Vol. 33, Nº 1, enero-junio, 2023

ISSN: 0258-9702

pp. 97-114

Artículo de revisión
Adaptación y mecanismo de la glándula salina

ADAPTACIÓN Y MECANISMO DE LA GLÁNDULA SALINA EN AVES MARINAS

ADAPTATION AND MECHANISM OF THE SALT GLAND IN SEA BIRDS

César Abdiel Amaya-Rodríguez, José Pompilio Young Castillo

[1] Universidad de Panamá. Facultad de Ciencias Naturales, Exactas y Tecnología. Departamento de Fisiología y Comportamiento Animal. Panamá. e-mail: cesar.amaya@up.ac.pa; josep.young@up.ac.pa
https://orcid.org/0000-0003-1916-573X ; https://orcid.org/0000-0002-0832-4347

Autor de correspondencia: cesar.amaya@up.ac.pa

Recepción	Aprobación
02-08-2022	19-11-2022

Resumen

Las glándulas salinas han permitido a las aves marinas afrontar el gran reto fisiológico de vivir en ambientes costeros donde cuentan con poca disponibilidad de agua dulce. Esta adaptación al medio salino ha permitido a las aves marinas ingerir agua del mar sin afectar su equilibrio osmótico. En este artículo de revisión se abordó desde el origen evolutivo, su anatomía, la función de la bomba Na^+-K^+-ATPasa y el contransportador Na^+- K^+- $2Cl^-$ involucrados en el mecanismo celular para eliminar las altas concentraciones de los iones de Na^+ y Cl^- en el plasma sanguíneo a través de la glándula salina, la regulación mediante los receptores muscarínicos acoplados a proteína G, del péptido instetinal vasoactivo.

Palabras claves: Aves marinas, glándula salina, Na^+-K^+-ATPasa, Na^+-K^+-$2Cl^-$, osmorregulación.

Abstract

Salt glands have allowed seabirds to face the great physiological challenge of living in coastal environments where there is little freshwater available. This adaptation to the saline environment has allowed seabirds to ingest sea water without affecting their osmotic balance. In this article, the evolutionary origin, its anatomy, the function of the Na^+-K^+-ATPase pump and the Na^+- K^+- $2Cl^-$ countertransporter involved in the cellular mechanism to eliminate the high concentrations of Na^+ and Cl^- ions in the blood plasma through the saline gland, the regulation by the G protein-coupled muscarinic receptors of the vasoactive intestinal peptide, and the regulation by the G protein-coupled muscarinic receptors of the vasoactive intestinal peptide were discussed.

Keys words: Seabirds, salt gland, Na^+-K^+-ATPase, Na^+-K^+-$2Cl^-$, osmoregulation.

Introduction

Water plays a fundamental role in living beings, it represents 70% of body weight; its consumption is important in homeostasis processes and in the ionic balance of animals (Minieri, 1979; Woodin et al., 2008). Particularly seabirds and other vertebrates are osmoregulatory organisms; maintaining blood concentrations of 250-300 mOsm/L (Gutiérrez et al., 2015) allowing the different cells to perform their functions in a stable manner. The search for freshwater in seabirds can be complicated when freshwater is limited, so dehydration could be an important survival factor (Randall et al., 1998). Throughout evolutionary history, seabirds have adapted to the saline environment because they can ingest seawater due to their osmoregulatory mechanism through salt glands (Sabat, 2000). This mechanism compensates for the limited capacity of avian renal physiology to produce concentrated or hyperosmotic urine. For this reason, a bibliographic review of the adaptation of salt glands in seabirds is carried out and the cellular mechanism that operates in these glands is described.

For the search of information on the adaptation of the salt gland in seabirds, primary sources of original articles published in journals such as: American Journal of Physiology, Journal of General Physiology, Journal of Comparative Physiology, Journal of Experimental Biology, Comparative Physiology and Biochemistry, Endocrinology, among others, were used; in the secondary sources, search engines such as Google Scholar, NCBI and finally tertiary sources such as specialized text books were used. To optimize the search for information, keywords such as Birds, Salt glands, osmoregulation were used, and it was decided to use the English language because most of the literature is written in this language.

