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Drugging aquaporins

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ABSTRACT

Water is essential for all life because it is required for the proper functioning of the cells and tissues of all organisms. It crosses biological membranes down osmotic gradients through the pores of aquaporin membrane channels at rates of up to 3 billion molecules per second. In the twenty years since Peter Agre was awarded the 2003 Nobel Prize in Chemistry for the discovery of the aquaporin family, aquaporin structure and function have become established in the literature. As a consequence, we understand in fine detail the mechanism by which aquaporins facilitate membrane water flow while excluding protons. We also know that some aquaporins facilitate the permeation of other small neutral solutes, ions or even unexpected substrates across biological membranes. The thirteen aquaporins in the human body have been implicated in pathologies including oedema, epilepsy, cancer cell migration, tumour angiogenesis, metabolic disorders and inflammation. Surprisingly, however, there is no aquaporin-targeted drug in the clinic. Some scientists have therefore concluded that aquaporins are intrinsically non-druggable targets. Discovering medicines to treat disorders of water homeostasis is thus an enduring challenge for the aquaporin field. Success in this endeavour will meet the urgent clinical need of millions of patients suffering from a range of life-threatening conditions and for whom no pharmacological interventions are currently available.

1. Introduction

As recently as the early 1990s, it was assumed that water entered or exited cells by diffusing across the lipid bilayer of cell membranes. However, this assumption of simple diffusion did not explain the high water permeation rates that were necessary for some cells to perform their biological functions or to respond to rapidly changing environmental conditions. This prompted a search for membrane water channels by several independent scientific groups [1]. The body of experimental evidence generated by these investigators meant that by the late 1980s, the existence of membrane proteins that could facilitate the diffusion of water across biological membranes was indisputable, although their identity remained elusive [2]. In 1992, Peter Agre demonstrated the appearance of membrane water channels in *Xenopus laevis* oocytes that were expressing a human red blood cell protein (CHIP28), which was later renamed aquaporin-1 (AQP1) [3]. This year marks the twentieth anniversary of Peter's award of the 2003 Nobel Prize in Chemistry for that discovery [4].

Since the discovery of AQP1, thirteen human aquaporins (AQP0–12) have been identified and several of them have been implicated in human diseases [5,6] (Fig. 1). Indeed, in all kingdoms of life, myriad aquaporins have been shown to play essential roles in stress responses; particularly well studied are those in plants [7] and microbes [8]. Thirty-four unique structures of aquaporin family members (as defined by the mpstruc database [https://blanco.biomol.uci.edu/mpstruc/]) have been deposited in the Protein Data Bank (https://www.rcsb.org). These unique structures include bovine and ovine AQP0, bovine and human AQP1, human AQP2, human and rat AQP4, human AQP5, human AQP7 and human AQP10. The high resolutions of several of these structural models, and the fine detail revealed by the 0.88 Å structure of yeast Aqy1 [9], mean that we now understand the precise mechanism by which aquaporins facilitate the transmembrane flow of water while excluding protons, and how aquaglyceroporin sub-family members facilitate the permeation of other small neutral solutes, ions or even unexpected substrates across biological membranes [5].

The aquaporin field is now regarded by many as being mature, but key knowledge gaps still exist as exemplified by a notable lack of any aquaporin-targeted drug in the clinic. This has prompted some scientists to conclude that aquaporins are intrinsically non-druggable targets. Others, however, have recognised that finding medicines to treat the wide range of disorders of water homeostasis is urgently needed to give hope to the millions of patients for whom no targeted interventions are currently available. This has prompted the search for alternative approaches to blocking the aquaporin pore.

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2. Targeting aquaporin regulation

The structural biology of the aquaporin family (and our consequent mechanistic understanding of aquaporin function) is well established in the scientific literature. Family members share many features such as their common passive mechanism, homo-tetrameric assembly and narrow pore, although in many cases structural information is lacking on the amino- and carboxy-termini, where key regulatory interactions occur. As a consequence, aquaporin regulation is far from comprehensively understood.

It has been known for many years that AQP2 in the mammalian kidney collecting duct rapidly relocalises from intracellular vesicles to the plasma membrane in response to the anti-diuretic hormone, arginine vasopressin, signaling via the vasopressin V2 receptor [10]. This hormone-induced relocalisation response is still widely considered an idiosyncrasy of AQP2; other AQPs are assumed to be constitutively localised to the plasma membrane, a misunderstanding that still persists, in part, today. Notably, stimuli such as hypoxia or hypotonicity directly induce intracellular calcium ion elevations through transient receptor potential channels, triggering sub-cellular relocalisation via intracellular vesicles of the endocytic system for human AQP1, 3, 4 and 5 [11]. In the case of AQP4, a pathological hypoxic stimulus was shown to trigger AQP4 relocalisation to the astrocyte cell surface, exacerbating central nervous system oedema in vivo following injury [12]. Pharmacological inhibition of this mechanism in vivo promoted full functional recovery just 2 weeks after the intervention suggesting that targeting the trafficking of aquaporin proteins to the plasma membrane may well be a viable alternative drug target to direct inhibition of the water-conducting pore [13]. More broadly, subcellular relocalisation of mammalian AQP1, AQP3, AQP4, AQP5, AQP7, AQP8, AQP9 and AQP10 has been reported [14], suggesting that a more dynamic view of aquaporin localisation is required to understand cellular responses to constantly changing extracellular environments.

