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ORIGINAL ARTICLE

Upregulation of prolactin receptor in proximal tubular cells was induced in cardiac dysfunction model mice

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Abstract
Background In order to clarify the interaction between cardiac dysfunction and sodium homeostasis in the kidney, we used a murine model of cardiac dysfunction and investigated the effect on sodium transporters in renal tubular cells.
Methods Cardiac function was deteriorated by abdominal aortic banding, and the gene expression of sodium transporters in the kidneys was evaluated by real-time RT-PCR and compared with that in the kidneys of control mice.
Results Gene expression of all three variants of the murine prolactin receptor was enhanced by aortic banding. Upregulated prolactin receptor was distributed in the proximal tubular cells of the pars recta in the deep inner cortex and the outer stripe of the outer medulla. Prolactin has been reported to be a natriuretic hormone that inhibits proximal tubular Na^+/K^+-ATPase activity, resulting in reduced sodium reabsorption and the acceleration of natriuresis. Inhibition of endogenous prolactin secretion by bromocriptine administration decreased the urine sodium excretion in both aortic banding and control mice. On the other hand, excess exogenous prolactin administration enhanced urine potassium excretion in aortic banding mice. Furthermore, a high-sodium diet accelerated urinary sodium excretion, which was also significantly decreased by inhibition of endogenous prolactin secretion in aortic banding mice.
Conclusion We reported that the prolactin receptor was upregulated by aortic banding treatment. Prolactin-prolactin receptor interaction in the proximal tubular cells of the pars recta should involve a different mechanism of kaliuresis other than inhibition of Na^+/K^+-ATPase.

Keywords Sodium transport · Cardio-renal · Bromocriptine

Introduction

Cardio-renal interaction has been intensively investigated, and many pieces of evidence on this interaction have been reported. Direct and indirect effects of each dysfunctional organ can initiate and perpetuate the combined disorder of the two organs through a complex combination of neurohormonal feedback mechanisms [1]. For example, in addition to the low cardiac output and renal hypoperfusion as the pathophysiology of renal dysfunction in cardio-renal syndrome, several mechanisms have been proposed including renal congestion, chronic overactivation of the renin-angiotensin-aldosterone system and sympathetic nervous system, nitric oxide-reactive oxygen species dysbalance, and increased levels of inflammatory substances and hormonal factors in both the circulation and the target organs [1–3]. In addition to these established findings, we focused on the possible interaction between impaired cardiac function and sodium homeostasis, and investigated the

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influence of cardiac dysfunction on the sodium transporters in the kidney by analyzing a murine cardiac failure model induced by abdominal aortic banding (Ab).

Upon exposure to pressure overload induced by abdominal Ab, cardiac hypertrophy gradually develops as an adaptive response to preserve function by normalizing chamber wall stress; however, prolonged cardiac hypertrophy results in a dilated heart with decreased cardiac function. Cardiac dysfunction appears 4–8 weeks after aortic banding, and this model has been adopted as an animal model of chronic heart failure [4–6]. We investigated the expression of sodium transporters in the proximal tubular cells of the kidney affected by abdominal Ab and discovered that prolactin receptor (PrlR) was upregulated.

Prolactin is involved in water and electrolyte balance in almost all classes of vertebrates, although its precise function and target tissues have changed during evolution [7]. PrlR is still present in the kidney, and prolactin regulates Na^+/K^+-ATPase in fish [8] and mammals [9–11]. In mouse kidney, PrlR has been reported to be distributed on the luminal membrane of the proximal tubular cells and the parietal epithelial cells of Bowman's capsule [12]. In the present study, we demonstrated the upregulation of PrlR as a model of cardio-renal interaction and clarified the role of prolactin-PrlR interaction.

Materials and methods

Animal models

Male C57BL/6J mice (8 weeks old) weighing about 30 g were purchased from Charles River Japan (Yokohama, Japan) and maintained in our animal facility. Animal care was in accordance with the guidelines of Niigata University. Abdominal Ab was conducted by constricting the abdominal aorta at the infrarenal level with 7-0 silk sutures together with a blunted 27-gauge needle, which was pulled out thereafter. The sham operation was performed by isolation of the aorta without ligation. Eight weeks after Ab, the ejection fraction of each mouse was measured by transthoracic echocardiography with Prosound SSD-3500SV (ALOKA). Mouse serum cystatin C and prolactin concentrations were determined by ELISA using mouse cystatin C ELISA (BioVendor, Brno, Czech Republic) and prolactin mouse ELISA (Abcam, Cambridge, MA), respectively. Renal ischemia-reperfusion (IR) injury was induced under anesthesia by occlusion of the left renal artery with a non-traumatic vascular clamp for 45 min, and then the clamp was removed and renal blood flow was re-established. Twenty-four hours after renal IR injury, all mice were anesthetized again and bilateral kidneys were removed. The left kidney was used as an IR-injured kidney and the right kidney as a control one. For a high-sodium-loaded model, mice were fed an 8 % NaCl diet for more than 7 days.

Quantitative real-time PCR analysis

Total RNA of kidney, heart, liver, spleen, skeletal muscle, and lung was extracted from Ab and control mice 8 weeks after the operation. They were treated with DNase before PCR. Real-time PCR analysis was performed using Thermal Cycler Dice Real Time System II with One Step SYBR PrimeScript Plus RT-PCR Kit (Takara Bio, Shiga, Japan) following the manufacturer's protocol. Target amplicons and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were reverse-transcribed and quantified using the same template RNA for relative quantification analysis. The primer sequences are shown in Table 1.

