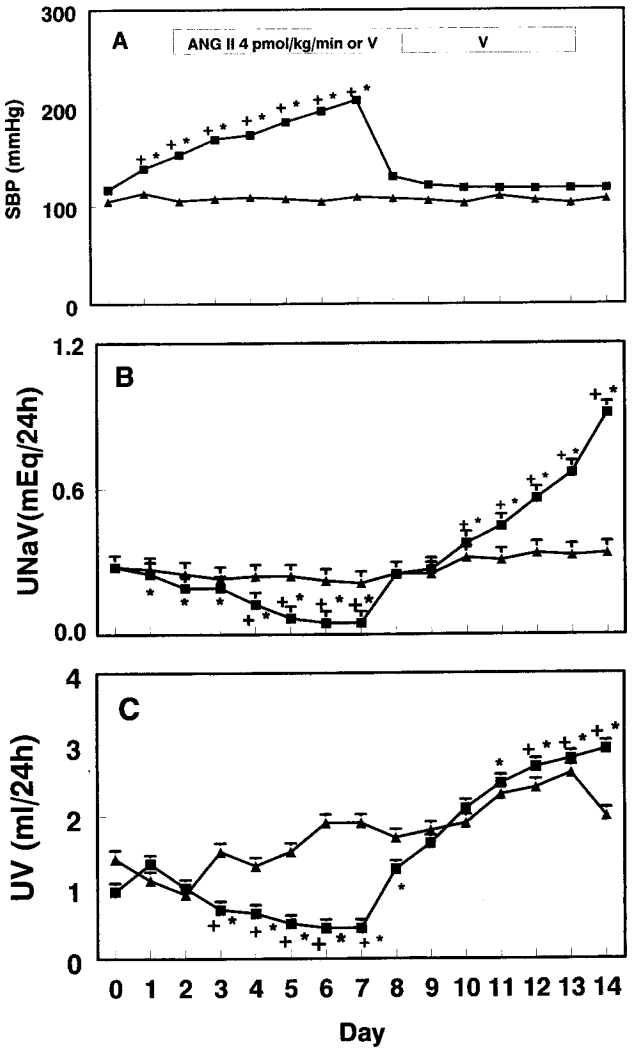

FIG. 3. SBP (*A*), 24-h urine sodium excretion ($U_{Na}V$) (*B*), and 24-h urine volume (UV) (*C*) of mice ($n = 10$) lacking the subtype-2 (AT_2) angiotensin receptor (squares) and WT mice ($n = 10$; triangles) during vehicle infusion (day 0), during sustained s.c. infusion of ANG II (days 1–7, solid line) and during vehicle (V) infusion in the postexperimental control period (days 8–14). Baseline data for AT_2-null mice infused with vehicle (V) throughout the 14-day period were 104 ± 3 mmHg (SBP), 0.23 ± 0.04 milliequivalent/24 h ($U_{Na}V$), and 2.4 ± 0.6 ml/24 h (UV). There was no change from baseline for any of these parameters on any day of the 14-day vehicle infusion (*P* was not significant). *, $P < 0.0001$ from vehicle control (day 0); +, $P < 0.0001$ from WT mice.

