Long-term aldosterone treatment induces decreased apical but increased basolateral expression of AQP2 in CCD of rat kidney

Sophie de Seigneux, Jakob Nielsen, Emma T. B. Olesen, Henrik Dimke, Tae-Hwan Kwon, Jørgen Frøkiær and Søren Nielsen

Am J Physiol Renal Physiol 293:F87-F99, 2007. First published 20 March 2007;
doi:10.1152/ajprenal.00431.2006

You might find this additional info useful...

This article cites 37 articles, 27 of which can be accessed free at:

<http://ajprenal.physiology.org/content/293/1/F87.full.html#ref-list-1>

This article has been cited by 8 other HighWire hosted articles, the first 5 are:

Effect of high and low sodium intake on urinary aquaporin-2 excretion in healthy humans

Carolina Cannillo Graffe, Jesper Nørgaard Bech and Erling Bjerregaard Pedersen
Am J Physiol Renal Physiol, January 15, 2012; 302 (2): F264-F275.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Altered urinary excretion of aquaporin 2 in IgA nephropathy

Maria Teresa Rocchetti, Grazia Tamma, Domenica Lasorsa, Ida Valentina Suriano, Annamaria D'Apollò, Massimo Papale, Lisa Mastrofrancesco, Giuseppe Grandaliano, Maria Svelto, Giovanna Valenti, Loreto Gesualdo and Salvatore Di Paolo
Eur J Endocrinol, October, 2011; 165 (4): 657-664.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Protein-enriched diet increases water absorption via the aquaporin-2 water channels in healthy humans

Thomas Guldager Lauridsen, Henrik Vase, Jørn Starklint, Jesper N. Bech and Erling B. Pedersen
Nephrol. Dial. Transplant., August, 2010; 25 (8): 2502-2510.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Direct effect of methylprednisolone on renal sodium and water transport via the principal cells in the kidney

Thomas G Lauridsen, Henrik Vase, Jesper N Bech, Søren Nielsen and Erling B Pedersen
Eur J Endocrinol, May, 2010; 162 (5): 961-969.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Protein-enriched diet increases water absorption via the aquaporin-2 water channels in healthy humans

Thomas Guldager Lauridsen, Henrik Vase, Jørn Starklint, Jesper N. Bech and Erling B. Pedersen
Nephrol. Dial. Transplant., March 17, 2010; .

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high resolution figures, can be found at:

<http://ajprenal.physiology.org/content/293/1/F87.full.html>

Additional material and information about *AJP - Renal Physiology* can be found at:

<http://www.the-aps.org/publications/ajprenal>

This information is current as of July 11, 2012.

Long-term aldosterone treatment induces decreased apical but increased basolateral expression of AQP2 in CCD of rat kidney

Sophie de Seigneux,^{1,2} Jakob Nielsen,^{1,2} Emma T. B. Olesen,^{1,2}
Henrik Dimke,^{1,2} Tae-Hwan Kwon,^{1,3} Jørgen Frøkiær,^{1,4} and Søren Nielsen^{1,2}

¹The Water and Salt Research Center and ²Institute of Anatomy, University of Aarhus, Aarhus C;

³Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Taegu, Korea; and ⁴Institute of Clinical Medicine, Aarhus University Hospital, Aarhus N, Denmark

Submitted 30 October 2006; accepted in final form 16 March 2007

de Seigneux S, Nielsen J, Olesen ET, Dimke H, Kwon T-H, Frøkiær J, Nielsen S. Long-term aldosterone treatment induces decreased apical but increased basolateral expression of AQP2 in CCD of rat kidney. *Am J Physiol Renal Physiol* 293: F87–F99, 2007. First published March 20, 2007; doi:10.1152/ajprenal.00431.2006.—The purpose of the present studies was to determine the effects of high-dose aldosterone and dDAVP treatment on renal aquaporin-2 (AQP2) regulation and urinary concentration. Rats were treated for 6 days with either vehicle (CON; $n = 8$), dDAVP (0.5 ng/h, dDAVP, $n = 10$), aldosterone (Aldo, 150 $\mu\text{g/day}$, $n = 10$) or combined dDAVP and aldosterone treatment (dDAVP+Aldo, $n = 10$) and had free access to water with a fixed food intake. Aldosterone treatment induced hypokalemia, decreased urine osmolality, and increased the urine volume and water intake in ALDO compared with CON and dDAVP+Aldo compared with dDAVP. Immunohistochemistry and semiquantitative laser confocal microscopy revealed a distinct increase in basolateral domain AQP2 labeling in cortical collecting duct (CCD) principal cells and a reduction in apical domain labeling in Aldo compared with CON rats. Given the presence of hypokalemia in aldosterone-treated rats, we studied dietary-induced hypokalemia in rats, which also reduced apical AQP2 expression in the CCD but did not induce any increase in basolateral AQP2 expression in the CCD as observed with aldosterone treatment. The aldosterone-induced basolateral AQP2 expression in the CCD was thus independent of hypokalemia but was dependent on the presence of sodium and aldosterone. This redistribution was clearly blocked by mineralocorticoid receptor blockade. The increased basolateral expression of AQP2 induced by aldosterone may play a significant role in water metabolism in conditions with increased sodium reabsorption in the CCD.

aquaporin-2; mineralocorticoid; water; salt; trafficking; cortical collecting duct

ALDOSTERONE AND VASOPRESSIN are major hormones in the regulation of extracellular fluid volume. A potential direct role of aldosterone in water metabolism is still unclear. As previously demonstrated, arginine vasopressin (AVP) increases the osmotic water permeability (P_f) in isolated, perfused collecting ducts from normal rabbits and rats (3, 23). The increased P_f by AVP is mediated by the vasopressin-regulated water channel aquaporin-2 (AQP2) expressed in connecting tubule (CNT) cells and collecting duct principal cells (10, 27, 28). Pretreatment of rabbits with mineralocorticoid (in vivo, deoxycorticosterone) enhanced the AVP-induced increase in P_f by AVP in the isolated, perfused cortical collecting duct (CCD) (3), sug-

gesting that mineralocorticoids may play some role in the regulation of water balance in some species.

Previous studies have demonstrated effects of aldosterone on AQP2 regulation. In vitro studies show that aldosterone has a synergistic effect with vasopressin on stimulation of AQP2 expression in a mouse CCD cell line after >24 h of exposure to the hormone (13). Animal models of aldosterone-deficient vs. aldosterone-replaced adrenalectomized rats presented no changes in whole-kidney AQP2 expression (18), although segmental differences cannot be excluded. In addition to regulation of protein expression, AQP2 is regulated by trafficking from intracellular vesicles to the apical plasma membrane, thereby increasing P_f (27). Acutely, aldosterone has been described to stimulate a rise in intracellular calcium (12) in CCD cells and increase cAMP in inner medullary collecting duct cells (31), suggesting that acute exposure to aldosterone could be involved in the regulation of AQP2 trafficking. In adrenalectomized rats, aldosterone deficiency has no apparent effect on the subcellular distribution of AQP2 in the inner medullary collecting duct (18). However, recent evidence demonstrates that chronic high-dose aldosterone infusion in rats with diabetes insipidus (e.g., lithium-induced nephrogenic diabetes insipidus in Brattleboro rats) induces an increase in urine output and a marked change in subcellular redistribution of AQP2, mainly in the CNT and CCD (26), further supporting a potential role of aldosterone in AQP2 regulation and body water homeostasis.

In normal rats, the effect of long-term aldosterone infusion and possible interactive effects with dDAVP on AQP2 protein expression and subcellular localization of AQP2 in the CCD have not previously been studied. Here, we investigate whether long-term aldosterone treatment alone or together with dDAVP infusion affects 1) water homeostasis, 2) the protein expression of AQP2, and 3) the subcellular distribution of AQP2 and Ser²⁵⁶ phosphorylated AQP2 (p-AQP2) compared with normal untreated control rats and rats treated with dDAVP. The main focus of this article is on the cortical part of the collecting duct, the part of the collecting duct most sensitive to the effects of aldosterone.

