

Mineralocorticoids induce polyuria by reducing apical aquaporin-2 expression of the kidney in partial vasopressin deficiency

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Article

Keywords:

Posted Date: May 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1676160/v1>

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Abstract

The symptoms of diabetes insipidus can be masked by the concurrence of adrenal insufficiency and emerge after the administration of hydrocortisone, occasionally at high doses. To elucidate the mechanism underlying polyuria induced by the administration of high-dose corticosteroids in deficiency of arginine vasopressin (AVP), we first examined the secretion of AVP in three patients in whom polyuria was observed only after the administration of high-dose corticosteroids. Next, we examined the effects of dexamethasone or aldosterone on water balance in wild-type and familial neurohypophysial diabetes insipidus (FNDI) model mice. The hypertonic saline test showed that AVP secretion was partially impaired in all patients. In one patient, there were no apparent changes in AVP secretion before and after the administration of high-dose corticosteroids. In FNDI mice, unlike dexamethasone, the administration of aldosterone increased urine volumes and decreased urine osmolality. The immunohistochemical analyses showed that, after the administration of aldosterone in FNDI mice, aquaporin-2 expression was decreased in the apical membrane and increased in the basolateral membrane in the collecting duct. These changes were not observed in wild-type mice. The present data suggest that treatment with mineralocorticoids induces polyuria by reducing aquaporin-2 expression in the apical membrane of the kidney in partial AVP deficiency.

Introduction

Arginine vasopressin (AVP), an antidiuretic hormone, is secreted from the posterior pituitary and acts on V2 receptors in the kidney to promote reabsorption of free water^{1–3}. Diabetes insipidus (DI), characterized by polyuria and polydipsia, is caused by a deficiency in either AVP release (central DI) or the renal action (nephrogenic DI)⁴. Occasionally, the symptoms of DI are masked by the concurrence of adrenal insufficiency. This condition is termed masked DI, and polyuria emerges after glucocorticoid replacement. Thus far, the precise mechanisms by which polyuria is induced after the administration of glucocorticoids in patients with masked DI have not been fully clarified. However, previous studies suggested the involvement of both AVP-dependent and AVP-independent mechanisms: glucocorticoid deficiency resulted in increases in the plasma levels of AVP in Sprague–Dawley rats⁵, and water permeability of the renal collecting ducts was increased after adrenalectomy compared with sham operation in Brattleboro rats, which are genetically AVP-deficient⁶.

There are some case reports in which polyuria was induced after administration of high-dose hydrocortisone, which has both glucocorticoid and mineralocorticoid action, in patients with partial DI^{7–10}. In these studies, the investigators speculated that the patients had masked DI, and that the polyuria was induced by the administration of glucocorticoids. However, aldosterone replacement reportedly improved a defect in the urinary diluting ability in adrenalectomized Brattleboro rats¹¹. Moreover, it has been shown that the administration of aldosterone increases urine volumes in a rat model of DI¹². Thus, it is unclear which action, i.e., glucocorticoid or mineralocorticoid action, is involved in the polyuria after administration of high dose of hydrocortisone in partial DI.

In this study, we firstly reported three clinical cases in which polyuria was developed during the administration of high-dose corticosteroids. Secondly, we examined the effects of dexamethasone (a glucocorticoid) or aldosterone (a mineralocorticoid) on water balance in familial neurohypophyseal diabetes insipidus (FNDI) model mice, in which AVP secretion was partially impaired¹³.

