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TRANSLATIONAL PHYSIOLOGY

Physiological insights into novel therapies for nephrogenic diabetes insipidus

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Sands JM, Klein JD. Physiological insights into novel therapies for nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol* 311: F1149–F1152, 2016. First published August 17, 2016; doi:10.1152/ajprenal.00418.2016.—Fundamental kidney physiology research can provide important insight into how the kidney works and suggest novel therapeutic opportunities to treat human diseases. This is especially true for nephrogenic diabetes insipidus (NDI). Over the past decade, studies elucidating the molecular physiology and signaling pathways regulating water transport have suggested novel therapeutic possibilities. In patients with congenital NDI due to mutations in the type 2 vasopressin receptor (V2R) or acquired NDI due to lithium (or other medications), there are no functional abnormalities in the aquaporin-2 (AQP2) water channel, or in another key inner medullary transport protein, the UT-A1 urea transporter. If it is possible to phosphorylate and/or increase the apical membrane accumulation of these proteins, independent of vasopressin or cAMP, one may be able to treat NDI. Sildenifil (through cGMP), erlotinib, and simvastatin each stimulate AQP2 insertion into the apical plasma membrane. Some recent human data suggest that sildenafil and simvastatin may improve urine concentrating ability. ONO-AE1-329 (ONO) stimulates the EP4 prostanoid receptor (EP4), which stimulates kinases that in turn phosphorylate AQP2 and UT-A1. Clopidogrel is a P2Y12-R antagonist that potentiates the effect of vasopressin and increases AQP2 abundance. Metformin stimulates AMPK to phosphorylate and activate AQP2 and UT-A1, and it increases urine concentrating ability in two rodent models of NDI. Since metformin, sildenafil, and simvastatin are commercially available and have excellent safety records, the potential for rapidly advancing them into clinical trials is high.

nephrogenic diabetes insipidus; water reabsorption; metformin; aquaporin 2

FUNDAMENTAL KIDNEY PHYSIOLOGY research can provide important insight into how the kidney works and suggest novel therapeutic opportunities to treat human diseases. This is especially true for a relatively rare condition, nephrogenic diabetes insipidus (NDI). Over the past decade, studies elucidating the molecular physiology and signaling pathways regulating water transport have suggested novel therapies, in many cases involving the repurposing of existing and commercially available medications. In this perspectives article, we will review several of these advances.

Water reabsorption occurs in collecting duct principal cells. When a person or animal needs to conserve water, the posterior pituitary secretes vasopressin (also called antidiuretic hormone or ADH). Vasopressin binds to type 2 vasopressin receptors (V2R) on the basolateral membrane of principal cells, activates adenylyl cyclase, generates cyclic AMP, and activates a kinase or kinases. The kinase is generally thought to be protein kinase A (PKA) since it is inhibited by H-89. However, H-89 can also inhibit other kinases, so direct evidence that PKA is the kinase is lacking. The kinase(s) that is/are activated ultimately phosphorylates and causes the insertion of aquaporin 2 (AQP2) water channels into the apical membrane (24). Water enters the cell through AQP2, and exits via AQP3 and AQP4 in the basolateral membrane. Failure of any of these steps can result in diabetes insipidus (Fig. 1).

Central diabetes insipidus results from failure of the posterior pituitary to make or secrete vasopressin. Central diabetes insipidus can be effectively treated with desmopressin, a selective V2R agonist, to replace the missing hormone. NDI results from end-organ (kidney) insensitivity to vasopressin. The primary mechanisms leading to a failure of the kidney to respond to vasopressin can be predicted from the physiology of water reabsorption: a defect in the V2R preventing activation of the downstream signaling cascade; or a defect in AQP2 (reviewed in Refs. 22, 24). NDI can result from genetic abnormalities: 90% of families have a mutation in the V2R; and AQP2 mutations account for most of the other families. It can also result from acquired causes, such as chronic lithium therapy, amphotericin, foscarnet, and demeclocycline, which reduce adenylyl cyclase activity, thereby decreasing the effect of vasopressin on the collecting ducts. Lithium is the most common cause of acquired NDI and its mechanism includes inhibition of glycogen synthase 3β (GSK3β). GSK3β inhibition increases cyclooxygenase 2 (COX2) and reduces adenylyl cyclase activity, cyclic AMP production, and AQP2 phosphorylation, thereby leading to polyuria and NDI (18, 19). Patients with NDI produce very large quantities of dilute urine. In the most severe forms of congenital NDI, patients can produce 20 liters of urine daily, 24/7/365, and must drink a comparable amount of water to avoid severe dehydration. At present, therapy for congenital NDI includes a thiazide diuretic, a very low-salt diet, and indomethacin, and is only minimally effective.