METHODS

Experimental Protocols

All animal protocols were approved by the boards of the Institute of Anatomy and Institute of Clinical Medicine, University of Aarhus,

Address for reprint requests and other correspondence: S. Nielsen, The Water and Salt Research Center, Institute of Anatomy, Bldg. 233/234, Univ. of Aarhus, DK-8000 Aarhus C, Denmark (e-mail: sn@ana.au.dk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

according to the licenses for use of experimental animals issued by the Danish Ministry of Justice. Experiments were performed using male Wistar-Hannover rats (Taconic Europe, Eiby, Denmark) or Sprague-Dawley rats (Møllegaard Breeding Center, Skensved, Denmark) in *protocol 2*. For all protocols, rats were kept individually in metabolic cages and were allowed to acclimate for at least 3 days before initiation of the experiments, including implantation of osmotic minipumps. The rats were fed once daily in the morning and ate all of the offered food during the course of the day. During the entire experiment, there were a 12:12-h artificial light-dark cycle and a temperature of $21 \pm 2^\circ\text{C}$. Before subcutaneous implantation of osmotic minipumps in the neck region and intraperitoneal injection, rats were anesthetized with isoflurane and given subcutaneous injections of buprenorphine (Temgesic, Schering-Plough, Brussels, Belgium) for pain relief. Osmotic minipumps (models 2001 and 2002, Alzet, Palo Alto, CA) were prepared and equilibrated in saline for at least 4 h at 37°C before implantation.

Protocol 1: long-term infusion of aldosterone and/or dDAVP in rats receiving a fixed amount of food with free access to water. Male Wistar-Hannover rats (220–240 g) were implanted with osmotic minipumps (model 2001, Alzet) containing 1) saline mixed with DMSO [30% vol/vol, control, $n = 3$ (set 1) + 6 (set 2)]; 2) dDAVP (0.5 ng/h) in saline and DMSO (30% vol/vol, dDAVP, $n = 4 + 6$); 3) aldosterone (150 $\mu\text{g}/\text{day}$) in saline and DMSO (30% vol/vol, Aldo, $n = 4 + 6$); or 4) dDAVP (0.5 ng/h) and aldosterone (150 $\mu\text{g}/\text{day}$) in saline and DMSO (30% vol, Aldo+dDAVP, $n = 4 + 6$), respectively. The rats were kept in metabolic cages during the following 6 days until death. They had free access to water but a fixed daily food intake of $17 \text{ g} \cdot \text{rat}^{-1} \cdot \text{day}^{-1}$ (normal rat chow, Altromin 1320, Petersen, Ringsted, Denmark). For practical reasons, we established two sets of experimental animals in this protocol. The two sets were run in exactly the same way, and the data were compiled. In the second set, one control rat had to be excluded due to a markedly lower food intake for more than 2 consecutive days.

Protocol 2: long-term infusion of lower dose aldosterone in rats receiving fixed amounts of food and water intake and spironolactone treatment. An experimental set of rats which received lower dose aldosterone with fixed food and water intake was examined to determine whether the dose of aldosterone and the increased water intake could explain the observations made in *protocol 1*. This set of rats was also used in our previous study (25). Ten male Sprague-Dawley rats ($n = 14$, body weight 190–210 g) were implanted with osmotic minipumps (model 2002) delivering $50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ aldosterone (A6628, Sigma) dissolved in DMSO (25% vol/vol) and sterile saline (75% vol/vol). Four control rats received pumps with vehicle only. The rats received daily food rations of a food mix consisting of 15 g rat chow (no. 1320, Altromin) and 30 ml tap water, and they ate all the food given. The rats were then placed back into metabolic cages and kept during the following 7 days until death. Five rats also treated with spironolactone in addition to aldosterone infusion had $120 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ spironolactone (3378, Sigma) added to the food. After 7 days in metabolic cages, the rats were killed.

Protocol 3: long-term infusion of aldosterone in rats with clamped plasma angiotensin II level. As aldosterone infusion could possibly lead to a downregulation of angiotensin II levels in vivo, we performed aldosterone infusion in rats with clamped angiotensin II levels. Twelve male Wistar-Hannover rats (220–250 g) were implanted with osmotic minipumps (model 2002, Alzet) containing angiotensin II ($50 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) diluted in Ringer lactate, a suppressor dose in unrestrained rats after 1 wk (29). Two days after the implantation of the first minipump, each rat had a second minipump (model 2001) implanted that delivered either vehicle ($n = 6$, saline and DMSO, 30% vol/vol) or aldosterone (150 $\mu\text{g}/\text{day}$) in saline and DMSO (30% vol/vol, $n = 6$), as described in *protocol 1*, with free water intake and a fixed food intake of $17 \text{ g} \cdot \text{rat}^{-1} \cdot \text{day}^{-1}$. The rats were then monitored

for the following 6 days and were killed. One rat in the control group was excluded due to an insufficient food intake.

Protocol 4: low-potassium diet-induced hypokalemia in rats with low levels of plasma aldosterone. Given the presence of hypokalemia in the aldosterone-treated rats and the potential effect of this factor on AQP2 regulation, we investigated the distribution of AQP2 in the CCD of hypokalemic rats induced by potassium restriction, who have a low plasma aldosterone level (8). Kidney sections from 4-day hypokalemic rats were used. Regulation of other transporters was previously described in these rats (8). Rats were randomized into two groups matched for body weight: a hypokalemic group ($n = 12$) and a control group ($n = 12$). To produce hypokalemia, rats were fed a potassium-deficient diet (C1037, potassium content: 0.18 g/kg chow, Altromin) for 4 days. In the control group, rats were fed control chow (C1000, potassium content: 7 g/kg chow, Altromin) and offered the amount of food corresponding to the mean intake of food in the hypokalemic group of rats during the previous day. Thus the food intake was matched between the two groups. Rats in both groups had free access to water throughout the experiment (8).

Protocol 5: acute aldosterone administration in rats. To understand whether aldosterone has an acute effect on AQP2 redistribution in the CCD, we studied rats acutely treated with a single dose of

Fig. 1. Time course of urine osmolality and output in *protocol 1* [control ($n = 8$); dDAVP ($n = 10$); aldosterone (Aldo; $n = 10$); Aldo-dDAVP ($n = 10$)]. Data from the 2 sets of rats in *protocol 1* were pooled. Values are means $\pm$ SE. A: time course of urine output from day 0 to day 6 in 4 treatment groups. dDAVP rats presented rapidly a decrease in the urine output compared with controls. Aldo rats presented a progressive rise in their urine output. Aldo-dDAVP rats presented an initial decrease in their urine output before slowly coming back to control levels. B: time course of urine osmolality from day 0 to day 6 in 4 treatment groups. dDAVP rats present an increase in their urine osmolality, Aldo rats present a decrease in their osmolality, and Aldo-dDAVP first present an increase in their osmolality before a return to the control level. $P < 0.05$ is considered significant: *different from controls; †different from Aldo-dDAVP; ‡different from dDAVP; #different from dDAVP.

aldosterone. Twelve male Wistar-Hannover rats (230–250 g) were given an intraperitoneal injection of either 150 μg aldosterone/kg body wt (solubilized in ethanol, then diluted in sterile saline, $n = 6$) or sterile saline with ethanol ($n = 6$). This dose of aldosterone has previously been demonstrated to induce subcellular changes in localization of H^+ -ATPase after 30 min in mice (37). After the injection, the rats were returned to their normal cages. Thirty minutes after injection, 6 control rats and 6 aldosterone-treated rats were killed.

