

Accessory to kidney disease

Malcolm Hunter

The protein that is mutated in a human disorder of the kidney and ear turns out to be an accessory subunit for a chloride ion channel. The discovery explains the symptoms of the disease.

Kidneys are filters: they remove waste substances from the bloodstream, producing urine. In so doing, they also regulate various qualities of the blood, such as its salt level. When kidneys malfunction the results can be debilitating, as occurs in Bartter's syndrome, a salt-wasting disorder that affects humans. Bartter's syndrome was first described nearly 40 years ago¹, and several subtypes, caused by different genetic defects, have since been identified. One of these, Bartter's syndrome type IV, is also associated with deafness². The type IV syndrome is quite rare, affecting just one in a million live births. Patients are generally identified at birth or in early childhood, with the main symptoms — apart from deafness — being increased urine flow, a great thirst (to cope with the loss of fluid in urine), and a failure to thrive. The gene mutated in the type IV syndrome was identified earlier this month, and encodes a small membrane protein christened barttin³. Estévez and colleagues⁴ have now found out what this protein does, as they describe on page 558 of this issue.

Each human kidney is made up of roughly half a million hollow tubules called nephrons. Blood plasma is filtered into the

nephrons and is then modified by the selective absorption or secretion of various ions or molecules by the cells that make up the walls of the tubules. The fluid that remains in the tubules forms the urine. The function of the tubule cells varies along the length of a nephron. For example, one part of the nephron — the thick ascending limb — actively absorbs sodium and chloride ions, creating an osmotic gradient that allows water to be reabsorbed into the body if needed⁵. Defects in the ion-transporting proteins in cells of the thick ascending limb lead to Bartter's syndrome⁶.

The energy for ion transport in cells of the thick ascending limb comes from the breakdown of ATP molecules — the cellular energy store — by the ubiquitous Na^+, K^+ -ATPase. This protein sits in the cell membrane on the external surface of the tubule (that is, on the 'basolateral' surface of the cell; Fig. 1). It uses energy from ATP to transport Na^+ ions out of the cell into the external tissue fluid, and K^+ ions into the cell from the tissue fluid. This reduces the intracellular Na^+ concentration and raises the intracellular K^+ concentration relative to that in the surrounding fluids.

The resulting concentration gradient of

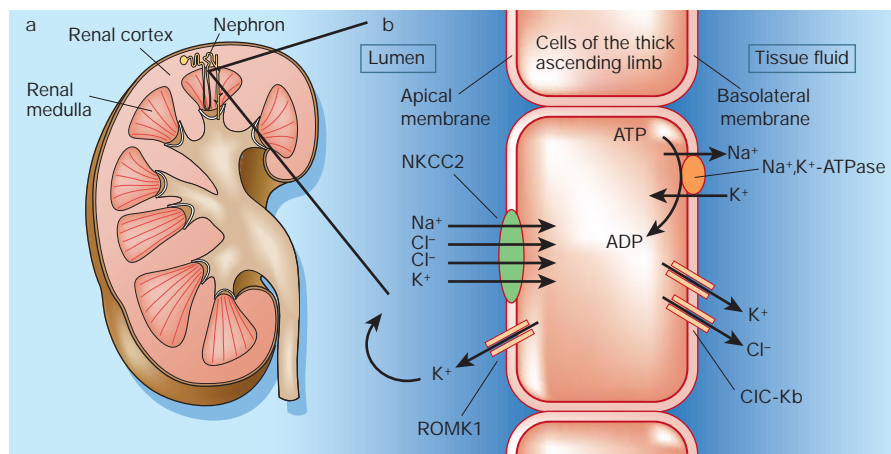


Figure 1 Ion transport in the kidney. **a**, The kidney. The renal cortex and medulla are made up of some half a million nephrons — the hollow tubules through which blood fluid flows to be filtered. One part of the nephron is the thick ascending limb. **b**, As fluid flows through the thick ascending limb, ions are extracted and returned to the body through the cells that form the tubule walls. Using energy supplied by hydrolysis of ATP, the Na^+, K^+ -ATPase (right-hand side) imports K^+ ions from the tissue fluid and exports Na^+ ions. The resulting concentration gradients allow another ion transporter, NKCC2 (left), to import Na^+ , Cl^- and K^+ ions from the fluid in the tubule. K^+ and Cl^- ions may then leave the cell by diffusing through relevant channels (including ROMK1 and CIC-Kb channels). K^+ ions merely recycle across the apical and basolateral membranes. But the net result is that Na^+ and Cl^- ions are concentrated in the tissue fluid and returned to the blood.