In patients with NDI due to V2R mutations or lithium, there are no functional abnormalities in AQP2, or in another key inner medullary transport protein, the UT-A1 urea transporter (23). This suggests the hypothesis that if it is possible to phosphorylate these proteins and/or increase their apical membrane accumulation and function, independent of vasopressin or cAMP, one may be able to treat NDI. If these treatments reduced urine output, even if not to normal levels, they would still make a major improvement in the quality of life for

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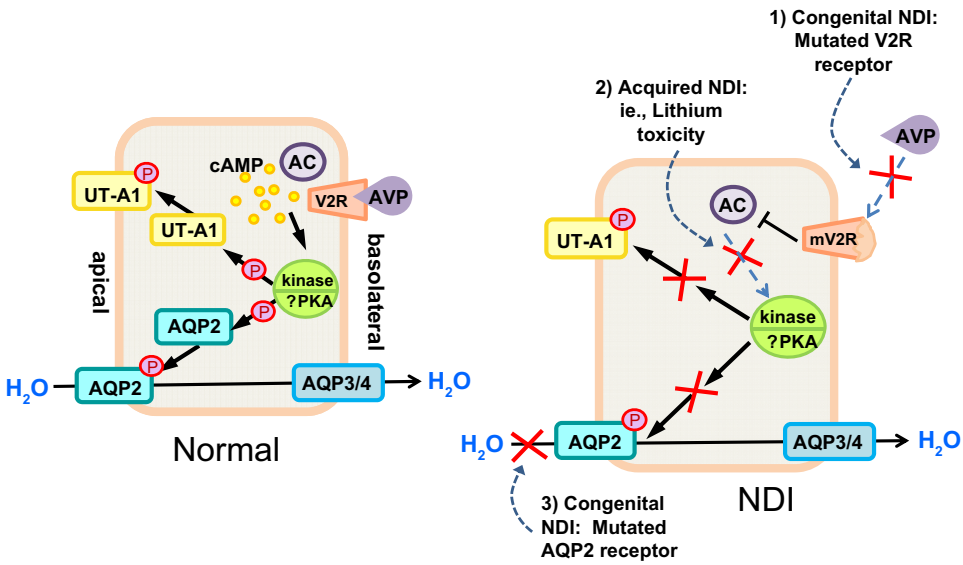

Fig. 1. Known causes of nephrogenic diabetes insipidus (NDI). *Left*: in a normal principal cell, vasopressin (AVP) binds to the type 2 vasopressin receptor (V2R), activates adenylyl cyclase (AC), increases cyclic adenosine monophosphate (cAMP) production, and activates a kinase. This results in the phosphorylation of AQP2 and UT-A1, and an increase in their accumulation in the apical membrane. Water is reabsorbed transcellularly by AQP2 in the apical membrane and aquaporin (AQP)3 and 4 in the basolateral membrane. *Right*: 3 mechanisms for NDI: *1*) congenital NDI due to mutation of V2R receptor that results in decreased AC activity; *2*) acquired NDI due to lithium (and other medications, not shown) that interrupts AC activity thus decreasing kinase activity; and *3*) congenital NDI due to AQP2 mutations resulting in an inability to transport water. All 3 mechanisms interfere with a key step in vasopressin-stimulated water reabsorption.

patients with NDI. Recent physiological studies suggest that this may be possible (Fig. 2).

The initial studies testing non-cAMP pathways used the phosphodiesterase inhibitor, sildenafil, to increase cyclic GMP. Sildenafil is used clinically to treat pulmonary hypertension and erectile dysfunction. Sildenafil increased the apical membrane accumulation of AQP2, both in vitro and in vivo, in Brattleboro rats (4). Brattleboro rats are a model of central diabetes insipidus, rather than NDI; however, the increase in AQP2 accumulation was a promising proof of concept. There was no increase in urine osmolality in the Brattleboro rat, possibly due to effects of sildenafil to increase medullary blood flow, which would reduce urine concentrating ability (4). However, sildenafil does reduce polyuria in rats with lithium-induced NDI (21). Recently, a case was reported of a child with X-linked NDI who was treated with sildenafil. The child had decreased urine volume and increased urine osmolality compared with prior treatment with the standard combination of hydrochlorothiazide, amiloride, and indomethacin (2).