In situ hybridization and immunohistochemistry

In situ hybridization was carried out using the Ribomap kit and Discovery Automatic Staining Module (Ventana Medical Systems, Tucson, AZ) following the manufacturer's protocol. Complementary RNA probes for mouse PrlR were synthesized by in vitro transcription using cDNA (507 bp corresponding to the coding region spanning nucleotides

Table 1 Sequences of primers used in the RT-PCR

Gene	Sense (5′–3′)	Antisense (3′–5′)
GAPDH	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG
PrlR (variant 1)	GCCAGACCATGGATACTGGAGTAGA	AGAACGGCCACAATGATCCAC
PrlR (variant 2)	GTGGATCATTGTGGCCGTTC	TGGATCCACCTTGTATTTGCTTG
PrlR (variant 3)	GGATCATTGTGGCCGTTCTC	ACTCAGTTGTTGGAATCTTCACCAG
Drd1A	TTAAGATGTGCATCGAGGTGAATGA	GAGCAGCCGGCTTGGTTAGA
Atp1a1	GATCAGCATGGCCTATGGACAG	ACCGTTCTCAGCCAGAATCACA
Scl5a1	CCTCGTGGTGCTGATGTTGAC	GGACCTCAGGCTAATGATTTCAATG
Slc9a3r1	CCAGGACCGAATTGTGGA	CCTGGGATGGGATCACTTTG

GAPDH glyceraldehyde-3-phosphate dehydrogenase, *PrlR* prolactin receptor, *Drd1A* dopamine receptor D1A, *Atp1a1* ATPase, Na+/K+ transporting, alpha 1 polypeptide, *Scl5a1* solute carrier family 5, member 1, *Slc9a3r1* solute carrier family 9, member 3 regulator 1

1,036–1,542 bp containing a common sequence of all three variants and 503 bp corresponding to the coding region spanning nucleotides 2,184–2,683 bp as a PrlR variant 1-specific sequence of the mouse PrlR variant 1 cDNA sequence, NM_011169.5). For immunohistochemistry, PrlR and megalin were detected in the paraffin-embedded kidney specimens using sheep anti-PrlR antibody (AbD Serotec, Raleigh, NC) and mouse anti-megalin antibody (Novus Biologicals, Littleton, CO), respectively. Detection of immune complex was performed using anti-sheep HRP-DAB cell & tissue staining kit (R & D Systems, Minneapolis, MN) for PrlR and alkaline phosphatase-conjugated rabbit anti-mouse IgG antibody (Dako, Glostrup, Denmark) for megalin, followed by visualization with new fuchsin (Nichirei, Tokyo, Japan).

Bromocriptine, prolactin, and G129R-prolactin administration

Bromocriptine mesylate (24 mg/kg/day, Sigma-Aldrich) suspended in 600 µl of distilled deionized water or the same amount of solvent was orally administered 7 weeks after the aortic banding or its sham operation for 8 days, divided into three times a day. Seven days after the initiation of bromocriptine administration, each mouse was placed in a metabolic cage to collect a 24-h urine sample, and then the mice were killed under anesthesia to collect the blood. Two hundred fifty micrograms of sheep prolactin antagonist G129R (Protein Laboratories Rehovot, Rehovot, Israel), 200 µg of sheep prolactin (Sigma-Aldrich, St. Louis, MO), or its solvent was intraperitoneally administered 8 weeks after the aortic banding or its sham operation. Then, each mouse was placed in a metabolic cage to collect a 24-h urine sample. In order to collect the blood, the mice were killed under anesthesia 12 h after the prolactin administration. The urine concentrations of sodium and potassium were determined by an ion-selective electrode method, and the creatinine concentration was measured by an enzymatic method.

Statistical analysis

Values are expressed as mean ± SD. The statistical significance of differences was calculated by Student's *t* test. $P < 0.05$ was considered significant.

Results

Evaluation of cardiac and renal function

Eight weeks after Ab or its sham operation, cardiac functions of Ab and control mice were evaluated by the ejection fraction measured by echocardiography. Figure 1a shows that Ab induced decreased cardiac function in Ab mice; on the other hand, serum cystatin C levels were equal between Ab and control mice (Fig. 1b). We assumed the influence of cardiac dysfunction upon sodium homeostasis and evaluated the gene expression of sodium transporters in the kidney in Ab and control mice.

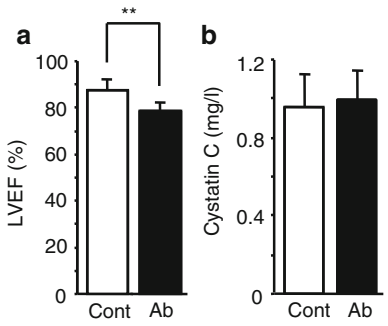


Fig. 1 Biomarkers in cardiac dysfunction model and control mice. **a** Ejection fraction of left ventricle (LVEF) was measured in each mouse to evaluate cardiac function 8 weeks after the Ab or its sham operation ($n = 5$). **b** The serum cystatin C level was measured in each mouse to evaluate renal function at the same time ($n = 5$). Values are mean ± SD. $^{**}P < 0.01$. *Cont* control, *Ab* aortic banding