Na^+ ions provides the driving force to import Na^+ , K^+ and two Cl^- ions from the fluid in the thick ascending limb, on an ion 'co-transporter' called NKCC2. In this way the intracellular Cl^- and K^+ levels are raised above electrochemical equilibrium and can leave the cell by diffusion through ion channels. K^+ ions recycle across both cell surfaces through channels, such as ROMK channels when recycling back into the thick ascending limb. Cl^- ions leave across the basolateral membrane through CIC-Kb channels. The result is the net transport of NaCl out of the thick ascending limb and into the tissue fluid for reabsorption. Mutations in NKCC2, ROMK or CIC-Kb can cause Bartter's syndrome⁶.

Surprisingly, the ion-transport processes of cells in the ear's stria vascularis⁷ are similar to this (Fig. 2). Here, intracellular ion concentrations comparable to those of the thick ascending limb are achieved by the concerted action of the Na^+, K^+ -ATPase and a different form of the co-transporter, NKCC1. Potassium ions then leave the cells through KCNQ1/KCNE1 channels, generating a high concentration of K^+ ions in one of the fluid-filled chambers of the ear (the endolymph). The high K^+ concentration aids in the mechanism whereby sound waves are converted to a voltage signal suitable for relay to the brain. Mutations in NKCC1, KCNE1 and KCNQ1 are all associated with deafness (see ref. 4 for references).

In both cases, Cl^- channels are vital: the accumulation of Cl^- ions within the cell cannot continue indefinitely because it would oppose further ion uptake, and all ion transport would cease. The channels that allow the efflux of Cl^- ions are members of the CIC-K family, which are expressed exclusively in the kidneys and ears. In kidneys, CIC-Ka is expressed in the thin ascending limb and CIC-Kb in the adjacent thick ascending limb. In the ear, both channel types are expressed in the stria vascularis, as Estévez *et al.* show⁴.

As mentioned above, mutations of CIC-Kb result in Bartter's syndrome. Defects in CIC-Ka in humans have not yet been reported, although disruption of the mouse counterpart causes renal insufficiency (progressive loss of kidney function) and diabetes insipidus (an inability to concentrate urine)⁸. But the absence of either CIC-Ka or CIC-Kb would not be expected to cause deafness, as the other channel would presumably compensate. Indeed, the CIC-Kb mutations that cause Bartter's syndrome lead only to kidney malfunction, not to deafness.

Presumably, however, the barttin protein³ has a pivotal role in both ears and kidneys, as it is mutated in people with type IV Bartter's syndrome. But what does it do? Estévez *et al.*⁴ aimed to find out. They showed that barttin is expressed together with CIC-K channels in certain mouse tissues, including

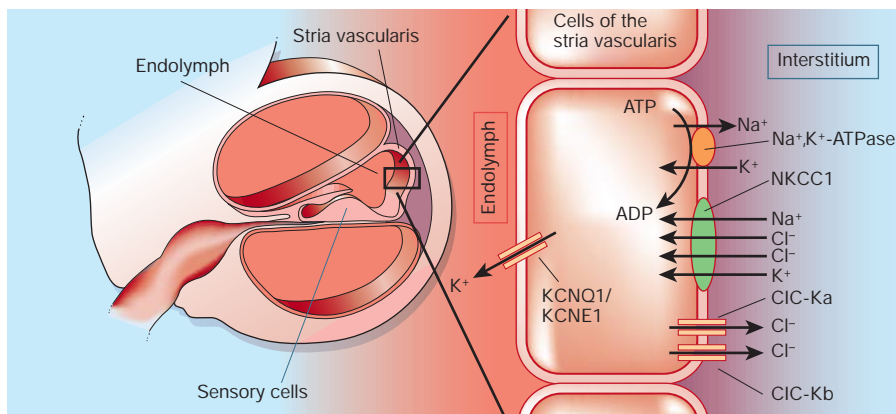


Figure 2 Ion transport in the inner ear. The balance of activities of various ion channels and ion transporters (including Na^+ , K^+ -ATPase, NKCC1, KCNQ1, KCNE1 and the CIC-K channels) ensures that the concentration of K^+ ions is high inside the endolymph of the ear. This aids in the mechanism by which sensory cells convert sound waves to voltage signals for transmission to the brain.

the stria vascularis, thin and thick ascending limbs, and other kidney cells that are known to express Cl^- channels. Moreover, barttin is needed for these channels to function: injecting RNA that encodes CIC-Ka or CIC-Kb into frog eggs did not result in measurable Cl^- currents, but also injecting RNA that encodes barttin resulted in Cl^- currents characteristic of those in ear or kidney cells. In addition, Estévez *et al.* introduced into barttin specific mutations that are seen in patients with type IV Bartter's syndrome. The mutations reduced the ability of the protein to support the expression of CIC-K channels. The authors' proposal that barttin is an accessory subunit (a ' β -subunit') of CIC-K channels seems the most reasonable explanation of these findings.

