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Review

Physiological roles of AQP7 in the kidney: Lessons from AQP7 knockout mice

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Abstract

The aquaporin7 (AQP7) water channel is known to be a member of the aquaglyceroporins, which allow the rapid transport of glycerol and water. AQP7 is abundantly present at the apical membrane of the proximal straight tubules in the kidney. In this paper, we review the physiological functions of AQP7 in the kidney. To investigate this, we generated AQP7 knockout mice. The water permeability of the proximal straight tubule brush border membrane measured by the stopped flow method was reduced in AQP7 knockout mice compared to wild-type mice (AQP7, $18.0\pm0.4\times10^{-3}$ cm/s vs. wild-type, $20.0\pm0.3\times10^{-3}$ cm/s). Although AQP7 solo knockout mice did not show a urinary concentrating defect, AQP1/AQP7 double knockout mice showed reduced urinary concentrating ability compared to AQP1 solo knockout mice, indicating that the contribution of AQP7 to water reabsorption in the proximal straight tubules is physiologically substantial. On the other hand, AQP7 knockout mice showed marked glycerol in their urine (AQP7, 1.7 ± 0.34 mg/ml vs. wild-type, 0.005 ± 0.002 mg/ml). This finding identified a novel pathway of glycerol reabsorption that occurs in the proximal straight tubules. In two mouse models of proximal straight tubule injury, the cisplatin-induced acute renal failure (ARF) model and the ischemic–reperfusion ARF model, an increase of urine glycerol was observed (pre-treatment, 0.007 ± 0.005 mg/ml; cisplatin, 0.063 ± 0.043 mg/ml; ischemia, 0.076 ± 0.02 mg/ml), suggesting that urine glycerol could be used as a new biomarker for detecting proximal straight tubule injury.

Keywords: Aquaporin7; Glycerol; Water; Urea; Kidney; Proximal straight tubules

Contents

1. Introduction

Aquaporins (AQPs) are membrane proteins that facilitate water transport across the cell membrane [1,2]. Until now, at least 13 AQPs have been identified in mammals [3]. The AQPs that allow rapid transport of glycerol, as well as water, are

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classified as belonging to the aquaglyceroporin family [4]. AQP7 is an aquaglyceroporin that is abundantly expressed in the kidney, adipocytes, and testis [5,6]. In addition, AQP7 is also known to facilitate urea and arsenite transport [5,7]. In the kidney, AQP7 is expressed on the apical membrane of the proximal straight tubules (S3 segment) [5], where AQP1 is also expressed [8]. Studies of AQP1 knockout mice have demonstrated that AQP1 plays a major role in proximal tubular water transport [9,10]. In fact, AQP1 knockout mice show polyuria due to both defective water reabsorption in the proximal tubules and a dysfunctional countercurrent mechanism in the inner medulla [11]. Other renal AQPs, such as AQP2, AQP3, and AQP4, are expressed in the collecting ducts, and are directly involved in water reabsorption to generate the final concentrated urine [12–14]. Among them, AQP3, another aquaglyceroporin expressed in kidney, localized to basolateral membrane of the collecting duct cells.

In this paper, we review the physiological functions of AQP7 in the kidney, depending on the report we recently published [15]. Although the contribution of AQP7 to the water permeability of the brush border membrane of proximal straight tubules was identified to be small compared with that of AQP1, we identified a new pathway of glycerol reabsorption that is important for preventing glycerol from being excreted into urine. Based on this finding, we investigated the possibility that the measurement of urine glycerol could be a new biomarker for the detection of proximal straight tubule injury in vivo.

