The Journal of Physiology

Many kinases for controlling the water channel aquaporin-2

Enno Klussmann and Andrii Kharin
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The following individual(s) involved in review of this submission have agreed to reveal their identity: Lene N. Nejsum (Referee #1)

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Senior Editor: Laura Bennet

Reviewing Editor: Helle Praetorius

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Dr Klussmann,

Re: JP-SR-2023-284100 "Many kinases for controlling the water channel aquaporin-2" by Enno Klussmann and Andrii Kharin

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert referees and we are pleased to tell you that it is potentially acceptable for publication following satisfactory major revision.

Please advise your co-authors of this decision as soon as possible.

The referee reports are copied at the end of this email.

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We look forward to receiving your revised submission.

If you have any queries, please reply to this email and we will be pleased to advise.

Yours sincerely,

Professor Laura Bennet
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EDITOR COMMENTS

Reviewing Editor:

Thank you for accepting the commission and for submitting your manuscript to the Journal of Physiology. Your manuscript has now been in expert review, and as you can see below, the reviewers have been very positive about the influence of your work. However, the reviewers alert to the manuscript very quickly, focusing on the topic at hand and that it may appeal to a broader audience if the introduction of kinase regulation of AQP2-mediated epithelial transport into a broader perspective of renal function. In addition, there are several more specific comments to consider, including changes in figures and graphical abstracts, where the notification of the apical and basolateral membrane is reversed.

Senior Editor:

Thank you for your submission. While the reviewers have found merit in your submission, they have highlighted several areas that should be addressed in you revision.

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For readers outside of the kidneys field, the introduction would be easier to read and appreciate with a schematic of the kidney with the localization of the different AQPs.

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A figure showing the dynamic phosphorylation of the four C-terminal sites following cAMP increase would be insightful.

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1. Page 3, seventh line from the end: Through AQP2, AQP3, and AQP4, the authors hypothesized that AVP plays a role in water absorption in the collecting duct. It is unclear, however, whether AQP4 is regulated by AVP. The levels of AQP1 and AQP4 in rats were unaffected by a prolonged infusion of AVP, according to a previous study (Am J Physiol 271: F414-F422, 1996). Did the authors confirm that, in addition to its effects on AQP2 and AQP3, AVP also regulates AQP4?

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8. Page 8 - 10, in the section of diabetes insipidus: please add the role of "glycogen synthase kinase 3" in lithium-induced NDI.

_____

END OF COMMENTS

Confidential Review **29-Mar-2023**

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Berlin, 9.06.2023

JP-SR-2023-284100: Many kinases for controlling the water channel aquaporin-2

Dear Prof. Bennet

We would like to thank you, the Reviewing Editor and the Referees for the helpful comments on our manuscript. We have addressed the points raised and hope that the manuscript is now suitable for publication in The Journal of Physiology.

Please find below a point-by-point response.

Yours sincerely,

Enno Klussmann

EDITOR COMMENTS

Reviewing Editor:
Thank you for accepting the commission and for submitting your manuscript to the Journal of Physiology. Your manuscript has now been in expert review, and as you can see below, the reviewers have been very positive about the influence of your work. However, the reviewers alert to the manuscript very quickly, focusing on the topic at hand and that it may appeal to a broader audience if the introduction of kinase regulation of AQP2-mediated epithelial transport into a broader perspective of renal function. In addition, there are several more specific comments to consider, including changes in figures and graphical abstracts, where the notification of the apical and basolateral membrane is reversed.

Response
We have broadened the introduction. We have, for example, included a paragraph to generally emphasize the relevance of kinases and protein phosphorylation for signal transduction and appropriate cellular responses. We also included new figures. The graphical abstract was corrected and the further comments by the reviewers addressed.

Senior Editor:
Thank you for your submission. While the reviewers have found merit in your submission, they have highlighted several areas that should be addressed in you revision.