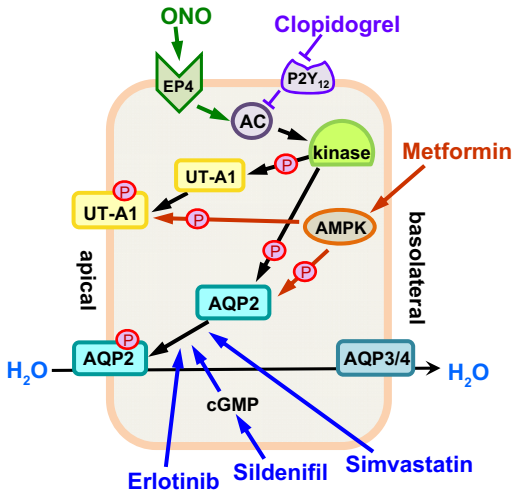

Fig. 2. Potential nonvasopressin treatments for NDI. Diagram of an inner medullary collecting duct cell showing alternative stimulation of water reabsorption pathways. In green, ONO-AE1-329 (ONO) stimulates the EP4 prostanoid receptor (EP4) which stimulates AC that phosphorylates urea and AQP2 transporters; and in purple, clopidogrel inhibits the P2Y12-R receptor resulting in increased AC activity and increases AQP2 abundance. In brown, metformin stimulates AMPK to phosphorylate and activate AQP2 and urea transporter UT-A1. In blue, erlotinib, simvastatin, and sildenifil (through cGMP) stimulate insertion of AQP2 into the apical plasma membrane.

Another approach that was based on the physiology of water transport was altering the cholesterol content of the apical membrane to increase AQP2 accumulation. Methyl-β-cyclodextrin is a cholesterol-depleting drug that induces the apical membrane accumulation of AQP2 by inhibiting endocytosis (20). Simvastatin is used clinically to lower serum cholesterol. Simvastatin was tested in Brattleboro rats and shown to enhance the apical membrane expression of AQP2 through downregulation of Rho GTPase activity and inhibition of endocytosis, and to transiently increase urine osmolality (15). Although simvastatin has not been tested in patients with NDI, hypercholesterolemic patients treated with simvastatin show an increase in urinary AQP2, suggesting that it may be useful in NDI (17). Fluvastatin is another cholesterol-lowering agent that is used clinically. Administration of fluvastatin, along with secretin, increases urine concentrating ability in a V2R knockout mouse, which is a model of X-linked congenital NDI in humans (16).

Erlotinib is a selective epidermal growth factor receptor (EGFR) inhibitor that is used clinically to treat advanced nonsmall cell lung cancer and pancreatic cancer (3). Erlotinib increases AQP2 expression in the apical membrane of collecting duct principal cells and reduces urine volume by nearly 50% in mice with lithium-induced NDI (5). This beneficial effect was sustained for 5 days. Erlotinib increases AQP2 membrane expression both by increasing exocytosis and decreasing endocytosis. The effect of erlotinib was not through cyclic AMP or cyclic GMP (5). While the beneficial effects of erlotinib on AQP2 and urine osmolality are encouraging, erlotinib has several side effects, including diarrhea, fatigue, skin rash, and rarely, interstitial pneumonitis (3). These side effects may preclude erlotinib's use as a treatment for NDI.

Another approach based on the physiology of the collecting duct was to determine whether the principal cells might express

another Gs-coupled receptor that could activate adenylyl cyclase, as an alternative to activation via the V2R. The EP4 prostanoid receptor is highly expressed in mouse medullary collecting duct cells (13). AQP2 abundance, apical membrane targeting, and urine osmolality are reduced in mice with genetic knockout of the EP4 receptor in the collecting duct (9). ONO-AE1-329 (ONO) is a selective EP4 PGE_2 agonist that increases urine osmolality in V2R knockout mice (13). The effect was greatest at *day 1* but did show some improvement out to *day 6*. Unfortunately, ONO has limited solubility and is relatively unstable in aqueous solution (13). Since activation of the EP4 receptor increases AQP2 abundance and apical membrane targeting, it is a potential therapy for both congenital and lithium-induced NDI (9). However, development of a more stable EP4 receptor agonist will be needed before this approach can be translated to patients.

