Osmoregulation during Long-Term Fasting in Lungfish and Elephant Seal: Old and New Lessons for the Nephrologist

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Key Words

Arginine vasopressin · Aldosterone · Kidney function · Osmoregulation · Renin angiotensin system · Salt balance · Water balance

Abstract

Vertebrates control the osmolality of their extra- and intra-cellular compartments despite large variations in salt and water intake. Aldosterone-dependent sodium reabsorption and vasopressin-dependent water transport in the distal nephron and collecting duct play a critical role in the final control of sodium and water balance. Long-term fasting (no eating, no drinking) represents an osmotic challenge for survival. Evolution has found very different solutions to meet this challenge. To illustrate this point, I will discuss osmoregulation of a mammal (elephant seal pup) and of a fish (lungfish) that are able to survive long-term fasting for months or even years. Homer W. Smith taught us how informative comparative anatomy and physiology of the kidney could help physiologists and nephrologists to better understand how the kidney works. In recent years, comparative genomics, transcriptomics and proteomics across the tree of life have led to the emergence of a new discipline, evolutionary medicine. In the near future, physiologists and nephrologists will benefit from this new field of investigation, thanks to its potential for the identification of novel drug targets and therapies.

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Aldosterone-Dependent Sodium Transport and Vasopressin-Dependent Water Transport in the Kidney

The control of sodium and water balance in mammals allows the precise regulation of the osmolality of the intra- and extra-cellular milieu [1]. As beautifully described by Homer Smith in his book 'From Fish to Philosopher' [2], higher cognitive functions of primates could not develop without the appearance during the evolution of one critical homeostatic process, the precise control of the extracellular sodium concentration (i.e. osmolality), appearing with fish and then maintained in amphibians, reptiles, birds and mammals. In humans, osmoregulation is achieved by the dual and tight control of plasma Na concentration ($140 \text{ mM} \pm 2\%$) and plasma osmolality ($290 \text{ mOsmol/l} \pm 1\%$) in the extracellular fluid despite large variations in salt (0.5 g to 30 g/day) and/or water intake (0.5 liter to >10 liters/day) [1]. Osmoregulation of the extra- and intra-cellular fluid is mainly under the hor-

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monal control of the vasopressin-V2-type receptor-aquaporin axis and the renin angiotensin aldosterone system (RAAS) controlling water and sodium reabsorption in the distal nephron and collecting duct of the kidney, the aldosterone sensitive distal nephron (ASDN) and the vasopressin sensitive distal nephron (VSDN). The 2 systems control water and sodium homeostasis independently of each other when challenged by water or salt restriction respectively. Under simultaneous water and salt restriction, a strong synergism between the 2 pathways takes place; vasopressin stimulates both water and sodium transport in principal cells [3], whereas aldosterone stimulates sodium [4] and water transporters in the same cells [5, 6]. ASDN overlaps with VSDN in the principal cells expressed in connecting tubule and collecting ducts.

RAAS: An Evolutionary Perspective

How RAAS has evolved has been recently and extensively reviewed by Fournier et al. [7] and Rossier et al. [1]. Using recent genomic data obtained from a variety of species (bacteria, archaea, unicellular eukaryotes, multicellular organisms and animals), it is possible to construct phylogenetic trees of genes involved either in RAAS or in the action of aldosterone on its target cell in ASDN. Of special interest is to study the genes whose expression is limiting in a given signaling cascade. For RAAS, such analysis was performed for the genes coding for renin (REN), angiotensinogen (AGT), the angiotensin 1 converting enzyme (ACE), the angiotensin 2 receptor (AGTR1) and the aldosterone synthase (CYP11B2) [7]. For the effects of aldosterone on its target cell, the main limiting steps are the 11β -OH-steroid-dehydrogenase type 2 (HSD11B2), the mineralocorticoid receptor MR (NR3C2), the sodium pump Na,K-ATPase (an heterotrimeric membrane protein made of α , β , and γ subunit) (ATP1A1, ATPA1B1, FXYD2) and the epithelial sodium channel ENaC (α , β and γ subunit) (SCNN1A, SCNN1B and SCNN1G) [1]. Looking at orthologous genes (i.e. genes with identical functions), a great variability in the appearance of orthologs during evolution is observed. For instance, the ATP1A1 gene (α subunit of Na,K-ATPase) has orthologs in bacteria, whereas the ATPA21B1 gene (β subunit) appears later linked to the appearance of multi-cellularity, epithelial polarity and the extracellular compartment [1]. The FXYD2 (γ subunit) appears much later at the time of tetrapod emergence [1]. Similar observations can be made for the MR and its ligand (aldosterone). The assembly of a functional signaling cascade