2. Water permeability in the brush border membrane vesicles obtained from the outer medulla of AQP7 knockout mice

AQP7 knockout mouse were generated as previously reported [15]. To investigate whether AQP7 contributes to the water permeability of the brush border membranes in the proximal straight tubules, we measured the water permeability of the brush border membrane vesicles derived from the outer medulla of the kidney. Water permeability was measured over the time course of the ninety degree scattered light intensity in response to a rapidly imposed osmotic gradient (Fig. 1B). The water permeability of the vesicles derived from the outer medulla of AQP7 knockout mice ($18.0\pm0.4\times10^{-3}$ cm/s, $n=4$) was slightly but significantly lower than that of wild-type mice ($20.0\pm0.3\times10^{-3}$ cm/s, $n=4$, $P<0.001$). These results indicate that AQP7 contributes partially to the water permeability of the proximal straight tubules. To compare the contribution of AQP7 with that of AQP1, we also checked the water permeability of the brush border membrane vesicles taken from AQP1/AQP7 double knockout mice. In the AQP1/AQP7 knockout mice, the water permeability of the vesicles was decreased more than in the AQP1 knockout mice. AQP1/AQP7 double knockout mice showed significantly lower water permeability, compared to AQP1 knockout mice (AQP1, $3.3\pm0.11\times10^{-3}$ cm/s vs. AQP1/AQP7, $2.4\pm0.14\times10^{-3}$ cm/s, $n=5$, $P<0.001$). Based on these results and previously published data [9], the estimated contribution of AQP7 to the water permeability in the proximal

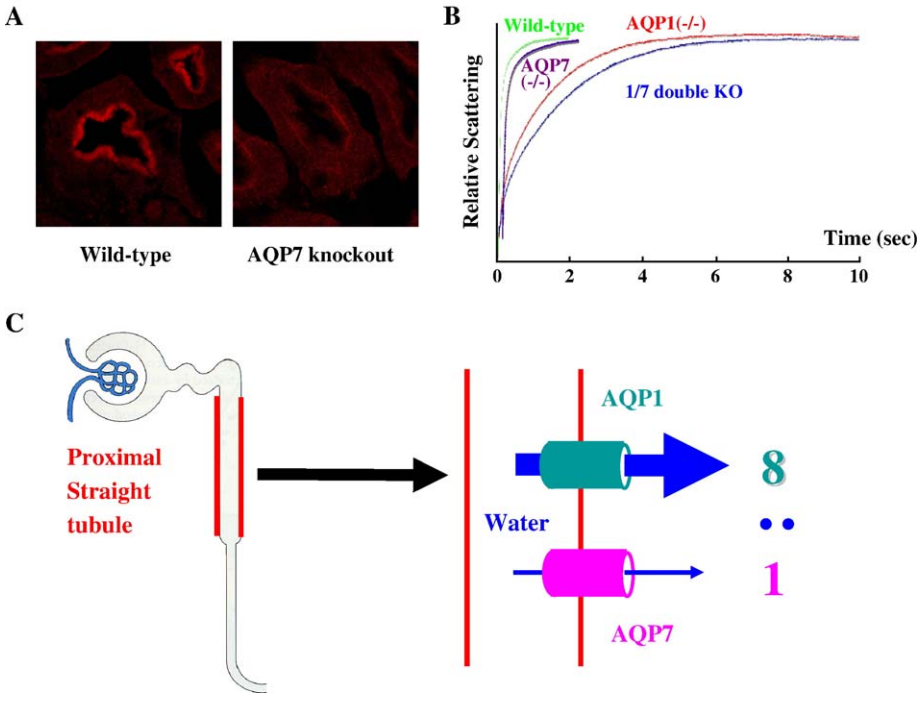

Fig. 1. (A) Immunohistochemistry of AQP7 in the S3 segment of the proximal tubules of wild-type and AQP7 knockout mice. (B) The time course of the light scattering intensity. Increase in light scattering intensity was slower in vesicles from AQP7 knockout mice than in vesicles from wild-type knockout mice. Similarly, the increase of scattering intensity of vesicles from AQP1/AQP7 double knockout mice was also slower, compared to those from AQP1 knockout mice, indicating the contribution of AQP7 to water permeability is substantial. (C) Schema of the contribution of AQP1 and AQP7 to water permeability in the proximal straight tubules. The estimated contribution of AQP7 to water permeability in the proximal straight tubules is one eighth that of AQP1.

straight tubules was calculated to be one eighth that of AQP1 (Fig. 1C).