Results

Case reports

Case 1

A 75-year-old man diagnosed with IgG4-related autoimmune pancreatitis 4 years earlier was admitted to hospital due to hyponatremia. Blood testing showed elevated IgG4 levels, and magnetic resonance imaging revealed a strongly contrasted enlarged pituitary gland, collectively leading to the diagnosis of IgG4-related hypophysitis. The plasma level of adrenocorticotrophic hormone (ACTH) was 2.8 pg/mL and the serum level of cortisol was 0.5 µg/dL, indicating secondary adrenal insufficiency. The plasma aldosterone level was 53.0 pg/mL and plasma renin activity was 0.4 ng/mL/h. Following the initiation of treatment with 15 mg/day hydrocortisone, the patient complained of decreased visual acuity and bitemporal hemianopsia; hence, treatment was switched from 15 mg/day hydrocortisone to 40 mg/day prednisolone, of which glucocorticoid and mineralocorticoid activity are equivalent to 160 and 32 mg hydrocortisone, respectively¹⁴. Subsequently, he complained of thirst, polydipsia, and polyuria. On day 14 of hospitalization (12 days after the initiation of treatment with prednisolone), pituitary gland enlargement was reduced on magnetic resonance imaging and ocular symptoms improved, although the symptom of DI continued (Fig. 1A). A hyperintense signal in the posterior pituitary gland was absent on T1-weighted magnetic resonance imaging (data not shown). On day 23 of hospitalization, when the patient presented with polyuria receiving 25 mg/day prednisolone, a hypertonic saline infusion test revealed insufficient vasopressin secretion (Fig. 2A). Based on the diagnosis of central DI, the administration of oral desmopressin (DDAVP) (60 µg/day) was initiated. As the dose of prednisolone was decreased, the symptoms of DI were resolved and DDAVP was discontinued. During follow-up examination 2 years later, when polyuria was not evident, a hypertonic saline infusion test showed persistent insufficient vasopressin secretion (Fig. 2B).

Case 2

A 53-year-old man visited our hospital with a complaint of fatigue. He had been treated with levothyroxine following the diagnosis of hypothyroidism based on the results of blood tests (thyrotropin: 5.47 µIU/mL; free thyroxine: 0.625 ng/dL, anti-thyroperoxidase antibody: 17.5 IU/mL); nevertheless, his symptoms had not been ameliorated. Additional blood testing revealed that the plasma level of ACTH was 1.8 pg/mL and the serum level of cortisol was 0.1 µg/dL, indicating secondary adrenal insufficiency. The plasma aldosterone level was 137.0 pg/mL and plasma renin activity was 4.5 ng/mL/h. Polyuria developed after

initiating treatment with 30 mg/day hydrocortisone (Fig. 1B). T1-weighted magnetic resonance imaging demonstrated the loss of a hyperintense signal in the posterior pituitary gland (data not shown). DDAVP was administered on day 4, and urine volumes were decreased on day 5. As the dose of hydrocortisone was tapered, the urine volume decreased and the administration of DDAVP was discontinued. A hypertonic saline infusion test performed during treatment with 15 mg/day hydrocortisone showed insufficient vasopressin secretion (Fig. 2C).

Case 3

A 59-year-old woman who had received chemotherapy, including pembrolizumab (an immune checkpoint inhibitor), for lung adenocarcinoma presented to the emergency department with fever. She was diagnosed with septic shock due to leg cellulitis. Despite the clinical improvement of infection by antibacterial treatment for 10 days, persistent unexplained hypotension was noted. Endocrinological examinations revealed that the plasma levels of ACTH were 6.9 pg/mL and the serum levels of cortisol in the morning were 0.3 µg/dL. These levels did not show increases in the corticotropin-releasing hormone stimulation test (data not shown). Thus, she was diagnosed with ACTH deficiency induced by pembrolizumab. Neither the plasma aldosterone level nor plasma renin activity were measured. Treatment with high-dose hydrocortisone was initiated, which led to stabilization of circulation dynamics. During treatment with 50–150 mg/day hydrocortisone, polyuria was observed and fluid infusion volumes were increased (Fig. 1C). A hyperintense signal in the posterior pituitary gland was absent on T1-weighted magnetic resonance imaging (data not shown). However, as the dose of hydrocortisone was tapered, the urine volume was decreased to < 40 mL/kgBW/day (Fig. 1C). On day 17 after the initiation of steroid replacement, in the absence of polyuria, a hypertonic saline infusion test was performed, which showed insufficient AVP secretion (Fig. 2D).

