Endocrine (2012) 42:63–68
DOI 10.1007/s12020-012-9634-y

MINI REVIEW

The evolutionary origin of the vasopressin/V2-type receptor/aquaporin axis and the urine-concentrating mechanism

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Received: 5 December 2011 / Accepted: 8 February 2012 / Published online: 29 February 2012

Abstract In this mini-review, current evidence for how the vasopressin/V2-type receptor/aquaporin axis developed co-evolutionary as a crucial part of the urine-concentrating mechanism will be presented. The present-day human kidney, allowing the concentration of urine up to a maximal osmolality around 1200 mosmol kg^{-1}—or urine to plasma osmolality ratio around 4—with essentially no sodium secreted is the result of up to 3 billion years evolution. Moving from aquatic to terrestrial habitats required profound changes in kidney morphology, most notable the loops of Henle modifying the kidneys from basically a water excretory system to a water conserving system. Vasopressin-like molecules has during the evolution played a significant role in body fluid homeostasis, more specifically, the osmolality of body liquids by controlling the elimination/reabsorption of fluid trough stimulating V2-type receptors to mobilize aquaporin water channels in the renal collector tubules. Recent evidence supports that all components of the vasopressin/V2-type receptor/aquaporin axis can be traced back to early precursors in evolutionary history. The potential clinical and pharmacological implications of a better phylogenetic understanding of these biological systems so essential for body fluid homeostasis relates to any pathological aspects of the urine-concentrating mechanism, in particular deficiencies of any part of the vasopressin-V2R-AQP2 axis causing central or nephrogenic diabetes insipidus—and for broader patient populations also in preventing and treating disturbances in human circadian regulation of urine volume and osmolality that may lead to enuresis and nocturia.

Keywords Arginine vasopressin · V2-R · AQPs · Evolution

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Introduction

The evolutionary process that ultimately lead to the development of the urine-concentrating mechanism was originally proposed by Homer Smith, the 'Father of Renal Physiology' [1], and is now generally accepted: First crucial step was salt water vertebrates' simple nephron developing into nephrons with large glomerular capillaries and proximal and distal tubules in fresh water vertebrates, allowing the excretion of the vast amounts of excess water that enters osmotically across the body surfaces in fresh water habitats (Fig. 1). The next great leap forward was the development of smaller glomerular capillaries in amphibians and reptiles, an adaptation to habitats with varying access to water by adjusting the rate of filtration at the glomerulus. The final step was the development of nephrons with longer loops of Henle in both birds and mammals, creating an interstitial concentration gradient from the cortex to the medulla of the kidneys. In mammals, short-looped and long-looped nephrons were developed with an emergence of thin ascending limb of Henle's loop reflecting a differentiation of the inner medulla in mammalian kidney.

However, the urine-concentrating mechanism came into being not only by changes in kidney morphology. The evolution of several functional molecules was also needed for the adaptation to limited water supply. In particular, vasopressin-like molecules has during the evolution played

