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Genetic AVP deficiency abolishes cold-induced diuresis but does not attenuate cold-induced hypertension

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Sun, Zhongjie. Genetic AVP deficiency abolishes cold-induced diuresis but does not attenuate cold-induced hypertension. *Am J Physiol Renal Physiol* 290: F1472–F1477, 2006. First published January 5, 2006; doi:10.1152/ajprenal.00430.2005.—Chronic cold exposure causes hypertension and diuresis. The aim of this study was to determine whether vasopressin (AVP) plays a role in cold-induced hypertension and diuresis. Two groups of Long-Evans (LE) and two groups of homozygous AVP-deficient Brattleboro (VD) rats were used. Blood pressure (BP) was not different among the four groups during a 2-wk control period at room temperature (25°C, warm). After the control period, one LE group and one VD group were exposed to cold (5°C); the remaining groups were kept at room temperature. BP and body weight were measured weekly during exposure to cold. Food intake, water intake, urine output, and urine osmolality were measured during *weeks 1, 3*, and *5* of cold exposure. At the end of *week 5*, all animals were killed and blood was collected for measurement of plasma AVP. Kidneys were removed for measurement of renal medulla V_2 receptor mRNA and aquaporin-2 (AQP-2) protein expression. BP of LE and VD rats increased significantly by *week 2* of cold exposure and reached a high level by *week 5*. BP elevations developed at approximately the same rate and to the same degree in LE and VD rats. AVP deficiency significantly increased urine output and solute-free water clearance and decreased urine osmolality. Chronic cold exposure increased urine output and solute-free water clearance and decreased urine osmolality in LE rats, indicating that cold exposure caused diuresis in LE rats. Cold exposure failed to affect these parameters in VD rats, suggesting that the AVP system is responsible for cold-induced diuresis. Cold exposure did not alter plasma AVP in LE rats. Renal medulla V_2 receptor mRNA and AQP-2 protein expression levels were decreased significantly in the cold-exposed LE rats, suggesting that cold exposure inhibited renal V_2 receptors and AVP-inducible AQP-2 water channels. We conclude that *1*) AVP may not be involved in the pathogenesis of cold-induced hypertension, *2*) the AVP system plays a critical role in cold-induced diuresis, and *3*) cold-induced diuresis is due to suppression of renal V_2 receptors and the associated AQP-2 water channels, rather than inhibition of AVP release.

cold exposure; inbred Brattleboro rats; vasopressin; V_2 receptor; aquaporin-2

CHRONIC EXPOSURE TO COLD induces hypertension and cardiac hypertrophy within 1–3 wk (4, 16, 19–22). Cold-induced hypertension (CIH) represents a prototypical model of environmentally induced hypertension. A number of hormonal responses occur during cold exposure, each of which may contribute to cold-induced elevation of blood pressure (BP). It seems reasonable that CIH is not the result of one discrete alteration but, rather, is as multifactorial and complicated as essential hypertension. Chronic cold exposure may increase secretion of several stress-induced hormones, including arginine vasopressin (AVP). In addition to its antidiuretic properties, AVP is a very potent vasoconstrictor. We hypothesized that AVP plays a role in the development of CIH. Thus the first objective of this experiment was to test this hypothesis using genetic AVP-deficient rats chronically exposed to cold. Our previous studies showed that the renin-angiotensin system (RAS) may be involved in the pathogenesis of CIH (19–21, 25, 27). Therefore, aortic angiotensin II (ANG II) content was measured to determine whether AVP deficiency affects the RAS in cold-exposed rats.

Chronic cold exposure also induces diuresis, as evidenced by increased urine output and decreased urine osmolality in cold-exposed animals (22, 23). However, the mechanism mediating cold-induced diuresis is not clear. Because an increase in AVP release is expected in cold-exposed rats, we hypothesized that cold-induced diuresis is not mediated by the AVP system. Thus the second objective of this study was to determine whether the AVP system plays a role in cold-induced diuresis by measuring urine output, urine osmolality, and solute-free water clearance in AVP-deficient rats during exposure to cold.

Aquaporin-2 (AQP-2) is the AVP-regulated water channel that mediates water transport across the apical plasma membrane of the renal collecting duct (5). The number of AQP-2 water channels in the apical membranes of the collecting ducts determines the water permeability of the apical membrane and is principally regulated by AVP via V_2 receptors (12, 14, 32). Therefore, the effect of chronic cold exposure on the regulation of renal V_2 receptors and AQP-2 water channels is evaluated in this study.

