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Tokyo Institute of Technology

Mayo Clinic

Press Release

Absorbing sodium from fresh water in exchange with waste

– Identification of a Na^+/NH_4^+ exchanger in teleost gill –

- Freshwater teleosts absorb Na^+ from low-salt environments in the body as a nutrient.
- Na^+ is considered to be absorbed in exchange with ammonium ions. Here, we identified the molecule that mediates Na^+/NH_4^+ exchange.
- This study will contribute to elucidate the mechanism by which teleost fish adapt to freshwater, and may provide clues for understanding the acid waste system of the human kidney.

Summary

A collaborative team from the Tokyo Institute of Technology and Mayo Clinic identified a Na^+/NH_4^+ exchanger for the first time in the gill of freshwater fish and characterized its activity. Freshwater fish absorb Na^+ from low-salt environments from the gill. Approximately 70 years ago, Na^+ was suggested to be absorbed in exchange with NH_4^+; however, the molecule that mediates the Na^+/NH_4^+ exchange has not been identified. Here, we analyzed the function of a Na^+/H^+ exchanger (NHE3), which is expressed in zebrafish gill, and found that NHE3 also acts as a Na^+/NH_4^+ exchanger. This result indicated that freshwater fish use NHE3 as an energy saving system that mediates the exchange of NH_4^+ (waste) and Na^+ (nutrient). This study will contribute to elucidate the mechanism by which teleost fish adapt to freshwater. In humans, NHE3 is involved in the acid waste system in the kidney. However, there is no good system that analyzes the NH_4^+-transport activity of human NHE3. Our results may provide clues for the analysis of the NH_4^+-transport activity of human NHE3.

Background

Na^+ is the major cation in extracellular fluid, and the plasma of human and teleosts contains 140–170 mM Na^+. Freshwater teleosts live in environment that contains less than 1 mM of Na^+. Therefore, they are always at risk to of losing ions. However, freshwater fish can absorb Na^+ from their gills and maintain the ion balance of their body fluid (Fig. 1). August Krogh, a Danish physiologist, found such a phenomenon and proposed the hypothesis that Na^+ in freshwater is absorbed in exchange with internal ammonium ion (NH_4^+) approximately 70 years ago (Krogh, 1939). However, the protein that mediates the Na^+/NH_4^+ exchange has not been identified.

In the gill, there are cells called ionocytes that are dispersed over the gill surface (Fig. 1). Ionocytes have functionally similar characteristics to renal tubular cells, which mediate the ion balance of the body. In fish larvae, ionocytes are present on the body surface (skin). In 2003, a collaborative team from the Tokyo Institute of Technology (Hirata, Hirose), The University of Tokyo, the National Cerebral and Cardiovascular Center in Japan, and Case Western Reserve University (Romero) analyzed the gills of Japanese dace living in an acidic lake (pH 3.5) and found for the first time that a Na^+/H^+ exchanger, NHE3, is highly expressed in ionocytes on the freshwater side of the plasma membrane (apical membrane) (Hirata et al., 2003). Subsequently, NHE3 was also reported to be expressed in the ionocytes of freshwater teleosts such as zebrafish, tilapia, medaka, and rainbow trout and of an elasmobranch (Atlantic stingray) (Choe et al., 2005), causing NHE3 to be recognized playing a major role in the Na^+ absorption system of freshwater fish. However, these experiments did not prove that NHE3 can absorb Na^+ from freshwater as opposed to NHE3 leaking Na^+. Thus, this issue created a conundrum in fish physiologists for over a decade.

Why should medical researchers care about or work with fish or other model/lower organism solute transporters? This is the true beauty of physiology being the scientific basis of clinical medicine: organs, cell types, and solute transporter genes/function are often highly conserved. NHE3 in the mammalian and human kidney allows Na^+ to be absorbed from the forming urine, whereas simultaneously discards H^+ waste that is generated by normal cell metabolism. This same NHE3 protein performs a similar function in the epithelia of the intestine, pancreas, liver, lungs, and reproductive systems (Kato and Romero, 2011). The kidney normally adjusts its absorptive and secretory activities to maintain a balance of all ions, including H^+ (pH) and Na^+. Whole-body Na^+ is partially controlled by hormones such as angiotensin and aldosterone that are the part of the renin–angiotensin–aldosterone system (RAAS). Defects in the RAAS often result in hypertension, which can be mediated by inappropriate activity of NHE3 in the kidney and intestine. The Na^+/H^+ exchange activity of NHE3 is activated in the state of human diseases such as metabolic or respiratory acidosis. Cells in the kidney proximal tubule respond by a process called ammoniagenesis, which generates cellular NH_3, allowing the kidney to excrete more acid (H^+ and NH_4^+) and facilitates absorbtion of more pH buffer (HCO_3^-) into the blood. Because cellular NH_3 and NH_4^+ are generated in this process, scientists have hypothesized for over 2 decades that the Na^+/H^+ exchanger in the proximal tubule (NHE3, Slc9a3) is capable of Na^+/NH_4^+ exchange to accomplish this additional discard of H^+ waste. Nevertheless, this Na^+/NH_4^+ exchange activity has not been previously shown experimentally to be a property of any NHE3.