Response
Hopefully, we have addressed all concerns adequately.

REFEREE COMMENTS

Referee #1:

Comment to the Author

The manuscript by Kharin and Klussmann addresses the complex regulation of the renal water channel AQP2 by various kinases. The review is timely and addresses an important area of renal physiology.

Major comments:

Comment to the Author

For readers outside of the kidneys field, the introduction would be easier to read and appreciate with a schematic of the kidney with the localization of the different AQPs.

Response

We have extended the introduction and prepared a new figure depicting schematically a nephron with the AQP expression (Figure 1). Also, we extended the introduction by introducing kinases and protein phosphorylation more generally.

Comment to the Author

AQP structure is not unique for AQP2 and should be presented with the AQP introduction. The introduction assumes a previous knowledge from the reader regarding AQPs and should be expanded and more detailed.

Response

We have included a paragraph to more generally introduce AQPs. In addition, a new figure 2 was included.

Comment to the Author

Regarding the PKA KO cell study it is essential to mention the significant reduction in AQP2 expression levels and what implications this has for phosphorylation.

Response

The sentence has been revised:

In PKA-knockout mpkCCD mouse cells, the AQP2 expression was lost, underpinning the relevance of PKA in maintaining AQP2 expression. When AQP2 expression was reconstituted through transfection, it was still phosphorylated in the PKA-knockout cells, indicating an involvement of kinases other than PKA in AVP-induced AQP2 phosphorylation of S256. The relevant kinase/s has/have not been identified (Isobe et al., 2017; Datta et al., 2020). Protein kinase G (PKG) might be a candidate. Its consensus site (R/K2-3-X-S/T) is

similar to that of PKA and PKG phosphorylated S256 of AQP2 in LLC-PK1 epithelial cells (Bouley *et al.*, 2000) and primary cultured inner medullary collecting duct cells (Klokkers *et al.*, 2009).

Comment to the Author

A figure showing the dynamic phosphorylation of the four C-terminal sites following cAMP increase would be insightful.

Response

The sequence of the C terminus of AQP2 with phosphorylation sites is shown in a new figure 2B and the phosphorylations and kinase phosphorylating the sites are shown in Figures 3A and B in the revised version of the manuscript.

Comment to the Author

It is still unclear if F-actin is a barrier to all AQP2 vesicles or only a subpopulation.

Response

We have revised the paragraph entitled **Kinase network downstream of V2R and links to F-actin**. This point has been addressed in this paragraph in the context of AURKA and the F-actin cytoskeleton. We apologise not to have included this thought in the original submission. It is also included in the legend to figure 3.

The phosphorylation of AQP2 at S256 increased the interaction of AQP2-bearing vesicles with F-actin in cytosolic domains, and F-actin was considered to provide the tracks for AQP2 trafficking to the plasma membrane in response to AVP (Nedvetsky *et al.*, 2007; Sasaki *et al.*, 2014; Holst *et al.*, 2021). Therefore, the inhibitory effect of Aurora-A inhibitor I on the AVP-induced redistribution of AQP2 may be explained by the removal F-actin stress fibre tracks for trafficking. ROCK not only catalyses the AQP2 phosphorylation of S269 (see above) but also induces F-actin formation through phosphorylation of cytoskeletal elements. Inhibition of ROCK or its upstream activator RhoA caused an AVP-independent F-actin depolymerisation and plasma membrane accumulation of AQP2 in primary IMCD and rabbit collecting duct cells. Thus, peripheral F-actin may serve as a physical barrier preventing AQP2-bearing vesicles from reaching the plasma membrane under resting conditions (Klussmann *et al.*, 2001; Tamma *et al.*, 2001; Nedvetsky *et al.*, 2007; Sasaki *et al.*, 2014). The barrier function would explain the alisertib-caused inhibition of the redistribution of AQP2 to the plasma membrane. However, not all AQP2-bearing vesicles reside below or in front of the peripheral F-actin layer, a sub-pool of AQP2-bearing vesicles resides between the F-actin layer and the plasma membrane and may constitute the pool readily available for fusion with the plasma membrane (Holst *et al.*, 2021).