METHODS

Animals. Two groups of Long-Evans (LE) and two groups of genetic AVP-deficient (VD) rats (6 rats/group) were used. Systolic BP was measured three times and body weight was measured twice during a 2-wk control period at room temperature (25°C, warm). Systolic BP was measured from the tail of each unanesthetized rat by the tail-cuff method with slight warming (28°C), but not heating, of the tail. All rats were handled frequently (3 times/day, 5 min each time) to minimize handling stress. Animals did not appear stressed during BP measurement. The tail-cuff method has been commonly used by us (19–20, 24, 25) and others (16, 33) to delineate cold-induced elevation of BP. The noninvasive tail-cuff method has been confirmed by intra-arterial cannulation to be effective and reliable for monitoring systolic BP in rats exposed to cold (4, 26).

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Experimental protocol. After the control period, one group of LE rats and one group of VD rats were moved into a cold climate-controlled walk-in chamber (5 ± 2°C), while the remaining groups were maintained at room temperature (warm, 25 ± 2°C) and served as control. Relative humidity was controlled automatically at 45 ± 5% in both thermal environments. All rats were housed individually in wire mesh cages without bedding throughout the experiment. The temperature inside the cage was confirmed to be the same as that of the chamber. All the rats were provided with laboratory chow (No. 5001, Purina, St. Louis, MO) and tap water. For all rats, lights were on from 0700 to 1900. BP and body weight were measured weekly in all rats during exposure to cold. Food intake, water intake, and urine output were measured for 3 consecutive days during the control period and during the *weeks 1, 3*, and *5* of cold exposure. Food containers and fluid containers are spill resistant and have been used in previous studies (21–23). Urine was collected in volumetric beakers and saved for measurement of osmolality with a vapor pressure osmometer as described previously (22, 23). Evaporation was prevented by light mineral oil (Fisher). At the end of *week 5* of cold exposure, all rats were killed by decapitation. Blood was collected in EDTA for measurement of plasma AVP concentration. Plasma AVP was measured using radioimmunoassay as described previously (22, 23). Plasma osmolality also was measured as described previously (22, 23) for calculation of solute-free water clearance. The heart and kidneys were removed and weighed. V_2 receptor mRNA expression and AQP-2 protein expression were measured in the renal medulla using quantitative real-time RT-PCR and Western blot analysis, respectively. The aorta was removed for measurement of tissue ANG II content as described previously (19).

Calculation of solute-free water clearance. Solute-free water clearance, or free water clearance, represents the ability of the kidney to excrete water. It was calculated from rats exposed to cold for 5 wk using the following equation: free water clearance = urine output − (urine osmolality ÷ plasma osmolality) × urine output. A positive value indicates that the kidney adds free water to the urine; a negative value means that the kidney reabsorbs free water from the urine. A negative free water clearance also is called free water reabsorption, which represents the ability of the kidney to maintain water.

Quantitative real-time RT-PCR. Renal medulla V_2 receptor mRNA was quantified using quantitative real-time RT-PCR as described previously (20, 23, 27). Briefly, the kidney inner medulla was dissected and homogenized. Quantitative real-time RT-PCR was performed with a sequence detector (model 5700, PE Biosystems, Foster City, CA). Specific quantitative assays for rat V_2 receptor mRNA and 36B4 were developed using Primer Express software (PE Biosystems) following the recommended guidelines based on cDNA sequences from GenBank. The rat V_2 receptor primers were 5′-CCATGGTTCTGCAAATCGGG-3′ (antisense) and 5′-TAGGTCATCATCAACCACCCCA-3′ (sense). The precise amount of total RNA added to each reaction (based on optical density) and its quality were checked by quantification of the endogenous RNA control 36B4 (also known as RPLP0). Each sample was then normalized on the basis of its 36B4 content.

Western blot analysis. Renal medulla AQP-2 protein expression was quantified by Western blot as described in our previous studies (27, 30, 31). Rabbit anti-rat AQP-2 antibody (1:1,000 dilution) and goat anti-rabbit IgG-horseradish peroxidase (1:2,000 dilution; Santa Cruz Biotechnology) were used to reveal rat AQP-2. Each band was normalized with β-actin.