3. Identifying specific aquaporin modulators

Aquaporins are established drug targets for a variety of disorders associated with disrupted water homeostasis, including brain oedema following stroke or trauma, epilepsy, cancer cell migration, tumour angiogenesis, metabolic disorders and inflammation. Despite this, drug discovery for aquaporins (which has focused predominantly on inhibiting the water pore) has made little progress.

One of the most investigated targets is AQP4 [15]. In the brain and spinal cord, AQP4 is the predominant water channel and its function is exerted at astrocyte endfeet, which encompass the perivascular spaces of the blood-brain barrier (Fig. 2). The degree of AQP4 perivascular enrichment differs between brain regions, but the molecular basis and physiological consequences of these differences are not understood [16]. The role of AQP4 itself remains an area of ongoing debate. Early publications suggested that the clearance of waste products from the sleeping brain was AQP4-independent [17], but a 2018 study [18] established the AQP4-dependence of the glymphatic system. However, the mechanism by which AQP4 exerts its effect remains controversial as prevailing osmotic gradients are likely to limit the ability of AQP4 to mediate brain water flow; water may instead be co-transported by diverse solute transporters [19].

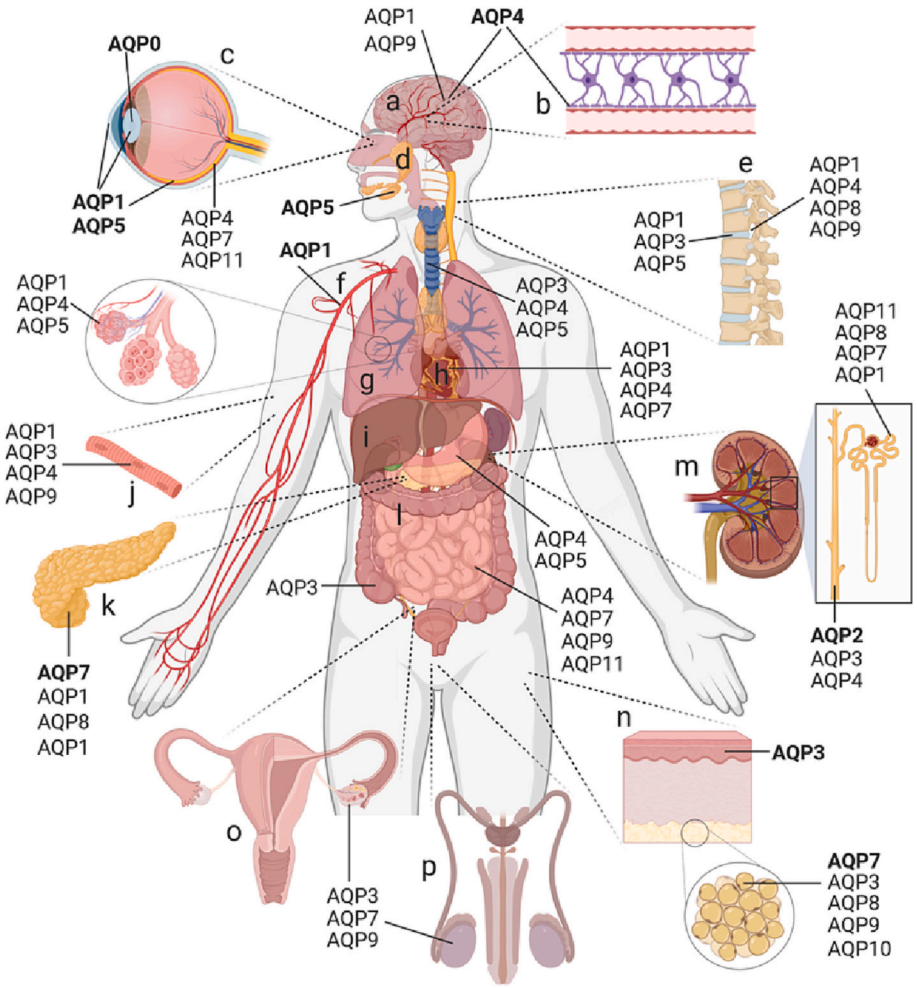

Fig. 1. Aquaporin distribution in the human body. Location of each of the thirteen human aquaporins is shown in (a) brain, (b) blood–brain barrier, (c) eye, (d) exocrine glands, (e) inner ear, (f) cardiovascular system, (g) spine, (h) heart, (i) respiratory tract (trachea and lung; inset showing alveoli), (j) skeletal muscle, (k) pancreas, (l) liver, (m) gastrointestinal tract, (n) kidney, (o) skin (inset showing adipose tissue), and (p) female as well as (q) male reproductive tracts. Minor aquaporin subtypes are omitted for clarity. Bold text is used to highlight the major aquaporins studied in the selected tissues.
Created with BioRender.com and originally published in reference [6].