The purinergic receptor P2-Y12R is a Gi-coupled receptor that is expressed in collecting duct principal cells (29). Activation of the P2Y12-R results in reduced cAMP levels, suggesting that the P2Y12-R antagonist clopidogrel may increase cAMP levels and improve urine concentration. Clopidogrel decreased polyuria, increased urine osmolality, and increased AQP2 abundance in mice with lithium-induced NDI (29). Clopidogrel did not increase urine osmolality in Brattleboro rats, indicating that it works by augmenting the effect of vasopressin (29).

Phosphorylation state is not only a result of phosphorylation by kinases but also reflects the dephosphorylation activity of phosphatases. Although not specifically linked to NDI, several studies show that protein phosphatase 1 (27), protein phosphatase 2A (11), and calcineurin (10, 14) are able to impact the membrane association of AQP2, suggesting a potential area for future therapy development.

As mentioned above, conventional therapy for NDI includes thiazide diuretics, amiloride, and a very low-sodium (0.5 g) diet. A nonsteroidal anti-inflammatory drug is often added, but these are nephrotoxic. Thiazides inhibit the sodium-chloride cotransporter NCC expressed in the distal convoluted tubule, and they induce a mild hypovolemia. This leads to activation of the renin-angiotensin-aldosterone system, and an increase in proximal tubule sodium and water reabsorption, thereby decreasing distal delivery and the amount of tubular fluid available to become urine. However, in mice lacking NCC, thiazides still reduce polyuria in lithium-treated mice, suggesting that there may be an additional mechanism by which thiazides act (25). In mice with lithium-induced NDI, treatment with either acetazolamide or thiazide/amiloride resulted in a similar increase in AQP2 protein abundance and urine osmolality, and a reduction of polyuria, and did so with fewer side effects (6). Acetazolamide has not been tested in patients with lithium-induced NDI, nor has it been tested in patients or animal models of congenital NDI.

The most recent approach to treating congenital X-linked NDI is the activation of adenosine monophosphate kinase (AMPK) by metformin (7, 12). AMPK is an energy-sensing serine/threonine kinase that phosphorylates the Na-K-2Cl cotransporter NKCC2 in the outer medulla (8). Metformin is an AMPK activator that is widely used to treat patients with diabetes mellitus or polycystic ovary syndrome. Metformin is thought to directly target the mitochondrial respiratory-chain complex 1, thereby reducing cellular energy status, resulting in an indirect activation of AMPK (1, 28). We recently showed metformin increases osmotic water permeability by increasing AQP2 phosphorylation and accumulation in the apical plasma membrane (12). Metformin also increases urea permeability, but does so by increasing the phosphorylation of UT-A1 that is already present in the membrane; Metformin did not increase UT-A1 accumulation in the apical plasma membrane (12). In two rodent models of NDI, rats treated with tolvaptan to inhibit the V2R and in V2R knockout mice, metformin increased both AQP2 and UT-A1 protein abundances in the inner medulla, and increased urine osmolality (7). Perhaps most promising for metformin's potential to be a therapeutic option for patients with NDI, the effect of metformin was sustained for 10 days in tolvaptan-treated rats (7). Tolvaptan is approved for the treatment of autosomal dominant polycystic kidney disease (ADPKD) in some countries, and can result in iatrogenic NDI in patients (26). The finding that metformin improved urine concentrating ability in tolvaptan-treated rats suggests that metformin may lessen the polyuria in patients treated with tolvaptan (Figs. 1 and 2). Given that metformin is commercially available with an excellent long-term safety record, including patients with polycystic ovary syndrome (and without diabetes mellitus), the opportunity for translation into patients is high.