Protocol 6: effect of sodium intake on AQP2 redistribution by aldosterone. To understand the relative role of sodium reabsorption and aldosterone in AQP2 redistribution, we studied AQP2 redistribution in the CCD of sections of rats given a chronic aldosterone infusion along with either a sodium-deficient or sodium-replete diet. These rats have been previously described elsewhere (18). Six male Munich-Wistar rats were implanted with osmotic minipumps (model 2002, Alzet) delivering 200 μg aldosterone (A6628, Sigma) a day. The minipump infusion was maintained throughout the entire time course (7 days) in all rats. The sodium intake was initially maintained at a low level (0.32 meq Na/day) using synthetic low-sodium powdered food (Altromin no.1321, Petersen) in a mixture of low-sodium food (15 g/220 g body wt) and water (30 ml/220 g body wt). On day 4, one-half of the rats ($n = 3$) were switched to a higher sodium intake (2.0 mmol Na $\cdot$ 200 g body wt $^{-1}\cdot$ day $^{-1}$) with the same amount of food (15 g/220 g body wt) and water (30 ml/220 g body wt), whereas the remaining rats ($n = 3$) were continued on the 0.32 mmol $\cdot$ 200 g body wt $^{-1}\cdot$ day $^{-1}$ sodium intake.

Inhalation Anesthesia and Sampling of Blood and Tissue

Normal room air mixed with isoflurane was inhaled for anesthesia. When the rats were sedated, a large laparotomy was performed and the abdominal aorta was cannulated. Blood was collected from the inferior vena cava, and subsequently the right renal artery and vein were clamped and the right kidney was removed. Kidneys were dissected into three different zones [cortex and outer stripe of outer medulla (cortex/OSOM), inner stripe of outer medulla (ISOM) and inner medulla (IM)] and were processed for immunoblotting as described below. The left kidneys were fixed by retrograde perfusion as described below.

Plasma Samples

The heparinized blood was centrifuged for 15 min at 4,000 g to remove the blood cells, and subsequently the plasma was analyzed

for sodium, potassium, urea, and creatinine using a Vitros 950 (Johnson&Johnson). Osmolality was measured with an automatic cryoscopic osmometer (Osmomat 030-D, Gonotec, Berlin, Germany). Plasma aldosterone concentrations were determined using a commercially available radioimmunoassay kit (Coat-A-Count, Diagnostic Products, Los Angeles, CA).

Primary Antibodies

Affinity-purified polyclonal rabbit anti-rat AQP2 (H7661) was developed against the same sequence which we have used for LL127AP (27, 28) and has recently been characterized (26). A commercially available mouse monoclonal antibody against rat calbindin D-28K (Research Diagnostics, Flanders, NJ) was purchased, and an antibody previously characterized against p-AQP2 (phosphorylated in the PKA phosphorylation consensus site Ser-256) (5) was used.

Semiquantitative Immunoblotting

The dissected renal cortex/OSOM, ISOM, and IM were homogenized and prepared as previously described in detail (6, 26).

Kidney Fixation and Immunohistochemistry

Kidneys were fixed by perfusion via the aorta with 3% paraformaldehyde, in 0.1 M cacodylate buffer pH 7.4. Immunolabeling was performed on sections from a paraffin-embedded preparation (2- μm thickness) using methods described previously in detail (6, 26).

Immunoelectron Microscopy

For immunoelectron microscopy, small pieces of kidney cortex were cut from slices of fixed kidney, cryoprotected in 2.3 M sucrose, and frozen in liquid nitrogen and prepared and stained for AQP2 as described previously (4).

Semiquantitative Analysis of AQP2 Immunofluorescent Labeling Intensity in CCD

We used two different methods for analyzing the AQP2 immunofluorescent labeling intensity in CCD.

Method a. We studied five aldosterone-treated rats and five control rats. Five CCDs in each rat were acquired randomly. All the immunofluorescent images in both groups were taken on the same day with

Table 1. Serum and urinary values from protocol 1

	Control ($n = 8$)	dDAVP ($n = 10$)	dDAVP-Aldo ($n = 10$)	Aldo ($n = 10$)
P_{Na} , mmol/l	135 $\pm$ 0.5	130 $\pm$ 0.7*	134 $\pm$ 0.8†	135 $\pm$ 0.2†‡
P_{K} , mmol/l	4.6 $\pm$ 0.2	4.8 $\pm$ 0.2	3 $\pm$ 0.2*†	3.1 $\pm$ 0.1*‡
P_{urea} , mmol/l	4.8 $\pm$ 0.3	6.2 $\pm$ 0.2	5.1 $\pm$ 0.6	5 $\pm$ 0.4
P_{osm} , mosmol/kgH ₂ O	298 $\pm$ 1.2	289 $\pm$ 1.4*	294 $\pm$ 1.9	298 $\pm$ 1.5‡
Cl_{Cr} , ml/min	3.6 $\pm$ 0.7	3.4 $\pm$ 0.9	2.8 $\pm$ 0.7	3.1 $\pm$ 0.8
$\text{P}_{\text{aldosterone}}$, pg/ml	270 $\pm$ 27	296 $\pm$ 21	2,098 $\pm$ 84*†	2,130 $\pm$ 76*†
FE_{Na} , %	0.29 $\pm$ 0.06	0.3 $\pm$ 0.05	0.35 $\pm$ 0.05	0.43 $\pm$ 0.08
FE_{K} , %	0.74 $\pm$ 0.12	0.85 $\pm$ 0.14	0.94 $\pm$ 0.12	0.96 $\pm$ 0.14
U_{NaV} , mmol	1.54 $\pm$ 0.10	1.31 $\pm$ 0.06	1.43 $\pm$ 0.06	1.76 $\pm$ 0.08†
U_{KV} , mmol	4.15 $\pm$ 0.14	3.67 $\pm$ 0.17	3.79 $\pm$ 0.12	4.15 $\pm$ 0.21
U_{Na} , mmol/l	125 $\pm$ 10	240 $\pm$ 14*	123 $\pm$ 6†	78 $\pm$ 3*†‡
U_{K} , mmol/l	335 $\pm$ 16	670 $\pm$ 35*	328 $\pm$ 19†	187 $\pm$ 11*†‡
$\text{U}/\text{P}_{\text{osm}}$	5.1 $\pm$ 0.3	9.1 $\pm$ 0.9*	5.19 $\pm$ 0.4†	3.13 $\pm$ 0.39*†‡
Water intake, ml	31.3 $\pm$ 1	25.24 $\pm$ 0.4	32.1 $\pm$ 1.9†	43.4 $\pm$ 2.2*†‡
U_{osm} , mosmol/kgH ₂ O	1,516 $\pm$ 96	2,995 $\pm$ 162*	1,543 $\pm$ 100†	934 $\pm$ 105*†‡
Water balance, ml	16.7 $\pm$ 4.2	19.5 $\pm$ 0.6	20.4 $\pm$ 2.6	22.4 $\pm$ 2.7
Urine output, $\mu\text{l}/\text{min}$	8.7 $\pm$ 0.5	4.0 $\pm$ 0.3*	8.4 $\pm$ 0.8†	15.9 $\pm$ 1.2*†‡

Values are means $\pm$ SE from plasma collected at the time of death and urine collected in the final 24 h. n , No. of rats; Aldo, aldosterone; P_{Na} , P_{K} , P_{urea} , P_{osm} , $\text{P}_{\text{aldosterone}}$: plasma sodium, potassium, urea, osmolality, and aldosterone, respectively. Cl_{Cr} , creatinine clearance. FE_{Na} and FE_{K} , fractional excretion of sodium and potassium, respectively. U_{NaV} , U_{KV} , U_{Na} , U_{K} , and U_{osm} : urinary sodium excretion, potassium excretion, sodium, potassium, and osmolality, respectively. * $P < 0.05$ compared with controls. † $P < 0.05$ compared with dDAVP. ‡ $P < 0.05$ compared with dDAVP-Aldo.

identical microscope settings (laser light intensity, same offset and gain on the photomultiplier, sampling period, and averaging and optimal focus) in blinded ways; thus the observer was not aware of the treatment of the individual rats. Double-labeled sections for AQP2 and calbindin D-28K were used to ensure that CCDs were chosen, not CNTs. As demonstrated in Fig. 6, every other labeled cell was marked cross sectionally using Leica LSM software and the profile of the immunofluorescence intensity on each cross section (from the region of apical plasma membrane to the region of the basolateral plasma membrane) was obtained.