Background

The first recorded studies on salt glands are the work done by Jacobson in 1813, where he compared them with Stenson's gland in the nasal cavity of mammals. Jobert in 1869 was the first to demonstrate that in birds there are two pairs of salt glands, using ducks as an experimental model. By the end of the 19th century several researchers (Nitzsch, 1820; Kolliker, 1860; Ganin, 1890; Gadow, 1890) claimed that the saline gland had a link with the vemeronasal or Jacobson's organ, while Born, (1879) and Mihalkovics, (1898) claimed that the saline gland had no relationship with Jacobson's organ, This conflict had its outcome with the investigations of Cohn, (1903) using chicken embryos, who concluded that the internal duct of the saline gland does not resemble the Jacobson's organ in its development, therefore, there is no relation with the Jacobson's organ.

However, the first studies on the function of the salt gland date back to the early 20th century, where Marples (1932) suggested that, seabirds obtained water through their feeding, meanwhile; Murphy in his work on seabirds in South America in 1936, was one of the first researchers to mention that seabirds can drink seawater, a question that remained until the 1950s and was resolved when the function of the salt gland was described (Schmidt-Nielsen and Fange, 1958) which is responsible for secreting excess NaCl in seabirds when ingesting seawater (Fänge, et al., 1958; Doyle, 1960; Schmidt-Nielsen, 1960).

Evolutionary origin of the salt gland

The salt gland is currently found in five taxa: crocodilians, turtles, snakes, lizards, and occur in about 40 families in birds and in almost all orders, with the exception of the order Passeriformes (Peaker and Linzell, 1975; Hackett et al., 2008; Scanes, 2015). Several authors suggest that this structure has evolved in multiple times, curiously there are similarities in the location of the gland, in the position of the duct and the possible embryological origin of the five existing groups, it is hypothesized that the salt glands were present in the ancestor of all diapsids (Fernández and Gasparini, 2000; Babonis and Brischoux, 2012).

Wang et al. (2018) mention that there are records of salt glands in extinct birds from the Early Cretaceous, around 125 million years ago, located in the supraorbital fossa of the reported taxa (*Hesperornis regals* and *Parahesperornis alexi*) from the Late Cretaceous.

Salt gland anatomy

The position of the salt gland, mostly in pelagic seabirds with estuarine habitats, is located supra- or infra-orbitally (Ibañez et al., 2010); they are more developed in cormorants, herring gulls, gannets, pelicans, albatrosses and penguins (Reshag et al., 2016); with the size being directly proportional to the NaCl concentrations to which it is subjected (Woodin et al., 2008; Reshag et al., 2016).

Histological studies have shown that saline gland cells undergo hypertrophy by increasing their cell size (Benson and Phillips, 1964; Peaker, 1973; Reshag et al., 2016; Ali and Reshag, 2021). Schmidt-Nielsen and Fange (1958) indicate that the salt gland of the Brown Pelican (*Pelecanus occidentalis*, Linnaeus, 1766) (Pelecanidae) is oblong or pear-shaped with a length of 2.6-3 cm and a width of 0.6-0.8 cm. Another example is in flamingos (*Phoenicopterus roseus*, Pallas, 1811) (Phoenicopteridae), where the salt gland is approximately 20.8 ± 0.2 mm long and 3.9 ± 0.2 mm in diameter, with a weight of 0.56 ± 0.3 g, these values are matched by the salt gland of the domestic duck (*Anas platyrhynchos*, Linnaeus, 1758) (Anatidae) (Reshag et al*.,* 2016).