Statistical analysis. Data for BP, body weight, food intake, water intake, urine output, and urine osmolality were analyzed by a two-way ANOVA (temperature and strain) followed by a one-way ANOVA repeated in time. Data for organ weights, ratio of water to food intake, free water clearance, V_2 receptor mRNA, AQP-2 protein, and plasma AVP were analyzed by a two-way ANOVA followed by a one-way ANOVA. Newman-Keuls procedure was used to assess the significance of differences between means. Significance was set at the 95% confidence limit.

RESULTS

BP and body and organ weight. The basal BP of the four groups did not differ during the control period at room temperature (Fig. 1*A*). BP of the LE-Cold and VD-Cold groups increased significantly ($P < 0.05$) above that of their counterparts kept at room temperature by *week 2* of cold exposure. BP of these two groups increased to a high level at *week 5* of cold exposure ($P < 0.01$ vs. their counterparts at room temperature). Cold exposure increased BP in VD rats at approximately the same rate and to the same degree as in LE rats (Fig. 1*A*). BP of the LE-Cold and VD-Cold groups did not differ significantly ($P > 0.05$) throughout exposure to cold. BP of LE-Warm and VD-Warm groups did not differ, nor did they change across the experiment ($P > 0.05$).

Body weight was generally greater ($P < 0.05$) in the LE than in the VD rats, although no difference was found in the initial measurement (Fig. 1*B*). Cold exposure did not change body weight gain significantly ($P > 0.05$) in LE or VD rats.

Heart and kidney weights of LE-Cold and VD-Cold groups were increased significantly ($P < 0.01$) compared with their counterparts kept at room temperature (Fig. 2). Cold exposure

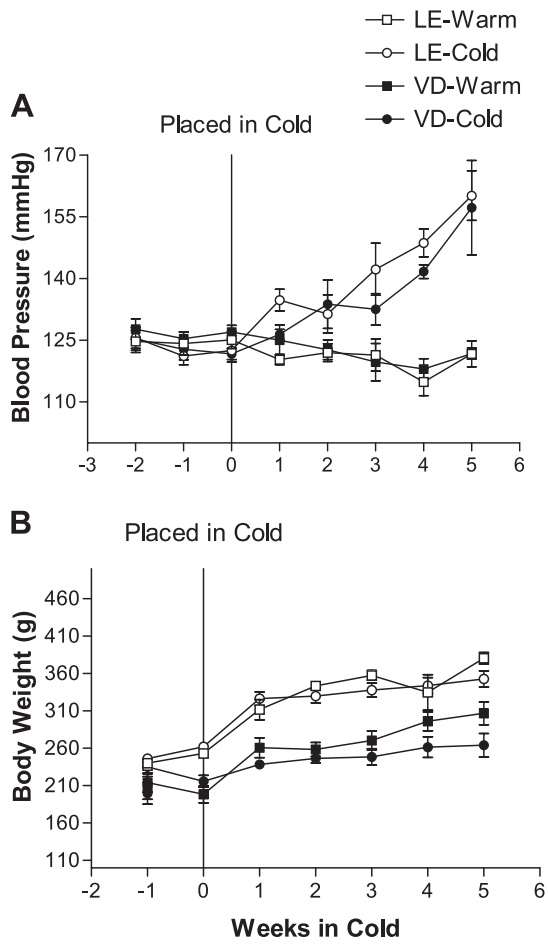

Fig. 1. Systolic blood pressure (*A*) and body weight (*B*) of AVP-deficient (VD) and Long-Evans (LE) rats exposed to cold (5°C) or maintained at room temperature (25°C, warm). Values are means ± SE ($n = 6$).