Method b. The same images as used in *method a* were analyzed. Using Image-Pro Plus 5 software (version 4.5.1, Media Cybernetics, Silver Spring, MD), we performed semiquantitative analysis of AQP2 labeling intensity in the CCD of aldosterone-treated rats vs. control rats, as previously described (26). The sum of pixel intensity in each tubule was measured. Each cell from each tubule was then divided into two parts at the level of the middle of the nucleus by manual tracing (i.e., apical and basolateral cell compartments). Then, the sum of pixel intensity on each half of the tubule was measured and the ratio of the sum of pixel intensity in the apical or basolateral compartments in each tubule vs. the sum of pixel intensity of the whole area of each tubule was obtained. Cells that were partly sectioned were not included in the analysis.

Statistical Analyses

Values are presented as means $\pm$ SE. Comparisons between four groups were made by ANOVA followed by Tukey's multiple comparisons test. Multiple comparisons tests were only applied when a significant difference ($P < 0.05$) was determined in the ANOVA. Two-group comparisons were made by an unpaired *t*-test. *P* values < 0.05 were considered significant.

RESULTS

Long-Term Aldosterone Infusion is Associated With Increased Urine Output and Hypokalemia, While dDAVP Infusion Decreases Urine Output and Induces Hyponatremia (Protocol 1)

Long-term aldosterone infusion in rats with free access to water (*protocol 1*) was associated with a progressively increased urine output and decreased urinary osmolality after 2 days of treatment (Fig. 1, Table 1). Water intake was also significantly elevated in aldosterone-treated rats at *day 1* and from *day 3* until the end of the experiment (data not shown). Long-term aldosterone infusion alone or together with dDAVP

Fig. 2. Semiquantitative immunoblotting of homogenized rat kidney tissue from the cortex and outer stripe of the outer medulla (CTX/OSOM), inner stripe of outer medulla (ISOM), and inner medulla (IM) obtained from control, dDAVP, Aldo, and Aldo-dDAVP rats in *protocol 1*. **A**: representative immunoblot of samples from *protocol 1* from 5 control and 6 dDAVP, 6 Aldo-dDAVP, and 6 Aldo rats incubated with anti-aquaporin-2 (AQP2) antibody showing a distinct band at ~ 27 kDa and a more diffuse band at 33–40 kDa. **B**: densitometric analysis of all animals in *protocol 1* showed that the abundance of AQP2 was increased in dDAVP rats (filled bars) in all zones compared with controls (open bars). In Aldo-dDAVP rats (dark grey bars), AQP2 abundance was slightly increased in ISOM and IM compared with controls but was significantly lower than in dDAVP rats. In Aldo rats (light grey bars), AQP2 abundance was lower than control in ISOM segment only. $P < 0.05$ is considered significant: *different from controls; ‡different from Aldo-dDAVP; #different from dDAVP.

induced hypokalemia compared with controls (Table 1). In contrast, dDAVP infusion decreased urine output and increased urinary osmolality during the whole experimental period (Fig. 1). In rats cotreated with aldosterone and dDAVP, urine output decreased during the first day but then slowly went back to control levels. The rats infused with dDAVP alone developed hyponatremia, which was not observed when they were cotreated with aldosterone (Table 1). The urinary excretion rates of sodium and potassium as well as their fractional excretion were unchanged by aldosterone alone or aldosterone+dDAVP compared with controls (Table 1). The measurements were performed at the last day of the experiments, corresponding to a steady-state period.

Decreased Protein Expression of AQP2 in ISOM of Aldosterone-Treated Rats (Protocol 1)

Semiquantitative immunoblotting revealed that the protein expression of AQP2 in the ISOM of aldosterone-treated rats was significantly decreased compared with controls (Fig. 2). In contrast, in the cortex and inner medulla, no significant changes in AQP2 expression were noted in aldosterone-treated rats compared with controls. In dDAVP-treated rats, AQP2 expression in the three kidney zones (cortex/OSOM, ISOM, and IM) was significantly increased compared with controls (Fig. 2). In rats cotreated with aldosterone and dDAVP, AQP2 expression was significantly increased in the ISOM and IM compared with controls, but not to the same degree as in the rats treated with dDAVP alone (Fig. 2).

Long-Term Aldosterone Infusion is Associated With Increased AQP2 Labeling Intensity in Basolateral Domains of CCD, While Apical AQP2 Labeling Decreases (Protocols 1 and 2)

In the CCD principal cells of normal rat kidneys, AQP2 is known to be localized at the apical plasma membrane as

well as in the subapical intracellular vesicles (10, 28). Consistently, immunohistochemistry revealed that most AQP2 was localized at the apical domains of the CCD principal cells in controls (Fig. 3A). In dDAVP-treated rats, the overall labeling intensity of AQP2 was increased particularly at the apical plasma membrane, but also in the basolateral plasma membrane domain (Fig. 3C). The apical labeling intensity of AQP2 in the CCD principal cells of the aldosterone-treated rats was decreased compared with control rats, but there was a distinctly increased AQP2 labeling at the basolateral plasma membrane domains (Fig. 3B). Cotreatment with aldosterone and dDAVP was also associated with decreased apical AQP2 labeling and increased AQP2 labeling intensity at the basolateral plasma membrane domains compared with dDAVP alone (Fig. 3, B and D). Moreover, in aldosterone-treated rats, immunoperoxidase labeling of p-AQP2 also revealed strong basolateral labeling and an apparent decrease in apical labeling of CCD principal cells compared with controls (Fig. 4).

Since the CNT is known to exhibit basolateral AQP2 even in the basal state (4), double immunofluorescence labeling using antibodies against calbindin D-28K and AQP2 was undertaken (Fig. 5) to confirm that the observed tubules were CCDs and not CNTs. In the cortex, tubules that were negatively or only weakly labeled with calbindin D-28K (inset in Fig. 5, A–D), but strongly positive for AQP2, were considered CCDs, whereas tubules positive for both AQP2 and calbindin D-28K were considered CNTs (2) (Fig. 6, A–D). The double immunofluorescent labeling confirmed that CCDs of rats treated with aldosterone alone showed prominent basolateral staining of AQP2 and decreased apical staining (Fig. 5B), whereas chronic dDAVP infusion was associated mainly with strong apical AQP2 labeling and some increase in basolateral labeling (Fig. 5C). In contrast to the CCDs, CNTs had basolateral AQP2 labeling in addition to the apical AQP2 labeling in all groups

Fig. 3. Representative micrographs showing immunohistochemical localization of AQP2 in cortical collecting ducts (CCDs) in sections of paraffin-embedded kidneys from control rats (A), Aldo rats (B), dDAVP rats (C), and Aldo-dDAVP rats (D). In control rats, the labeling for AQP2 is mainly apical, whereas in Aldo rats, there is a high component of basolateral AQP2 staining together with lighter apical staining. In dDAVP rats, AQP2 staining is stronger and mainly apical, with some basolateral staining apparent as well. In Aldo-dDAVP rats, there is also a clear appearance of staining on the basolateral side of the principal cells.

