Effect of ANG II type I receptor antagonist and ACE inhibitor on vitamin D receptor-null mice

Juan Kong and Yan Chun Li

Department of Medicine, University of Chicago, Chicago, Illinois 60637

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Kong, Juan and Yan Chun Li. Effect of ANG II type I receptor antagonist and ACE inhibitor on vitamin D receptor-null mice. Am J Physiol Regul Integr Comp Physiol 285: R255–R261, 2003. First published March 13, 2003; 10.1152/ajpregu.00517.2002.—We recently showed that vitamin D receptor (VDR) inactivation results in deregulated stimulation of the renin-angiotensin system (RAS). To address further the relation between RAS activation and the abnormalities in electrolyte and volume homeostasis, we studied the effect of the ANG II type I receptor antagonist losartan and the angiotensin-converting enzyme inhibitor captopril on VDR-null mice. Treatment with losartan or captopril normalized the water intake and urine excretion of VDR-null mice. However, the increase in salt excretion in VDR-null mice was not affected by either drug, suggesting that this abnormality is independent of the RAS. Northern blot and immunohistochemical analyses revealed that both drugs caused a drastic stimulation of renin expression in wild-type and VDR-null mice, but renin expression remained much higher in the treated VDR-null mice than in the treated wild-type mice, suggesting that the ANG II feedback mechanism remains intact in the mutant mice. These data firmly established a causative relation between RAS overstimulation and the abnormal volume homeostasis in VDR-null mice and demonstrated that vitamin D repression of renin expression is independent of the ANG II feedback regulation in vivo.

renin; captopril; losartan

THE RENIN-ANGIOTENSIN SYSTEM (RAS) plays an essential role in blood pressure, electrolyte, and extracellular volume homeostasis. The rate-limiting component of the RAS is renin, a protease produced predominantly by the juxtaglomerular (JG) cells in the kidney. The main function of renin is to convert angiotensinogen to ANG I, which is then converted to ANG II by the ANG-converting enzyme (ACE). ANG II is the central effector of the RAS and plays diverse physiological roles through interactions with its receptors (3). At least two types of ANG II receptors with unique biological functions have been identified in mammalian cells. The ANG II type I (AT1) receptor is believed to mediate such physiological effects as vasoconstriction, cell proliferation, and antinatriuresis, whereas the type II (AT2) receptor largely counterbalances the effects of the AT1 receptor and is involved in vasodilation, cell apoptosis, and natriuresis (4).

As the rate-limiting enzyme in the RAS, renin is well known to be regulated by multiple physiological stimuli, such as the renal perfusion pressure (mediated through the baroreceptor mechanism in JG cells), the renal sympathetic nerve activity (mediated by the β-adrenergic receptors in JG apparatus and the glomerulus), and the tubular sodium load (mediated by the macula densa) (2, 3, 8). Renin is also believed to be regulated by ANG II feedback suppression (10, 25). Clinically, inappropriate stimulation of the RAS represents a major risk factor for hypertension, heart attack, and stroke. For this reason, different classes of inhibitors targeting the ACE and ANG II receptors have been developed. For instance, losartan is a specific AT1 receptor antagonist, whereas captopril is an ACE inhibitor and blocks the conversion of ANG I to ANG II.

We recently demonstrated that 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] functions as a potent endocrine suppressor of renin expression. Mice lacking the vitamin D receptor (VDR) maintain an elevated renin expression and plasma ANG II production, develop hypertension, and exhibit increased water consumption and urinary salt excretion (17, 18). To correlate directly the physiological abnormalities with the overstimulation of the RAS and to address the relation between vitamin D repression and ANG II feedback inhibition in renin regulation, in the present study we investigated the effect of losartan and captopril treatment on VDR-null mice.

MATERIALS AND METHODS

Animals. Wild-type and VDR(−/−) littermates were produced through breeding of VDR(+/−) mice and identified by PCR with tail genomic DNA as the template. The mutant mice used in the study were originally generated in a C57BL6–129/sv mixed background (19) and backcrossed into the C57BL6 background for at least six generations. The animals were fed normal rodent chow and housed in a barrier facility with a 12:12-h dark-light cycle. The use of animals in this study was approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Animal treatment. Fourteen pairs of age-2–3 mo old) and gender-matched VDR(−/−) and wild-type littermates were used in the drug treatment study. Pretreatment data, including body weight, water and food consumption, and urinary

Address for reprint requests and other correspondence: Y. C. Li, Dept. of Medicine, University of Chicago, MC 4076, 5841 S. Maryland Ave., Chicago, IL 60637 (E-mail: cyao@medicine.bsd.uchicago.edu).