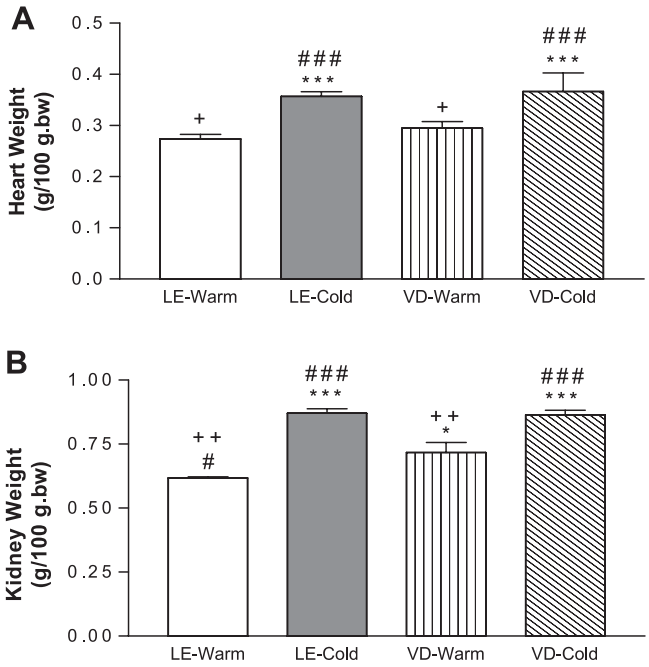

Fig. 2. Heart weight (*A*) and kidney weight (*B*) of VD and LE rats exposed to cold or kept at room temperature. bw, Body weight. Values are means ± SE ($n = 6$). *$P < 0.05$; ***$P < 0.001$ vs. LE-Warm. +$P < 0.05$; ++$P < 0.01$ vs. LE-Cold. ###$P < 0.001$ vs. VD-Warm.

increased heart and kidney weights to approximately the same extent in VD and LE rats ($P > 0.05$). Kidney weight was greater ($P < 0.05$) in the VD-Warm than in the LE-warm group, indicating that AVP deficiency caused renal hypertrophy.

Food intake, water intake, urine output, and urine osmolality. Food intake was not different ($P > 0.05$) among the four groups during the control period at room temperature (Fig. 3), indicating that AVP deficiency did not affect food intake. Chronic cold exposure increased food intake significantly in LE and VD rats ($P < 0.01$ vs. their counterparts at room temperature) at *weeks 1, 3*, and *5*. Water intake (Fig. 4) and urine output (Fig. 5) were increased significantly ($P < 0.001$) in the VD rats compared with the LE rats throughout the

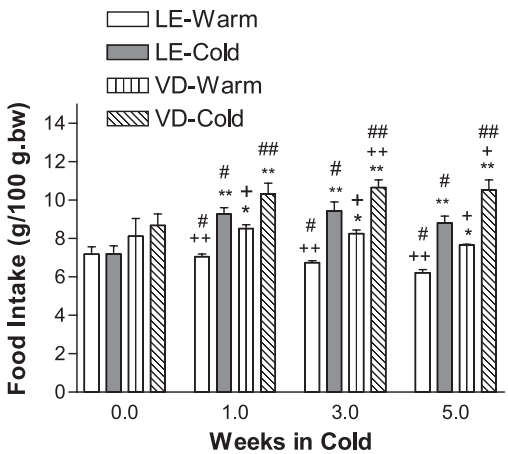

Fig. 3. Daily food intake of VD and LE rats exposed to cold or kept at room temperature. Values are means ± SE ($n = 6$). *$P < 0.05$; **$P < 0.01$ vs. LE-Warm. +$P < 0.05$; ++$P < 0.01$ vs. LE-Cold. #$P < 0.05$; ##$P < 0.01$ vs. VD-Warm.

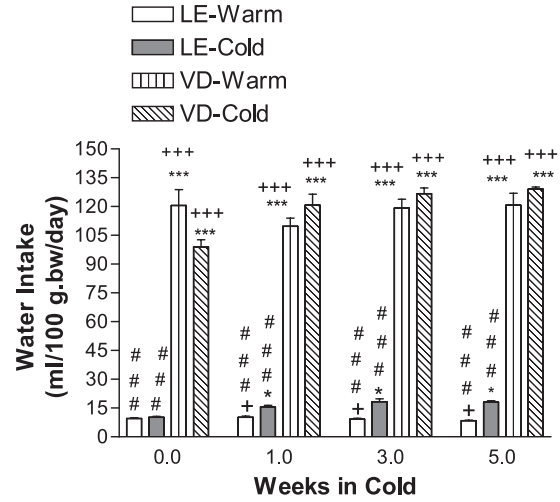

Fig. 4. Daily water intake of VD and LE rats exposed to cold or kept at room temperature. Values are means ± SE ($n = 6$). *$P < 0.05$; ***$P < 0.001$ vs. LE-Warm. +$P < 0.05$; +++$P < 0.001$ vs. LE-Cold. #$P < 0.05$ vs. VD-Warm.