Fig. 4. Representative micrographs showing immunoperoxidase staining for Ser²⁵⁶-phosphorylated AQP2 (Ser²⁵⁶-p-AQP2) in a straight segment of CCD in sections of paraffin-embedded kidneys from control (A) and Aldo rats (B) in *protocol 1*. In the control rat, Ser-p-AQP2 is mainly visible on the apical part of the CCD, whereas in the Aldo rat, note the appearance of strong basolateral staining for Ser²⁵⁶-p-AQP2 and decreased apical staining.

(Fig. 6A). In rats treated with aldosterone, however, basolateral staining of AQP2 in CNTs appeared more prominent and apical AQP2 labeling appeared slightly weaker compared with control rats (Fig. 6B). In the CNTs of aldosterone- and dDAVP-cotreated rats, AQP2 labeling was seen in both apical and basolateral domains (Fig. 6).

To examine whether this effect was specific to the high (supraphysiological) aldosterone dose and/or was related to the high water intake in *protocol 1*, we also examined the kidney sections of rats which received a lower dose of aldosterone and fixed water intake (25) (*protocol 2*, Fig. 7). Consistent with *protocol 1*, immunohistochemistry showed a clear increase in AQP2 labeling in the basolateral domains of CCDs (Fig. 7B) in aldosterone-treated rats compared with controls (Fig. 7A). In *protocols 1* and 3, the height and volume of CCD cells of aldosterone-treated rats were increased compared with controls or dDAVP-treated rats (Figs. 3, 5, and 7).

To test whether the effects of aldosterone on AQP2 distribution were mediated by activation of the mineralocorticoid receptor, we examined the effect of spironolactone (a mineralocorticoid receptor antagonist) cotreatment of aldosterone-infused rats (*protocol 2*). In aldosterone-infused rats cotreated with spironolactone, the observed aldosterone-induced increase in basolateral AQP2 labeling in the CCD was largely

blocked (Fig. 7C), and the labeling pattern was more similar to untreated controls (Fig. 7A). Moreover, the aldosterone-induced changes in CCD cell morphology (increased volume and height) were also largely blocked by spironolactone and the CCD appeared similar to the untreated control rats (Fig. 7, A vs. C).

AQP2 in Basolateral Cell Domain was Situated on the Basolateral Membrane (Protocol 1)

To investigate whether the observed basolateral AQP2 in the CCD of aldosterone-treated rats was associated with the basolateral membrane, we investigated control and aldosterone-treated rats from *protocol 1* by immunoelectron microscopy using kidney cortex tissue sections stained for AQP2. By electron microscopy, we observed that most basolaterally located gold-labeled AQP2 in the CCD was situated on the basolateral membrane in aldosterone-treated rats (Fig. 8).

Semiquantitative Analysis of AQP2 Immunofluorescence Labeling in CCDs (Protocol 1)

To quantify the apparent changes in AQP2 labeling intensity at the apical and basolateral plasma membrane domains, we performed semiquantitative analysis of AQP2 immunofluorescence labeling in the CCDs in control rats vs. aldosterone-treated rats from *protocol 1* (Fig. 9).

We used Leica software (*method a*) to measure the pixel intensity on the cross sections of CCD cells in the two groups (Fig. 9, A–E). The cross-sectional pixel intensity of CCD cells in control rats typically showed one tall peak located close or at the apical plasma membrane domains in control rats (Fig. 9C) with none or a very small peak on the basolateral side of the cells. Compared with control rats, the aldosterone-infused rats had a significantly lower peak at the apical plasma membrane but a second taller and broader peak on the basolateral side of the cell (Fig. 9D). The average height of the apical and basolateral intensity peaks in each rat was estimated and used to compute the mean for each group (Fig. 9E). This method will tend to underestimate the basolateral redistribution of AQP2 on the basolateral side of the cell because the width of the peaks, which was broader in aldosterone-treated rats, was not taken into account. We therefore used a second semiquantitative method to confirm our results.

The same images as for *method a* were used. Using the software program Image-Pro 5 as described in *method b*, we measured the sum of the pixel intensity on the apical half of the cells compared with the total pixel intensity of the cells. The ratio of the pixel intensity of the apical half of the tubule to the total tubule pixel intensity was $81.6 \pm 0.9\%$ for control rats vs. $50.9 \pm 2.1\%$ for aldosterone-treated rats ($P < 0.001$) (Fig. 9F), with the rest of the labeling located on the basolateral part of the cells in each group. Therefore, long-term aldosterone treatment was associated with increased AQP2 labeling intensity in the basolateral domains of CCD with a concomitant decrease in apical labeling.

Aldosterone-Induced Increase in Basolateral AQP2 was not due to Decreased Angiotensin II Level (Protocol 3)

In vivo and in vitro evidence suggest a potential role of angiotensin II in the regulation of AQP2 expression and trafficking (17, 19). Since long-term aldosterone infusion could reduce

Fig. 5. Representative micrographs showing double immunofluorescence labeling for AQP2 and calbindin in CCD in section of paraffin-embedded kidneys. CCDs presented no staining for calbindin (A–D, top right insets). AQP2 was mainly apical in control rats (A). dDAVP (C) rats had a higher staining intensity and presented apical staining, although basolateral AQP2 was also increased. In Aldo (B) and Aldo-dDAVP rat sections (D), the basolateral component of AQP2 staining was even more visible and was more important relative to apical labeling.

the plasma angiotensin II levels in vivo in *protocols 1* and *2*, we made a new protocol to avoid the potential role of altered levels of angiotensin II in the aldosterone-induced increase in basolateral AQP2 expression. In *protocol 3*, we clamped plasma angiotensin II levels before the aldosterone or vehicle treatment in rats. Immunohistochemistry showed that aldosterone infusion in the presence of angiotensin II clamping was persistently associated with an increased basolateral labeling intensity of AQP2 in the principal cells of the CCDs compared with rats only treated with angiotensin II (Fig. 10, A and D).

Aldosterone-Induced Increase in Basolateral Labeling was not Present in Hypokalemia Without Elevated Aldosterone (Protocol 4)

Given the presence of hypokalemia in all rats receiving chronic infusion of aldosterone, we examined whether hypokalemia per se could play a role in the decreased apical AQP2 labeling intensity and increased basolateral AQP2 labeling intensity observed in the CCD. Ten-day exposure to low potassium diet-induced hypokalemia has been demonstrated to decrease renal AQP2 expression and decreased urinary concentration (20). As aldosterone-treated rats might have suffered hypokalemia for a shorter period, the immunohistochemistry of rats exposed to 4 days of a low-potassium diet was studied (8). Rats treated with a potassium-deficient diet for 4 days had weaker apical AQP2 labeling compared with control rats in CCDs (Fig. 10, B and E). However, most of the

AQP2 labeling in rats on the potassium-deficient diet was seen at the apical domains, and no labeling was observed in the basolateral domains in the principal cells of CCD. This indicates that hypokalemia induced by chronic aldosterone infusion is not likely to play a major role in the increased basolateral AQP2 expression observed in aldosterone-treated rats, whereas it could play a role in the decreased expression of the apical plasma domain.

Absence of Apparent Changes in Subcellular Localization of AQP2 in Response to Short-Term Aldosterone Infusion (Protocol 5)

In addition to the protocols with long-term aldosterone infusion, we examined the effect of short-term aldosterone treatment on the subcellular redistribution of AQP2. Immunohistochemistry showed no increase in the basolateral AQP2 labeling 30 min after a single injection of aldosterone compared with vehicle-injected rats (Fig. 10, C and F).