Unlike dexamethasone, aldosterone induced polyuria in FNDI mice

There were no significant differences in urine volumes or water intake for 5 days during the administration of vehicle, dexamethasone, and aldosterone in WT mice (Figures 3A, B). In FNDI mice, there were no significant differences in urine volumes or water intake between vehicle and dexamethasone groups. However, 2–5 days after the initiation of treatment, these parameters were significantly increased in the aldosterone group compared with the vehicle group (Figures 3C, D).

Aldosterone-induced hypotonic polyuria in FNDI mice without a decrease in AVP secretion or hypokalemia

In FNDI mice, the urine osmolality was significantly lower in the aldosterone group versus the vehicle group on day 5 after the initiation of treatment (Figure 4A). However, there were no significant differences in the concentrations of urine AVP between the vehicle and aldosterone groups (Figure 4B). On day 5, the levels of potassium in the serum were not significantly different between the vehicle and aldosterone groups (vehicle: 6.91 ± 0.21 mEq/L; aldosterone: 6.72 ± 0.42 mEq/L; $P=0.67$). These results demonstrated that the increase in urine volumes was not associated with a decrease in AVP secretion or hypokalemia.

Aldosterone altered the distribution of AQP2 and reduced its expression in the apical membrane in the collecting ducts

The protein abundance of AQP2 in the inner medulla was significantly lower in FNDI mice than in WT mice under basal conditions (Supplementary Figure 1). However, there were no significant differences in AQP2 between the vehicle and aldosterone-treated groups in WT (Figure 5A) or FNDI mice (Figure 5B). Nevertheless, the fluorescence intensities of AQP2 were decreased in the apical membrane and increased in the basolateral membrane in the aldosterone-treated group compared with the vehicle-treated group in FNDI mice (Figures 5E, F, I, J, M, N). Semiquantitative analyses revealed that the ratio of AQP2 intensity in the apical membrane to that in the basolateral membrane was significantly decreased in aldosterone-treated FNDI mice versus vehicle-treated FNDI mice (Figure 5P). In WT mice, treatment with aldosterone did not affect the distribution of AQP2 (Figures 5C, D, G, H, K, L, O).

Discussion

In the three patients of this study, polyuria was only observed following the administration of high-dose corticosteroids. Hypertonic saline testing showed that AVP secretion was impaired even in the absence of polyuria. In FNDI mice, unlike dexamethasone, the administration of aldosterone increased urine volumes and decreased urine osmolality. The immunohistochemical analyses showed that AQP2 expression was decreased in the apical membrane but increased in the basolateral membrane of the collecting duct following the administration of aldosterone in FNDI mice. These changes were not observed in WT mice treated with aldosterone.

When DI is accompanied by either primary or secondary adrenal insufficiency, polyuria is masked, which is attributed to decreases in glucocorticoid deficiency. Indeed, decreased urine volumes in masked DI are usually increased after substitution of physiological dose of glucocorticoids. However, there are some cases, including 3 cases in this study, who showed polyuria only after high dose of corticosteroid. In case 1 of this study, hypertonic saline tests were performed both when polyuria was evident and urine volumes were in the normal range, showing similar results. These findings demonstrated that the administration of high-dose corticosteroids induced polyuria in an AVP-independent manner, at least in case 1 of this study. On the other hand, while the three patients of this study were diagnosed with DI based on hypertonic saline tests¹⁵, basal plasma AVP levels were detectable in all cases. This may explain why the three patients did not require DDAVP treatment unless high-dose corticosteroids were administered.

As both hydrocortisone and prednisolone have mineralocorticoid as well as glucocorticoid activities¹⁴, it was unclear which activity was responsible for increases in urine volumes in the three patients of this study. A previous study reported that treatment with high-dose aldosterone increased urine volumes in a rat model of DI¹². However, AVP secretion was not examined, and high-dose mineralocorticoids were administered in the previous study, which could decrease the levels of potassium in the serum¹⁶. In this study, we assessed AVP secretion and the levels of potassium in the serum of FNDI mice treated with a relatively low dose of aldosterone. The results demonstrated that urine volumes were increased following

the administration of aldosterone without affecting AVP secretion or the levels of potassium in the serum. In comparison, dexamethasone did not affect urine volumes in FNDI mice in this study. Thus, this suggests that mineralocorticoids, but not glucocorticoids, were responsible for polyuria during treatment with high-dose corticosteroids in partial DI.