Quantitative RT-PCR of sodium transporters

Quantitative RT-PCR was performed with a comparison of Ab and control mice to verify the influence of cardiac dysfunction on the gene expression related to sodium transport in the tubular cells. We evaluated the gene expression level of dopamine receptor D1A (Drd1A), ATPase, Na^+/K^+ transporting, alpha 1 polypeptide (Atp1a1), solute carrier family 5, member 1 (Scl5a1), solute carrier family 9, member 3 regulator 1 (Slc9a3r1), and PrlR variant 1. Figure 2 shows that the gene expression of PrlR variant 1 was significantly upregulated in Ab mice. In addition to this long-isoform PrlR variant 1, we also investigated the gene expression of short-isoform PrlR variants 2 and 3, which have a shorter intracellular domain [13]. Figure 3a demonstrates that PrlR variants 2 and 3 were also significantly upregulated in Ab mice. The endogenous serum prolactin concentration was not affected by Ab (Fig. 3b). Both long and short PrlRs are reported to be distributed in most of the organs of humans and rodents, but they play different roles in different organs [13, 14]. We also investigated the mRNA expression of long PrlR in the heart, liver, spleen, skeletal muscle, and lung of Ab and control mice and revealed that upregulation of long-PrlR expression was kidney-specific (Fig. 3c).

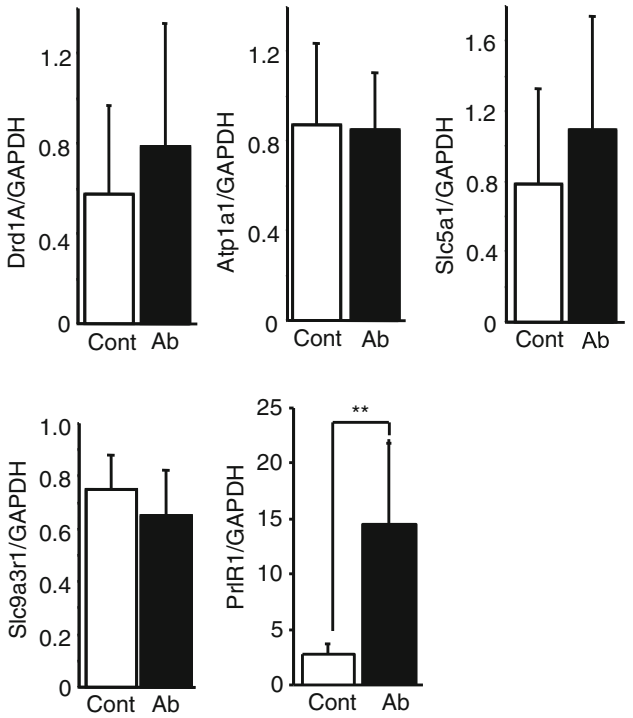


Fig. 2 Real-time quantitative PCR for sodium transporters. Total RNA purified from each kidney 8 weeks after Ab or its sham operation was used as a template for PCR reaction of dopamine receptor D1A (*Drd1A*), ATPase, Na^+/K^+ transporting, alpha 1 polypeptide (*Atp1a1*), solute carrier family 5, member 1 (*Slc5a1*), solute carrier family 9, member 3 regulator 1 (*Slc9a3r1*), and prolactin receptor variant 1 (long isoform, PrlR1) ($n = 10$). Each PCR product was quantified and standardized with the amount of PCR product for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and is shown as fold change. No significant differences in GAPDH expression were observed among each sample. Values are mean ± SD. $^{**}P < 0.01$. *Cont* control *Ab* aortic banding

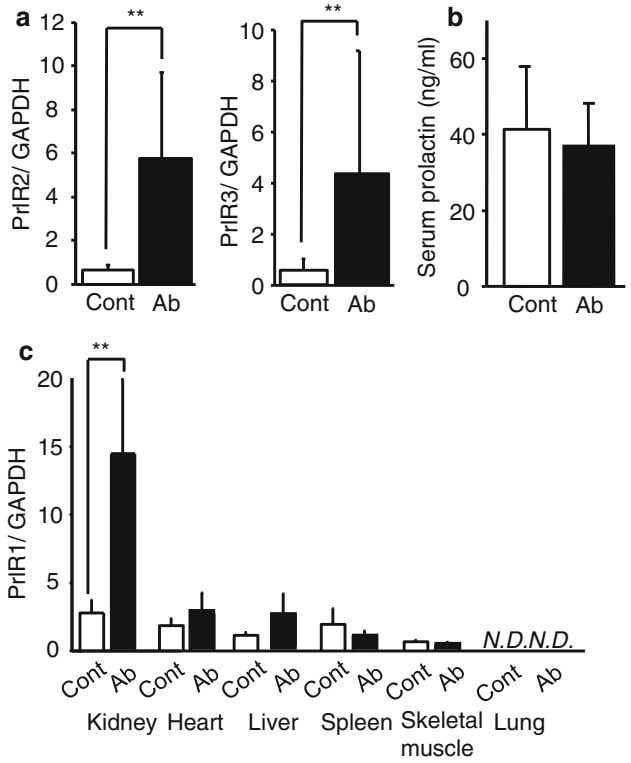


Fig. 3 Expression of prolactin receptor isoforms and its organ specificity. **a** Total RNA purified from each kidney 8 weeks after Ab or its sham operation was used as a template for PCR reaction of prolactin receptor variants 2 and 3 (short isoform, PrlR2 and PrlR3). Each PCR product was quantified and standardized with the amount of PCR product for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and is shown as fold change ($n = 5$). **b** Serum concentration of prolactin in mice with each treatment 8 weeks after Ab or its sham operation ($n = 5$). **c** Prolactin receptor variant 1 mRNA expression induced by Ab in several other organs ($n = 5$). No significant differences in GAPDH expressions were observed among each sample. Values are mean ± SD. $^{**}P < 0.01$. *Cont* control, *Ab* aortic banding, *N.D.* not detected