Comment to the Author

Vasopressin is mentioned, however, other extracellular stimuli that initiate phosphorylation events are also relevant to include.

Response

We feel that an extensive discussion of further stimuli would rather be an extra-topic and beyond the scope of this manuscript. However, in our conclusion section, we refer to a recent review by Dennis Brown who discussed many different stimuli controlling AQP2 (Cheung *et al.*, 2020).

All the influences are effective through stimulation of different plasma membrane receptors, which all initiate different downstream signalling cascades. Renal principal cells express amongst others prostaglandin, angiotensin II, EGF and Ca^{2+}-sensing receptors, exemplifying the range of stimuli integrated by the signalling systems of principal cells. Downstream signalling in response to most, if not all, stimuli involves activation of kinases and crosstalk between kinases (Cheung *et al.*, 2020). Such pathways may all contain potential targets, not just kinases, for the treatment of diabetes insipidus.

Comment to the Author

The studies with AMPK/metformin are only mentioned in one sentence and referencing the 2016 papers. This needs to be elaborated with the subsequent studies targeting this pathway in NDI models.

Response

We have added subsequent studies:

Activation of AMPK with metformin did also induce the plasma membrane localisation of AQP2 in mice (Efe *et al.*, 2016; Klein *et al.*, 2016). Metformin and another compound, NDI-5033, which activates AMPK improved the urine concentrating ability in rat and mouse models of acquired (tolvaptan- or lithium-induced) and congenital (V2R knockout) NDI. Thus, targeting AMPK seems a promising therapeutic approach for the treatment of diabetes insipidus (Klein et al., 2021; Tas & Sancak, 2021).

Comment to the Author

The figure seems to mainly summarize findings from the author's laboratory - the figure should be expanded to summarize the current knowledge described in the manuscript.

Response

We have split the figure in part A and B, whereby A more generally depicts AVP-induced AQP2 trafficking and B focuses more on PKA compartmentalisation with relevance to AQP2. Both parts summarise work from many laboratories. However, including all kinases mentioned throughout the text, especially in the light of the fact that their roles are often not yet clear, would make a figure too crowded. Therefore, we would prefer to show only selected components of signalling cascades.

Comment to the Author

Page 3: the authors wrote "Ion permeability of AQP6 increased at acidic pH of 4.0, which is in...". both water and ion permeability of AQP6 are increased at pH lower than 5.5

Response

This is corrected.

Comment to the Author

Page 3: the author wrote "It contains an Asp-Ser-Cys (NPC) box instead of a conserved Asp-Ser-Ala box shared by other aquaporins". In fact AQP7 has NAA and NPS motifs.

Response

This sentence was now included into the more general introduction of AQP2. We also included as Figure 2A an alignment of all mammalian AQPs highlighting those motifs.

Referee #2:

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The present study provides a comprehensive overview of various kinases that regulate the water channel AQP2. The manuscript was well written, however, I have several comments:

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1. Page 3, seventh line from the end: Through AQP2, AQP3, and AQP4, the authors hypothesized that AVP plays a role in water absorption in the collecting duct. It is unclear, however, whether AQP4 is regulated by AVP. The levels of AQP1 and AQP4 in rats were unaffected by a prolonged infusion of AVP, according to a previous study (Am J Physiol 271: F414-F422, 1996). Did the authors confirm that, in addition to its effects on AQP2 and AQP3, AVP also regulates AQP4?

Response

The sentence is revised:
AQP2, AQP3 and AQP4 are expressed in collecting duct principal cells. Through the principal cells, around 10 % of the water from primary urine is reabsorbed. This water reabsorption is controlled by the peptide hormone, AVP (Vukicevic *et al.*, 2016; Centrone *et al.*, 2022; Klussmann, 2023).
Since the focus of the manuscript is on AQP2 and its phosphorylation and due the word limit, a discussion on AQP3 and AQP4 expression was omitted.