Table 1. Ability to maximally concentrate urine in a selection of mammalian species

Species	Common name	U_{osm}	U/P_{osm}	Reference
<i>Oryctolagus cuniculus</i>	European rabbit	1,390	4.6	[9, 25]
<i>Homo sapiens</i>	Human	1,430	4.8	[9]
<i>Macaca mulatta</i>	Rhesus macaque	1,600	5.3	[9]
<i>Mirounga angustirostris</i>	Northern elephant seal	2,000	5.9	[9, 10]
<i>Rattus norvegicus</i>	Brown rat	2,850	9.5	[9]
<i>Camelus dromedarius</i>	Dromedary camel	3,170	10.5	[9]
<i>Mus musculus</i>	Mouse	6,000	20	[9]
<i>Jaculus jaculus</i>	Jerboa	6,500	22	[8, 9]
<i>Psammomys obesus</i>	Sand rat	6,300	21	[8]
<i>Chaetodipus penicillatus</i>	Desert pocket mouse	8,600	29	[8]
<i>Notemis alexus</i>	Australian hopping mouse	9,370	31	[9]

takes time implying many trials and errors before being selected across the tree of life. For a long time it was thought that the amphibians were the first to adapt to a dry and terrestrial environment, thanks to RAAS and an aldosterone-signaling pathway, allowing efficient sodium reabsorption by the kidney, the skin or the bladder. As discussed below, this view has to be reconsidered in light of new genomic and functional data.

Two Examples of Efficient Osmoregulation during Long-Term Fasting

Elephant Seal

In mammals, the ability to survive in the midst of complete fasting (total food and fluid restriction) depends on the ability to reabsorb salt and water in the ASDN and VSDN. Under salt and water restriction, the mammalian kidney is able to concentrate urine, thanks to an efficient countercurrent mechanism. Under water deprivation, the ability to maximally concentrate urine depends on the size of the animal, the anatomy of renal medulla, the site of the countercurrent mechanism and the type of food ingested, dry (<10%) or rich (>80%) in water [8, 9]. As shown in table 1, the ability to concentrate urine (estimated by the ratio U/P_{osm}) varies greatly from species to species. Typically, U/P_{osm} in humans can reach a value of 4.5 and 9.5 for the rat, while the Australian hopping mouse is the champion in this category (maximum U/P_{osm} 31). Notably, there are species that can survive complete fasting for weeks or months and yet they do not have a very high capacity for concentrating their urine.

The camel ($U/P_{\text{osm}} 10.5$) and the northern elephant seal ($U/P_{\text{osm}} 5.9$) are good examples of osmoregulation achieved without a particularly high ability to concentrate urine. Osmoregulation in marine mammals has been investigated for over a century [10]. In this respect, the pin-niped elephant seal provides extensive information.

(1) Seal maintained their water balance only from pre-formed water in the diet and endogenous water metabolically produced from lipid oxidation. The amount of water-derived, sea-water drinking is negligible.

(2) Elephant seal pups experience a prolonged and complete fasting (no eating, no drinking) just after weaning and for a period of 2–3 months. Before weaning, pups drink milk from their lactating mother for about 2 months. This milk has one of the highest lipid content (up to 60%) known among mammals. The pups will then accumulate a large amount of fat.

(3) Water and salt conservation by the kidney is highly effective during long-term fasting. Dehydration and increased plasma osmolality are minimal, yet the urine-concentrating ability remains modest despite an increased renal medullary thickness, an anatomical feature prerequisite for an efficient countercurrent mechanism.

(4) There is experimental evidence for a functional RAAS and electrolyte balance is regulated by aldosterone. Water reabsorption across the collecting duct is mediated by arginine vasopressin (AVP) as in terrestrial mammals. Surprisingly, plasma AVP remains low during fasting and plasma osmolality remains fairly constant, suggesting that endogenous water production is sufficient to compensate water losses through the lung.

Lungfish

Lungfish have attracted the attention of anatomists, taxonomists, paleontologists, animal biologists, lung and renal physiologists. In many aspects, lungfish are unique animals.

(1) Lungfish share a number of important characteristics with tetrapods. Among these characters are tooth enamel, separation of pulmonary blood flow from body blood flow, arrangement of the skull bones, and the presence of 4 similarly sized fins with the same position and structure as the 4 tetrapod limbs. Amazingly, a recent study [11] suggests ‘that some fundamental features of tetrapod locomotion, including pelvic limb gait patterns and substrate association, probably arose in sarcopterygians before the origin of digitated limbs or terrestriality’.