To clarify the mechanism through which mineralocorticoids induced polyuria in FNDI mice, we examined the expression of AQP2 in the kidney. It is established that AQP2 trafficking to the apical membrane promotes water reabsorption in the collecting ducts^{17,18}. The present data showed that mineralocorticoids reduced AQP2 expression in the apical membrane in FNDI mice. On the other hand, aldosterone did not affect AQP2 expression or water balance in WT mice in this study. It has been shown that AVP increases AQP2 expression in the apical membrane^{3,19}. Therefore, it is possible that the expression levels would be determined by the balance between AVP and aldosterone. Our data suggested that aldosterone could interfere with the water reabsorption only when AVP release is partially impaired.

In this study, we did not compare the effects of glucocorticoids and aldosterone on urine volumes in patients with DI. Thus, it is unclear whether the findings observed in FNDI mice are applicable to patients. This is the limitation of the present study.

In conclusion, our analyses in patients with DI and animal models suggest that treatment with mineralocorticoids induces polyuria in partial DI in an AVP-independent manner.

Methods

Clinical data collection

All clinical data were collected at the Nagoya University hospital. Hypertonic saline infusion tests were performed and assessed as previously described¹⁵.

Animals

The generation of FNDI mice heterozygous for the mutant Avp gene (Cys98stop) has been previously described¹³. Wild-type (WT) mice (C57BL/6J) were purchased from Japan SLC, Inc. (Shizuoka, Japan). In all animal experiments, 2-month-old male FNDI and WT mice were used. Mice were maintained under controlled conditions (23.0±0.5°C, lights on from 09:00 to 21:00).

Measurement of urine volumes, urine AVP concentrations, urine osmolality, and serum potassium levels

WT mice and FNDI mice were implanted with osmotic minipumps (Alzet model 2002; Muromachi Kikai Co., Tokyo, Japan) containing: 1) dimethyl sulfoxide (DMSO) (WT or FNDI + vehicle group); 2) aldosterone (A9477; Sigma-Aldrich, St. Louis, MO, USA; 0.025 µg/g body weight [BW]/day) in DMSO (WT or FNDI + aldosterone group); or 3) dexamethasone (D2915; Sigma-Aldrich; 0.2 µg/g BW/day) in DMSO (WT or FNDI + dexamethasone group). All groups had free access to water and food. Mice were housed in metabolic cages, and the 24-h pooled urine was collected for 5 days. Urine AVP concentrations were

measured using the Arg8-Vasopressin ELISA Kit (Enzo Life Sciences, Farmingdale, NY, USA) following the instructions provided by the manufacturer. Urine osmolality was determined using the cryoscopic method (Oriental Yeast Co., Ltd., Tokyo, Japan). The levels of potassium in the serum were determined using the ion-selective electrode method (Oriental Yeast Co., Ltd.).

Western blotting analysis

Homogenates of the inner medullae separated from the kidneys of mice were prepared. Semiquantitative immunoblotting was carried out to assess the relative expression levels of proteins of interest, as previously described²⁰. The blots were probed with the following primary antibodies: rabbit anti-aquaporin-2 (anti-AQP2, SPC-503D; StressMarq Biosciences, Victoria, British Columbia, Canada; 1:1,000) and rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH; ab181602; Abcam, Cambridge, UK; 1:10,000). Horse radish peroxidase-conjugated donkey anti-rabbit immunoglobulin G (IgG) (NA934; GE Healthcare, Little Chalfont, UK; Research Resource Identifier: AB_772206) was used as the secondary antibody. Can Get Signal Immunoreaction Enhancer Solution (TOYOBO, Osaka, Japan) was used for the dilution of the primary and secondary antibodies. ECL Prime Western Blotting Detection Reagent (GE Healthcare, Chicago, IL, USA) was used to detect signals. The intensities of bands in western blots were quantified with the ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Immunohistochemistry and immunofluorescence analysis