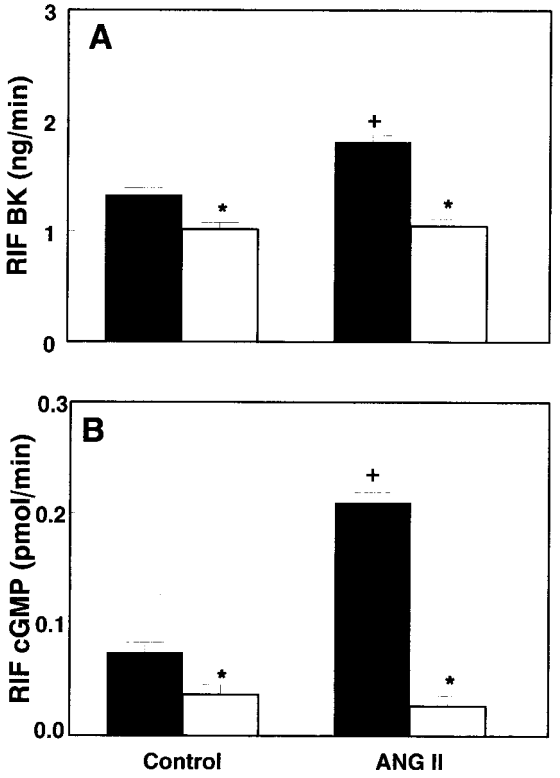

FIG. 4. RIF, BK (*A*), and cGMP (*B*) in mice ($n = 10$) lacking the subtype-2 (AT_2) angiotensin receptor (open bars) and WT mice ($n = 10$; closed bars) during the vehicle control period (Control) and after 7 days of continuous infusion of ANG II. *, $P < 0.0001$ from WT mice; +, $P < 0.0001$ from vehicle control (day 0).

ings: failure to generate BK and cGMP in response to ANG II. Taken together, these findings strongly suggest a protective role of the AT_2 receptor as a counter-regulatory receptor to the pressor and sodium-retaining actions of ANG II.

Two previous studies reported increased pressor responsiveness to a pharmacological injection of ANG II in AT_2-null mice (7, 8). In the present study, we confirmed these observations by demonstrating an approximate doubling of BP in response to an acute pharmacologic dose of ANG II. Using an infusion rate of ANG II that was predicted to increase circulating ANG II concentrations within the physiologic range, we further demonstrated progressive and sustained sodium retention and hypertension over a 1-week period in mice lacking the AT_2 receptor. In contrast, no changes in either BP or sodium excretion were observed in WT mice. These results indicate that the AT_2 receptor is important physiologically and also possibly pathophysiologically in counteracting these detrimental effects of ANG II.

At present, it is generally accepted that the vast majority of known biological actions of ANG II are mediated by the subtype-1 (AT_1) receptor (1, 2). However, it recently has been shown that the AT_2 receptor mediates renal BK and nitric oxide formation in the rat (3, 4), and Gohlke *et al.* (6) have shown that ANG II stimulates aortic cyclic GMP in stroke-prone hypertensive rats by stimulating BK via the AT_2 receptor. The data of the present study strongly support the concept that the AT_2 receptor stimulates BK and nitric oxide production. AT_2-null mice had low baseline renal BK and cGMP. In response to both increased endogenous ANG II (sodium restriction) and exogenous ANG II, both during a 7-day period, AT_2-null mice failed to mount a normal increase in the renal production of these vasodilator and natriuretic autacoids. Thus, the AT_2 receptor mediates physiologically the renal production of BK and nitric oxide. From the present study, it is unclear whether BK and nitric oxide contribute to AT_2 receptor-mediated vasodilation and natriuresis independently, as they have the capacity to do, or whether BK increases nitric oxide production as the final pathway (9).

The demonstration of a potential mechanism for sustained pressor hypersensitivity to ANG II, namely decreased activity of the AT_2 receptor, has important implications in the pathogenesis of human essential hypertension. Essential hypertension recently has been linked to the angiotensinogen gene in which a gene-dose effect on blood pressure has been demonstrated (10). In addition, some patients with essential hypertension have been reported to have increased blood pressure and adrenal sensitivity to ANG II (11). The results of the present study indicate that one potential mechanism whereby ANG II hypersensitivity could lead to sustained blood pressure elevation is AT_2 receptor deficiency. Determining whether this is the case will require receptor studies in hypertensive humans.

Another interesting finding in the present study was the sustained antinatriuresis in response to ANG II. This observation was unanticipated for two independent reasons. In the reported studies with continuous administration of ANG II, escape from the sodium retaining action of ANG II is apparent within 24–48 h (12). Second, in our study, the antinatriuresis was associated with a rise in blood pressure, but an increase in pressure should be associated with a natriuretic response ("pressure natriuresis") (13). In the present study, then, the increase in pressure engendered by ANG II in AT_2-null mice was probably related to a combination of systemic vasoconstriction and renal sodium retention. The sustained antinatriuresis with ANG II may indicate the importance of BK and nitric oxide as compensatory natriuretic autacoids. In the presence of deficiency of these substances, as in AT_2-null mice, escape from the sodium-retaining actions of ANG II may not be possible. The reason(s) for the dramatic rebound natriuresis after cessation of ANG II administration in AT_2-null mice is (are) not clear. Histological examination of the kidneys at the end of the experiment revealed no differences between WT and AT_2-null mice.