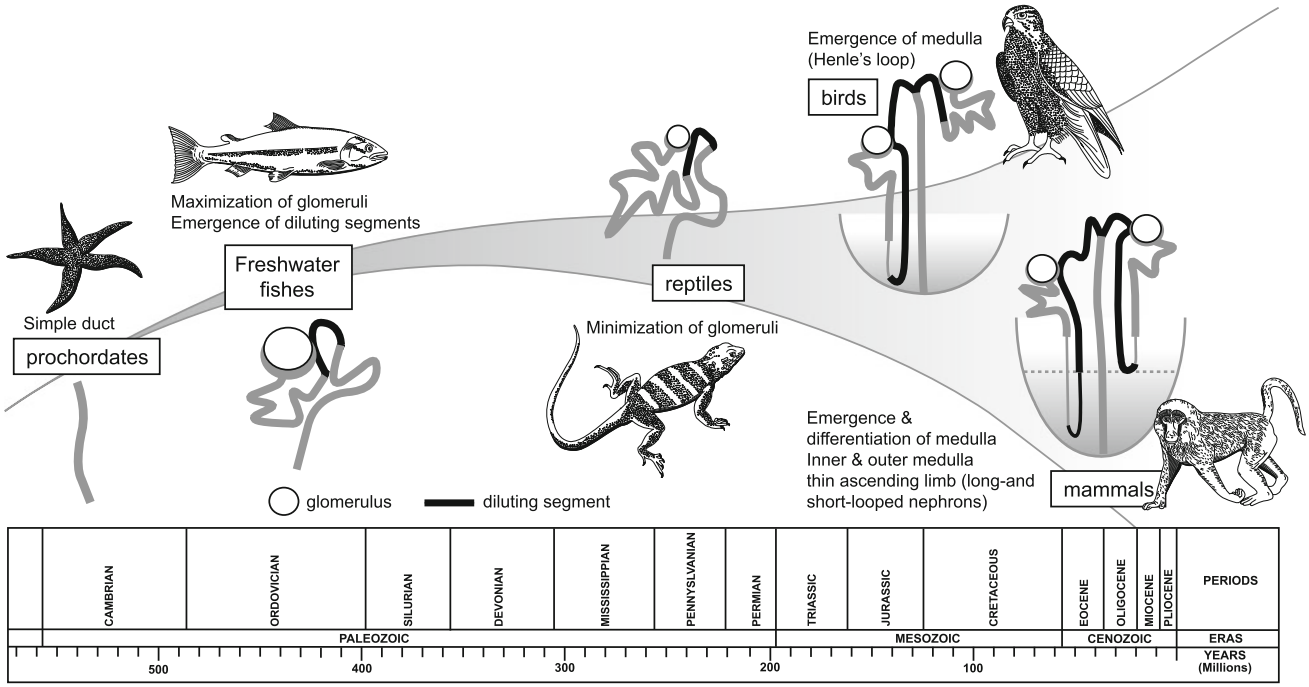

Fig. 1 The evolution of the kidney. Modified with kind permission from Springer Science + Business Media: Kondo Y, Morimoto T, Nishio T, Aslanova UF, Nishino M, Farajov EI, Sugawara N, Kumagai N, Ohsaga A, Maruyama Y, Takahashi S. Phylogenetic, ontogenetic, and pathological aspects of the urine-concentrating mechanism. Clin Exp Nephrol. 2006 Sep;10(3):165–74. Fig. 2 [44]

a significant role in body fluid homeostasis, more specifically, the osmolality of body liquids by controlling the elimination/reabsorption of fluid trough stimulating V2-type receptors to mobilize aquaporin water channels in the renal collector tubules. Since tight body fluid homeostasis is critical for the survival of virtually any living organism in order to maintain stable osmolality in extracellular fluids, the evolutionary origins of neural, humoral, and renal mechanisms involved in fluid homeostasis, among them most important the renal-neurohypophysial vasopressin–vasopressin receptor system and the aquaporin water channels, can be traced back to precursors developed in simpler organisms very early in evolutionary history.

In order to fully understand the ability of present-days human kidney to produce hypertonic urine (with urine to plasma osmolality ratio over 1.0[2]) and to modify urinary osmolality within a wide range of 30–1200 mosmol kg^{-1} in order to maintain serum osmolality within the stable range 280–300 mosmol kg^{-1} [3], it is important to recognize the increasing phylogenetic evidence on how mammalian urine-concentrating mechanism may have evolved from early predecessors. In this mini-review, current evidence for how the vasopressin/V2-type receptor/aquaporin axis developed co-evolutionary as a crucial part of the urine-concentrating mechanism will be presented.

PubMed was searched using the following keywords: Vasopressin; V2-type Receptor; Aquaporin; Urine-Concentration; Evolutionary Origin; Molecular Phylogeny, additional relevant literature were identified by citation chasing. No limitations by date of publication was applied, however, only English language literature was included, unless a non-English paper was deemed of particular relevance.

Evolutionary origin of the vasopressin/oxytocin hormone superfamily

The vasopressin/oxytocin hormone superfamily includes vasopressin, oxytocin and related peptides, and is present both in vertebrates and invertebrates[4].The member of the superfamily that regulates the urine concentrating mechanism, vasopressin, is a semi-cyclic peptide hormone, composed of nine amino acids (Table 1)[5]. The endocrine vasopressin/oxytocin signalling systems are involved not only in the physiology of fluid balance, but also in carbohydrate metabolism, thermoregulation, immunity and reproduction, underlining the superfamily's functional significance in the evolution [6].

Genes encoding vasopressin-like peptides and receptors probably evolved as far back in time as more than 700 million years ago [7], and the typical architecture of the precursors vasopressin-like molecules was present already in the Archaemetazoa, a stem group from which both vertebrates and invertebrates diverged about 600 million

Table 1 Antidiuretic hormones across vertebrate species

	Hormone	Species
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH2	Vasopressin	Mammals[a]
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH2	Lysipressin	Pigs, hippopotamuses, warthogs, some marsupials
Cys-Phe-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH2	Phenypressin	Some marsupials
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly-NH2	Vasotocin[b]	Non-mammals

[a] Vasopressin is not found in some marsupials, pigs, and some other mammals

[b] Vasotocin is the progenitor of the vertebrate neurohypophysial hormones. Only vasotocin is found in hagfish and lampreys

years ago [8, 9]. While all invertebrates express only a single member of the oxytocin/vasopressin superfamily, vertebrate species in contrast, except for jawless fishes, contain at least one vasopressin and one oxytocin family member [10]. Since the most primitive jawless fishes only have vasotocin (Table 1), the evolutionary progenitor of vasopressin, it is now generally accepted that genes coding for oxytocin and vasopressin have arisen by duplication of a common ancestral gene after the radiation of jawless fishes that occurred between 500 and 450 million years ago, largely during the Ordovician period (Fig. 1) [11, 12].