Sodium is Important for Redistribution of AQP2 to the Basolateral Membrane and for Structural Changes in the CCD in the Presence of Elevated Aldosterone Levels (Protocol 6)

A reorganization of CCD cell morphology is a well-described effect of chronic aldosterone treatment in rats with normal food intake (33). However, the morphological changes

Fig. 6. Representative micrographs showing double immunofluorescence staining for AQP2 and calbindin in connecting tubule (CNT) in sections of paraffin-embedded kidneys of control (A), Aldo (B), dDAVP (C), and Aldo-dDAVP (D) rats in *protocol 1*. Calbindin was used as a marker for CNT (A–D, top right insets). In all animals, basolateral AQP2 was clearly observed in CNT. The apical fraction of AQP2 appeared higher in control and dDAVP rats than in Aldo and Aldo-dDAVP rats.

seem dependent not only on the presence of aldosterone but also on the presence of sodium (36). Indeed the aldosterone-induced morphological changes (increase in cell volume and in basolateral membrane area) described in CCD principal cells are prevented by sodium restriction (33, 36). Here, we investigated whether the basolateral expression of AQP2 in chronic aldosterone-treated rats was also dependent on sodium intake (*protocol 6*). In aldosterone-treated rats, the basolateral redistribution of AQP2 was clearly reduced during sodium restriction (Fig. 11) compared with normal sodium intake. Thus the basolateral redistribution of AQP2 also appears dependent on sodium.

DISCUSSION

The current study demonstrates that long-term aldosterone infusion in rats with free access to water is associated with hypokalemia, increased water intake, increased urinary excretion, and decreased urinary concentration. Moreover, in CCD principal cells, long-term aldosterone infusion induces an increase in AQP2 labeling intensity in the basolateral domain and a decrease in the apical domain of CCD principal cells relative to controls. The increased basolateral AQP2 in CCDs in response to long-term aldosterone infusion was still present when angiotensin II levels were clamped and was not seen in the presence of hypokalemia without elevated aldosterone. Moreover, the aldosterone-induced redistribution of AQP2

appeared also dependent on sodium. Basolateral AQP2 expression was not seen acutely or in the presence of mineralocorticoid receptor blockade, suggesting a mineralocorticoid receptor-mediated genomic mechanism.

Long-Term Aldosterone Infusion was Associated With Increased Urine Output

Long-term aldosterone-infused rats presented with a decreased urine concentration and increased urine output when the animals had free access to water, hence increased water intake, but not when they were placed on a fixed-water diet (25). Similar effects of aldosterone infusion on urine production have been described in aldosterone-infused rats with both central and lithium-induced diabetes insipidus (26) and can here be generalized to normal rats with free water intake. The increased urine output in aldosterone-treated rats can involve either a central defect with an increase in thirst drive (polydipsia), and/or a renal effect with a decreased reabsorptive capacity of the kidney, resulting in increased water loss and secondary increase in water intake. A central contribution is very likely in this situation, given the absence of change of plasma osmolality. Aldosterone is not known directly to increase thirst; however, aldosterone-treated animals present with hypokalemia, which is very well known to induce a central defect leading to increased water intake (1), which we also observed here. A renal defect, either primary or secondary, cannot be

Fig. 7. Representative micrographs of rat CCDs labeled for AQP2 in control rats (A), Aldo rats (B), and Aldo+spironolactone (Aldo-spiro; C) rats in protocol 4. In the paired water intake group treated with aldosterone, there is also the appearance of clear basolateral staining for AQP2 in CCDs and lesser apical staining. The addition of spironolactone to the food of the treated rats reversed the effect on AQP2 subcellular distribution (C).

excluded in this situation. Indeed, AQP2 expression was also decreased in the ISOM and the apical staining of AQP2 was decreased in aldosterone-infused rats in CCDs compared with control rats, possibly contributing to the decrease in urine concentration observed. Therefore, a mixed effect of decreased renal concentration ability and mostly an increased water

intake is probably the source of the observed increased urine output in aldosterone-treated rats, with a maintained plasma osmolality.

In rats cotreated with dDAVP and aldosterone, urinary concentrating ability was also decreased compared with dDAVP rats. Here also the participation of the observed increased water intake in rats cotreated with dDAVP and aldosterone compared with dDAVP rats cannot be excluded, although one would expect an increased water intake to further lower plasma osmolality in this situation, which was not the case. Therefore, the observed decrease in AQP2 expression in rats cotreated with dDAVP and aldosterone relative to dDAVP rats might be important in the higher urine output of aldosterone- and dDAVP-cotreated rats compared with dDAVP rats.

Aldosterone Decreases AQP2 Immunolabeling Density in Apical Membrane Domains of the CCD

In this study, a decrease in the apical expression of AQP2 in the CCD and to a lesser extent in the CNT was observed in rats undergoing long-term aldosterone infusion. This is a surprising observation, since aldosterone acutely induces an increase in cAMP in inner medullary collecting duct cells (32), which could possibly be involved in an increase in the apical targeting of AQP2. Moreover, a more than 24-h exposure of a mouse CCD cell line to aldosterone and dDAVP resulted in an increased total expression of AQP2 (13). Therefore, the observed downregulation of apical AQP2 might be linked to a more chronic and possibly indirect effect of aldosterone infusion in vivo. First, the mineralocorticoid-induced hypokalemia is likely to contribute to the decreased apical expression of AQP2, as demonstrated in rats exposed to 4 days and previously to 10 days of a hypokalemic diet (20). Long-term aldosterone infusion could also directly, via an unknown mechanism or the synthesis of other local or systemic factors, be involved in this long-term downregulation of apical AQP2. For example, an increase in urine output of reduced osmolality has also been described following long-term angiotensin II infusion in rats. The increased urine output was associated with increased PGI₂ production (7), possibly mediating the decreased CCD water permeability (15). Finally, the downregulation of apical AQP2 may serve to limit the water reabsorption in the CCD secondary to increased sodium reabsorption. These potential long-term regulatory mechanisms deserve further study as they might be important in vivo for blood pressure regulation. The same hypotheses are true for the relative downregulation of AQP2 in cortex/OSOM and ISOM in aldosterone- and dDAVP-cotreated rats compared with dDAVP rats.

Long-Term Aldosterone Infusion Induces an Increase in Basolateral AQP2 Expression

In both control rats and dDAVP-treated rats, a high-dose infusion of aldosterone clearly increased basolateral AQP2 and expression in the CCD. Immunoelectron microscopy demonstrated that most of the basolaterally located AQP2 was located in the basal membrane itself. In addition, increased expression AQP2 phosphorylated at Ser²⁵⁶, which is normally associated with increased expression in the apical plasma membrane (21, 24), was also observed in the basolateral cell domain of CCD

Fig. 8. Representative electron microscopic images of AQP2 immunogold labeling in cortical collecting duct from control rats (A and C) and aldosterone-treated rats in *protocol 1* (B and D) at the level of the apical membrane (A and B) and basolateral membrane (C and D). In aldosterone-treated rats, fewer gold particles were located in the apical membrane (B) compared with controls (A). In aldosterone-treated rats, the basolateral membrane had more infoldings and more gold particles (D) compared with controls (C). m, Mitochondria. Scale bar: 500 nm (A, C, and D) and 200 nm (B).

cells following long-term aldosterone treatment. This suggests that the Ser²⁵⁶ phosphorylation of AQP2 also may play a role in the basolateral trafficking of AQP2.

