Ion channelopathies in endocrinology: recent genetic findings and pathophysiological insights

Canalopatias em endocrinologia: achados genéticos recentes e fisiopatologia

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SUMMARY

Ion channels serve diverse cellular functions, mainly in cell signal transduction. In endocrine cells, these channels play a major role in hormonal secretion, Ca^{2+}-mediated cell signaling, transepithelial transport, cell motility and growth, volume regulation and cellular ionic content and acidification of lysosomal compartments. Ion channel dysfunction can cause endocrine disorders or endocrine-related manifestations, such as pseudohypoaldosteronism type 1, Liddle syndrome, Bartter syndrome, persistent hyperinsulinemic hypoglycemia of infancy, neonatal *diabetes mellitus*, cystic fibrosis, Dent's disease, hypomagnesemia with secondary hipocalcemia, nephrogenic *diabetes insipidus* and, the most recently genetically identified channelopathy, thyrotoxic hypokalemic periodic paralysis. This review briefly recapitulates the membrane action potential in endocrine cells and offers a short overview of known endocrine channelopathies with focus on recent progress regarding the pathophysiological mechanisms and functional genetic defects. Arq Bras Endocrinol Metab. 2010;54(8):673-81

Keywords

Ion channel; channelopathy; endocrine channelopathy

SUMÁRIO

Canais iônicos auxiliam diferentes funções celulares, principalmente na transdução de sinal. Nas células endócrinas, esses canais têm funções importantes na secreção hormonal, sinalização do Ca^{2+}, transporte transepitelial, regulação da motilidade, volume e conteúdo iônico celular e da acidificação do compartimento lisossomal (pH). Como esperado, as alterações nos canais iônicos podem causar distúrbios endocrinológicos, como pseudo-hipoaldosteronismo tipo 1, síndrome de Liddle, síndrome de Bartter, hipoglicemia hiperinsulinêmica da infância, diabetes melito neonatal, fibrose cística, doença de Dent, hipomagnesemia com hipocalcemia secundária, diabetes insípido nefrogênico e paralisia periódica tirotóxica hipocalêmica. Este artigo propõe uma breve revisão das canalopatias endócrinas conhecidas, com foco particular nos recentes progressos no conhecimento dos mecanismos fisiopatológicos adquirido a partir das alterações funcionais encontradas. Arq Bras Endocrinol Metab. 2010;54(8):673-81

Descritores

Canal iônico; canalopatia; canalopatia endócrina

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INTRODUCTION

Electrophysiological properties, once thought to be exclusive to neuronal and muscle cells, have been found to apply to endocrine tissues. In fact, endocrine cells carry electrical impulses in the same way other excitable cells do, relying on membrane transport proteins, such as pores, gated channels and pumps, to react to signals. Mutations in any of these channel proteins can cause dysfunctions that are collectively referred to as endocrine channelopathies. Pores are ion-specific passages that allow specific ions to diffuse across the plasma membrane along the concentration gradient.

Gated-channels are a group of ion-specific transport proteins that open or close in response to ligand binding (ligand-gated), voltage changes (voltage-gated) or mechanical deformation (mechanically-gated). In general, hormones modify ligand-gated channels located at the cell surface, which allows the influx of Ca^{2+} into the cell for downstream cascade signaling. Pumps counteract the ionic flow from pores and gated channels to reestablish the resting membrane potential by inverting the ionic concentration differences on both sides of the membrane (1). At rest, the inner surface of the membrane is more highly negative than the outer surface, typically -70 mV, resulting in a difference in potential that can be disrupted by a depolarizing action potential.