Kidneys were fixed by perfusion (through the left ventricle) with periodate lysine (0.2 M) and paraformaldehyde (2%) in phosphate-buffered saline. Tissue samples were soaked for several hours in 20% sucrose in phosphate-buffered saline, embedded in Tissue-Tek O.C.T. compound (Sakura Finetechnical, Tokyo, Japan), and stored at -80°C until sectioning. Kidneys were cut into sections (thickness: 10 µm) using a cryostat at -20°C, thaw mounted on Superfrost Plus microscope slides (Matsunami, Tokyo, Japan), and stored at -80°C until immunohistochemical analysis, which was performed as previously described²¹. Briefly, the sections were incubated with the primary antibody anti-AQP2 (ab199975; Abcam; 1:3,000) overnight at 4°C. The sections were rinsed and incubated with the secondary antibody Alexa Fluor 488-conjugated goat anti-mouse IgG (H + L) highly cross-adsorbed (1:1,000; A11029; Invitrogen, San Diego, CA, USA) for 2 h at room temperature. Immunofluorescence images were captured by a laser-scanning confocal microscope (LSM 5 Pascal; Carl Zeiss, Oberkochen, Germany). The distribution of AQP2 was semiquantitatively analyzed. The fluorescence intensities of AQP2 in the kidneys were quantified with the ImageJ software, according to previous studies²²⁻²⁴. The AQP2 intensities were measured from the apical to the basolateral membrane. These data were obtained using principal cells constructing tubule segments (n=9 collecting ducts from three animals per group).

Statistical analysis

The statistical significance of differences among groups was analyzed using the unpaired *t*-test, one-way analysis of variance (ANOVA), or two-way ANOVA, with repeated measures followed by the Bonferroni test

as appropriate. Results are expressed as the mean ± standard error, and P-values <0.05 denoted statistically significant differences.

Declarations

Study approval

The Ethics Committee of Nagoya University Graduate School of Medicine does not ask approval for case reports. Written informed consent for the collection and use of retrospective clinical data was provided by the patients. All animal procedures were approved by the Animal Experimentation Committee of the Nagoya University Graduate School of Medicine and performed in accordance with institutional guidelines for animal care and use.

Acknowledgments

This work was supported by the Suzuken Memorial Foundation (to HA) and a research grant from Aichi Kidney Foundation (to HT and JK).

We thank Michiko Yamada and the staff of the Division of Experimental Animals, Nagoya University Graduate School of Medicine, for their technical support.

Author Contributions

HT and HA designed the project. JK and HT performed the experiments and analyzed the data with technical help and advice from TM, YK, YH, TT, DH, T. Kobayashi, YY, MS, TO, SI, HS, RB, and T. Katsuki. JK, HT, FA, SU, and HA wrote the manuscript.

Competing interests

The authors declare no competing interests.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Figures