Localization of prolactin receptor in the kidney

The localization of PrlR-mRNA-expressing cells in the kidney was examined by in situ hybridization using a complementary RNA probe corresponding to the common sequence of all three PrlR variants' mRNA. In control mice, gene expression of PrlR was sporadically observed in the proximal tubular cells of pars recta, but not those of proximal convoluted tubular segments (Fig. 4a–c). In Ab mouse kidney, PrlR gene expression was clearly upregulated mainly in the proximal tubular cells of the pars recta located in the deep inner cortex and the outer stripe of the outer medulla, and to a much lesser extent in the proximal convoluted tubular cells (Fig. 4d–f). PrlR variant 1-specific probe also detected upregulation of gene expression in the same segments (Fig. 4g–i), although PrlR variant 2- and variant 3-specific gene expression could not be verified because their specific mRNA sequences were too short in length. PrlR expression was also examined with an antibody specific for the PrlR extracellular domain preserved in all three isoforms, and it was distributed in the megalin-positive proximal tubular cells (Fig. 4j, k). No morphological changes were observed in the proximal tubular cells among Ab and control mice (Fig. 4l).

Prolactin receptor expression in renal ischemia-reperfusion model

Considering the anatomical distribution of PrlR expression induced by Ab, we hypothesized that PrlR expression might be induced by hypoxic challenge, since the proximal tubular cells of the pars recta in the deep inner cortex and the outer stripe of the outer medulla of normally perfused kidney are characterized by low oxygen tension because of countercurrent exchange and the shunting of oxygen [15, 16], and the outer medulla remains profoundly hypoxic because of persistent intrarenal vasoconstriction and congestion of vessels [17, 18]. We investigated the PrlR