In summary, the knowledge gained from fundamental physiological research into the mechanisms of transcellular water transport and the urine concentrating mechanism has led to new insights into potential therapies for treating patients with NDI. Several potential therapies involve repurposing existing medications (metformin, sildenafil, simvastatin, clopidogrel). Since these medications are commercially available and have excellent safety records, the potential for rapidly advancing them into clinical trials is high. Even if these therapies do not normalize urine output in NDI patients, a significant reduction in urine output would markedly improve their quality of life and reduce the risk of dangerous levels of dehydration.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.M.S. drafted manuscript; J.M.S. and J.D.K. edited and revised manuscript; J.M.S. and J.D.K. approved final version of manuscript; J.D.K. prepared figures.

REFERENCES

1. **An H, He L.** Current understanding of metformin effect on the control of hyperglycemia in diabetes. *J Endocrinol* 228: R97–106, 2016.
2. **Assadi F, Sharbaf FG.** Sildenafil for the treatment of congenital nephrogenic diabetes insipidus. *Am J Nephrol* 42: 65–69, 2015.
3. **Bou Matar RN, Malik B, Wang XH, Martin CF, Eaton DC, Sands JM, Klein JD.** Protein abundance of urea transporters and aquaporin 2 change differently in nephrotic pair-fed vs. non-pair-fed rats. *Am J Physiol Renal Physiol* 302: F1545–F1553, 2012.
4. **Bouley R, Pastor-Soler N, Cohen O, McLaughlin M, Breton S, Brown D.** Stimulation of AQP2 membrane insertion in renal epithelial cells in vitro and in vivo by the cGMP phosphodiesterase inhibitor sildenafil citrate (Viagra). *Am J Physiol Renal Physiol* 288: F1103–F1112, 2005.
5. **Cheung PW, Nomura N, Nair AV, Pathomthongtaweechai N, Ueberdiek L, Jenny Lu HA, Brown D, Bouley R.** EGF receptor inhibition

by erlotinib increases aquaporin 2-mediated renal water reabsorption. *J Am Soc Nephrol*. JASN ASN.2015080903; published ahead of print March 9, 2016, doi:10.1681/ASN.2015080903.