Figure 1

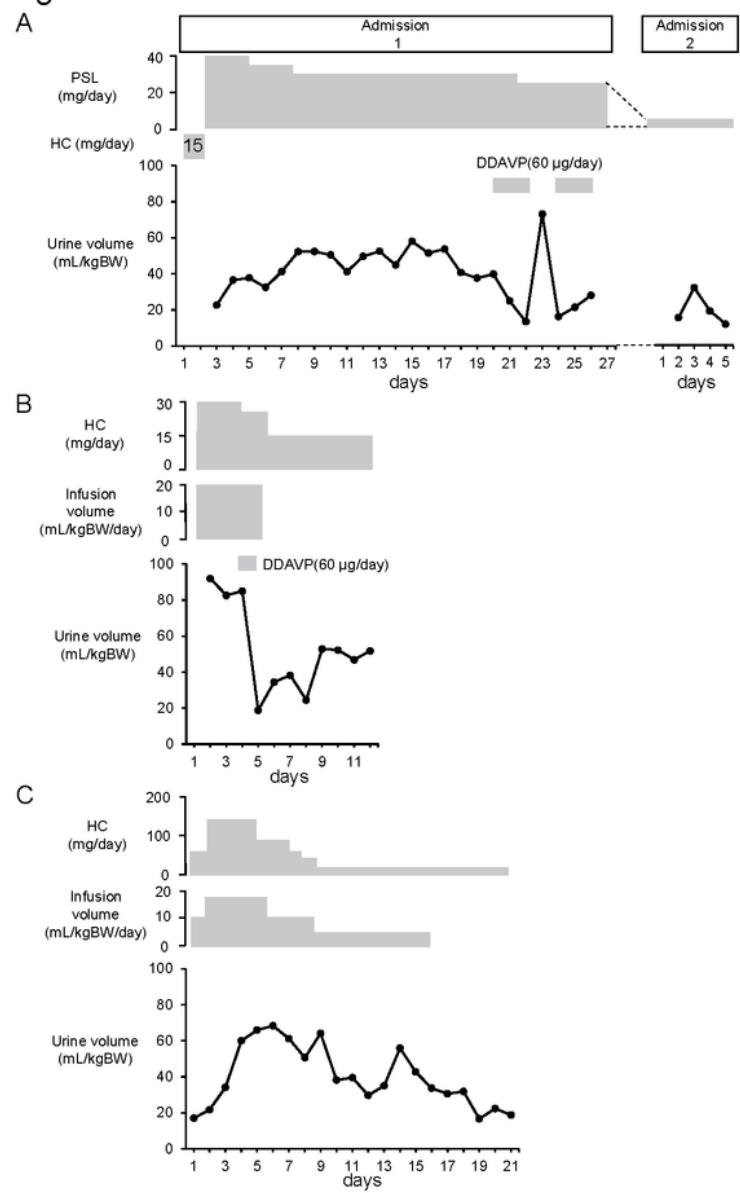


Figure 1

Clinical courses and changes in urine volume of the three cases presented in this article. Urine volumes of case 1 during first and second hospitalizations (A), case 2 (B), and case 3 (C). The x-axis shows the number of days after the initiation of treatment with steroids during hospitalization.

BW, body weight; DDAVP, desmopressin; HC, hydrocortisone; PSL, prednisolone.

Figure 2

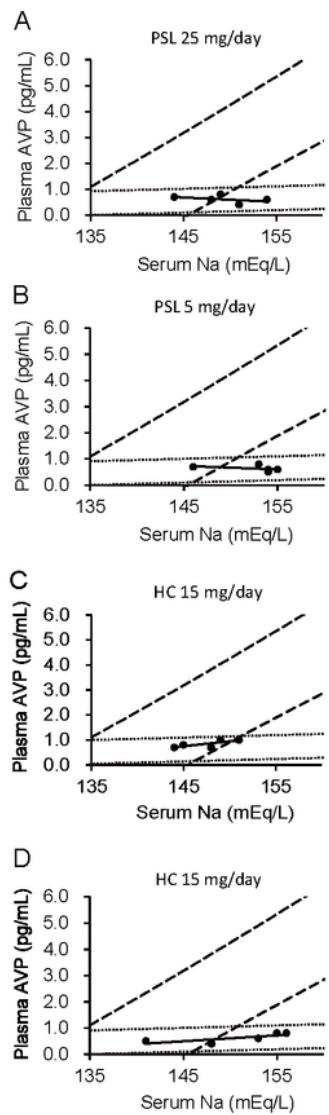


Figure 2

Changes in the concentrations of serum sodium and plasma AVP during hypertonic saline infusion tests. Results of hypertonic saline infusion tests during treatment with 25 mg of prednisolone (A) and 15 mg of hydrocortisone (B) in case 1. Results of hypertonic saline infusion tests during treatment with 15 mg of hydrocortisone in cases 2 (C) and 3 (D). The 95% prediction intervals for the concentrations of plasma

AVP during a hypertonic saline test in healthy controls are shown by dashed lines. The counterparts in patients with central diabetes insipidus are shown by dotted lines.

AVP, arginine vasopressin; HC, hydrocortisone; PSL, prednisolone.