Lo *et al.* (14) recently reported that AT_2 receptors blunted pressure natriuresis in the rat. If this were the case, then disruption of the AT_2 receptor should augment pressure natriuresis. In the present study, however, deletion of the AT_2 receptor was strongly linked to inhibition of pressure natriuresis. In contradistinction to the data of Lo *et al.* (14), our data suggest that AT_2 receptors physiologically increase pressure natriuresis and that this effect is sustained over a prolonged period. Although it is possible that ANG II increased aldosterone or atrial natriuretic peptide secretion in our study, it is highly unlikely that this was the cause of the absence of pressure natriuresis for the following reasons. ANG II would be expected to increase aldosterone secretion via the AT_1 receptor to a similar degree in both AT_2-null and WT mice, but only AT_2-null mice had sodium retention. Also, any increase in ANP should have been reflected in an increase in cGMP, which did not occur in AT_2-null mice as a result of ANG II infusion.

AT_2-null mice in the present study were able to conserve sodium normally in response to dietary sodium deprivation, a physiologic stimulus to the renin–angiotensin system. Indeed, there was no evidence from our study that AT_2-null mice decreased urinary sodium excretion more rapidly than did WT mice in the absence of BK and nitric oxide or in response to unopposed AT_1 receptor stimulation as expected. The reason for this finding is not clear and will require further examination.

There are several limitations of the present study. First, renal blood flow and glomerular filtration rate could not be measured because technical considerations in the conscious mouse, and, therefore, the fraction of sodium excretion caused by hemodynamic change was not available. Second, we were unable to obtain plasma ANG II measurements because of the relatively large volume of blood required, which would have caused volume depletion in these mice. Third, we were unable to measure BK and cGMP in nonrenal tissues, such as the peripheral vasculature. However, we believe that our results with renal autacoids are likely to be reflected in the vasculature based on the work of Gohlke *et al.* (5) in the rat.

The renin–angiotensin system may regulate fluid, electrolyte, and blood pressure homeostasis, at least in part, through receptor subtype interaction. Evidence for cross-talk between AT_1 and AT_2 receptors has been reported for neuronal cells and in the heart and gastrointestinal tract (15–17). Recently, nitric oxide has been shown to down-regulate AT_1 receptor messenger RNA (18). According to concepts drawn from the present study, the AT_2 receptor stimulates nitric oxide production, which down-regulates the AT_1 receptor, further limiting detrimental effects of ANG II, such as sodium retention and vasoconstriction. We and others have recently demonstrated up-regulation of the renal AT_2 receptor protein by sodium restriction and/or ANG II (19, 20). Thus, when ANG II is increased, up-regulation of the AT_2 receptor may lead to increased nitric oxide production and down-regulation of the AT_1 receptor. However, receptor binding studies have not yet been performed to validate this concept.

The present study extends previous work using the AT_2 receptor antagonist PD 123319, showing that acute AT_2 receptor blockade increases blood pressure in the rat (21, 22). To our knowledge, no information is available on long-term effects of AT_2 receptor blockade with PD 123319. The present study extends these observations and provides information on blood pressure, sodium excretion, and renal autacoid responses to ANG II in the absence of the AT_2 receptor.

In summary, the present study suggests that (*i*) the AT_2 receptor protects against the sodium retaining and hypertensive actions of ANG II; (*ii*) this effect is mediated by an autacoid cascade that includes BK and nitric oxide; and (*iii*) absence of the AT_2 receptor leads to sustained sodium retention and hypertension in the presence of a physiological increase in ANG II. These studies strongly suggest that the AT_2 receptor plays a functional role in long-term blood pressure regulation.

This work was supported by Grants HL-47669 and HL-57503 (to H.M.S.) and HL-49575 and HL-59948 (to R.M.C.) from the National Institutes of Health. H.M.S. is the recipient of Research Career Development Award K04-HL-03006 from the National Institutes of Health.

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