Cells that secrete hormones or sense hormonal regulation usually produce chemical messengers that are transported through the circulatory system, and require seconds, minutes or even hours to act. Neuronal cells, on the other hand, transmit messages much more quickly and require only thousands of a second for this action. Differently from a "wired" neuronal transmission in which the rapid propagation works along the synaptic cleft, endocrine transmission occurs slowly throughout the body because hormones are secreted into the blood stream and delivered to distant target sites, as if in a "wireless" system (2). Both endocrine and neuronal cells, however, can produce electrical impulses, or action potentials (AP). Endocrine cells that respond to stimuli with action potentials include chromaffin, pancreatic and adenohypophyseal cells (3). With the exception of β-cells that depolarize by closing K_{ATP} channels in response to increases in the intracellular ratio of ATP/ADP after a meal, endocrine cell APs are mainly generated by voltage-dependent ion currents (4,5).

In general, APs bring Ca^{2+} into endocrine cells. This influx is crucial for refilling the endoplasmic reticulum (ER) Ca^{2+} pool after depletion by hormone-induced calcium release (6), which is a mechanism known as stimulus-secretion coupling. The upstroke of the action potential is the result of the fast activation of the voltage-gated Ca^{2+} channels (VDCC). Slow activation of K^+ channels along the second half of phase 1 (Figure 1) leads to the downstroke. Interestingly, spontaneous action potentials can also occur in the absence of agonists, such as in excitable gonadotroph cells; such model of spontaneous oscillations was first proposed in 1995 by Li and cols. (7). The most important ion currents responsible for altering the membrane potential are those derived from delayed rectifying K^+ channels, L- and T- type Ca^{2+} channels and calcium activated potassium (K_{Ca}) channels (8,9). Importantly, K_{Ca} channels only work when intracellular Ca^{2+} content is high, especially during the release of Ca^{2+} from the ER (10). Figure 1 shows a schematic diagram of the AP phases of a representative endocrine cell and summarizes the major ion currents throughout the waveform (phases 1-4). It is worth emphasizing that cells in the endocrine system require an action potential with a long duration rather than a fast one.
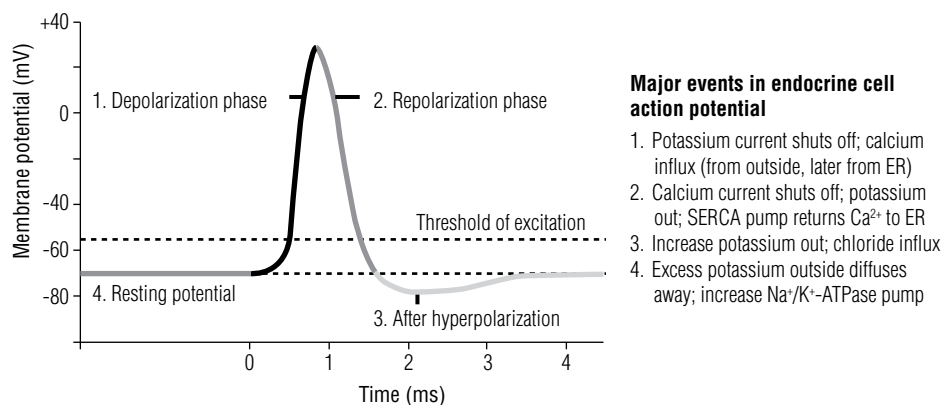
Thyrotoxic hypokalemic periodic paralysis (TPP)

TPP is an urgent medical condition where patients suffer from sudden and reversible loss of muscle strength in the limbs and thyrotoxicosis associated with hypokalemia. TPP is the most frequent form of acquired acute flaccid paralysis in adults. Although it is most prevalent in Asian populations, TPP can occur in individuals of any ethnicity (11). Hypokalemia does not indicate a depletion of the total potassium pool but an increased influx of the ion to the intracellular compartment (11). Early and definitive treatment of thyrotoxicosis should be aimed.