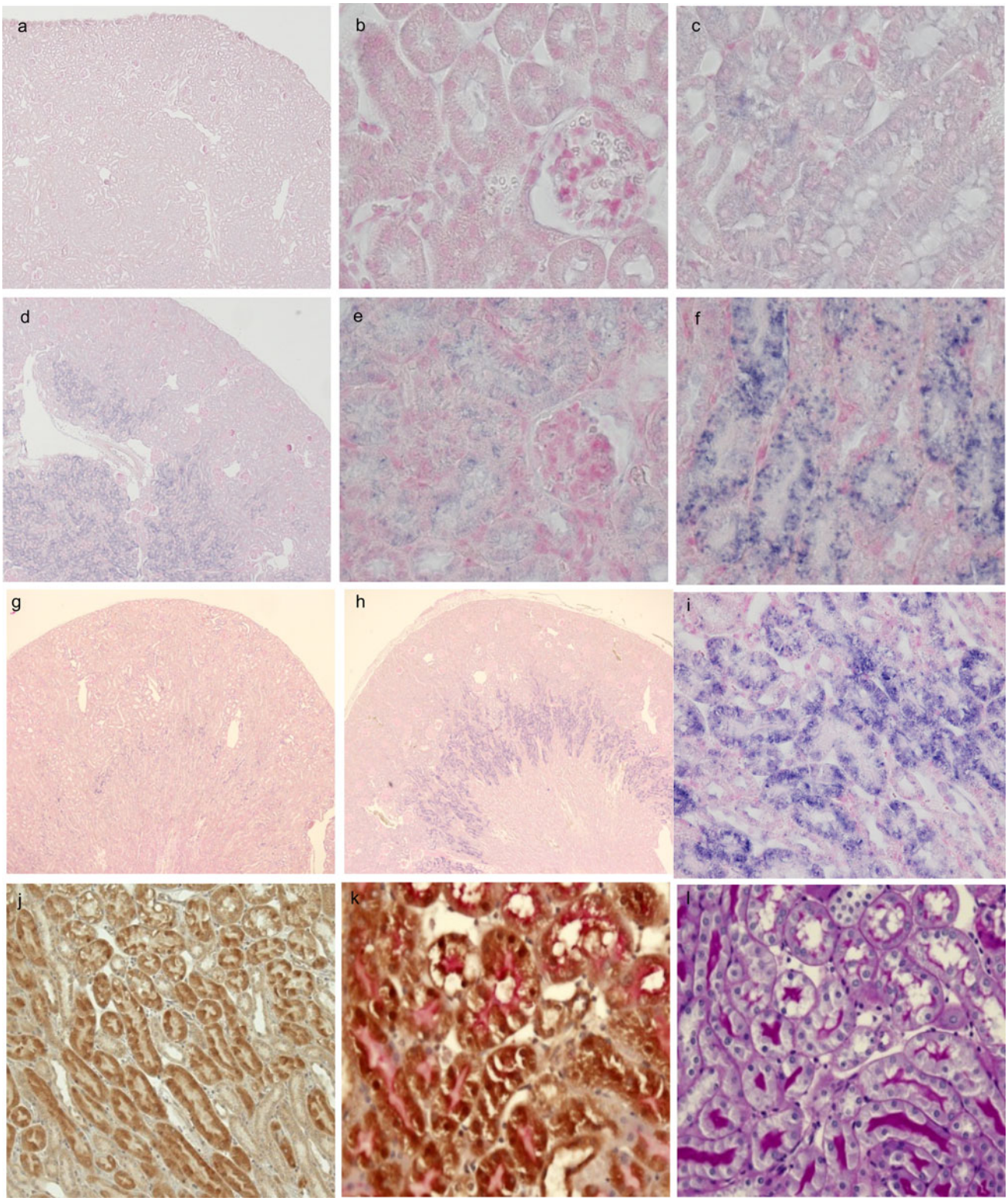


Fig. 4 Localization of prolactin receptor in the kidney induced by aortic banding. Kidney sections of control (**a**–**c**, **g**) and Ab (**d**–**f**, **h**–**l**) mouse 8 weeks after Ab or its sham operation. **a**–**f** Prolactin receptor mRNA with common sequences of all three variants was detected by in situ hybridization (*blue*). **g**–**i** Prolactin receptor variant 1 mRNA was detected by in situ hybridization (*blue*). **j** Prolactin receptor was detected by extracellular domain-specific antibody (*brown*). **k** Double staining of prolactin receptor (*brown*) and megalin (*red*). **l** Serial section of specimen **k** with Periodic acid-Schiff stain. **a**, **d**, **g**, **h** ×40; **j** ×200; **b**, **c**, **e**, **f**, **i**, **k**, **l** ×500 magnification (color figure online)

expression in a renal IR model as an acute renal ischemic model; however, mRNA expression of all three variants of PrlR in the IR-injured kidney was rather decreased (Fig. 5).

Effect of prolactin on urinary sodium and potassium excretion

To investigate the role of endogenous systemic prolactin, bromocriptine, an inhibitor of prolactin secretion, was administered in Ab and control mice. Eight days of bromocriptine treatment completely diminished the systemic serum prolactin to an undetectable level (Fig. 6a), without any effect on serum cystatin C (Fig. 6b). In both Ab and control mice, the urine sodium excretion level was significantly decreased by bromocriptine treatment; on the other hand, urine potassium excretion was not affected (Fig. 6c). As a result of the specific reduction of urine sodium excretion, the ratio of urinary sodium to urinary potassium was also significantly decreased in both Ab and control mice with bromocriptine treatment (Fig. 6d). To validate the specificity of bromocriptine treatment, prolactin antagonist G129R was administered in Ab and control mice, but G129R-prolactin administration did not affect urine sodium and potassium excretion (Fig. 6e). Basal urinary sodium excretion was rather decreased, albeit not significantly, in mice treated with bromocriptine. We surmise that, because bromocriptine was administered to mice as a suspension in water, forced excess water intake may

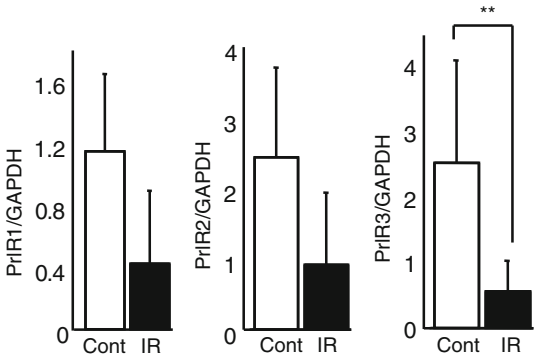
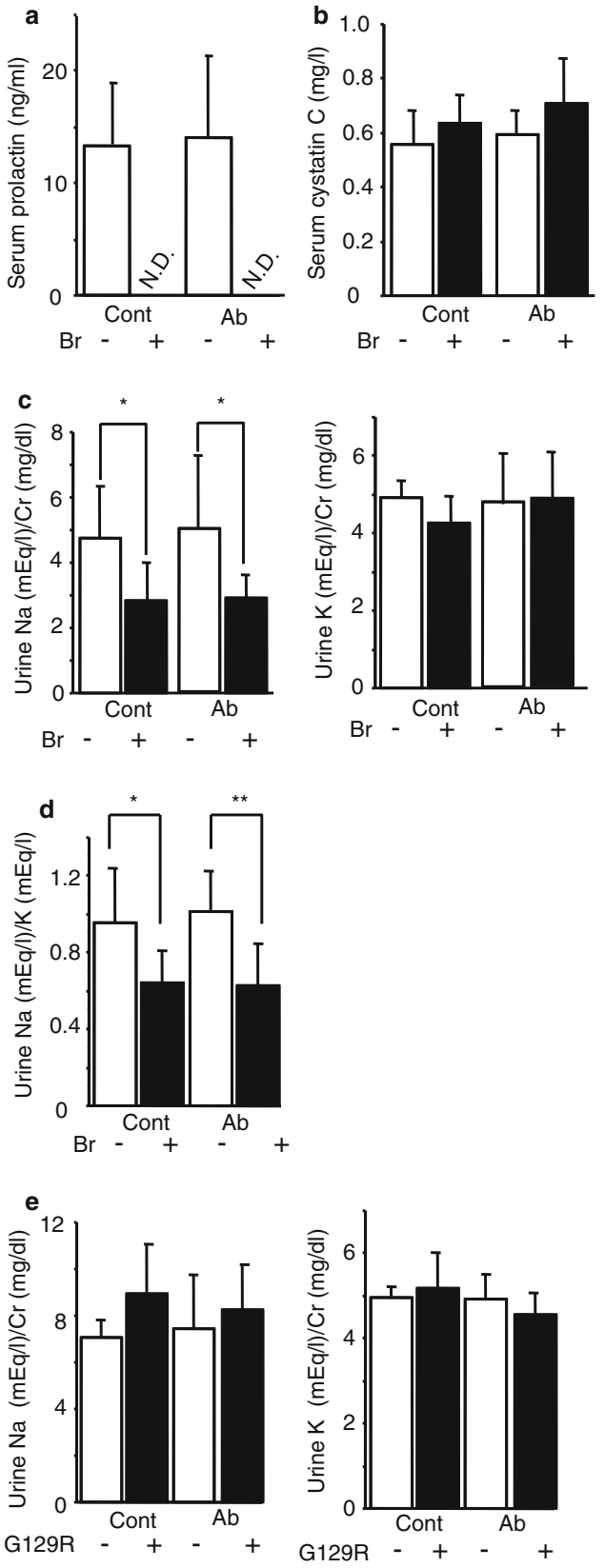