The mechanism involved in basolateral trafficking appears not to involve a direct role of hypokalemia or changes in plasma angiotensin II levels based on the data in the present study. Global changes in osmolality, which in the medulla have been associated with basolateral trafficking of AQP2 (34), are not known to occur in the cortex. Previously, vasopressin has also been shown to induce basolateral AQP2 expression. However, a previous study also demonstrated increased basolateral AQP2 expression in aldosterone-infused vasopressin-deficient Brattleboro rats (26). In the present study, basolateral AQP2 expression was observed in both normal rats treated with aldosterone only and in rats cotreated with aldosterone and dDAVP and could be blocked by spironolactone. These results further support that aldosterone mediates the increased basolateral expression of AQP2 in CCD. Finally, in the absence of sodium, the basolateral redistribution was in large part inhibited, indicating a likely sodium dependence of this phenomenon.

The presence of basolateral localization of AQP2 is well described in the CNTs of normal rats (4), a segment with high sodium reabsorptive capacities (22, 30). We here demonstrate that the increase in basolateral water channels extends into the CCDs with long-term aldosterone treatment in normal rats, in parallel with the known increase in the length of the basolateral membrane and modification of cell volume (35, 36), increase in sodium reabsorption (11, 14), and increased expression of the basolateral Na-K-ATPase (reviewed in Ref. 9) and AQP3 (18). All these phenomena,

including the relocation of AQP2, are potentially linked to the increased sodium reabsorption occurring in the CCD under high aldosterone levels. Indeed, the increased sodium reabsorption under aldosterone treatment is expected to lead to increased water reabsorption, hence the absence of hyponatremia with aldosterone treatment. Therefore, we hypothesize that in the case of the aldosterone-mediated increase in sodium reabsorption in the CCD, water will be reabsorbed through the apical membrane due to the solute drive, potentially participating in the observed cell swelling in the CCD. A higher permeability of the basolateral membrane, where the osmotic gradient is low, is then possibly needed to allow for sufficient water efflux and prevention of cell swelling. The observed redistribution of AQP2 and the increased expression of AQP3 (18) will potentially contribute to increase basolateral membrane permeability. Moreover, some very local changes of the osmolality at the basal side of the CCDs could occur due to the increased sodium reabsorption in this segment. This could then induce a secondary change in AQP2 localization as described in the medulla (34), although the global cortical osmolality does not change. Finally, increased basolateral expression of AQP2 in the CCD has also been described in nephrotic syndrome, another condition with markedly increased sodium reabsorption in the CCD (16).

The molecular mechanisms involved in this basolateral expression of AQP2 are currently unknown. Given the absence of acute AQP2 redistribution in response to aldosterone and its blockade by spironolactone, this effect is very likely to be genomic in origin, with possible synthesis and targeting of new aquaporin molecules to the basolateral

Fig. 9. Semiquantification of AQP2 by immunofluorescence. In *method a*, a profile of intensities in cross sections is obtained. In control rats (A and C), the staining is mainly limited to the apical side with a clear intensity peak. In Aldo rats (B and D), we can observe the appearance of a broad second basolateral peak. The intensity of the apical peak is also decreased compared with controls (E). In *method b*, the percentage of the sum of pixel intensity on each side of each tubule was calculated in each section using Image-Pro (F). By estimating the mean height of the apical and basal peak in each section, a general mean for all sections could be computed for each side, in each group. * $P < 0.05$.

membrane or redistribution of previously apical AQP2 to the basolateral membrane. This phenomenon is likely to be part of the global reorganization of the CCD principal cell, described under aldosterone treatment when sodium intake is sufficient (33). Whether the increased expression of AQP2 on the basolateral membrane is a regulated event or the consequence of nonregulatory expression (e.g., depending only on the changes in basolateral membrane surface area) cannot be answered here. However, in normal rats, very little basolateral AQP2 is seen in the CCD (4). A

further understanding of the signaling mechanisms involved in the increased basolateral AQP2 (and AQP3) in response to long-term aldosterone and its role in water and sodium homeostasis requires further studies.

In summary, we have demonstrated that long-term aldosterone infusion in normal rats and dDAVP-treated rats receiving water ad libitum was associated with hypokalemia, increased water intake, increased urine output, and decreased urine concentration with a decreased expression of AQP2 in ISOM and a decreased apical expression of

Fig. 10. Control experiments. Sections from Aldo-injected, clamped, angiotensin II-treated rats (A and D) are shown. Rats hypokalemic for 4 days (B and E) and rats acutely injected with aldosterone (C and F) were studied. Representative micrographs show immunoperoxidase staining for AQP2 in a straight segment of CCD in sections of paraffin-embedded kidneys of control (A) and Aldo (D) rats with clamped angiotensin II levels (*protocol 2*). In Aldo rats, basolateral AQP2 is also apparent compared with controls. Also shown are representative micrographs showing immunoperoxidase staining for AQP2 in a straight segment of CCD in sections of paraffin-embedded kidneys of control (B) and 4-day hypokalemic rats (HypoK; E; *protocol 3*). In control rats, the staining for AQP2 is mainly located apically. In HypoK rats, the staining is also located apically, with no evidence of increased basolateral labeling. The intensity of the staining also appears lower in HypoK rats. Also shown are representative micrographs showing immunoperoxidase staining for AQP2 in a straight segment of CCD in sections of paraffin-embedded kidneys of control and acutely aldosterone-treated rats 30 min after the injection (*protocol 5*). In rats that received aldosterone treatment acutely (F), no difference in AQP2 subcellular localization was obvious 30 min after the injection compared with their control (C).

AQP2 in the CCD. Aldosterone also induced an increase in basolateral expression of AQP2 mostly in CCDs in rats which appeared secondary to mineralocorticoid receptor activation. This effect on basolateral AQP2 was independent

of hypokalemia and plasma angiotensin II levels. Further knowledge of the functional role of basolateral AQP2 in the CCD in situations of increased sodium reabsorption will require further study.

Fig. 11. Role of sodium in basolateral AQP2 expression. AQP2 distribution in the CCD of chronically aldosterone-treated rats fed a sodium-replete (A) or sodium-deficient (C) diet were studied. B and D are higher magnifications of boxed areas in A and C. In the absence of a sodium-replete diet, the increase in cell volume as well as the increase in basolateral expression of AQP2 are markedly inhibited.

ACKNOWLEDGMENTS

The authors thank Helle Høyer, Lotte Vallentin Holbech, Ida Maria Jalk, Inger Merete Paulsen, Zhila Nikrozi, Mette Vistisen, Gitte Kall, and Dorte Wulff for expert technical assistance.

GRANTS

The Water and Salt Research Center at the University of Aarhus is established and supported by the Danish National Research Foundation (Danmarks Grundforskningsfond). S. de Seigneux was supported by the Swiss Foundation for Medicine and Biology (FSMB). Support for this study was provided by the Karen Elise Jensen Foundation, Novo Nordic Foundation, Danish Medical Research Council, European Commission (QRLT 2000 00778 and QRLT 2000 00987), Regional Technology Innovation Program of the MOCIE (RTI04-01-01), and Brain Korea 21 Project in 2006.