experiment. Cold exposure increased water intake and urine output significantly in LE rats ($P < 0.05$). In contrast, cold exposure failed to increase water intake and urine output significantly in VD rats ($P > 0.05$; Figs. 4 and 5). Urine osmolality was decreased significantly ($P < 0.01$) in VD rats compared with LE rats throughout the experiment (Fig. 6). Cold exposure decreased urine osmolality significantly ($P < 0.01$) in LE rats compared with the LE-Warm group. In contrast, cold exposure did not further decrease urine osmolality in VD rats.

Ratio of water to food intake and free water clearance. The ratio of water to food intake at *week 5* of cold exposure is shown in Fig. 7*A*. In VP rats, the ratio of water to food intake was increased significantly ($P < 0.001$) compared with the LE groups. However, cold exposure failed to alter the ratio of water to food intake in VD rats ($P > 0.05$). In contrast, cold exposure significantly increased ($P < 0.05$) the ratio of water to food intake in LE rats (Fig. 7*A*).

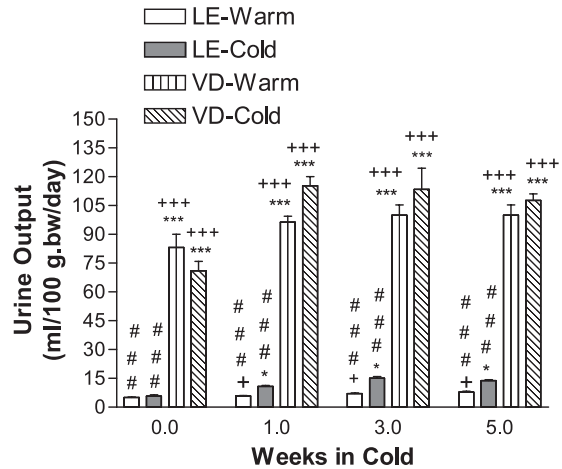

Fig. 5. Daily urine output of VD and LE rats exposed to cold or kept at room temperature. Values are means ± SE ($n = 6$). *$P < 0.05$; ***$P < 0.001$ vs. LE-Warm. +$P < 0.05$; +++$P < 0.001$ vs. LE-Cold. #$P < 0.05$ vs. VD-Warm.

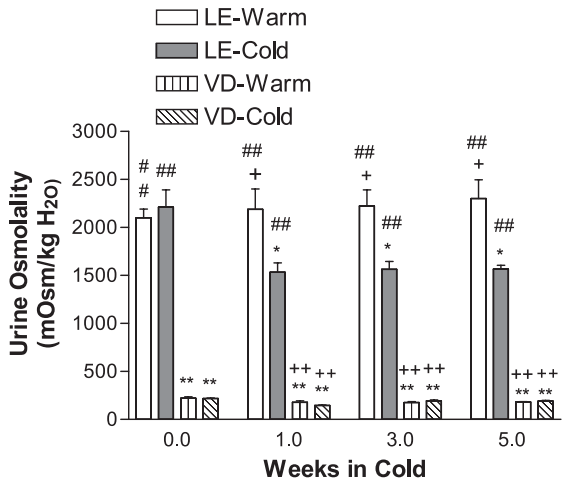

Fig. 6. Urine osmolality of VD and LE rats exposed to cold or kept at room temperature. Values are means ± SE ($n = 6$). *$P < 0.05$; **$P < 0.01$ vs. LE-Warm. +$P < 0.05$; ++$P < 0.01$ vs. LE-Cold. #$P < 0.05$; ##$P < 0.01$ vs. VD-Warm.

Free water clearance measured at *week 5* of cold exposure is shown in Fig. 7*B*. Cold exposure increased free water clearance, i.e., decreased free water reabsorption in LE rats ($P < 0.01$ vs. LE-Warm). AVP deficiency increased free water clearance significantly ($P < 0.001$ vs. LE-Warm). However, cold exposure did not affect free water clearance in VD rats ($P > 0.05$).