Fig. 5 Prolactin receptor mRNA expression in a renal ischemia-reperfusion model. Total RNA purified from each kidney 1 day after 45 min of left renal artery occlusion and reperfusion was used as a template for PCR reaction of prolactin receptor variants 1, 2, and 3. Total RNA from the left kidney was used for ischemia-reperfusion and that from the right kidney was used as a control. Each PCR product was quantified and standardized with the amount of PCR product for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) ($n = 6$). Values are mean ± SD. $^{**}P \leq 0.01$. *Cont* control, *IR* renal ischemia-reperfusion model



◀ **Fig. 6** Inhibition of endogenous prolactin secretion in Ab and control mice. **a** Bromocriptine (24 mg/kg/day) was orally administered in Ab and control mice for 8 days and serum prolactin concentrations were measured ($n = 5$). **b** Mouse serum cystatin C levels were measured 8 days after the bromocriptine administration ($n = 5$). **c** Sodium and potassium concentrations of 24-h urine were measured and standardized with the urine creatinine concentration 8 days after the bromocriptine administration ($n = 5$). **d** The sodium-to-potassium ratio of 24-h urine was decreased in accordance with diminished sodium concentration ($n = 5$). **e** G129R prolactin antagonist did not affect urine sodium and potassium excretion in control and Ab mice ($n = 4$). Values are mean ± SD. $*P < 0.05$. $**P < 0.01$. *Cont* control, *Ab* aortic banding, *Br* bromocriptine administration, *N.D.* not detected

have caused dilution of the urine sodium concentration. To investigate the effect of prolactin-PrlR interaction further, an excess amount of exogenous prolactin was administered to Ab and control mice. Exogenous prolactin administration did not have any effect on the serum cystatin C level (Fig. 7a), and the excess amount of systemic prolactin did not affect the urine sodium excretion; however, urine potassium excretion was significantly increased, specifically in Ab mice (Fig. 7b), without significantly influencing the urine sodium-to-potassium ratio (Fig. 7c).

Effect of prolactin in high-sodium-loaded model

Finally, to investigate the effect of prolactin in high-sodium-loaded conditions, control and Ab mice were fed a high-sodium diet containing 8 % sodium chloride. The sodium overload increased the amount of urine sodium excretion equally in both Ab and control mice, and the natriuretic response, as well as kaliuresis, was not blunted in the cardiac dysfunction mice (Fig. 8a). In control mice fed a high-sodium diet, the urine sodium excretion was reduced, albeit not significantly, by the inhibition of endogenous prolactin or the administration of exogenous prolactin (Fig. 8b). In contrast, urine sodium excretion was significantly decreased by the inhibition of endogenous prolactin excretion in Ab mice, without any influence on urine potassium excretion (Fig. 8c).

