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Cells in focus

Frog skin epithelium: Electrolyte transport and chytridiomycosis

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ABSTRACT

One unique physiological characteristic of frogs is that their main route for intake of water is across the skin. In these animals, the skin acts in concert with the kidney and urinary bladder to maintain electrolyte homeostasis. Water absorption across the skin is driven by the osmotic gradient that develops as a consequence of solute transport. Our recent study demonstrated that chytridiomycosis, an infection of amphibian skin by the fungal pathogen, *Batrachochytrium dendrobatidis*, inhibits epithelial Na^+ channels, attenuating Na^+ absorption through the skin. In frogs that become severely affected by this fungus, systemic depletion of Na^+, K^+ and Cl^- is thought to cause deterioration of cardiac electrical function, leading to cardiac arrest. Here we review the ion transport mechanisms of frog skin, and discuss the effect of chytridiomycosis on these mechanisms.

Cell facts

- Frog skin is an electrically tight epithelium, comprised primarily of principal cells, with a minority of mitochondria-rich cells interspersed.
- Principal cells of frog skin can absorb Na^+ against its concentration gradient.
- Superficial infection by *Batrachochytrium dendrobatidis* inhibits absorption of Na^+ across the skin, leading to depletion of plasma electrolytes and death.

1. Introduction

Fluid and electrolyte homeostasis in amphibians is maintained by fine balance of the activity of the kidneys, urinary bladder and skin. In these animals, the kidneys produce copious volumes of dilute urine, and the bladder serves mostly as a reservoir of water during terrestrial activity (Uchiyama and Konno, 2006). The unique properties of amphibian skin of having high permeability to water and electrolytes, therefore, allow this tissue to contribute to osmoregulation and electrolyte and fluid homeostasis. The outermost layer of frog skin, the *stratum corneum*, is composed of a thin layer of keratinized cells, offering very little resistance to movement of water between internal and external environments (Lillywhite, 2006). Consequently, terrestrial and semiterrestrial frogs are subject to water loss via evaporative dehydration (Lillywhite, 2006). Frogs do not exhibit primary drinking behavior for the purposes of relieving thirst or for rehydration. Instead, the main route for water intake is across the ventral skin, especially the highly vascularized pelvic patch (Parsons and Mobin, 1991).

Water intake is under tight control by various physiological factors, in particular the neurohypophyseal hormone, vasotocin (Uchiyama and Konno, 2006). At least two types of amphibian-specific vasotocin-sensitive aquaporin water channels, AQP-h2 and AQP-h3, have been identified in cells of the pelvic patch (Hasegawa et al., 2003). Water absorption across the skin occurs more quickly when frogs are in solutions containing NaCl, than when bathed with deionized water (Hillyard and Larsen, 2001). The rate of water absorption is linearly related to the rate of Na^+ influx, and is dependent upon the activity of the Na^+/K^+ ATPase (Larsen et al., 2009). Absorption of water, therefore, is coupled to Na^+ transport and energized by the activity of the pump (Larsen et al., 2009). Recent studies suggest that reduced Na^+ transport in frog skin is a key feature of the pathophysiology of chytridiomycosis (Voyles et al., 2009). This amphibian disease is caused by the lethal fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), and has been

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implicated in global declines in amphibian populations (Fisher et al., 2009b).

2. Cellular origin

The epidermal surface of frog skin is derived from ectoderm (Jones and Woodland, 1986). The basal layer of the epidermis, the *stratum germinativum*, is composed of columnar or cuboidal cells (Farquhar and Palade, 1965). These cells migrate superficially through the *stratum spinosum* and *stratum granulosum* layers as they mature, ultimately becoming keratinized in the *stratum corneum* (Farquhar and Palade, 1965). Principal cells comprise 90% of the *stratum granulosum*, the layer most prominently involved in active electrolyte transport (Larsen, 1991). Interspersed throughout, and comprising up to 10% of the epithelial volume of the *stratum granulosum*, are flask-shaped mitochondria-rich (MR) cells (Larsen, 1991).

3. Electrolyte transport in frog skin

Most of our knowledge about active Na^+ absorption in epithelial cells originated from the seminal studies by Hans Ussing on the isolated skin of *Rana temporaria*. Prior to Ussing, it had been established that frog skin can absorb electrolytes from pond water against a large chemical gradient (Krogh, 1937). The detail of the mechanism underlying this process was elucidated only later when Ussing developed a technique to quantitatively evaluate active transepithelial Na^+ absorption. Ussing's system allowed each side of the isolated frog skin to be exposed to different bathing solutions. He then used two different approaches to measure active Na^+ absorption. Firstly, he bathed both sides of the skin with symmetrical solutions and introduced the radioisotope, $^{24}Na^+$, into the solution bathing the pond-side membrane to measure net Na^+ flux (Ussing, 1949). He then used an electrophysiological method to determine the transepithelial potential difference, observing that the pond side of the skin was negative relative to the blood side (Ussing, 1949). Subsequently, Ussing applied a novel technique to determine the current required to drive the potential difference across the skin to 0 mV, which he then named the "short-circuit current". Since both sides of epithelium were exposed to symmetrical solutions, all passive transepithelial ion movement was eliminated. The short-circuit current measured under these conditions, therefore, is equal to net flux of ions transported via active mechanisms. Since the current predicted from the transepithelial $^{24}Na^+$ flux, was almost equivalent to the short-circuit current (Ussing and Zerahn, 1951), he concluded that frog skin actively transports Na^+ from pond water to blood plasma with negligible retrograde movement.