3. Urinary concentrating defect of AQP1/AQP7 double knockout mice

As expected, given the small decrease in water permeability seen in the AQP7 solo knockout mice, there was no difference in the urine concentrating ability between AQP7 solo knockout and wild-type mice even with dehydration. Thus, we compared the urinary concentrating ability of AQP1/AQP7 double knockout mice with that of AQP1 knockout mice because we suspected that the small contribution of AQP7 to water permeability would be more readily detected in a situation where the major water pathway via AQP1 was deleted. Even under normal conditions, AQP1/AQP7 double knockout mice showed a significantly increased urine volume compared to AQP1 solo knockout mice (AQP1/AQP7, 7.3 ±0.46 ml vs. AQP1, 5.7±0.31 ml, $n=6$, $P<0.03$). In addition, significantly greater weight loss after dehydration was observed in AQP1/AQP7 double knockout mice, compared to AQP1 knockout mice (AQP1/AQP7, 31.0%±0.54% vs. AQP1, 28.2%±0.46%, $n=6$, $P<0.01$). Similarly, urine osmolality in AQP1/AQP7 double knockout mice was significantly lower, compared to AQP1 knockout mice, both before water deprivation (AQP1/AQP7, 597±18 mOsm/kg.H_2O vs. AQP1, 715±39 mOsm/kg.H_2O, $P<0.03$) and after water deprivation (AQP1/AQP7, 626±65 mOsm/kg.H_2O vs. AQP1, 810± 16 mOsm/kg.H_2O, $P<0.03$, $n=6$).

Taking these results, we found that AQP7 made a small contribution to the water permeability of the brush border membranes in the proximal straight tubules. As expected given the restricted distribution of AQP7 compared to AQP1, the role of AQP7 in water reabsorption in the proximal tubules is minor, compared to that of AQP1. Thus, we generated AQP1/AQP7 double knockout mice, since we reasoned that the slight contribution of AQP7 to water permeability would be more easily detected when the major water transport pathway through AQP1 was eliminated. In fact, compared to AQP1 solo knockout mice, AQP1/AQP7 double knockout mice showed a significantly increased urine volume that was accompanied by a proportional decrease in urine osmolality. Like AQP7 solo knockout mice, AQP1/AQP7 double knockout mice excreted more glycerol in their urine. However, the urine glycerol level in AQP1/AQP7 double knockout mice was only 2.4 mM, and thus had a small effect on total urine osmolarity (~600 mOsm/kg.H_2O). In addition, urea excretion into the urine and urea accumulation in the papilla were not altered in AQP7 knockout mice. Thus, the increased urine volume in AQP1/AQP7 double knockout mice is most likely caused by an increased delivery of water to the distal nephrons.