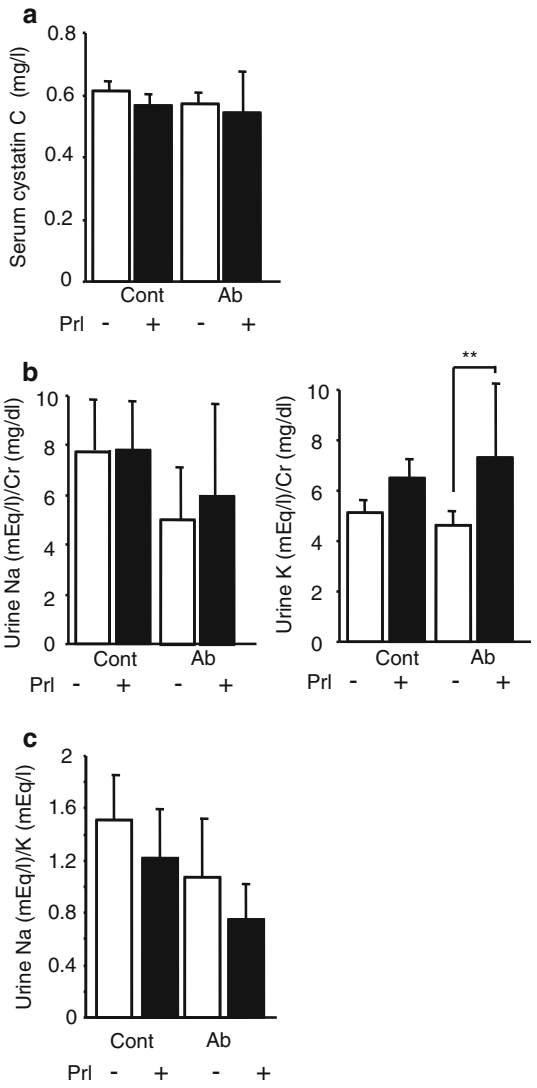


Fig. 7 Administration of exogenous prolactin in Ab and control mice. **a** Two hundred micrograms of prolactin was administered intraperitoneally in Ab and control mice and murine serum cystatin C levels were measured 12 h after the prolactin administration ($n = 5$). **b** Sodium and potassium concentrations of 24-h urine were measured and standardized with urine creatinine concentration after the prolactin administration ($n = 5$). **c** The sodium-to-potassium ratio of 24-h urine was not affected by exogenous prolactin administration. ($n = 5$). Values are mean ± SD. $**P < 0.01$. *Cont* control, *Ab* aortic banding, *Prl* prolactin administration

Discussion

In this study, we reported that the prolactin receptor was upregulated by Ab treatment. In addition to the natriuretic effect, kaliuresis was also accelerated by prolactin administration in this mouse model of cardiac dysfunction. We hypothesize that PrlR might be upregulated in the proximal tubular cells of the kidney under the effect of renal congestion and consequently induced chronic hypoxic challenge in Ab mice. In high-sodium-loaded conditions, the role of PrlR as a sodium transporter might be more dominant in Ab mice compared with other sodium transporters in the tubular cells. With respect to the PrlR upregulation induced by impaired cardiac function, this model would represent cardio-renal interaction, if not representing cardio-renal syndrome defined as impaired renal function secondary to cardiac failure.

Prolactin is a polypeptide hormone that is mainly synthesized in and secreted from the anterior pituitary gland, possessing many functions such as lactation, luteotrophic actions, reproductive and parental behavior, immune response, angiogenesis, and osmoregulation [19]. The

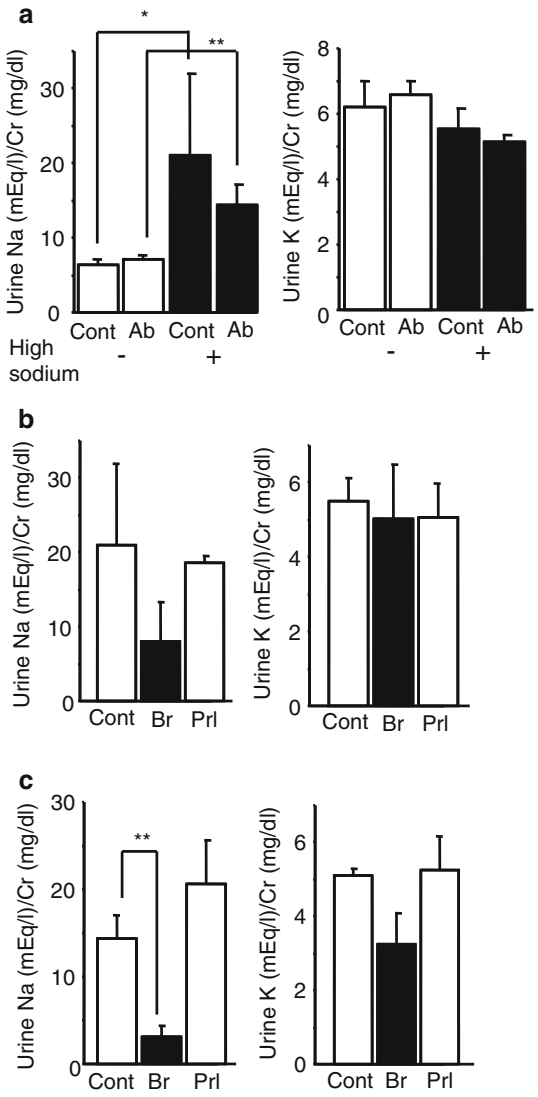


Fig. 8 Effect of high-sodium loading on sodium homeostasis. **a** Sodium and potassium concentrations of 24-h urine were measured and standardized with the urine creatinine concentration in control and Ab mice fed a high-sodium diet ($n = 4$). **b**, **c** Sodium and potassium concentrations of 24-h urine were measured and standardized with the urine creatinine concentration in control mice (**b**) or Ab mice (**c**) fed a high-sodium diet and treated with bromocriptine or prolactin ($n = 4$). Values are mean ± SD. $*P < 0.05$. $**P < 0.01$. *Cont* control, *Ab* aortic banding, *Br* bromocriptine was administered, *Prl* prolactin was administered