In subsequent studies, Ussing removed Cl^- from the solution on both sides to eliminate the contribution of the major anions to the short-circuit current. This approach allowed assessment of ion permeability of the membrane on either side of the epithelium. He observed that the pond side membrane of frog skin is primarily Na^+ selective, while the blood side was highly selective for K^+ (Koefoed-Johnsen and Ussing, 1958). These observations led to the formulation of the 'Two-Membrane Model of Epithelial Transport', one of the most important paradigms in epithelial physiology. In this model, activity of the Na^+/K^+ ATPase in the basolateral membrane of the principal cells exchanges cytosolic Na^+ for plasma K^+ (Fig. 1). By maintaining a low intracellular Na^+ concentration, Na^+ ions in the pond water passively moved into the cell via Na^+-selective channels. Excess intracellular K^+ generated by activity of the Na^+/K^+ ATPase is returned to the plasma across the basolateral membrane via K^+-selective channels (Nagel and Hirschmann, 1980; Larsen, 2011). The combination of a Na^+-selective apical membrane and K^+-selective basolateral membrane allows

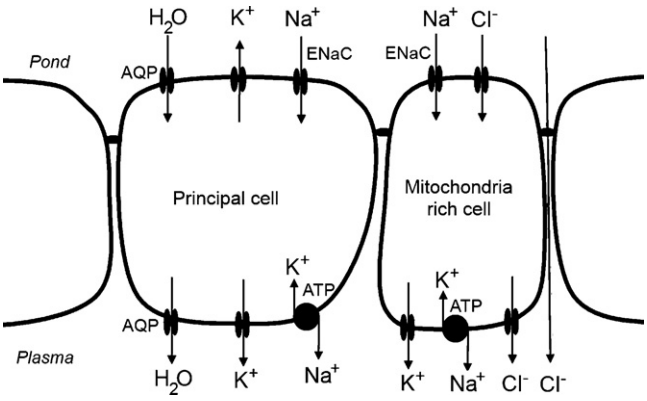

Fig. 1. Transport model of the principal and mitochondria-rich cells of the frog skin epithelium. Na^+ is moved from the pond solution via epithelial Na^+ channels (ENaC) in the apical membrane and extruded via the Na^+/K^+ ATPase in the basolateral membrane. Excess cytosolic K^+ generated by activity of the Na^+/K^+ ATPase is recycled to the plasma via K^+ channels in the basolateral membrane. The transepithelial potential difference generated by this mechanism drives absorption of Cl^- through mitochondria-rich cells and through paracellular pathways. NaCl absorption generates an osmotic gradient that drives absorption of water via aquaporin channels (AQP).

absorption of Na^+ to generate a large transepithelial potential difference (Koefoed-Johnsen and Ussing, 1958). In turn, this potential drives parallel uptake of Cl^- from pond water through Cl^- channels in mitochondria-rich cells (Voûte and Meier, 1978; Larsen, 2011) and paracellular pathways (Ussing and Windhager, 1964) (Fig. 1). The electroneutral, absorption of NaCl generates an osmotic driving force that facilitates uptake of water across the skin. The molecular identity of these Na^+-selective channels was not confirmed for almost 40 years after Ussing's studies (Canessa et al., 1994; Takada et al., 2006).

These epithelial Na^+ channels (ENaC) play an important role in active Na^+ absorption in a variety of epithelia. In mammals, the function of ENaC is important for the regulation of body fluid volume, blood pressure and maintenance of the depth of alveolar fluid (Garty and Palmer, 1997). Dysfunction of ENaC has been associated with disorders of Na^+ and fluid homeostasis, blood pressure, and lung fluid balance (Schild, 2004). It has also been reported that activity of ENaC in the lung is adversely affected by respiratory pathogens (Kunzelmann et al., 2004; Hee et al., 2011). Recent studies suggest that activity of ENaC in frog skin is inhibited by the fungus, *Bd*, leading to the fatal pathogenesis of chytridiomycosis (Voyles et al., 2009).

4. Associated pathologies: pathogenesis of chytridiomycosis

The global decline and, in some cases, extinction, of amphibian species has been attributed to the chytridiomycosis pandemic (Fisher et al., 2009b). Identified in 1998 (Berger et al., 1998), *Bd* belongs to the phylum Chytridiomycota, and is the only member of this fungal family known to parasitize vertebrate hosts with fatal effect (Longcore et al., 1999). *Bd* zoospores predominantly colonize the skin of the ventral abdomen and toes, but are rarely found on the dorsal skin of infected frogs (Berger et al., 2005b). Infection is contained within the superficial layers of the epidermis, and causes hyperkeratosis with cytoplasmic degeneration and vacuolation (Berger et al., 1998) (Fig. 2). Visible lesions of the skin, however, are not common and no histologically detectable changes in internal organs have been observed (Berger et al., 1998; Voyles et al., 2009).

Concomitant with the pathological changes in the superficial epidermis, frogs develop inappetance, lethargy, loss of righting