The following model describing the evolutionary origin of the vasopressin and its evolutionary progenitors was proposed by Archer[11, 13]: In early vertebrate evolution a single ancestral gene that encoded precursor vasopressin-like molecules underwent a gene duplication that resulted in the formation of the two lineages in the vasopressin/oxytocin superfamily: While the original gene continued to encode the vasopressin-like peptide, vasotocin, the duplicated gene encoded an oxytocin-like peptide. In the bony fishes, the oxytocin-like gene underwent further point mutations that produced isotocin. In early tetrapod evolution, the gene encoding isotocin mutated trough additional steps to encode oxytocin in mammals. Similarly, vasopressin was formed in mammals after point mutations in the gene encoding vasotocin, with a further subsequent point mutation in the pig family, in which lysine replaced arginine with the creation of lysine vasopressin (LVP) (Table 1). After the gene duplication that led to vasopressin and oxytocin genes, the vasopressin lineage did not function in reproduction, while oxytocin in mammals became associated with mammary gland milk ejection, uterine contractility, vas deferens ejaculation, prolactin secretion and reproductive behavior [4]. Interestingly, the pituitary gland, that produces these two hormones and others, exists only in vertebrates, and probably only evolved in early vertebrates after the large-scale vasopressin-oxytocin genome duplications.

Starting in the Silurian Period 440–410 million years ago (Fig. 1), shortly after the gene duplication, the gradual spread of fishes from brackish (or oceanic) to fresh waters, in other words hypo-osmotic habitats, required that the water–electrolyte metabolism and its regulation be remodelled [14]: While, fishes in seawater drink constantly to cope with osmotic loss of water across body surfaces, fishes in fresh water drink little and urinate abundantly to dispose off excess water that enters osmotically across the body surfaces [15].

Some of the extinct primitive fish species that first colonized fresh water had bony armor that was previously considered as a means of protection from predators, but may rather have served the purpose to protect against this constant environmental osmotic water pressure [1, 14]. The bony armor became obsolete, when newer freshwater species evolved with water-impermeable epithelium of the urinary bladder and other tissues. Interestingly, the urothelium at later stages of the evolution became water permeable again under certain circumstances (as prostaglandin secreted by cells are washed out). This on/off water permeability allowed for swift adaptation to change outer osmotic pressures.

The full transition from aquatic to terrestrial life required further physiological changes to maintain the body-fluid homeostasis: In amphibians, analogue to what developed later in mammalian kidney collecting duct, the water permeability of the urothelium became antidiuretic hormone sensitive [16]. Thus, frogs are able to use their urinary bladder to aid water conservation, when hypotonic urine in dry conditions is reabsorbed from the bladder. Also in humans and other mammals AQPs have recently been reported to be expressed in the urothelium [17], but the function and regulation of these newly discovered water-channels in the bladder epithelium remains unknown.

Thus, the evolution of a steadily more sophisticated interaction between vasopressin-like molecules and aquaporins, particularly in kidneys and bladder, allowed for a more flexible regulation of the osmotic permeability of epithelial cells in fresh water fishes. Evolutionary, this was a key component for the land invasion by amphibians allowing control, the entry of water in and out of the body in both dry and wet habitats.

Evolutionary origin of the V2 vasopressin receptor

With the divergence of the vasopressin/oxytocin hormone super family, as described above, these hormones'

receptors developed co-evolutionary [18]. In mammals two vasopressin receptor subtypes evolved: The V1 vasopressin receptors (V1aR, V1bR) that are involved in blood pressure regulation and adrenocorticotropin hormone secretion, but also in cognitive functions in the brain such as memory and learning. In contrast, the V2 vasopressin receptor (V2R), a 371 amino acid protein, developed with the exclusive function to control the urine-concentrating mechanism [19, 20]. A shared ancestry between the vertebrate vasopressin/oxytocin receptors is supported by a closer phylogenetically grouping of V1 vasopressin receptors with oxytocin receptors than with V2R [4].