Research outcome

In this study, we analyzed the function of zebrafish NHE3 that is expressed in *Xenopus* oocytes using the H^+, Na^+, and NH_4^+-ion selective microelectrode. We selected the *Xenopus* oocyte because its plasma membrane has low permeability to ions and ammonia. Therefore, we can observe the effects of the exogenous expression of an ion transporter. Normal oocytes showed only small changes in the intracellular

concentrations of H^+ and Na^+ when exposed to media with various Na^+ concentrations. However, oocytes expressing NHE3 showed large changes in the intracellular concentrations of H^+ and Na^+, confirming that NHE3 acted as a Na^+/H^+ exchanger correctly in *Xenopus* oocytes. In low-Na^+ media (0.5 mM Na^+), oocytes expressing NHE3 absorbed Na^+ from the media against the concentration gradient when the cytoplasm of the oocytes was artificially acidified by gas (CO_2) or a reagent (butyrate). When NH_4^+ was added to the media, we found that NHE3 also functioned as a Na^+/NH_4^+ exchanger.

Perspective

These results demonstrated for the first time that NHE3 acts as a Na^+/NH_4^+ exchanger and a Na^+/H^+ exchanger. In addition, the results indicated that NHE3 can act as an energy saving system that absorbs a nutrient (Na^+) in exchange with wastes (NH_4^+ or H^+ generated from CO_2) in the gills of freshwater fish (Fig. 1). In humans, renal NH_4^+ excretion is known to be the major acid wasting system, particularly when the body fluid is acidified (acidosis). Human NHE3 has been hypothesized to be the NH_4^+ excretion pathway in the renal proximal tubule. However, this putative Na^+/NH_4^+ exchange activity of NHE3 has not been directly demonstrated experimentally for NHE3. Our results showed that zebrafish NHE3 can transport NH_4^+, indicating that human NHE3 also has Na^+/NH_4^+ exchange activity.

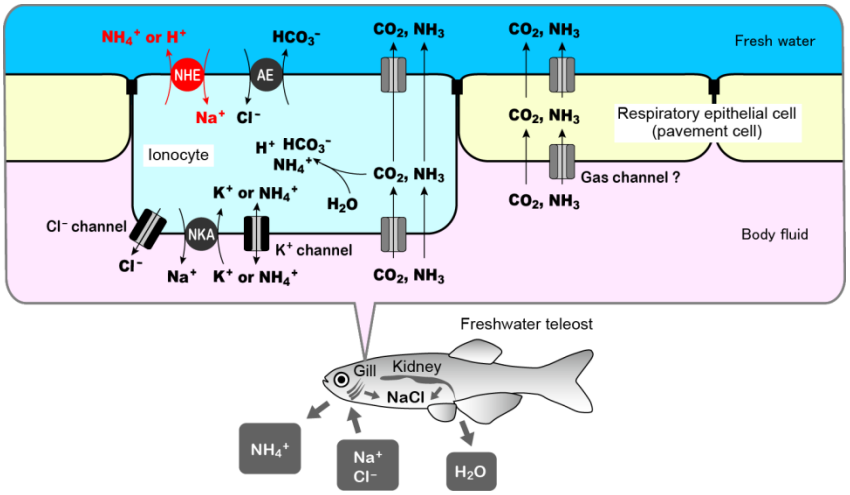

Fig. 1. Systems that absorb nutrient ions (Na^+, Cl^-) in exchange with wastes (NH_4^+, CO_2) in the gill of freshwater fish. Red indicates the transporter whose activity was analyzed in the present study; the other molecules are from other reports. NH_3 and CO_2 are excreted directly through the lipid bilayer or gas channels or they act as the driving force to absorb Na^+ and Cl^-. NH_4^+ is also considered to be absorbed by basolateral Na^+/K^+-ATPase and K^+ channels. NHE, Na^+/H^+ exchanger 3; AE, anion exchanger; NKA, Na^+/K^+-ATPase.

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News Release from Mayo Clinic

http://newsnetwork.mayoclinic.org/discussion/zebrafish-discovery-may-shed-light-on-human-kidney-function

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