Comment to the Author

2. Page 3, line 10: Is AQP5 expressed in the collecting duct of the kidney? Despite the fact that Procino et al. (Cell Physiol Biochem 28: 683-692, 2011) reported that AQP5 is expressed in the renal cortex at the apical membrane of pendrin-positive type B intercalated cells, it is known that AQP5 is barely detectable by Northern blot or immunoblot in the normal mouse and rat kidney.

Response

AQP5 expression is deleted in the revised version.

Comment to the Author

3. Yasui M (PMID: 10647010) should be cited regarding the ion permeability of AQP6 on page 3, line 12.

Response

The reference is now included.

Comment to the Author

4. Page 3, the last line and graphic abstract: vasopressin V2 receptors are localized on the basolateral surface of the collecting duct principal cells. However, the graphic abstract shows that V2R is expressed in the apical membrane, and AQP2 is expressed in the basolateral membrane. This should be corrected.

Response

Thank you for noticing. That is corrected now.

Comment to the Author

5. Page 4 and Fig 1: AC6 is the principal AC mediating these responses, as only AC6-deficient mice have urine concentration defects (Physiol Rep 2: e00277, 2014; J Am Soc Nephrol 21: 2059-2068, 2010; Am J Physiol Renal Physiol 302: F78-F84, 2012). Therefore, it would be preferable to delete AC3 and AC5 from Figure 1.

Response

We have deleted AC 1 and 3 from the figure – now figure 3A.

Comment to the Author

6. Page 4 - 5: The authors described the phosphorylation of AQP2 and the kinases involved on pages 4 and 5.

Readers may want to know more about

1) the role of AQP2 phosphorylation at the various serine sites in water reabsorption;

2) the protein-protein interaction of phosphorylated AQP2 with other proteins; and

3) the changes in AQP2 phosphorylation and kinase expression in disorders of water balance. Please provide more information if possible.

Response

We have tried to address these points to some degree within the framework of the allowed word count.

Ad 1)

In the paragraph **Phosphorylation of AQP2** we discuss the influence of the different phosphorylations of AQP2 on its localisation and have extended the discussion by including proteomics and phosphoproteomics studies. The last sentence of the first paragraph under this title relates phosphorylation to water reabsorption:

Such analyses identified four phosphorylation sites (Figures 2B), serine (S) 256, S261, S264 and S269, in the C terminus of AQP2 (Hoffert *et al.*, 2006) whose phosphorylation status changes upon AVP stimulation and which play key roles in the regulation of the AQP2 localisation and thus water reabsorption.

Ad 2) The role of protein-protein interactions are discussed mainly in the paragraphs **Location of kinases phosphorylating AQP2** and **Conclusions**.

Ad 3)
We have included some more information in the paragraph **Diabetes insipidus – potential treatment by targeting the kinase network?**
Targeting kinases of the network controlling AQP2 for modulating the localisation of AQP2 for therapeutic purposes would be attractive. Proteomics, phosphoproteomics and transcriptomics approaches have been used for analysis of animal models of diabetes insipidus (Mak *et al.*, 2023). Detected changes in signalling pathways that involve kinases require validation of potential targets.
There examples for interference with the kinase network…

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7. Page 8, 1st line: the authors described that both CDK18 and STUB1 control the plasma membrane insertion of AQP2. In a previous study (Cells 2020, 9(3), 673), the author's team demonstrated that CDK18 phosphorylated S261-AQP2, and CDK18 knockdown was associated with a decrease in AQP2 polyubiquitination. Since K270 of AQP2 is ubiquitinated, are there any effects of the decreased poly-ubiquitination of AQP2 at K270 under the condition of CDK knockdown on the phosphorylation of AQP2 at serine 269?