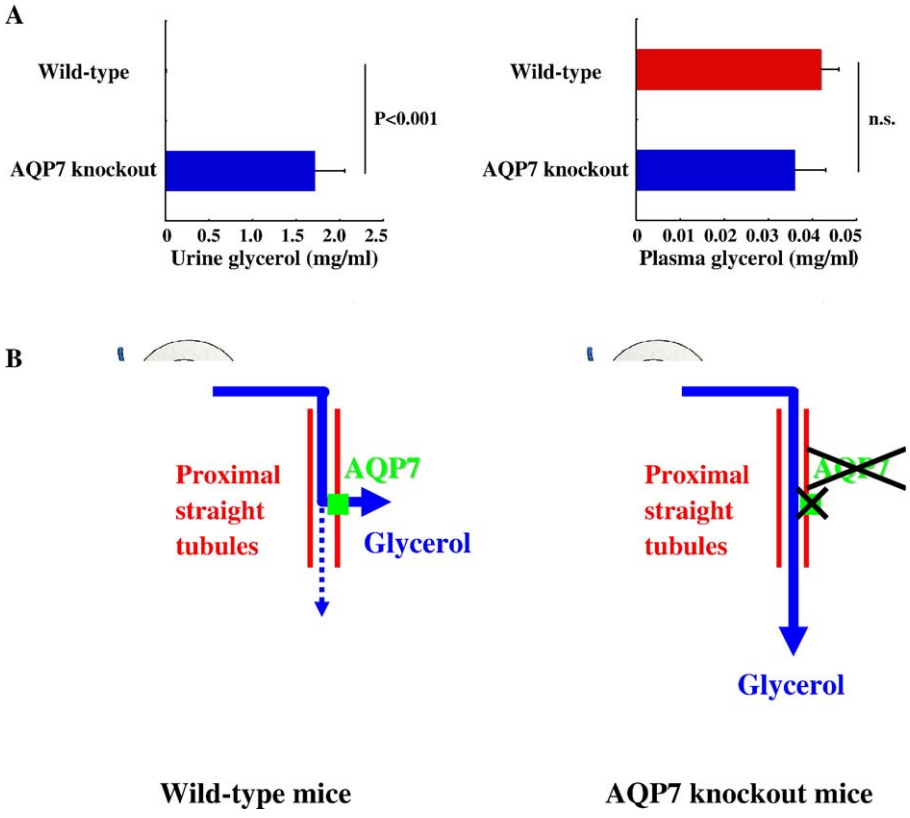

Fig. 2. (A) Urine and plasma glycerol levels in AQP7 knockout and wild-type mice. (B) Schema of glycerol reabsorption in the proximal straight tubules mediated by AQP7. Although wild-type mice did not have glycerol in their urine, AQP7 knockout mice did have glyceroluria, indicating that glycerol is reabsorbed mainly in the proximal straight tubules through AQP7.

Also, we confirmed that there was no compensatory increase in AQP1 and AQP7 expression in AQP7 and AQP1 knockout mice, respectively, using western blotting and quantitative RT-PCR. In addition, AQP7 in adypocyte is known to be increased in fasting condition, and to be suppressed by refeeding [6]. Taken together, the amounts of AQP7 expression might be controlled by the glycerol metabolism, not by water condition in the body.

4. Urea transport in the kidneys of AQP7 knockout mice

To investigate the involvement of AQP7 in kidney urea transport, we first measured the plasma and urine urea levels in AQP7 knockout mice. It has been proposed that the urea that is reabsorbed from the thick ascending limbs enters the neighboring proximal straight tubules; thus, complete recycling occurs between the descending limbs and ascending limbs of the loop, with AQP7 acting as a component of the pathway for urea in the proximal straight tubules [16,17]. Since this urea recycling pathway may be the mechanism by which a high urea content is maintained in the inner medulla, we therefore measured the urea content in the papilla of AQP7 knockout mice. The plasma urea level in AQP7 knockout mice was not different from that of wild-type mice (AQP7, 31.5±5.3 mg/dl vs. wild-type, 30.7±2.3 mg/dl, $n=4$). Similarly, the ratio of urine urea to urine creatinine level in AQP7 knockout mice was not significantly different from that in wild-type mice (AQP7, 22±9 vs. wild-type 28±10, $n=4$). There was also no difference between AQP7 knockout and wild-type mice in the urea content of the papilla taken under normal conditions (data not shown). To detect small differences in urea levels, the same experiments were performed using mice on a low protein diet (4% protein), which limits the urea supply to the kidney. AQP7 knockout mice fed a low protein diet for 20 days did not show an impairment in urea accumulation in the papilla even with dehydration (AQP7, 24.7±4.2 mg/g papilla wet weight vs. wild-type, 22.0±4.3 mg/g, $n=4$). They also did not show a urine concentrating defect with a low protein diet and dehydration.

5. In vivo reabsorption of glycerol in the proximal straight tubules mediated by AQP7

To investigate whether AQP7 has an important role in the transport of glycerol in the kidney, we measured the serum and urine glycerol levels of AQP7 knockout mice. The serum glycerol in AQP7 knockout mice was not significantly different from that of wild-type mice (AQP7, 0.036 ±0.007 mg/ml vs. wild-type, 0.042±0.004 mg/ml, $n=10$) (Fig. 2A). However, the urine glycerol level in AQP7 knockout mice was much higher than in wild-type mice (AQP7, 1.7±0.34 mg/ml vs. wild-type, 0.005±0.002 mg/ml, $n=10$, $P<0.0001$) (Fig. 2A). These results indicate that glycerol reabsorption is mediated by AQP7 in the proximal straight tubules, and that there might be no other glycerol reabsorbing system that can compensate for the deletion of the AQP7 protein in the more distal nephron segment (Fig. 2B).

