NALCN: a regulated leak channel

The literature on ion channels is extensive; searching PubMed with the term 'ion channel' results in close to 120,000 hits. Few of us have the energy—let alone the time—to keep up. However, should you wish to know everything about one functionally important channel, then read on; here might be the channel just for you! The Na^+ leak channel, non-selective (NALCN) was first cloned in 1999 (Lee *et al.*, 1999) but its true nature was not unveiled until 2007. To date, the entire literature on NALCN comprises fewer than ten papers, even when one considers its orthologues in *Drosophila melanogaster* and *Caenorhabditis elegans*.

NALCN is a member of the $4 \times 6\text{TM}$ family of ion channels, which comprise four homologous domains, each with six transmembrane (TM) segments. The family is encoded by 21 genes and includes voltage-gated Na^+ -selective and Ca^{2+} -selective ion channels (Lu *et al.*, 2007) that have crucial roles in almost every excitable cell of the body. *Nalcn* encodes a protein that has fewer positive charges in the voltage-sensing S4 segment than other members of the $4 \times 6\text{TM}$ family and that is responsible for a voltage-independent and tetrodotoxin (TTX)-resistant non-selective Na^+ 'leak' current.

The physiological significance of NALCN was highlighted by the finding that mice lacking the channel have abnormal respiratory rhythm and die within 24 h of birth. In addition, hippocampal neurons isolated from *Nalcn* knockout mice have reduced excitability. It seems that NALCN contributes to—but is almost certainly not the only channel responsible for—the elusive background leakage current often referred to as I_L , which was first postulated by Hodgkin & Huxley (1952) but has been difficult to identify due to the lack of selective antagonists. A recent study revealed that NALCN has a more active role than merely providing a passive—tonically active—leak conductance (Lu *et al.*, 2009). The authors showed that, in hippocampal neurons, neuropeptides such as neurotensin and Substance P (SP) stimulate the firing of action potentials by activating NALCN. This effect is mediated by the Src family of tyrosine kinases (SFK) but is independent of G-protein activation. The activation of NALCN by SFK also requires the protein mUNC80, which acts as a scaffold that recruits SFK to NALCN on receptor activation (Fig 1A).

In the August issue of *EMBO reports*, Swayne and colleagues report that NALCN, in addition to being expressed in the central nervous system, is present in the heart, pituitary and adrenal glands, and pancreatic islet cells (Swayne *et al.*, 2009). β -Cells are the main constituents of the pancreatic islets—comprising up to 80% of the cells—and secrete the plasma glucose-lowering hormone insulin. Pancreatic β -cells are electrically excitable; an increase in plasma glucose triggers insulin secretion through the induction of action potential firing. Glucose-induced closure of ATP-regulated K^+ -channels (K_{ATP} -channels) is a crucial event in glucose-sensing by the β -cell. The closure of the K_{ATP} -channels leads to membrane depolarization,

Fig 1 | The activation of NALCN. (A) Activation of Na^+ leak channel, non-selective (NALCN) by Substance P (SP) in hippocampal neurons. The SP receptor (SPR) and NALCN do not appear to interact physically, and channel opening is mediated by the activation of the Src family of tyrosine kinases (SFK) and through mouse UNC80, which interacts directly with NALCN. (B) Activation of NALCN by the binding of acetylcholine (ACh) to the M3 receptor (M3R). NALCN and M3R could form a functional complex, as suggested by co-immunoprecipitation experiments. Note that M3Rs can be modulated by SFK through an unknown mechanism.

opening of voltage-gated Ca^{2+} channels, and the Ca^{2+} -induced exocytosis of insulin-containing granules. Pancreatic islets are—as first described by Paul Langerhans—extensively innervated and contain cholinergic, peptidergic and adrenergic nerve endings. The neurotransmitters that are released from these nerve endings stimulate or inhibit insulin secretion and thereby fine-tune its release in response to the functional demand. Acetylcholine (ACh) is released by vagal nerve endings within the islet during feeding, binds to muscarinic M3 receptors (M3R; Henquin & Nenquin, 1988), enhances insulin secretion and prepares the body for the rise in plasma glucose (Gautam *et al.*, 2006).