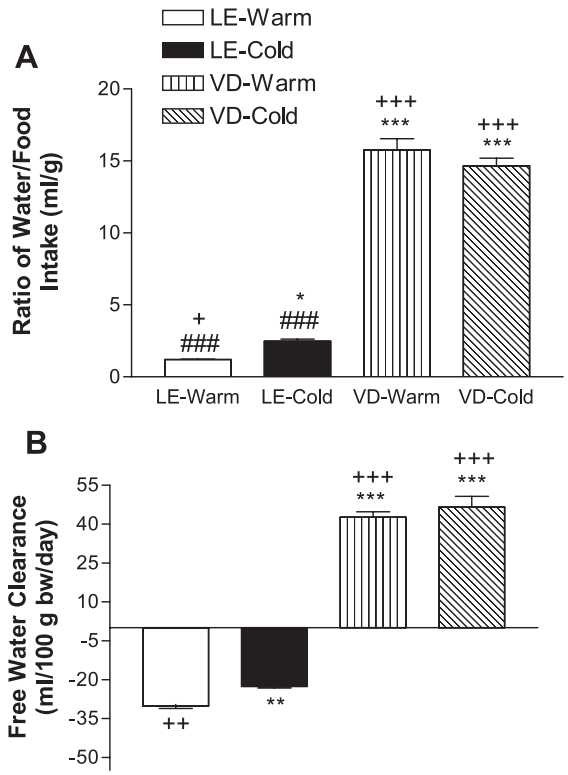

Fig. 7. Ratio of water to food intake (*A*) and free water clearance (*B*) of VD and LE rats exposed to cold or kept at room temperature. Values are means ± SE ($n = 6$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. LE-Warm. +$P < 0.05$; ++$P < 0.01$; +++$P < 0.001$ vs. LE-Cold. ###$P < 0.001$ vs. VD-Warm.

Table 1. *Plasma concentration of AVP and aortic ANG II content at week 5*

Group	Plasma AVP, pg/ml	Aortic ANG II, pg/g tissue
LE-Warm	4.70±0.68	71.95±6.41
LE-Cold	5.06±0.46	145.15±8.69*
VD-Warm	ND	72.51±5.62
VD-Cold	ND	149.68±7.84†

Values are means ± SE ($n = 6$). LE-Warm and LE-Cold, Long-Evans rats kept at room temperature (25°C) and exposed to cold (5°C); VD-Warm and VD-Cold, AVP-deficient rats kept at room temperature and exposed to cold; ND, not detectable. *$P < 0.001$ vs. LE-Warm. †$P < 0.001$ vs. VD-Warm.

Plasma AVP and aortic ANG II content. Cold exposure did not alter plasma AVP level in LE rats ($P > 0.05$; Table 1). Plasma AVP was not detectable in VD rats. Aortic ANG II content was increased significantly ($P < 0.001$) in both cold-exposed groups compared with their counterparts kept at room temperature (Table 1). No significant difference ($P > 0.05$) of aortic ANG II content was found between LE and VD groups in either thermal condition, indicating that AVP deficiency did not affect the cold-induced increase in vascular ANG II content.

Renal V_2 receptor mRNA and AQP-2 water channel protein. Cold exposure significantly decreased ($P < 0.001$) renal medulla V_2 receptor mRNA levels in LE and VD rats (Fig. 8*A*). V_2 receptor mRNA was significantly increased in the VD-Warm group ($P < 0.001$ vs. LE-Warm).

Renal medulla AQP-2 protein expression was significantly decreased ($P < 0.001$) in the LE-Cold group compared with the LE-Warm group (Fig. 8*B*). AQP-2 protein expression was decreased significantly in both VD groups ($P < 0.001$ vs. LE-Warm). AQP-2 protein expression level was not significantly different ($P > 0.05$) between the VD-Warm and the VD-Cold group.