Response
This is an interesting point. In the context of our CDK18/STUB1 studies we did not analyse the status of the S269 phosphorylation because we did not have a suitable antibody available. Therefore, we unfortunately cannot answer this question and have not addressed it since.

Comment to the Author
8. Page 8 - 10, in the section of diabetes insipidus: please add the role of "glycogen synthase kinase 3" in lithium-induced NDI.

Response
We have added:
Lithium inhibits GSK3β which, by modulating AC activity and decreasing cAMP, impaired AQP2 trafficking, reduced AQP2 mRNA and protein expression, and decreased its phosphorylation at S256 and altogether interfered with responses to AVP in the collecting duct principal cells (Rao *et al.*, 2010; Kishore & Ecelbarger, 2013; Kaiser & Edemir, 2020). However, GSK3β knockout mice were only mildly resistant to lithium-induced DI – as were AC6 knockout mice (Poulsen *et al.*, 2017).

References

Bouley R, Breton S, Sun T, McLaughlin M, Nsumu NN, Lin HY, Ausiello DA & Brown D. (2000). Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. *J Clin Invest* **106,** 1115-1126.

Centrone M, Ranieri M, Di Mise A, D'Agostino M, Venneri M, Ferrulli A, Valenti G & Tamma G. (2022). AQP2 trafficking in health and diseases: an updated overview. *Int J Biochem Cell Biol* **149,** 106261.

Cheung PW, Bouley R & Brown D. (2020). Targeting the Trafficking of Kidney Water Channels for Therapeutic Benefit. *Annu Rev Pharmacol Toxicol* **60,** 175-194.

Datta A, Yang CR, Limbutara K, Chou CL, Rinschen MM, Raghuram V & Knepper MA. (2020). PKA-independent vasopressin signaling in renal collecting duct. *FASEB J* **34,** 6129-6146.

Efe O, Klein JD, LaRocque LM, Ren H & Sands JM. (2016). Metformin improves urine concentration in rodents with nephrogenic diabetes insipidus. *JCI Insight* **1**.

Hoffert JD, Pisitkun T, Wang G, Shen RF & Knepper MA. (2006). Quantitative phosphoproteomics of vasopressin-sensitive renal cells: regulation of aquaporin-2 phosphorylation at two sites. *Proc Natl Acad Sci U S A* **103,** 7159-7164.

Holst MR, Jensen LG, Aaron J, Login FH, Rajkumar S, Hahn U & Nejsum LN. (2021). Regulated exocytosis: renal aquaporin-2 3D vesicular network organization and association with F-actin. *Am J Physiol Cell Physiol* **321,** C1060-C1069.

Isobe K, Jung HJ, Yang CR, Claxton J, Sandoval P, Burg MB, Raghuram V & Knepper MA. (2017). Systems-level identification of PKA-dependent signaling in epithelial cells. *Proc Natl Acad Sci U S A* **114,** E8875-E8884.

Kaiser M & Edemir B. (2020). Lithium Chloride and GSK3 Inhibition Reduce Aquaporin-2 Expression in Primary Cultured Inner Medullary Collecting Duct Cells Due to Independent Mechanisms. *Cells* **9**.

Kishore BK & Ecelbarger CM. (2013). Lithium: a versatile tool for understanding renal physiology. *Am J Physiol Renal Physiol* **304,** F1139-1149.

Klein JD, Khanna I, Pillarisetti R, Hagan RA, LaRocque LM, Rodriguez EL & Sands JM. (2021). An AMPK activator as a therapeutic option for congenital nephrogenic diabetes insipidus. *JCI Insight* **6**.

Klein JD, Wang Y, Blount MA, Molina PA, LaRocque LM, Ruiz JA & Sands JM. (2016). Metformin, an AMPK activator, stimulates the phosphorylation of aquaporin 2 and urea transporter A1 in inner medullary collecting ducts. *Am J Physiol Renal Physiol* **310,** F1008-1012.