(2) They can be considered ‘living fossils’ since the fossil records show their presence during the Devonian (410

million years ago). Lungfish burrows have been well documented in fossil records from early Devonian to Cretaceous eras [12]. Interestingly, recent phylogenies indicate that tetrapods are closer to lungfish than to coelacanths [13], a hypothesis recently supported by the discovery of a fossil of a primitive fish close to the last common ancestor of tetrapods and lungfish [14]. There are only three genera of lungfish alive today and each is found on a separate continent. *Neoceratodus* lives in Australia, *Leipodosen* lives in South America and *Protopterus* lives in Africa [15].

(3) African and South American lungfish are obligatory air-breathers. They have typical lung structures originating developmentally from the ventral part of the esophagus with separation of lung blood from the rest of circulation throughout the body [16].

(4) Lungfish live in environments characterized by marked alternations between wet and dry seasons [17]. During the dry season, African lungfish stranded in desiccating areas burrow themselves in the mud, encased in a cocoon made of mucous secretion from the skin, leaving a small orifice for breathing. They can survive for a very long period (2–3 years) until wet conditions interrupt estivation and allow the fish to swim in the water [17]. Recent functional studies indicate the presence of vasotocin-aquaporin [18, 19] and aldosterone-ENaC-signaling pathways [20, 21]. Thus, these signaling pathways have evolved in lungfish before the emergence of tetrapods (amphibians).

Main Differences and Possible Mechanisms

An interesting question is to try to understand why the lungfish is able to adapt so well to complete fasting in comparison with a mammalian species such as the elephant seal pup, the mammalian champion in its category. A summary of the main differences is shown in table 2. During evolution, functional adaptation can be achieved by very different mechanisms implying ‘molecular tinkering’ as proposed by Jacob [16]: lungfish drastically decrease their metabolism and shut down their glomerular filtration, activate their vasotocin-aquaporin pathway but not RAAS, and produce metabolic endogenous water from protein. Elephant seal pups can adapt to a complete fasting for more than 2 months and maintain a high rate of metabolism, by activating RAAS to retain salt and Vasopressin/AQP2 to retain water and compensating water losses by producing metabolic water from lipids.

Table 2. Mechanisms of adaptation to complete fasting: lungfish vs. elephant seal pup

Species	Lungfish	Elephant seal pup
Phenotype	6 months up to 4 years fasting No drinking No eating No evidence of dehydration	2 months fasting (post weaning) No drinking No eating No evidence of dehydration
Metabolism	Down	Up
O ₂ consumption	<15%	Not measured
Endogenously produced water	Up From protein	Up From lipid oxydation
P _{urea}	High	Low
P _{Na}	Normal	Normal
GFR	0 (anuria)	Normal
Countercurrent	No	Yes
U/P _{osm}	Not measurable	5.9–6.3
P _{aldo}	Low	Not measured
RAAS	No activation	Activated
VT or VP-AQP	Activated	No activation?
References	[17–21]	[10, 26]

Evolutionary Medicine: A New Field of Investigation

During evolution, many different solutions have been developed to answer the same functional question: how to get rid of the toxic or metabolic waste (i.e. urea) while retaining the ‘good stuff’ (i.e. sodium, water, glucose) under a variety of environmental challenges? ‘Let us intelligently design a new kidney!’ [22]. The comparison between lungfish and the elephant seal pup demonstrates how different the solutions can be. A wealth of recent data of comparative genomics, transcriptomics, and proteomics have added to old data of comparative physiology and anatomy. For the nephrologist, better molecular understanding of estivation (and de-estivation) may shed new insights into a number of questions: (a) what is the inducer? (b) what are the changes in metabolism? (c) how is GFR shut down without evidence of kidney failure? (d) what is the role of the nephron in its capacity to reabsorb water in an ‘glomerular’ kidney? (e) why is RAAS not activated? Similarly, it would be important to understand how the kidney functions in the elephant seal pup (i.e. the synergism between aldosterone and vasopressin) and why lipid metabolism is used to produce endogenous metabolic water rather than protein as in the lungfish.

Answering these questions should help the nephrologist to better understand human renal physiology and pathophysiology, to design new therapeutic strategies and to identify new drug targets for renal diseases such as acute kidney injury or chronic kidney disease. Evolutionary medicine is a new discipline using an evolutionary-based approach to understand mechanisms related to human diseases such as hypertension and kidney diseases, diabetes, obesity and recently cancer [23, 24].

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Disclosure Statement

The author declares no conflicts of interest.

Statement of Ethics

This study did not require informed consent nor review/approval by the appropriate ethics committee.

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