DISCUSSION

The present data clearly show that genetic AVP deficiency did not attenuate cold-induced elevation of BP and cardiac hypertrophy (Figs. 1 and 2). AVP is absent in VD rats (Table 1), confirming complete AVP deficiency. These results suggest, for the first time, that AVP may not be involved in the development of CIH. However, AVP plays a critical role in deoxycorticosterone acetate (DOCA)-salt hypertension (7, 9, 11, 18), because AVP deficiency attenuates DOCA-induced elevation of BP (9). Indeed, large doses of DOCA activate the AVP system (7, 9, 11, 18). In contrast, chronic cold exposure induced hypertension (Fig. 1*A*) without increasing the plasma AVP level in LE rats (Table 1). This result suggests that the mechanism of CIH is different from that of DOCA-salt hypertension, although they may share some common characteristics (28). CIH is a unique, "natural" form of experimental hypertension that does not require surgery, administration of large doses of hormones or drugs, or genetic manipulation. Numerous studies suggest that cold exposure activates the RAS (19, 21, 22, 24, 25). The overactive RAS may be responsible for the pathogenesis of CIH, because knockout of the angiotensinogen gene or the AT_{1A} receptor gene attenuated cold-induced elevation of BP (16, 27). Indeed, cold exposure increased aortic ANG II content in LE and VD rats to approximately the same extent (Table 1), explaining equal elevation of BP in the two

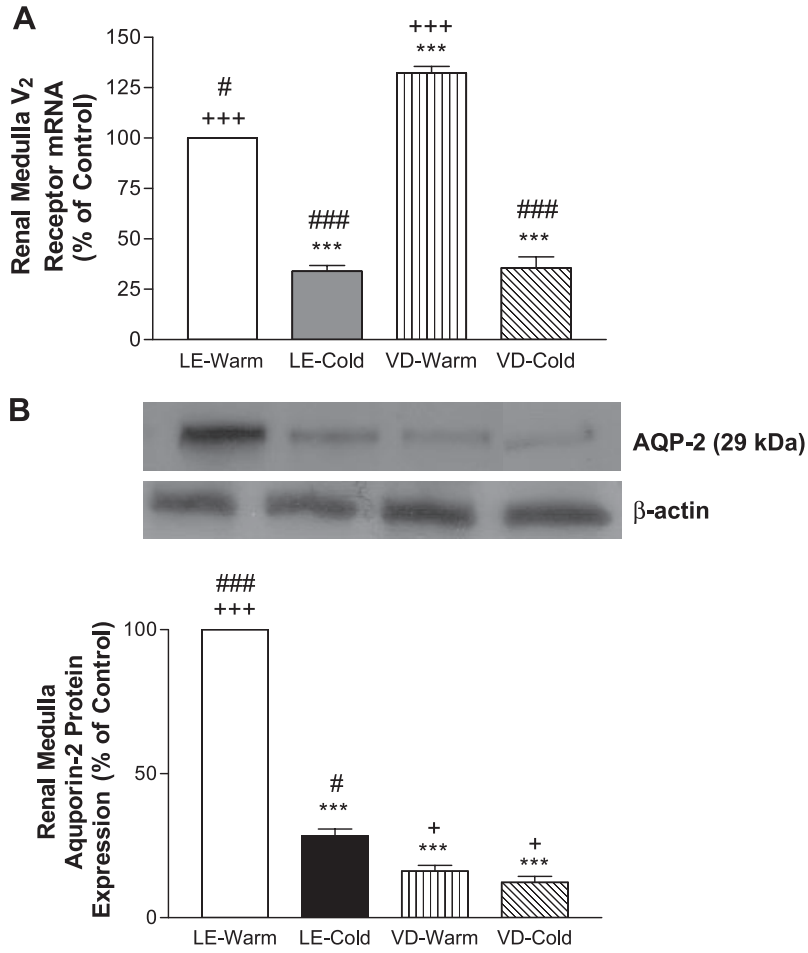

Fig. 8. Renal medulla V_2 receptor mRNA expression (*A*) and renal aquaporin-2 (AQP-2) protein expression (*B*) of VD and LE rats exposed to cold and kept at room temperature. Values are means ± SE ($n = 6$). ***$P < 0.001$ vs. LE-Warm. +$P < 0.05$; +++$P < 0.001$ vs. LE-Cold. #$P < 0.05$; ###$P < 0.001$ vs. VD-Warm.

strains of rats during exposure to cold. The present study also suggests that AVP-deficient rats are able to maintain normal BP, because AVP deficiency did not alter BP at room temperature (Fig. 1*A*).