The antidiuretic action of vasopressin through activation of V2R is caused by water reabsorption in the kidney, activated via the Gs protein/adenylyl cyclases/cAMP pathway causing mobilisation of water channel aquaporin (AQP2) to the luminal membrane of the distal convoluted tubule and the collecting duct, finally rendering the lipid bilayer of cell membranes permeable to water [21]. Recent research shows that the AQP2 water channels independent of antidiuretic hormonal stimulation recycles continuously between the cell surface and intracellular vesicles, and the effect of vasopressin is therefore likely due to a modification of this equilibrium between the plasma membrane and an intracellular pool of AQP2 channels [22–24]. In mammals, including humans, a deficiency of any part of this axis causes central or nephrogenic diabetes insipidus with polyuria and dilute urine [25–28].

A comparison of over 80 mammalian V2 vasopressin receptor (V2R) orthologs found a high degree of structural and functional conservation of V2R, with close to half of all amino acid residues in V2R remaining unchanged between mammalian species during 170 million years of mammalian evolution [18]. Interestingly, even if many mammalian species have unlimited access to water there is no evidence for a complete loss of V2R function, indicating that V2R activity is essential even in species, where the kidney's urine-concentrating mechanism would be an obsolete function if only access to abundant water was the determining factor. One possible reason could be the circadian need to increase urine osmolality and decrease volume during sleep to avoid nocturnal polyuria and sleep disruptions.

Evolutionary origin of AQP2

Classical aquaporins, the last crucial member of the vasopressin/V2-type receptor/aquaporin axis, are involved in water transport over permeable epithelial cell membrane [29]. The first aquaporin discovered were a type found in red blood cells, and for this discovery Peter Agre was awarded the Nobel Prize in 2003.

Aquaporins have an even older evolutionary history than the other components of the vasopressin/V2-type receptor/aquaporin axis and they have been documented in all kingdoms of life [30, 31]. The three-dimensional structures of the human AQP1 [32] is so similar to the bacterial aquaglyceroporin GlpF [33], that the basic structure of aquaporins must have been conserved over 2–3 billion years of evolution [34]. A mutational analysis suggested recently that AQPs may have evolved in two steps [35]: the initial formation of so-called asparagine–proline–alanine (NPA) boxes, that form pores in the cell membranes while preventing passage of inorganic cations, was followed by subsequent formation of a filter to shut off proton permeability. The first AQP may have had larger pores permitting the uptake of nutrients and release of waste products, but due to a range of modifications of the NPA box classical AQPs, specialized for water transport only, developed with the unique feature of allowing water transport across membranes independently of solute transport [36, 37].

Evolutionary comparisons among aquaporins from bacteria, yeast, plants and mammalians reveals that AQPs are less widespread in bacteria and archaea, in which water fluxes mainly occur through plasma membrane by passive diffusion or unspecific pores [38]. Complete absence of AQPs has been found in thermophilic archaea near submarine volcanoes where water channels may not be necessary with higher water diffusion at high temperatures [37]. In invertebrates (insects, ticks and nematodes), AQPs developed with physiological functions in common invertebrate phenomena such as high-volume liquid diets, cryoprotection and anhydrobiosis [39]. In mammals, including humans, 13 AQP proteins have been clustered into 3 functional groups according to their permeability characteristics in terms of water channels, aquaglyceroporins, and aquaporins with unknown substrate specificity [40]. AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8 are considered water channels, AQP9 being unique compared to other AQPs in that it mediates transport of other small molecules besides water [41], with only AQP2 (and in part AQP3, an aquaporin being expressed in kidney collecting duct, urinary bladder and a number of other epithelium tissue) being responsive to vasopressin as first shown by Nielsen in 1993[42].

V2R and AQP2 have only been found in tetrapods including amphibians, while in contrast, the presence of both V2R and AQP2 has not thus far been demonstrated in fishes [43]. Evolutionary, this indicates that these key components of water-reabsorption promoting the urine-concentrating mechanism were important in the transition from aquatic to terrestrial life forms.

Conclusion

In summary, this mini-review has highlighted important but in general little recognized evidence on how mammalian urine-concentrating mechanism may have evolved from phylogenetic predecessors of vasopressin-like hormones, V2 receptors and aquaporin water channels to facilitate present-days human kidneys urine-concentrating mechanism. The potential clinical and pharmacological implications relates to the pathological aspects of the urine-concentrating mechanism, in particular deficiencies of any part of the vasopressin-V2R-AQP2 axis causing central or nephrogenic diabetes insipidus. While these are potentially life-threatening but rare conditions, increasing knowledge of the evolutionary origin of the urine-concentrating mechanism also offers the prospects of allowing for better understanding of the human circadian regulation of urine volume and osmolality in order to prevent and treat enuresis, nocturnal polyuria and associated bothersome sleep disruptions in broader patient populations.

Acknowledgments The author would like to acknowledge and to express gratitude to my mentor, Professor Jens Peter Nørgaard, for encouraging this study and suggesting significant improvements of early drafts of this mini-review.

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