Mechanism

Most studies following the description of saline gland function have been by administering large concentrations of NaCl. Fletcher et al. (1967) mention, in their experiments with male Pekin ducks (*Anas platyrhynchos*, Linnaeus, 1758) (Anatidae), that the salt gland utilizes active Na^+ transport with a release of inorganic phosphate from ATP, however, in freshwater adapted ducks the activation of this mechanism was not high. Both Fletcher, et al. (1967) and Holmes and Stewart (1968) indicate that there is a two to six-fold increase in the normal size of the normal gland which allows them to increase NaCl secretion. Several studies have shown that ducks exposed to hypertonic solution increase the activity of Na^+-K^+-ATPase (NKA) pumps, NKA is found in large numbers in the basolateral membrane of salt gland cells, resulting in the passive secretion of Cl^- (Thesleff and Schmidt-Nielsen, 1962; Karlsson et al., 1971; Stewart et al., 1975; Cramp et al., 2010; El–Gohary et al., 2013). On the other hand, the Na^+- K^+- $2Cl^-$ cotransporter (NKCC2), (Fig. 1) in the basolateral membrane actively moving Cl^- ions from the blood into the cell, while in the apical membrane of the cells the secretion of Cl^- occurs, the secretion of Na^+ is given by the paracellular pathway employing the electrochemical gradient, this ionic movement across the membrane allows the secretion of high concentrations of NaCl into the lumen of the tubule of the salt gland (Shuttleworth, 1995; Cramp et al., 2010; Scanes, 2015).

The activation of the saline gland mechanism leads to the secretion of fluids out of the organism, these fluids are composed of various ions and salts in different concentrations, being K^+ and Cl^- the most excreted compounds by the saline gland (Table 1); in experiments on domestic ducks it is suggested that their tears can excrete a considerable amount of K^+ cations, however, it is not clarified (*A. platyrhynchos*) osmoregulatory capacity is unclear (Hughes and Ruch Jr, 1969; Peaker and Linzell, 1975) .

Regulation

The first investigations into how the salt gland is regulated were in experiments by Fänge et al. (1958) (Brown Pelican); Fletcher et al. (1967) (*A. platyrhynchos)* and Kühnel, (1972) (in geese, ducks and swans). They indicate that the nerves controlling the salt gland are parasympathetic in nature. Osmoreceptors located in the heart were thought to be responsible for regulating the salt gland, being activated when there is an increase in plasma tonicity (Schmidt-Nielsen, 1960; Hanwell et al., 1972; Stewart et al*.*, 1975; Hootman and Ernst, 1980; Butler, 2007). However, the hypothesis of the role of osmoreceptors was refuted in experiments on *A. platyrhynchos* (Gerstberger et al., 1984a,b), meanwhile other studies a modulatory role of osmoreceptors in extracellular volume changes produced by osmotic stress is proposed (Hammel et al., 1980). It is now known that the secretory response is regulated by two signaling pathways: 1). Activation of **G protein-coupled muscarinic receptors**, subtype Gq in saline gland cells, intracellularly increases diacylglycerol (DAG) activating protein kinase (PKC) and inositol tritosphate (IP3) which promotes Ca^{2+} release, both second messengers are involved in the regulation of NaCl secretion, cell growth and differentiation; 2). **G-protein-coupled vasoactive intestinal peptide** (VIP), subtype Gs stimulates adenylyl cyclase generating cyclic adenosine monophosphate (cAMP) which activates cAMP-dependent protein (PKA), involved in saline gland secretion and increased blood flow to the saline gland (Shuttleworth and Hildebrandt, 1999; Hildebrandt, 2001; Krohn and Hildebrandt, 2004).