Chronic cold exposure resulted in diuresis, as evidenced by increased output of low-osmolality urine in the LE-Cold group (Figs. 5 and 6). Cold-induced diuresis is independent of high BP, because prevention of CIH did not affect diuresis in cold-exposed rats (22). It is likely that cold-induced diuresis is caused by decreased renal concentrating ability, because cold exposure decreased urine osmolality (Fig. 6) and free water reabsorption (Fig. 7*B*) in LE rats. The increased ratio of water to food intake (Fig. 7*A*) suggests that thirst was increased in the LE-Cold group; this was probably caused by the increased water loss due to diuresis. Not surprisingly, AVP deficiency increased water intake and urine output and decreased urine osmolality. Cold exposure failed to increase the ratio of water to food intake (Fig. 7*A*), free water clearance (Fig. 7*B*), and urine output (Fig. 5) in VD rats. Moreover, cold exposure did not decrease urine osmolality in VD rats (Fig. 6). These results suggest that the AVP system is responsible for cold-induced diuresis, which disproves our hypothesis (see the introduction). However, cold-induced diuresis was not due to a decrease in AVP release, because plasma AVP level was not altered by cold exposure in LE rats (Table 1).

Cold exposure inhibited renal V_2 receptors, as evidenced by the decreased V_2 receptor mRNA expression in the renal medulla in the LE-Cold group (Fig. 8*A*). Cold exposure decreased the AVP-inducible AQP-2 water channel protein expression in the renal medulla in the LE-Cold group (Fig. 8*B*). This is the first study showing that chronic cold exposure may inhibit renal medulla AQP-2 protein expression. It was reported that selective blockade of V_2 receptors decreased AQP-2 expression and resulted in retrieval of AQP-2 from the apical membrane (12, 14, 32). Therefore, the decreased AQP-2 expression was probably due to the downregulation of V_2 receptors in the LE-Cold group. Similar to V_2 receptor antagonism, AVP deficiency attenuated AQP-2 protein expression to a minimum level in VD rats in both thermal environments (Fig. 8*B*). Although V_2 receptor expression was increased in the VD-Warm group, AQP-2 expression was decreased. This result supports the report that AQP-2 expression is AVP dependent (12). Therefore, inhibition of V_2 receptors and the associated decrease in AQP-2 water channel protein may mediate cold-induced diuresis in LE rats.

The decrease in the renal concentrating response to a V_2 receptor agonist in cold-exposed rats reported in our previous study (23) may be explained by the present finding that cold exposure downregulated expression of V_2 receptors (Fig. 8*A*). The increased V_2 receptor mRNA expression in the VD-Warm group (Fig. 8*A*) was likely due to the lack of AVP (Table 1). However, V_2 receptor mRNA expression was inhibited in the VD-Cold group. Cold exposure also downregulated V_2 receptors in LE rats (Fig. 8*A*), while plasma AVP level remained

unchanged (Table 1). The mechanism by which cold exposure inhibited V_2 receptors is not clear but is not related to AVP levels. Recent receptor physiology indicates that a receptor can be regulated by many factors in addition to its ligand. It has been reported that glucocorticoid hormones and catecholamines inhibit renal responses to exogenous administration of pitressin (an analog of AVP) in animals (3, 6). Renal α_2-adrenoceptors suppress AVP-stimulated cAMP accumulation, and α_2-adrenoceptor antagonism attenuates the diuretic response to acute cold exposure (1). The blood levels of both of these hormones are elevated during exposure to cold (15, 17, 21, 29). An additional study is required to test the hypothesis that an elevation of glucocorticoid hormones and/or catecholamines in the blood of cold-exposed rats inhibits or downregulates renal V_2 receptors.

Acute and chronic exposures to cold induced diuresis probably via different mechanisms: the former via a reduction in plasma AVP (2, 10, 13) and the latter by suppression of renal V_2 receptors without reduction of plasma AVP. Kidney weights of LE and VD rats were increased equally by cold exposure, suggesting that AVP may not be involved in cold-induced renal hypertrophy. However, the renal hypertrophy that developed in the VD-Warm group (Fig. 2*B*) is probably a compensatory renal response to the increased water excretion.

In summary, genetic AVP deficiency did not attenuate the cold-induced elevation of BP, indicating that AVP may not be involved in CIH and cardiac hypertrophy. However, the AVP system may play a critical role in cold-induced diuresis, because cold exposure failed to increase free water clearance and urine output in AVP-deficient rats. Cold-induced diuresis may be due to the downregulation of V_2 receptors and the associated decrease in AQP-2 water channel protein expression, rather than inhibition of AVP release.

GRANTS

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