Aquaporin-2 Inhibitors: Fishing in the Chemical Pool

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The critical contribution of aquaporin-2 (AQP) to water homeostasis in mammals is highlighted by the severe concentrating defects seen in humans with mutations in the AQP2 gene and in mice that are AQP2 null or have loss-of-function mutations in AQP2.1,2 The role of the antidiuretic hormone vasopressin and its receptor, V2R, in the signaling cascade that leads to membrane accumulation of AQP2 in collecting-duct principal cells, and to an increase in renal water reabsorption, is well established.3 The involvement of VP-induced cAMP elevation by adenyl cyclase activation, and subsequent protein kinase A–induced phosphorylation of AQP2, is also well known. Defects in any of the major components of this signaling cascade have the potential to lead to aberrant AQP2 trafficking and function, with an associated loss of urine-concentrating ability or an inappropriate increase in collecting-duct water retention.

Decreased water reabsorption leads to diabetes insipidus, either central (loss of vasopressin) or nephrogenic (loss of V2R or AQP2 function). Excessive AQP2 expression and plasma membrane localization are associated with such conditions as congestive heart failure, cirrhosis of the liver, and the syndrome of inappropriate antidiuretic hormone secretion. In such cases, inappropriate water re-absorption can lead to moderate or severe hyponatremia and its subsequent complications, sometimes fatal. Furthermore, many defects in water homeostasis result from acquired conditions, such as lithium-induced nephrogenic diabetes insipidus, a common consequence of the use of this compound as therapy for bipolar disorder.

Considerable effort has therefore been expended over the past several years to identify components of the AQP2 trafficking pathway that could be targeted by drugs or other therapeutic strategies in order to alleviate the consequences of these diseases. On the basis of current knowledge of the signaling and trafficking pathways that have been elucidated by several groups, different strategies have been explored. These include bypassing the V2R-mediated signaling pathway using alternative cyclic nucleotide-generating agents, such as the cyclic guanosine monophosphate phosphodiesterase inhibitor sildenafil;4,5 activating alternative pathways, including prostaglandin signaling;6 and modulating trafficking with drugs, including statins, that affect the actin cytoskeleton.7,8 A key factor in endo- and exocytotic events, as well as inhibiting endocytosis directly.9 Some of these interventions indeed result in increased membrane AQP2 expression, and they increase urinary-concentrating ability in animal models, including vasopressin-deficient Brattleboro rats and lithium-treated rats.2

In the current issue of JASN, Bogum et al.10 use a small-molecule screening approach to identify chemical compounds that inhibit cAMP-induced AQP2 membrane accumulation. Such compounds would function as aquaretics and counteract the effects of elevated vasopressin/cAMP or increased AQP2 levels in conditions that might lead to excessive fluid reabsorption, hypertension, and hyponatremia. Although this approach is expected to rediscover compounds that are already known to have such an inhibitory effect, the most interesting and exciting outcome of such an unbiased screen would be to uncover new and unexpected active compounds that might not have been predicted with a directed approach to this problem. This could potentially lead to the discovery of new pathways of AQP2 trafficking and regulation, as well as alternative reagents for intervening in the trafficking of AQP2.

The screening assay used by Bogum et al. is based on the known effect of forskolin (which maximally increases cAMP levels in cells) to induce translocation of AQP2 from a cytoplasmic vesicular location in epithelial cells to a predominantly plasma membrane localization. In this assay, MCD4 cells of collecting duct origin were used after stably transfecting them with human AQP2. An automated dispensing and washing system was used to immunostain cells for AQP2 after seeding them in 384-well plates and exposing them to forskolin in the presence or absence of almost 18,000 small chemical compounds from a commercially available library. An automated microscope system was used to image the AQP2 in cells from each well, and the distribution of AQP2 on the membrane or in the cytosol was expressed as a software-calculated ratio based on a comparison with the cellular localization of actin-phalloidin as a marker that reveals the position of the plasma membrane, and DAPI as a nuclear stain.

The screening strategy identified 17 small molecules that inhibit cAMP-induced AQP2 membrane localization in transfected MCD4 cells. These compounds were subsequently tested using inner medullary collecting duct cells expressing endogenous AQP2, and five of them successfully inhibited forskolin-induced AQP2 redistribution in these cells as well. One of
the molecules examined in greater detail in this study is 4-acetyldiphyllin, a known inhibitor of H+ ATPase (V-ATPase). This finding provides an important positive control for the utility of this novel screening assay because an earlier study showed that a different but more familiar V-ATPase inhibitor, bafilomycin, also inhibits AQP2 trafficking. The effect of 4-acetyldiphyllin on cAMP-induced AQP2 membrane accumulation was examined in more detail in the inner medullary collecting duct cell system; the authors found that although protein kinase A–mediated phosphorylation at the critical S256 position of AQP2 was inhibited, phosphorylation of S264 and, most important, S269, was not affected. These data indicate that in contrast to the results of a previous study, phosphorylation of S269 alone is not sufficient to induce AQP2 membrane accumulation. The reasons for this discrepancy are unclear. In addition to 4-acetyldiphyllin, the antifungal agent fluconazole was identified as another inhibitory compound, but its mechanism of action remains undetermined. Finally, Bogum et al. list several other small molecules with inhibitory action—including but their cellular modes of action also are unknown. The next step should be, of course, to examine the effects of these compounds on water handling in appropriate animal models, as well as their potential toxicity.

In all studies aimed at regulating intracellular protein and vesicle trafficking pathways, such as those involving AQP2 as well as other proteins whose defective trafficking leads to human disease, a general issue is one of specificity for the process that is dysfunctional in any given cell. Thus, although many of the cell biological elements that are involved in AQP2 trafficking are well known—which in microtubules, clathrin–coated vesicles, and heat shock proteins, Rab small guanosine triphosphatases and proteins of the SNARE complex to name but a few—developing treatments that are sufficiently specific for a particular trafficking process, in this case involving AQP2, can be considerably more difficult.

This approach requires a more detailed understanding of protein interaction mechanisms, receptor signaling pathways, and regulation of gene expression that are restricted to AQP2 in principal cells or the development of procedures that will deliver the therapeutic to these cells, to the exclusion of other potential targets in the kidney and indeed in the whole body.

That being said, the high-throughput screening assay described by Bogum et al. has the potential to serve not only as a supplement to conventional molecule–specific directed discovery processes relevant to the AQP2 pathway but also to contribute in a broader way by identifying compounds that target some essential common pathways that are important for the development of human diseases. For example, it could be used to determine the effects of identified inhibitory drugs on other trafficking processes—in the presence and absence of AQP2 expression—that are amenable to the elegant microscopic approach that these authors developed.

Finally, in searching for novel approaches to normalize AQP2 trafficking and collecting duct water reabsorption in disease conditions, the type of unbiased, basic approach described here is essential because it alone can provide the type of unpredictable breakthroughs that so often drive science and medicine in new and exciting directions. It is unfortunate that the increasing emphasis on directed translational science that is being advocated in some quarters seems to indicate that we may be losing sight of this fact.

**DISCLOSURES**

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**REFERENCES**