Figure 3

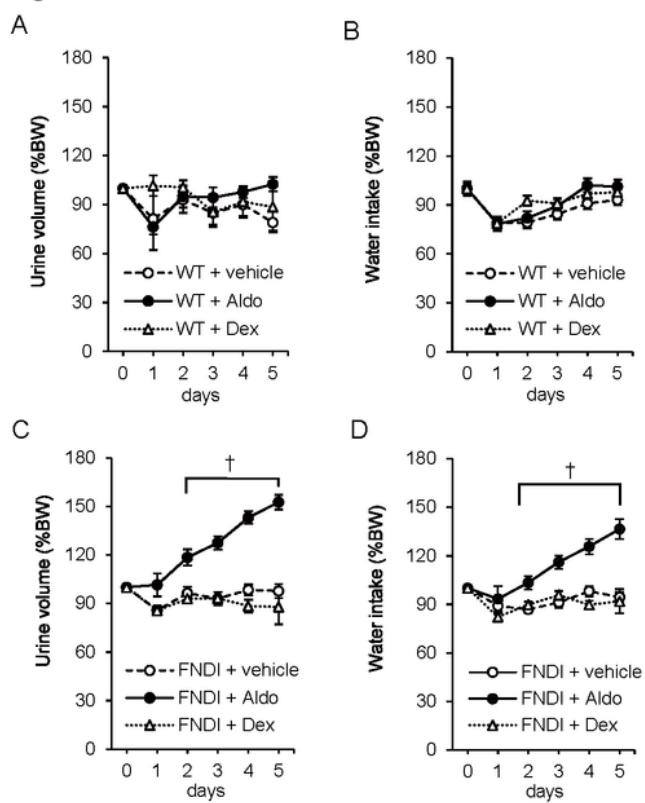


Figure 3

Changes in water balance during the administration of dexamethasone or aldosterone in WT and FNDI mice. Urine volume and water intake in WT (A, B) or FNDI (C, D) mice during the administration of vehicle (●), Aldo (●), and Dex (\triangle) for 5 days. Results are expressed as the mean \pm S.E.; n=7 WT mice per group; n=8–9 FNDI mice per group. $\dagger P<0.01$, compared with WT mice on the same day.

Aldo, aldosterone; BW, body weight; Dex, dexamethasone; FNDI, familial neurohypophyseal diabetes insipidus; S.E., standard error; WT, wild-type.

Figure 4

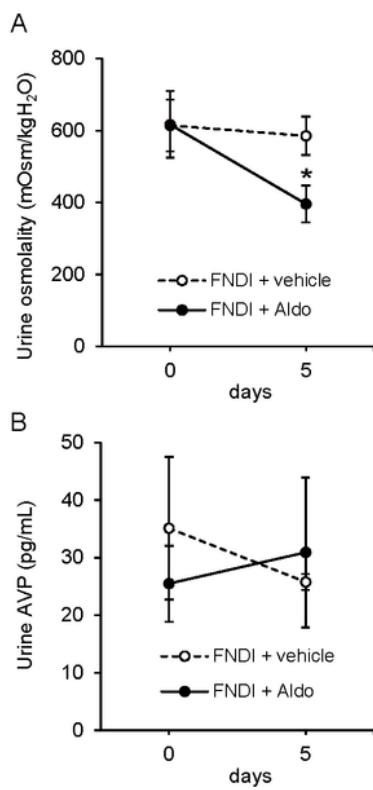


Figure 4

Aldosterone induced hypotonic polyuria in FNDI mice without decreasing AVP secretion. Changes in urine osmolality (A) and the concentration of urine AVP (B) in FNDI mice treated with vehicle (●) or Aldo (●) before (day 0) and 5 days after implantation of an osmotic pump. Results are expressed as the mean ± S.E.; n=6 per group with respect to urine osmolality; n=4–5 per group with respect to the concentration of urine AVP. * $P<0.05$, compared with mice which received vehicle.