The role of **angiotensin II** hormone (ANG II) in avian physiology has been extensively studied, as it inhibits the saline gland due to its Na^+ conserving function, stimulatesaldosterone production, while participating in the release of arginine vasotocin, and antidiuretic hormone, also stimulates water intake (Holmes et al., 1961; Crocker et al., 1971; Gerstberger et al., 1984; Gray et al., 1986; Heinz et al., 2001; Butler et al., 2006); in experiments in Pekin ducks, serum arginine-vasotocin hormone (AVT), has been shown to induce antidiuresis and is involved in afferent and central control of osmoregulation (Simon-Oppermann et al., 1980). The regulation of adaptive hypertrophy of the saline gland is provided by cholinergic nerves (Hanwell and Peaker, 1975). Studies in *A. platyrhynchos* on atrial natriuretic peptide (ANP) demonstrated an important role in the maintenance of salt and fluid balance in birds, this is because they promote diuresis, natriuresis and ANP has been documented to be an antagonist of the renin-angiotensin-aldosterone system (Gray et al., 1991ab, 1991a; Schütz et al., 1992; Marais and Gray, 2009).

It was thought (Ching et al., 1999) that the hormone melatonin (MT) had an inhibitory effect on salt gland secretion in Pekin ducks but not in gulls, however, studies by Hughes, et al. (2007) propose an osmoregulatory role in birds with salt glands as it increases urinary Na^+ concentrations; Müller et al. (2006) in their experiments with *A. platyrhynchos* describe the expression of aquaporins (AQP1 and AQP5) in different salt gland cells involved in the production of hypertonic fluid. A basal increase of the hormone corticosterone has been reported in birds that possess salt glands, different researchers suggest that the increase may be due to the energy expenditure that birds must make to excrete the elevated levels of salt in the body and the osmotic stress faced by the environment where they live (Gutiérrez et al., 2011; Brischoux et al., 2015).

Studies with the salt gland

Currently, salt gland investigations are focused as a model to study the dynamics of NKA in active ion transport through different techniques such as: Fourier transform infrared spectroscopy (Pratap et al., 2003), coupled fluorescence enzyme assays (Pratap et al., 2009) and total internal reflection fluorescence microscopy ((Pratap et al., 2011). These studies have focused on the conformational changes (*E1*) of NKA induced by ATP, the kinetics of ATP hydrolysis by the ATPase enzyme, isolated with the Cy3-melaimide compound, given through negative cooperativity has been elucidated (Pratap et al., 2003; Pratap et al., 2009; Pratap et al., 2011). Studies have also been conducted on the kinetic properties of NKA, with the aim of explaining whether the functional unit of the ATPase enzyme is a protomer-$\alpha\beta$ (Martin et al., 2000) or an oligomer, the latter being the most accepted at present (Taniguchi et al., 2001; Clarke et al., 2007). In addition, research has been conducted focusing on the sensitivity of NKA in reduction-oxidation changes, which is possibly determined by glutathionylation (Petrushanko et al., 2012; Dergousova et al., 2017; Dergousova et al., 2018).

Conclusions

The cellular mechanism involved in the secretion of high concentrations of NaCl through salt glands is mainly by activity of NKA and NKCC2 cotransporter located in the basolateral membrane. Due to their phylogeny, seabirds have been able to adapt to saline environments and consume seawater while maintaining the constancy of the internal environment.

Author contributions: Conceptualization, Amaya, C.; methodology, Amaya, C. and Young. J.; validation, Amaya, C. and Young. J.; formal analysis, Amaya, C. and Young. J.; research, Amaya, C. and Young. J.; writing - preparation of the original draft, Amaya, C. and Young. J.; writing - proofreading and editing, Amaya, C. and Young. J.; supervision, Young, J. All authors have read and accepted the published version of the manuscript.