Klokkers J, Langehanenberg P, Kemper B, Kosmeier S, von Bally G, Riethmuller C, Wunder F, Sindic A, Pavenstadt H, Schlatter E & Edemir B. (2009). Atrial natriuretic peptide and nitric oxide signaling

antagonizes vasopressin-mediated water permeability in inner medullary collecting duct cells. *Am J Physiol Renal Physiol* **297,** F693-703.

Klussmann E. (2023). Aquaporin-2 is not alone. *Kidney Int* **103,** 458-460.

Klussmann E, Tamma G, Lorenz D, Wiesner B, Maric K, Hofmann F, Aktories K, Valenti G & Rosenthal W. (2001). An inhibitory role of Rho in the vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. *J Biol Chem* **276,** 20451-20457.

Mak A, Sung CC, Pisitkun T, Khositseth S & Knepper MA. (2023). 'Aquaporin-omics': mechanisms of aquaporin-2 loss in polyuric disorders. *J Physiol*.

Nedvetsky PI, Stefan E, Frische S, Santamaria K, Wiesner B, Valenti G, Hammer JA, 3rd, Nielsen S, Goldenring JR, Rosenthal W & Klussmann E. (2007). A Role of myosin Vb and Rab11-FIP2 in the aquaporin-2 shuttle. *Traffic* **8,** 110-123.

Poulsen SB, Kristensen TB, Brooks HL, Kohan DE, Rieg T & Fenton RA. (2017). Role of adenylyl cyclase 6 in the development of lithium-induced nephrogenic diabetes insipidus. *JCI Insight* **2,** e91042.

Rao R, Patel S, Hao C, Woodgett J & Harris R. (2010). GSK3beta mediates renal response to vasopressin by modulating adenylate cyclase activity. *J Am Soc Nephrol* **21,** 428-437.

Sasaki S, Yui N & Noda Y. (2014). Actin directly interacts with different membrane channel proteins and influences channel activities: AQP2 as a model. *Biochim Biophys Acta* **1838,** 514-520.

Tamma G, Klussmann E, Maric K, Aktories K, Svelto M, Rosenthal W & Valenti G. (2001). Rho inhibits cAMP-induced translocation of aquaporin-2 into the apical membrane of renal cells. *Am J Physiol Renal Physiol* **281,** F1092-1101.

Tas HI & Sancak EB. (2021). Protective effect of metformin on lithium-induced nephrogenic diabetes insipidus: An experimental study in rats. *Adv Clin Exp Med* **30,** 1185-1193.

Vukicevic T, Schulz M, Faust D & Klussmann E. (2016). The Trafficking of the Water Channel Aquaporin-2 in Renal Principal Cells-a Potential Target for Pharmacological Intervention in Cardiovascular Diseases. *Front Pharmacol* **7,** 23.

Dear Dr Klussmann,

Re: JP-SR-2023-284100R1 "Many kinases for controlling the water channel aquaporin-2" by Enno Klussmann
Andrii Kharin

I am pleased to tell you that your Symposium Review article has been accepted for publication in The Journal of Physiology, subject to any modifications to the text that may be required by the Journal Office to conform to House rules.

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Thank you for an excellent review. One reviewer noted a few errors, for example page 4 paragraph one ca. 1-2% - ca. should probably be e.g. Next sentence, Water channels, aquaporins (AQP) mediate, would be better Water channels and aquaporins (AQP) mediate... next paragraph, aquaporin-1 (AQP-1) this has already been abbreviated in line one

These can be corrected at the proofing stage, so please thoroughly check and correct as promptly as possible.

REFEREE COMMENTS:

Referee #1:

The authors have addressed all comments.

Minor suggestions: please include cell types and subcellular localization when describing renal AQPs.

There are some typos and also "ca" should be e.g.

Referee #2:

The authors responded adequately to my concerns, and I do not have further comments.

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