ACh increases the release of insulin in several ways (Gilon & Henquin, 2001). One mechanism involves the stimulation of the electrical activity of β -cells through the activation of a small voltage-independent, G-protein-independent and Na^+ -dependent current (Rolland *et al.*, 2002). The molecular identity of the channels responsible for this current has remained an enigma. Swayne and colleagues now describe a G-protein-independent current that is activated by ACh in the mouse insulinoma cell line Min6 (Swayne *et al.*, 2009), the amplitude of which is reduced by short hairpin RNA knockdown of *Nalcn*. This current exhibited the biophysical and pharmacological properties expected for NALCN: its activation was prevented by the inhibition of SFK and the M3R antagonist 4-DAMP. Collectively, these findings raise the interesting possibility that NALCN represents

Fig 2 | NALCN-dependent currents in neurons and β -cells. **(A)** In neurons, Na^+ leak channel, non-selective (NALCN) gives rise to a tonic background current (NALCN-leak) that depolarizes the cell sufficiently to trigger action potentials. The application of Substance P (SP) results in further activation of NALCN (NALCN-SP) and increases action potential frequency. **(B)** In β -cells, NALCN contributes marginally to the resting membrane current. Acetylcholine (ACh) activates NALCN (NALCN-ACh) and induces the firing of action potentials when glucose is elevated, but not when glucose levels are low, because the magnitude of the K_{ATP} -channel current determines the influence of the ACh-activated depolarizing current on the β -cell membrane potential. The dotted horizontal lines indicate the zero-current level (that is, when no current is flowing across the plasma membrane).

the ACh-activated channel in β -cells. The co-expression of NALCN and M3R in a human embryonic kidney cell line (HEK293) was sufficient to reconstitute the ACh-activated current, irrespective of the presence of mUNC80. Co-immunoprecipitation experiments further suggested that M3R and NALCN interact to form a heteromultimeric protein complex. Therefore, it seems that the association between M3R and NALCN is closer than that observed between the SP and neurotensin receptors and NALCN (Fig 1A,B).

If these observations can be extended to primary β -cells, these data represent an important advance. As outlined above, the closure of K_{ATP} -channels is a crucial event in the sensing of glucose by the β -cell. However, the inhibition of K_{ATP} -channels alone does not explain the observed membrane depolarization; an additional depolarizing background conductance is necessary. It is tempting to propose that NALCN—by analogy to what is apparently the case in hippocampal neurons (Fig 2A; Lu *et al*, 2007)—represents this background current. However, an increase or decrease in the expression of NALCN did not affect the background leak conductance of Min6 or HEK293 cells. It will be interesting to see whether β -cells from *Nalcn* knockout mice (Lu *et al*, 2007) are hyperpolarized compared with wild-type cells and how the membrane potential is influenced by glucose.

Compounds that affect the activity of NALCN directly or indirectly through modulation by SFK might provide valuable leads for the development of new insulin-releasing drugs, as suggested by the observation that mice overexpressing M3Rs have improved glucose tolerance (Gautam *et al*, 2006). As the NALCN current is small in primary β -cells, such compounds could be superior to the sulphonylureas that are used at present. A disadvantage of sulphonylureas is that, by closing the K_{ATP} -channels regardless of the glucose concentration, they bypass the normal metabolic regulation of the β -cell; insulin secretion continues even when plasma glucose has fallen to abnormally—and dangerously—low levels. The ACh-induced activation of

NALCN would depolarize β -cells and stimulate insulin secretion only at normal and elevated blood glucose levels, when the K_{ATP} -channel activity is reduced. When glucose is low, the depolarizing effect of the small ACh-activated current would be too small to overcome the strong repolarizing action of increased K_{ATP} -channel activity (Fig 2B), minimizing the risk of hypoglycaemic episodes.

In order to move forward, it is now critical to ascertain whether NALCN is expressed in human β -cells and document its effects on primary β -cell electrical activity and insulin secretion. As NALCN is regulated by SFK, it might be worth investigating whether other factors that activate this family of tyrosine kinases—such as growth factors, cytokines and adhesion molecules—also modulate NALCN activity, β -cell electrical activity and insulin secretion. The results reported by the Monteil and Ren groups (Swayne *et al*, 2009; Lu *et al*, 2009) provide a firm molecular basis for such future studies.

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