Funding: This research did not receive external funding.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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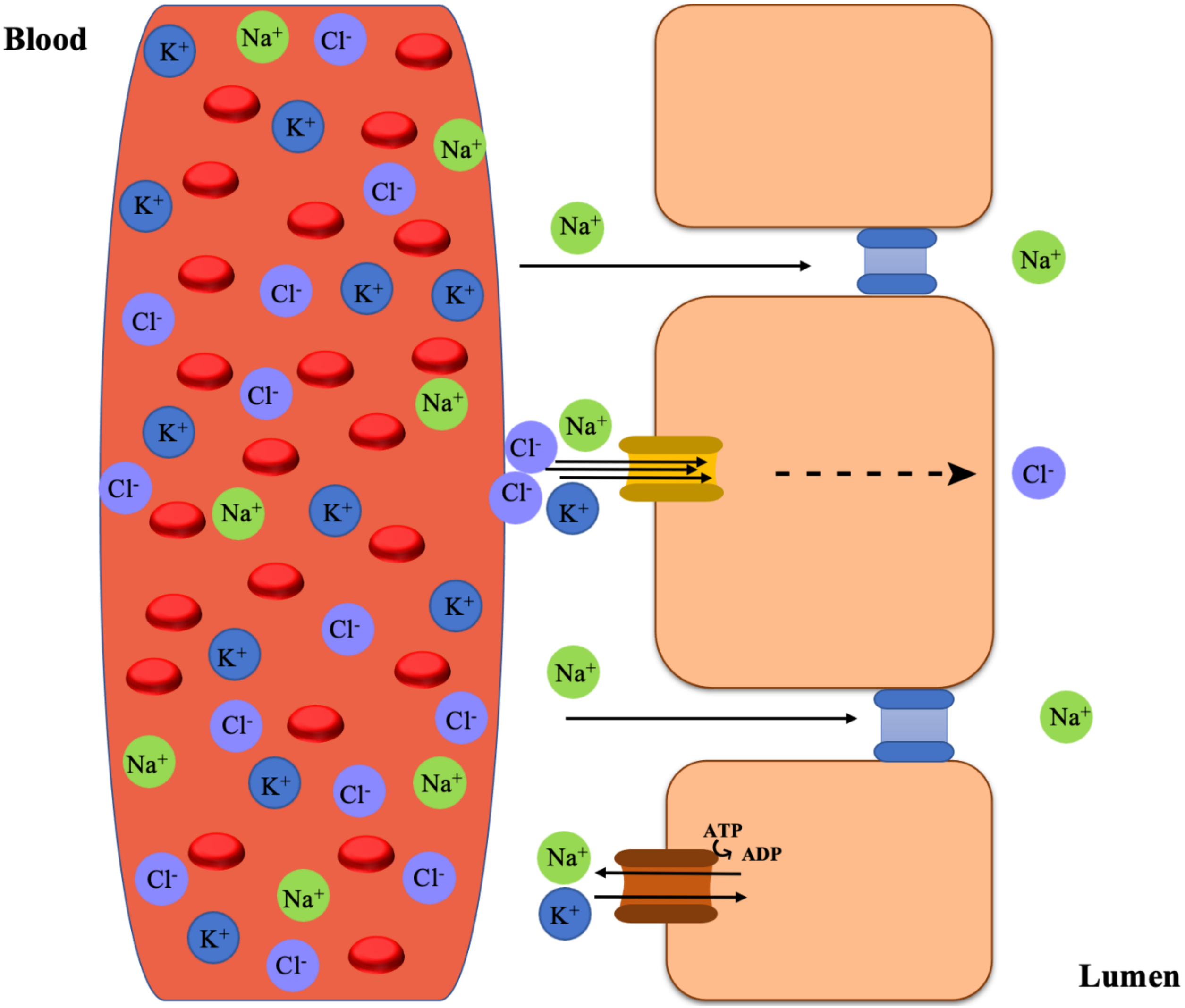

Fig. 1 The Na^+- K^+- $2Cl^-$ cotransporter located in the basolateral membrane of salt gland epithelium cells is responsible for secreting high concentrations of NaCl from the blood into the nasal passages of seabirds.

Table 1. Composition and concentration of fluids excreted through the salt gland.

Fluid composition	***Concentration [mmol 1 $^{-1}$]***
K^+	718
Na^+	24
Ca^{++}	1
Mg^{++}	1
Cl^-	720
$NaHCO_3$	13
SO_4^{2-}	0,35