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excretion, were collected before the treatment. Then the mice were randomly divided into two groups, with seven pairs in each group. One group was treated with captopril (100 mg·kg body wt⁻¹·day⁻¹; Par Pharmaceutical, Spring Valley, NY) and the other with losartan (30 mg·kg body wt⁻¹·day⁻¹; Merck, Whitehouse Station, NJ). Both drugs were dissolved in drinking water. After 5 days of treatment, posttreatment data were collected, the mice were killed, and the plasma and kidneys were harvested.

Metabolic cages were used to measure water intake and collect urine. To this end, mice were housed individually in metabolic cages with free access to food and water for 24 h before and after the drug treatment. Samples from all mice were collected at the same time, and the pre- and posttreatment data were derived from the same animals to minimize experimental variations.

To determine whether a change from the regular cage to the metabolic cage may affect the physiological and biochemical parameters that we measured in the study, in another experiment, VDR(−/−) and wild-type mice were housed individually in metabolic cages with free access to food and water for 4 days so that their food and water consumption was stabilized. Then over a 24-h period from day 4 to day 5, the food and water intake of each mouse was determined, and urine was collected for analyses. The data obtained in this experiment, including food and water intake, urine volume, and urinary sodium and potassium contents, were highly consistent with the data obtained in the experiment described above (not shown).

**Measurement of plasma and urinary parameters.** Plasma and urinary sodium, potassium, calcium, phosphorus, and creatinine concentrations were determined by using a Beckman CX5 Autoanalyzer, as described previously (18). Urinary data were presented as salt-to-creatinine ratios and as total salt excretion normalized to body weight, which was obtained by multiplying urinary salt concentration by total urinary volume.

**Measurement of aldosterone.** Plasma and urinary aldosterone levels were determined using a Coat-A-Count aldosterone radioimmunoassay kit (Diagnostic Products, Los Angeles, CA). Before they were assayed, urine samples were hydrolyzed with HCl and extracted with ethyl acetate according to the instruction provided by the manufacturer.

**Immunohistochemistry.** Freshly dissected kidneys were fixed in 4% formaldehyde in PBS (pH 7.2) for 4 h. After being processed, the samples were embedded in paraffin wax and cut into 6-μm sections with a microtome (model 2030, Leica). Sections were immunostained with an antiserum against mouse renin and visualized with a peroxidase substrate diaminobenzidine kit (Vector Laboratories, Burlingame, CA) as described elsewhere (18).

**RNA isolation and Northern blot.** Total RNA was extracted from freshly dissected tissues by using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA). The renin mRNA transcript was determined by quantitative Northern blot analysis as described elsewhere (18). Briefly, total RNAs were separated on 1.2% agarose gels and transferred onto nylon membranes. The membranes were hybridized with 32P-labeled renin cDNA probe. After hybridization, the mRNAs were detected by autoradiography and quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The membranes were then stripped and rehybridized with 32P-labeled 36B4 cDNA probe for internal loading control.

**Statistical analysis.** Values are means ± SD and were analyzed with Student’s t-test to assess significance. P ≤ 0.05 was considered statistically significant.

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**RESULTS**

**Effect of VDR ablation on renin expression in the submaxillary glands.** We previously reported that the increase in the blood pressure of VDR(−/−) mice was abolished by captopril treatment (18). To address whether the other abnormalities in electrolyte and volume homeostasis seen in VDR(−/−) mice are also due to overstimulation of the RAS, the mice were treated with losartan and captopril, respectively. Before the treatment, water intake and urine excretion were increased more than twofold in VDR(−/−) mice compared with wild-type mice (Fig. 2, A and B), consistent with our previous observations (18). Losartan or captopril treatment had no significant effect on the water consumption and urine excretion of wild-type mice but reduced the water intake and urine volume of VDR(−/−) mice to levels similar to those of wild-type mice (Fig. 2, A and B). There was no significant difference in food intake between wild-type and VDR(−/−) mice, which was not significantly affected by losartan or captopril treatment (Fig. 2C).