6. **de Groot T, Sinke AP, Kortenoeven MLA, Alsady M, Baumgarten R, Devuyst O, Loffing J, Wetzels JF, Deen PMT.** Acetazolamide attenuates lithium-induced nephrogenic diabetes insipidus. *J Am Soc Nephrol* 27: 2082–2091, 2016.
7. **Efe O, Klein JD, LaRocque LM, Ren H, Sands JM.** Metformin improves urine concentration in rodents with nephrogenic diabetes insipidus. *JCI Insight* 1: e88409, 2016.
8. **Fraser SA, Gimenez I, Cook N, Jennings I, Katerelos M, Katsis F, Levidiotis V, Kemp BE, Power DA.** Regulation of the renal-specific Na^+-K^+-$2Cl^-$ co-transporter NKCC2 by AMP-activated protein kinase (AMPK). *Biochem J* 405: 85–93, 2007.
9. **Gao M, Cao R, Du S, Jia X, Zheng S, Huang S, Han Q, Liu J, Zhang X, Miao Y, Kang J, Gustafsson JA, Guan Y.** Disruption of prostaglandin E2 receptor EP4 impairs urinary concentration via decreasing aquaporin 2 in renal collecting ducts. *Proc Natl Acad Sci USA* 112: 8397–8402, 2015.
10. **Gooch JL, Guler RL, Barnes JL, Toro JJ.** Loss of calcineurin Aα results in altered trafficking of AQP2 and in nephrogenic diabetes insipidus. *J Cell Sci* 119: 2468–2476, 2006.
11. **Jo I, Ward DT, Baum MA, Scott JD, Coghlan VM, Hammond TG, Harris HW.** AQP2 is a substrate for endogenous PP2B activity within an inner medullary AKAP-signaling complex. *Am J Physiol Renal Physiol* 281: F958–F965, 2001.
12. **Klein JD, Wang Y, Blount MA, Molina PA, LaRocque LM, Ruiz JA, Sands JM.** 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–F1012, 2016.
13. **Li JH, Chou CL, Li B, Gavrilova O, Eisner C, Schnermann J, Anderson SA, Deng CX, Knepper MA, Wess J.** A selective EP4 PGE2 receptor agonist alleviates disease in a new mouse model of X-linked nephrogenic diabetes insipidus. *J Clin Invest* 119: 3115–3126, 2009.
14. **Li SZ, McDill BW, Kovach PA, Ding L, Go WY, Ho SN, Chen F.** Calcineurin-NFATc signaling pathway regulates AQP2 expression in response to calcium signals and osmotic stress. *Am J Physiol Cell Physiol* 292: C1606–C1616, 2007.
15. **Li W, Zhang Y, Bouley R, Chen Y, Matsuzaki T, Nunes P, Hasler U, Brown D, Lu HAJ.** Simvastatin enhances aquaporin-2 surface expression and urinary concentration in vasopressin-deficient Brattleboro rats through modulation of Rho GTPase. *Am J Physiol Renal Physiol* 301: F309–F318, 2011.
16. **Procino G, Milano S, Carmosino M, Barbieri C, Nicoletti MC, Li JH, Wess J, Svelto M.** Combination of secretin and fluvastatin ameliorates the polyuria associated with X-linked nephrogenic diabetes insipidus in mice. *Kidney Int* 86: 127–138, 2014.
17. **Procino G, Portincasa P, Mastrofrancesco L, Castorani L, Bonfrate L, Addabbo F, Carmosino M, Di Ciaula A, Svelto M.** Simvastatin increases AQP2 urinary excretion in hypercholesterolemic patients: a pleiotropic effect of interest for patients with impaired AQP2 trafficking. *Clin Pharmacol Ther* 99: 528–537, 2016.
18. **Rao R, Patel S, Hao C, Woodgett J, Harris R.** GSK3beta mediates renal response to vasopressin by modulating adenylate cyclase activity. *J Am Soc Nephrol* 21: 428–437, 2010.
19. **Rao R, Zhang MZ, Zhao M, Cai H, Harris RC, Breyer MD, Hao CM.** Lithium treatment inhibits renal GSK-3 activity and promotes cyclooxygenase 2-dependent polyuria. *Am J Physiol Renal Physiol* 288: F642–F649, 2005.
20. **Russo LM, McKee M, Brown D.** Methyl-β-cyclodextrin induces vasopressin-independent apical accumulation of aquaporin-2 in the isolated, perfused rat kidney. *Am J Physiol Renal Physiol* 291: F246–F253, 2006.
21. **Sanches TR, Volpini RA, Massola Shimizu MH, Braganca AC, Oshiro-Monreal F, Seguro AC, Andrade L.** Sildenafil reduces polyuria in rats with lithium-induced NDI. *Am J Physiol Renal Physiol* 302: F216–F225, 2012.
22. **Sands JM, Bichet DG.** Nephrogenic diabetes insipidus. *Ann Intern Med* 144: 186–194, 2006.
23. **Sands JM, Layton HE.** Advances in understanding the urine-concentrating mechanism. *Annu Rev Physiol* 76: 387–409, 2014.
24. **Sands JM, Layton HE, Fenton RA.** Urine concentration and dilution. In: *Brenner and Rector's The Kidney*, edited by Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASL, and Brenner BM. Philadelphia: Elsevier, 2011, p. 326–352.
25. **Sinke AP, Kortenoeven ML, de Groot T, Baumgarten R, Devuyst O, Wetzels JF, Loffing J, Deen PM.** Hydrochlorothiazide attenuates lithium-induced nephrogenic diabetes insipidus independently of the sodium-chloride cotransporter. *Am J Physiol Renal Physiol* 306: F525–F533, 2014.
26. **Torres VE, Gansevoort RT, Czerwiec FS.** Tolvaptan in autosomal dominant polycystic kidney disease. *N Engl J Med* 368: 1259, 2013.
27. **Valenti G, Procino G, Carmosino M, Frigeri A, Mannucci R, Nicoletti I, Svelto M.** The phosphatase inhibitor okadaic acid induces AQP2 translocation independently from AQP2 phosphorylation in renal collecting duct cells. *J Cell Sci* 113: 1985–1992, 2000.
28. **Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F.** Cellular and molecular mechanisms of metformin: an overview. *Clin Sci* 122: 253–270, 2012.
29. **Zhang Y, Peti-Peterdi J, Muller CE, Carlson NG, Baqi Y, Strasburg DL, Heiney KM, Villanueva K, Kohan DE, Kishore BK.** P2Y12 receptor localizes in the renal collecting duct and its blockade augments arginine vasopressin action and alleviates nephrogenic diabetes insipidus. *J Am Soc Nephrol* 26: 2978–2987, 2015.