REFERENCES

- Berl T, Linas SL, Aisenbrey GA, Anderson RJ. On the mechanism of polyuria in potassium depletion. The role of polydipsia. *J Clin Invest* 60: 620–625, 1977.
- Bindels RJ, Hartog A, Timmermans JA, van Os CH. Immunocytochemical localization of calbindin-D28k, calbindin-D9k and parvalbumin in rat kidney. *Contrib Nephrol* 91: 7–13, 1991.
- Chen L, Williams SK, Schafer JA. Differences in synergistic actions of vasopressin and deoxycorticosterone in rat and rabbit CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F147–F156, 1990.
- Christensen BM, Wang W, Frøkiær J, Nielsen S. Axial heterogeneity in basolateral AQP2 localization in rat kidney: effect of vasopressin. *Am J Physiol Renal Physiol* 284: F701–F717, 2003.
- Christensen BM, Zelenina M, Aperia A, Nielsen S. Localization and regulation of PKA-phosphorylated AQP2 in response to V₂-receptor agonist/antagonist treatment. *Am J Physiol Renal Physiol* 278: F29–F42, 2000.
- De SS, Kim SW, Hemmingsen SC, Frøkiær J, Nielsen S. Increased expression but not targeting of ENaC in adrenalectomized rats with PAN-induced nephrotic syndrome. *Am J Physiol Renal Physiol* 291: F208–F217, 2006.
- Diz DI, Baer PG, Nasjletti A. Angiotensin II-induced hypertension in the rat. Effects on the plasma concentration, renal excretion, and tissue release of prostaglandins. *J Clin Invest* 72: 466–477, 1983.
- Elkjær ML, Kwon TH, Wang W, Nielsen J, Knepper MA, Frøkiær J, Nielsen S. Altered expression of renal NHE3, TSC, BSC-1, and ENaC subunits in potassium-depleted rats. *Am J Physiol Renal Physiol* 283: F1376–F1388, 2002.
- Feraillle E, Mordasini D, Gonin S, Deschenes G, Vinciguerra M, Doucet A, Vandewalle A, Summa V, Verrey F, Martin PY. Mechanism of control of Na,K-ATPase in principal cells of the mammalian collecting duct. *Ann NY Acad Sci* 986: 570–578, 2003.
- Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* 361: 549–552, 1993.
- Gross JB, Imai M, Kokko JP. A functional comparison of the cortical collecting tubule and the distal convoluted tubule. *J Clin Invest* 55: 1284–1294, 1975.
- Harvey BJ, Higgins M. Nongenomic effects of aldosterone on Ca²⁺ in M-1 cortical collecting duct cells. *Kidney Int* 57: 1395–1403, 2000.
- Hasler U, Mordasini D, Bianchi M, Vandewalle A, Feraillle E, Martin PY. Dual influence of aldosterone on AQP2 expression in cultured renal collecting duct principal cells. *J Biol Chem* 278: 21639–21648, 2003.
- Hawk CT, Schafer JA. Effects of AVP and deoxycorticosterone on Na⁺ and water transport in the Dahl salt-sensitive rat CCD. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F471–F478, 1991.
- Hebert RL, Regnier L, Peterson LN. Rabbit cortical collecting ducts express a novel prostacyclin receptor. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F145–F154, 1995.
- Kim SW, de SS, Sassen MC, Lee J, Kim J, Knepper MA, Frøkiær J, Nielsen S. Increased apical targeting of renal ENaC subunits and decreased expression of 11β-HSD2 in HgCl₂-induced nephrotic syndrome in rats. *Am J Physiol Renal Physiol* 290: F674–F687, 2006.
- Kwon TH, Nielsen J, Knepper MA, Frøkiær J, Nielsen S. Angiotensin II AT₁ receptor blockade decreases vasopressin-induced water reabsorption and AQP2 levels in NaCl-restricted rats. *Am J Physiol Renal Physiol* 288: F673–F684, 2005.
- Kwon TH, Nielsen J, Masilamani S, Hager H, Knepper MA, Frøkiær J, Nielsen S. Regulation of collecting duct AQP3 expression: response to mineralocorticoid. *Am J Physiol Renal Physiol* 283: F1403–F1421, 2002.
- Lee YJ, Song IK, Jang KJ, Nielsen J, Frøkiær J, Nielsen S, Kwon TH. Increased AQP2 targeting in primary cultured IMCD cells in response to angiotensin II through AT₁ receptor. *Am J Physiol Renal Physiol* 292: F340–F350, 2007.
- Marples D, Frøkiær J, Dorup J, Knepper MA, Nielsen S. Hypokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. *J Clin Invest* 97: 1960–1968, 1996.
- McDill BW, Li SZ, Kovach PA, Ding L, Chen F. Congenital progressive hydronephrosis (cph) is caused by an S256L mutation in aquaporin-2 that affects its phosphorylation and apical membrane accumulation. *Proc Natl Acad Sci USA* 103: 6952–6957, 2006.
- Meneton P, Loffing J, Warnock DG. Sodium and potassium handling by the aldosterone-sensitive distal nephron: the pivotal role of the distal and connecting tubule. *Am J Physiol Renal Physiol* 287: F593–F601, 2004.
- Morgan T, Berliner RW. Permeability of the loop of Henle, vasa recta, and collecting duct to water, urea, and sodium. *Am J Physiol* 215: 108–115, 1968.
- Nejsum LN, Zelenina M, Aperia A, Frøkiær J, Nielsen S. Bidirectional regulation of AQP2 trafficking and recycling: involvement of AQP2-S256 phosphorylation. *Am J Physiol Renal Physiol* 288: F930–F938, 2005.
- Nielsen J, Kwon TH, Frøkiær J, Knepper MA, Nielsen S. Maintained ENaC trafficking in aldosterone-infused rats during mineralocorticoid and glucocorticoid receptor blockade. *Am J Physiol Renal Physiol* 292: F382–F394, 2007.
- Nielsen J, Kwon TH, Praetorius J, Frøkiær J, Knepper MA, Nielsen S. Aldosterone increases urine production and decreases apical AQP2 expression in rats with diabetes insipidus. *Am J Physiol Renal Physiol* 290: F438–F449, 2006.
- Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci USA* 92: 1013–1017, 1995.
- Nielsen S, DiGiovanni SR, Christensen EI, Knepper MA, Harris HW. Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. *Proc Natl Acad Sci USA* 90: 11663–11667, 1993.
- Pelaez LI, Manriquez MC, Nath KA, Romero JC, Juncos LA. Low-dose angiotensin II enhances pressor responses without causing sustained hypertension. *Hypertension* 42: 798–801, 2003.
- Rubera I, Loffing J, Palmer LG, Frindt G, Fowler-Jaeger N, Sauter D, Carroll T, McMahon A, Hummler E, Rossier BC. Collecting duct-specific gene inactivation of alphaENaC in the mouse kidney does not impair sodium and potassium balance. *J Clin Invest* 112: 554–565, 2003.
- Sheader EA, Wargent ET, Ashton N, Balment RJ. Rapid stimulation of cyclic AMP production by aldosterone in rat inner medullary collecting ducts. *J Endocrinol* 175: 343–347, 2002.
- Sheader EA, Wargent ET, Ashton N, Balment RJ. Rapid stimulation of cyclic AMP production by aldosterone in rat inner medullary collecting ducts. *J Endocrinol* 175: 343–347, 2002.
- Stanton B, Janzen A, Klein-Robbenhaar G, DeFronzo R, Giebisch G, Wade J. Ultrastructure of rat initial collecting tubule. Effect of adrenal corticosteroid treatment. *J Clin Invest* 75: 1327–1334, 1985.
- van Balkom BW, van RM, Breton S, Pastor-Soler N, Bouley R, van der SP, Brown D, Deen PM. Hypertonicity is involved in redirecting the aquaporin-2 water channel into the basolateral, instead of the apical, plasma membrane of renal epithelial cells. *J Biol Chem* 278: 1101–1107, 2003.
- Wade JB, O'Neil RG, Pryor JL, Boulpaep EL. Modulation of cell membrane area in renal collecting tubules by corticosteroid hormones. *J Cell Biol* 81: 439–445, 1979.
- Wade JB, Stanton BA, Field MJ, Kashgarian M, Giebisch G. Morphological and physiological responses to aldosterone: time course and sodium dependence. *Am J Physiol Renal Fluid Electrolyte Physiol* 259: F88–F94, 1990.
- Winter C, Schulz N, Giebisch G, Geibel JP, Wagner CA. Nongenomic stimulation of vacuolar H⁺-ATPases in intercalated renal tubule cells by aldosterone. *Proc Natl Acad Sci USA* 101: 2636–2641, 2004.