**Effect of losartan and captopril on salt metabolism.** Figure 3 shows the urinary salt concentrations before and after losartan or captopril treatment. Urinary sodium and potassium excretion were significantly higher in untreated VDR(−/−) than in untreated wild-type mice. Neither losartan nor captopril had significant effects on sodium or potassium excretion in wild-type or VDR(−/−) mice; thus both drugs failed to normalize the urinary sodium or potassium level of VDR(−/−) mice (Fig. 3, A–D). Total urinary creatinine excretion was not significantly different between wild-type and VDR(−/−) mice and was not significantly

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**Fig. 1.** Effect of vitamin D receptor (VDR) inactivation on renin expression in submaxillary glands. Total RNAs were isolated from kidney and submaxillary glands of wild-type (+/+ ) and VDR(−/−) mice and analyzed by Northern blot (20 μg RNA/lane). Membranes were hybridized sequentially with 32P-labeled renin and 36B4 cDNA probes.
affected by losartan or captopril treatment (Fig. 3E). Despite the increase in urinary salt excretion, VDR(−/−) mice were not in a negative salt balance, because their plasma sodium and potassium levels were identical to those of wild-type mice before and after losartan or captopril treatment (Fig. 4). However, plasma potassium levels in wild-type and VDR(−/−) mice were increased to the same extent after losartan or captopril treatment (Fig. 4B) because of the hyperkalemic effect of these drugs (13, 28).

ANG II is known to stimulate aldosterone release and production from the adrenal glands. As expected, plasma and urinary aldosterone levels in VDR(−/−) mice were increased by >200% and 50%, respectively (Fig. 5). Captopril significantly reduced aldosterone levels in VDR(−/−) and wild-type mice, and the level in VDR(−/−) mice was still significantly higher after the treatment. However, losartan, at the dose used in the study, did not significantly suppress aldosterone production (Fig. 5).

Effect of losartan and captopril on renin expression. The effect of losartan or captopril treatment on renin expression in the kidney was assessed by Northern blot and immunohistochemical analyses. As reported pre-
viously (18), renin mRNA expression was increased more than threefold in VDR(−/−) mice than in wild-type mice (Fig. 6). After losartan or captopril treatment, renin mRNA levels in wild-type and VDR(−/−) mice were drastically increased (~5- to 8-fold higher than the respective untreated levels). Interestingly, the treated VDR(−/−) mice still expressed two to three times more renin mRNA transcripts than the treated wild-type mice (Fig. 6).

The level of renin protein, as assessed by renin-specific antibody in immunohistochemical staining, was consistent with the mRNA level. As shown previously (18), renin immunoreactivity in the afferent glomerular arterioles of the kidney was significantly increased in untreated VDR(−/−) mice (Fig. 7, A and B). Once again, losartan or captopril treatment led to a drastic stimulation of renin protein synthesis in wild-type and VDR(−/−) mice, but renin protein level remained significantly higher in the treated VDR(−/−) than in the treated wild-type mice (Fig. 7, C–F). It is known that, during maximal stimulation, upstream neighboring smooth muscle cells in the afferent glomerular arterioles are recruited to become renin-expressing cells (8). This transdifferentiation phenomenon was clearly seen in untreated VDR(−/−) mice (Fig. 7B) and was most prominent in the drug-treated VDR(−/−) mice (Fig. 7D and F, arrows). This was not unexpected, because renin expression in these treated VDR(−/−) mice was already ~20-fold higher than the physiologically normal level in untreated wild-type mice (see Fig. 6, B and D, for quantitation). Thus both the JG cells and the neighboring smooth muscle cells in the afferent arterioles contribute to the upregulation of renin expression in VDR(−/−) mice.

Fig. 5. Plasma and urinary aldosterone levels in wild-type and VDR knockout mice before and after losartan and captopril treatment. A: plasma aldosterone concentrations. B: urinary aldosterone concentrations. *P < 0.01 vs. corresponding +/+ values; **P < 0.05 vs. corresponding untreated values of the same genotype.

DISCUSSION

Epidemiological and clinical studies have long suggested a connection between low circulating 1,25(OH)2D3 levels or inadequate ultraviolet light exposure and high blood pressure (14, 23), and 1,25(OH)2D3 treatment has been shown to reduce blood pressure (11, 22). However, the mechanism is unknown. Our recent study demonstrated that 1,25(OH)2D3 functions as a negative endocrine regulator of the RAS by directly suppressing the renin gene expression (17, 18), which provides an insight into the mechanism.

Because of the lack of VDR-mediated vitamin D suppression, VDR(−/−) mice maintain a much higher renin and ANG II production than wild-type mice. The mutant mice develop hypertension and overdrinking behavior and exhibit increased urinary volume and salt excretion as well. These abnormalities were thought to be due to overstimulation of the RAS, inasmuch as ANG II is a potent vasoconstrictor and thirst inducer (3, 7), and overconsumption of water then results in increased urinary volume. To prove these points, we have shown that treatment of VDR(−/−) mice with captopril reverses the high blood pressure phenotype (18). In the present study, we used losartan and captopril, two drugs that block the RAS by different mechanisms, to treat the animals. As expected, both compounds normalized the water intake and urine volume of VDR(−/−) mice, strongly suggesting that these abnormalities are indeed caused by ANG II overproduction. It is well known that stimulation of thirst is one important mechanism whereby ANG II regulates extracellular volume. However, at this time, we cannot rule out the possibility that the increased...
drinking seen in VDR(-/-) mice may be secondary to obligatory urine loss in association with a defect in urinary concentrating capacity.