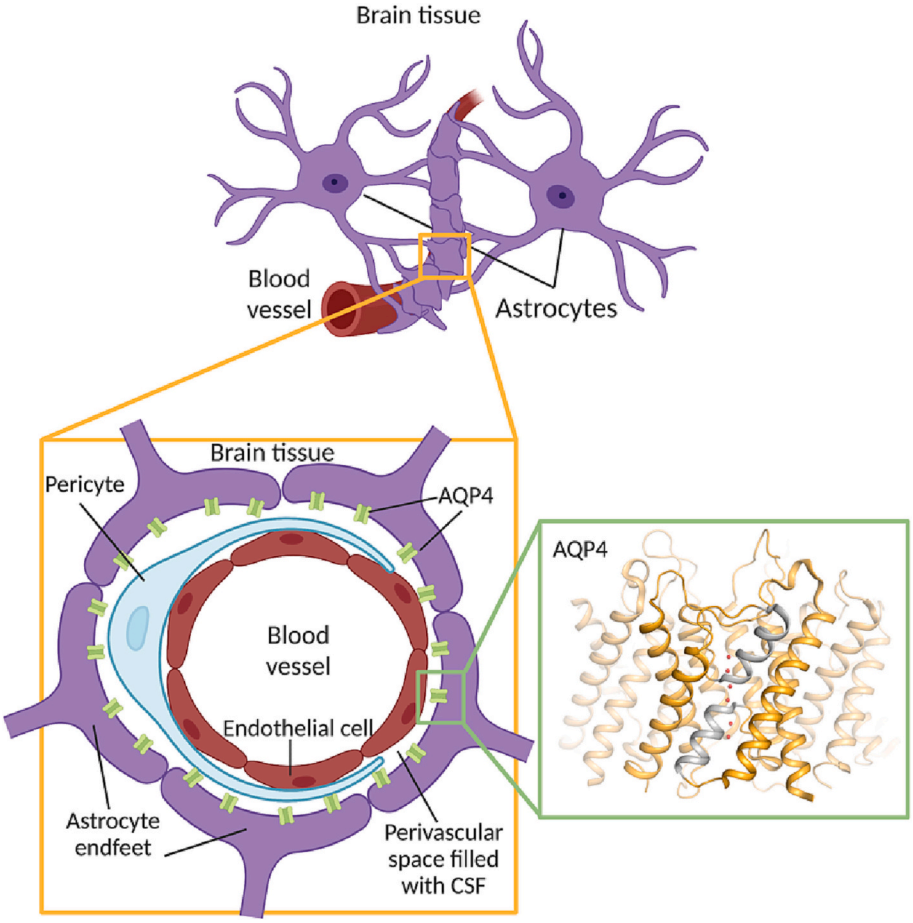

Fig. 2. AQP4 is enriched in the perivascular astrocyte endfeet of the blood-brain barrier. The endfeet of cerebral astrocytes (purple) wrap around the cerebral vasculature (endothelial cells are in red, pericytes are in pale blue) forming perivascular spaces that are filled with cerebrospinal fluid (CSF). AQP4 (green) is enriched at these perivascular endfeet and is a validated drug target for disorders of brain water homeostasis. Its precise role as a passive water channel remains unclear.
Created with BioRender.com.

One approach to conclusively addressing this open question is to use specific AQP4 modulators [20,21]. However, the inhibitory action of the majority of currently proposed molecules, including commercially available TGN-020, has been challenged [22]. Others, such as AER-270, have AQP4-independent effects on brain water transport. This limits their value when interpreting the AQP4-dependence or independence of a cellular process [5]. The identification of specific AQP4 modulators that either target localisation or block the pore is therefore a pressing challenge for the field.

4. Concluding remarks

Aquaporins are validated drug targets, but it is still unclear whether their pores are intrinsically undruggable or whether they can be blocked with small drug-like molecules. Targeting regulatory mechanisms such as aquaporin subcellular relocalisation provides an alternative approach for drug development, as demonstrated for AQP4 in central nervous system oedema models [12]. Identification of validated modulators will ensure the robust interpretation of aquaporin-dependent or independent effects. In the future, understanding the implications of aquaporin localisation as a regulatory mechanism may open up new, unanticipated roles for aquaporins.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: RMB reports financial support was provided by the Biotechnology and Biological Sciences Research Council (BB/T00746X/1 and BB/P025927/1) and Horizon Europe (Grant agreement 847419). RMB reports a relationship with Estuar Pharmaceuticals that includes equity or stocks. RMB is Executive Editor of BBA Biomembranes.

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