Barttin is the first known β -subunit of a Cl^- channel. But it may be that such subunits are the norm and that most ion channels

rely on them. Indeed, accessory subunits have been found to affect the expression and properties of several other channels, including various K^+ , Na^+ and Ca^{2+} channels⁹. The addition of β -subunits to the ion-channel armoury adds yet another layer of flexibility and diversity to ion-channel function. ■

Malcolm Hunter is at the School of Biomedical Sciences, Worsley Medical and Dental Building, University of Leeds, Leeds LS2 9NQ, UK.
e-mail: m.hunter@leeds.ac.uk

1. Bartter, F. C., Pronove, P., Gill, J. R. Jr & MacCardle, R. C. *J. Am. Soc. Nephrol.* **9**, 516–528 (1998).
2. Landau, D., Shalev, H., Ohaly, M. & Carmi, R. *Am. J. Med. Genet.* **59**, 454–459 (1995).
3. Birkenhager, R. *et al. Nature Genet.* **29**, 310–314 (2001).
4. Estévez, R. *et al. Nature* **414**, 558–561 (2001).
5. Greger, R. *Physiol. Rev.* **65**, 760–793 (1985).
6. Scheinman, S. J., Guay-Woodford, L. M., Thakker, R. V. & Warnock, D. G. *New Engl. J. Med.* **340**, 1177–1187 (1999).
7. Wangemann, P. H. & Schacht, J. in *The Cochlea* (eds Dallos, P., Popper, A. N. & Fry, R. R.) 130–185 (Springer, New York, 1996).
8. Matsumura, Y. *et al. Nature Genet.* **21**, 95–98 (1999).
9. Hille, B. *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, 2001).

Materials science

A broader view of membranes

Roderic Lakes

Membranes that get fatter when they are stretched are considered counterintuitive, but may be more common than we think. They might even turn up in human tissue.

Stretch a rubber band and it becomes thinner; squeeze a rubber eraser and it becomes fatter. Most common, and not-so-common, materials share this property, even those that are so stiff you cannot see the deformation. The opposite effect — becoming fatter when stretched — is a property of some specially designed materials, notably foams with unusual structures, as well as certain single crystals. Writing in *Physical Review Letters*, Bowick and co-workers¹ show that a broad class of membrane structures also becomes fatter when

stretched. These structures include certain types of naturally occurring membranes, so if we look closely we may find that some biological cells deform in unexpected ways.

Elastic materials deform easily when under strain but return to their original dimensions when the force is removed — they resist changes to both shape and volume. Rubbery materials, which easily change shape but not volume, become notably thinner in cross-section when stretched. This is described by Poisson's ratio, the ratio of transverse contraction to longitudinal

extension during stretching. For rubber, Poisson's ratio is close to the theoretical upper limit of 0.5; for most other common materials it is between 0.25 and 0.35. Because all these materials become thinner when stretched, Poisson's ratio is always positive. The reason for the thinning is that interatomic bonds tend to align when deformed.

A negative Poisson's ratio, which requires a transverse expansion (thickening) on stretching, is considered counterintuitive; indeed such materials were once thought not to exist or even to be impossible. But materials with negative Poisson's ratio do occur², and have been called anti-rubber, auxetic or dilational. For example, two-dimensional honeycomb structures have been developed with 'inverted' cells^{3,4}, which unfold when stretched (Fig. 1, overleaf). (Regular honeycomb lattices, like most materials, have a positive Poisson's ratio.) Similarly, some foam materials with a three-dimensional microstructure of 'inwardly bulging' cells⁵ also get fatter when stretched. These foams and honeycombs have such unusual elastic behaviour because of non-uniform unfolding or deformation of the microstructure⁶. Such materials are usually tougher and more resilient than most conventional materials, and so would make good knee pads, seat cushions, biomaterials and air filters that are easily tuned or cleaned.

Bowick and co-workers¹ have shown that structures known as 'fixed connectivity' membranes have a negative Poisson's ratio. Such membranes are assumed to have fixed connections: their bonds do not break. They occur in some biological^{7,8} and synthetic⁹ systems, and are a two-dimensional counterpart to one-dimensional molecular chains of polymers¹⁰. Like polymers they have a fractal-like structure, but unlike polymers, which are always crumpled, polymerized membranes can undergo a phase transformation from a crumpled phase at high temperatures to a 'flat' but locally rough phase at low temperatures.

The negative Poisson's ratio in fixed-connectivity membranes¹¹ is due to changes in entropy⁸ (a measure of thermal disorder) that occur with deformation. Stretching the membrane tends to flatten out undulations arising from thermal fluctuations (Fig. 2, overleaf), producing expansion in two directions. This deformation, as with the honeycomb and foam, is non-uniform. But the honeycomb expands in the same plane as that in which its microstructure unfolds. By contrast, the thermally induced undulations of the polymerized membrane are perpendicular to the surface of the membrane. Some cubic single crystals¹² can also expand in one direction and constrict in another, although a polycrystalline version of the cubic material has 'normal' elasticity. In polycrystalline materials the elastic properties of the individual crystals are averaged,