Thirst is induced mainly through ANG II acting on the central nervous system. Our observation that losartan, a AT1 receptor-specific antagonist, normalized the overdrinking behavior of VDR(-/-) mice suggests that ANG II-induced water drinking is mediated by the AT1 receptor, consistent with an early report demonstrating that the AT1b receptor mediates the ANG II-induced effects on thirst (5). Previous studies have shown that dehydration induces c-fos expression in...
specific neurons in the brain (6, 20, 21). Further studies are required to understand the neurological changes in the brain of VDR(−/−) mice.

Consistent with our early report (18), we show here that VDR(−/−) mice have increased urinary salt excretion while maintaining normal plasma electrolyte concentrations. Because their food intake is not higher than that of wild-type mice, it is speculated that VDR(−/−) mice might have a higher intestinal salt absorption to maintain the electrolyte balance. Superficially, some aspects of the abnormal salt metabolism may be explained by the higher ANG II and aldosterone levels found in VDR(−/−) mice, inasmuch as ANG II may induce sodium appetite and intestinal sodium absorption (9, 15), and aldosterone is known to stimulate urinary potassium excretion. However, several lines of evidence obtained in this study suggest that the abnormal salt metabolism of VDR(−/−) mice is independent of ANG II and aldosterone: 1) aldosterone is a major hormone with sodium-retaining capacity by its action on the distal nephron to decrease sodium excretion [this contradicts the elevated aldosterone level and higher sodium excretion in VDR(−/−) mice]; 2) neither losartan nor captopril, which block ANG II and its receptor, can normalize urinary sodium and potassium excretion in VDR(−/−) mice; 3) no reduction in urinary potassium excretion was observed in the treated animals with hyperkalemia, which is believed to be a consequence of drug-induced reduction in potassium excretion (28); and 4) there is no correlation between reduced aldosterone levels seen in captopril-treated mice and potassium excretion. Therefore, vitamin D may regulate salt metabolism by mechanisms unrelated to the RAS. There are several possible explanations for the higher salt excretion in VDR(−/−) mice. One is the secondary hyperparathyroidism developed in VDR(−/−) mice. The plasma parathyroid hormone level in adult VDR(−/−) mice is increased as much as 150-fold (18, 19). The high level of parathyroid hormone can dramatically reduce proximal tubular sodium reabsorption and, consequently, cause a higher sodium excretion (1, 12, 24). Another possible explanation comes from our recent DNA microarray study, which demonstrates that the expression pattern of a large number of genes in the kidney, including a number of ion channels and transporters involved in sodium and potassium transport, is altered in VDR(−/−) mice (16). Changes in those genes may well affect salt excretion.

ANG II feedback suppression of renin synthesis is believed to be an important regulatory loop in the RAS to ensure an appropriate renin level in the body. AT1 receptor-mediated inhibition of renin expression by ANG II has been shown in rats (25), and an upregulation of renin expression has been reported in angiotensinogen and AT1a gene knockout mice, likely because of the lack of feedback inhibition by ANG II (26, 27). These results support a direct, AT1 receptor-mediated ANG II suppression of renin expression in vivo. Our recent finding that vitamin D functions as a renin suppressor suggests that vitamin D, as an endocrine hormone, provides another layer of defense in the body to prevent renin overproduction. However, there is a possibility that the elevation of renin production in VDR(−/−) mice may be caused by impairment of other regulatory mechanisms (such as the ANG II feedback inhibition), which needs to be ruled out before the role of vitamin D can be definitively established. In this regard, we have demonstrated that renin upregulation in VDR(−/−) mice is independent of calcium metabolism and of the salt- or volume-sensing mechanisms (18). Here we show that losartan and captopril treatment drastically stimulate renin expression in wild-type as well as in VDR(−/−) mice, and the magnitude of the stimulation is almost identical in wild-type and VDR(−/−) mice. The increase in renin expression in the treated animals is due to the disruption of the ANG II feedback regulation caused by the drug treatment, although changes in other physiological parameters (such as perfusion pressure, sympathetic output, and/or tubular sodium load) that may also affect renin expression cannot be excluded at this time. These results indicate that the regulatory mechanisms for renin production, including the ANG II feedback regulation, are functionally intact in VDR(−/−) mice. Thus ANG II feedback repression and vitamin D repression of renin expression are independent negative regulatory pathways to maintain the homeostasis of the RAS.

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REFERENCES