Aldo, aldosterone; AVP, arginine vasopressin; FNDI, familial neurohypophysial diabetes insipidus; S.E., standard error.

Figure 5

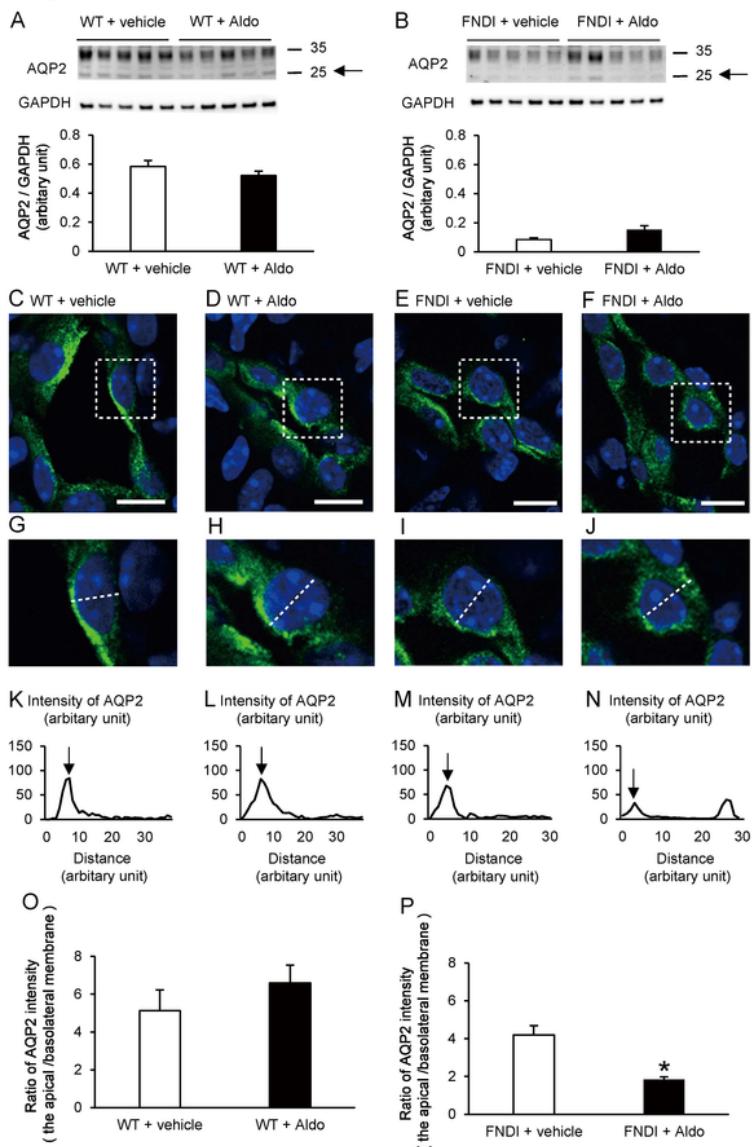


Figure 5

Aldosterone altered the distribution of AQP2 and reduced its expression in the apical membrane. Semiquantitative immunoblotting of total AQP2 using homogenates from the inner medullae of mouse kidneys. Densitometry analysis of total AQP2 (non-glycosylated AQP2 bands; 25 kDa) between the vehicle and aldosterone groups in WT (A) or FNDI (B) mice. Representative images of kidney sections immunohistochemically stained for AQP2 (C-F). Scale bars: 10 µm. (G), (H), (I) and (J) are higher

magnifications of dashed boxed areas in (C), (D), (E) and (F), respectively. AQP2 intensity of each white dotted line from the apical membrane to the basolateral membrane (K–N). Black arrow: the peak of AQP2 intensity in the apical membrane. Semiquantitative analyses of the peak of AQP2 intensity in the apical membranes of three animals per group (O, P); n=9 collecting ducts per group. Results are expressed as the mean \pm S.E. $\square P<0.05$, compared with mice which received vehicle.

Aldo, aldosterone; AQP2, aquaporin-2; FNDI, familial neurohypophyseal diabetes insipidus; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WT, wild-type

Supplementary Files

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