biological effects of prolactin are mediated by its interaction with PrlR, which is a member of the cytokine receptor superfamily, and this receptor is present in nearly all organs and tissues [13, 14]. Although the PrlR gene is unique in each species, alternative splicing generates different isoforms, which are identical in their extracellular domains but differ in the lengths and sequences of their intracellular domains. PrlR exists as seven recognized isoforms in humans, and in mice, one long and two short isoforms have been identified as proteins, and the short forms only differ by a few amino acids in the C-terminal part of the cytoplasmic tail [13]. Short-form PrlRs are reported to have independent biological activity and to have a signaling pathway distinct from that of the long-form PrlR. In addition, different PrlR isoforms could generate heterodimers, which are an inactive complex [13, 20]. From this perspective, short types of PrlR would possess some opposite effects to PrlR-1 or could behave in a dominant-negative manner. The long isoform of PrlR has been described as being predominant in the renal cortex, whereas the short isoforms have been reported to be predominant especially in the proximal convoluted tubular cells [10]. In this Ab model, PrlR gene expression of all three variants was elevated and the localization was almost limited to the proximal tubular cells in the deep inner cortex and the outer stripe of the outer medulla. In the present study, interaction among these three PrlR isoforms in proximal tubular cells was not clarified and should be investigated in further study.

Prolactin induces dose-dependent inhibition of proximal tubular Na^+/K^+-ATPase activity, resulting in the decreased reabsorption and facilitation of urinary excretion of sodium along with kaliuresis without affecting the glomerular filtration rate or the arterial blood pressure [9–11]. Our data about the natriuretic effect of prolactin verified by the inhibition of endogenous systemic prolactin (Fig. 6) were in accordance with previous reports; however, this natriuretic effect was also observed in control mice, suggesting that this natriuresis should have been induced by PrlR in proximal convoluted tubular cells or the ascending limb of Henle's loop as they possess a relatively large amount of Na^+/K^+-ATPase [21] and were not affected by Ab. In contrast, kaliuresis was accelerated by exogenous prolactin administration specifically in Ab mice, without significantly affecting the sodium-to-potassium ratio (Fig. 7), suggesting that PrlR in the proximal tubular cells of the pars recta in the deep inner cortex and the outer stripe of the outer medulla might contain a different mechanism of kaliuresis other than the inhibition of Na^+/K^+-ATPase activity, although this kaliuretic effect was not affected by a diminished systemic prolactin level. These contradictory findings could be explained by the hypothesis that kaliuresis would already be compensated for by other transporters and the prolactin-PrlR interaction would not function in the physiological state, but the upregulated PrlR could respond to an excess level of systemic prolactin and could contribute to additional kaliuresis in Ab mice.

On the other hand, inhibition of endogenous prolactin significantly reduced urinary sodium excretion in Ab mice, but not significantly in control mice, loaded with high-sodium. This specific inhibition of natriuresis would indicate the predominance of PrlR upregulated with decreased cardiac function upon natriuresis in the high-sodium-

loaded condition. Prolactin antagonist G129R was also used to validate the specificity of the bromocriptine experiment, but its effect on urinary sodium and potassium excretion was not observed. The properties of G129R-prolactin were reported to depend on assay sensitivity and vary from the weak agonist, partial agonist, to full antagonist [22]. We conjecture that this ineffectiveness would be due to the variation of G129R-prolactin properties.

In the present study, the upregulation of PrlR expression induced by Ab was mainly observed in the proximal tubular cells of the pars recta in the deep inner cortex and the outer stripe of the outer medulla, which correspond to the S3 segment of rat proximal tubule. One of the characteristic features in the outer medulla of normally perfused kidney is the low oxygen tension as described in the Results section. Renal congestion is reported to be accompanied by dilated peritubular capillaries [23], which indicate hypoperfusion and stagnation of blood flow in peritubular capillaries [24]. In addition to hypoperfusion caused by decreased cardiac function, renal perfusion pressure would also be decreased, resulting in chronic hypoxia. This anatomical susceptibility leads us to surmise that PrlR expression might have been induced by chronic hypoxia due to renal congestion. We also adopted an IR model as an acute hypoxia model, but mRNA expression of PrlR was rather reduced by IR (Fig. 5). We surmise that this acute and drastic IR caused cellular death, especially in proximal tubular cells expressing PrlR, and the relative expression of PrlR mRNA in the whole kidney was reduced. We also hypothesize that mild and chronic hypoxia would be necessary to induce PrlR upregulation in the proximal tubular cells. To the best of our knowledge, no direct evidence of hypoxic responsive elements on the regulatory domain of the PrlR gene has been reported; however, transcriptional regulation of the PrlR gene is accomplished by three different tissue-specific promoter regions. Promoter I is specific for the gonads and promoter II for the liver; promoter III, which is present in both gonadal and nongonadal tissue [15], binds CCAAT-box/enhancer-binding protein β (C/EBPβ) and Sp1 [25]. Sp1s are reported to interact with hypoxia-inducible factor (HIF) 1α [26, 27], which is stabilized in acute and chronic hypoxic conditions [28]. It is possible that the promoter III activity of PrlR might be regulated by the HIF1α–Sp1 interaction.

Considering these findings, we hypothesize that PrlR gene expression would be induced by chronic hypoxic challenge to the proximal tubular cells, specifically in this segment of the kidney in Ab mice, probably because of decreased renal perfusion and renal congestion. To the best of our knowledge, this study is the first to discover the upregulating condition of PrlR in the renal tubular cells. We consider that the upregulation of PrlR in the proximal tubular cells in patients with cardiac failure warrants further investigation.

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Conflict of interest All the authors have declared no competing interests.

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