These findings identified a new glycerol reabsorption pathway mediated by AQP7. AQP7 knockout mice had glyceroluria, though their plasma glycerol levels were not increased. According to the review by Lin, urine glycerol in mammals is completely reabsorbed in the nephron [18], but the reabsorption pathway had not yet been identified. This is the first study to identify the glycerol reabsorption pathway in the kidney. Our AQP7 knockout mice did not show a significant difference in serum glycerol concentration. This indicates that glyceroluria seen in AQP7 knockout mice was not due to an increase in plasma glycerol levels, but was due to the reduction or the elimination of the pathway for glycerol reabsorption in the kidneys. At present, it is not clear whether AQP7 is the only glycerol reabsorption pathway that exists along the nephron, since, for example, AQP3, another aquaglyceroporin, is localized in the collecting ducts. In fact, the fractional excretion of glycerol to creatinine (FE glycerol) in AQP7 knockout mice was less than 100% (~50%). This suggests that other minor glycerol reabsorption pathways might exist, or that glycerol could be metabolized along the nephron. Nevertheless, the fact remains that AQP7 effectively prevents glycerol from being excreted into the urine and that the other glycerol reabsorption pathway(s), if any, cannot completely compensate for the absence of AQP7. The glycerol that is reabsorbed in the proximal straight tubules could also be metabolized in situ, since the kidney is an organ that is rich in glycerol kinase. Glycerol must be converted to *sn*-glycerol 3-phosphate by glycerol kinase before it is utilized in other metabolic pathways, such as gluconeogenesis and in the generation of glyceride lipids. Thus, AQP7 knockout mice may have a defect in some stage of glycerol metabolism located within the kidney.

6. Glycerol leakage in the urine of mouse models of proximal straight tubule injury

The finding of this new glycerol reabsorbing pathway, which is restricted to the proximal straight tubules, suggested the hypothesis that glyceroluria could be a new biomarker for proximal straight tubular injury. To investigate whether glycerol could leak into urine due to proximal tubule dysfunction, we measured urine glycerol levels in two mouse models of proximal straight tubular injury: the cisplatin-induced acute renal failure (ARF) model and the ischemic–reperfusion ARF model. It has been reported that cells of the proximal straight tubule are especially sensitive to cisplatin and ischemia and undergo extensive necrosis when challenged [19–22]. In both of these models of S3 injury, the mice showed significantly higher urine glycerol levels compared to pre-treatment mice (pre-treatment, 0.007±0.005 mg/ml; cisplatin, 0.063±0.043 mg/ml; ischemia, 0.076±0.02 mg/ml; control, 0.010±0.007 mg/ml). We also checked the urine glycerol concentrations of control mice that had been starved for 24 h because these mice are known to show higher plasma glycerol levels than mice under normal conditions [6,23]. The control mice showed significantly lower urine glycerol concentrations compared to S3 injury model mice, although their plasma glycerol levels were not significantly different from those in S3 injury model mice (pre-treatment,

0.039±0.013 mg/ml; cisplatin, 0.061±0.035 mg/ml; ischemia, 0.055±0.006 mg/ml; control, 0.071±0.013 mg/ml).

As we expected, the mice of S3 injury models showed higher urine glycerol concentrations than did non-treated or fasted control mice. Plasma glycerol levels could not explain these changes, indicating that the urine leakage of glycerol was due to the failure of glycerol reabsorption. Therefore, the measurement of urine glycerol levels could be used as a new method for detecting proximal straight tubule injury. This new method could be more sensitive that the currently used conventional markers.

Our analysis on renal phenotype of AQP7 knockout mice clearly identified some of the physiological roles that AQP7 has in the kidney. Though AQP7 plays a minor role in water transport, AQP7 constitutes a major glycerol reabsorbing pathway in the kidney.

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