For a decade, we have been searching for TPP candidate genes. Our group and others have demonstrated that mutations in the *CACN1AS* and *SCN4A* genes, present in familial forms of hypokalemic paralyses (FHypokPP), are not present in patients with TPP (11-14). Kir2.x paralogues have constitutive thyroid hormone-responsive *cis*-elements (TREs) in their regulatory regions, therefore we screened the coding sequence of the *KCNJ12* (Kir2.2), *KCNJ4* (Kir2.3) and *KCNJ14* (Kir2.4) genes. We performed low-stringency PCR and direct sequencing of Kir2.2 and found a new paralogue, Kir2.6. This novel channel, encoded by *KCNJ18* (17p11.1-2), is a functional potassium channel. This channel is expressed in skeletal muscle and is transcriptionally regulated by T3 (15). Six mutations in Kir2.6 have been found to be associated with TPP: R205H, T354M, K366R, R399X, Q407X and I144fs (15). The two most common Kir2.6 mutations (15), R399X and Q407X, are located at the C-terminus of the channel and lack the PDZ binding domain boundaries that interfere with the assembly either as homotetramers or heterotetramers, and trafficking of the complex to the membrane (16). We proposed that TPP pathophysiological mechanism is the loss of function of the Kir channel reducing the outward potassium current, which leads to depolarization and subsequent loss of muscle excitability (Figure 2).

Table 1. Ion channel defects associated with endocrine disorders. Data from OMIM (Online Mendelian Inheritance in Man), NCBI (National Center for Biotechnology Information), HGMD (Human Gene Mutation Database) and Ensembl websites

Channel	Gene	Chromosome	RefSeq ID (NCBI)	Disorder	N. of mutations (HGMD)	Functional defect	First published reference
ENaCα	SCNN1A	12p13	NM_001038	Pseudohypoaldosteronism type 1	23	Renal salt loss	Chang and cols., 1996 (13)
ENaCβ	SCNN1B	16p12.2-p12.1	NM_000336	Pseudohypoaldosteronism type 1 Pseudoaldosteronism (Liddle syndrome)	37	Renal salt loss increased Renal sodium uptake	Shimkets and cols., 1994 (14)
ENaCγ	SCNN1G	16p12	NM_001039	Pseudohypoaldosteronism type 1 Pseudoaldosteronism (Liddle syndrome)	15	Renal salt loss increased Renal sodium uptake	Hansson and cols., 1995 (15)
TRPM6	TRPM6	9q21.13	NM_017662	Hypomagnesemia with secondary hypocalcemia	35	Renal magnesium loss Decrease serum concentration of calcium	Schlingmann and cols., 2002 (20) Walder and cols., 2002 (19)
Kir1.1	KCNJ1	11q24	NM_000220	Bartter syndrome type II	51	Renal salt loss	Simon and cols., 1996 (23)
hClC-Kb	CLCNKB	1p36.13	NM_000085	Bartter syndrome type III	37	Renal salt loss	Simon and cols., 1997 (24)
Kir6.2	KCNJ11	11p15.1	NM_000525	Persistant hyperinsulinemic hypoglycemia of infancy Neonatal diabetes mellitus	82	Insulin hypersecretion Insulin hyposecretion	Thomas and cols., 1996 (29)
SUR1	ABCC8	11p15.1	NM_000352	Persistant hyperinsulinemic hypoglycemia of infancy Neonatal diabetes mellitus	243	Insulin hypersecretion	Thomas and cols., 1995 (28)
CFTR	CFTR	7q31.2	NM_000492	Cystic fibrosis	1489	Altered epithelial transport	Riordan and cols., 1989 (35)
CLC-5	CLCN5	Xp11.23-p11.22	NM_000084	Dent's disease	134	Impaired endosome acidification	Fisher and cols., 1995 (42)
CLCN7	CLCN7	16p13	NM_001287	Osteopetrosis	57	Impaired bone resorption	Kornak and cols., 2001 (46)
AQP2	AQP2	12q12-q13	NM_000486	Nephrogenic diabetes insipidus	46	Renal water loss	van Lieburg and cols., 1994 (53)
Kir2.6	KCNJ18	17p11.1-2	NW_003315950	Thyrotoxic hypokalemic Periodic Paralysis	6	Hypokalemia due to T3-excess inducing increased K$^+$ shift	Ryan and cols., 2010 (8)

Major events in endocrine cell action potential

1. Potassium current shuts off; calcium influx (from outside, later from ER)
2. Calcium current shuts off; potassium out; SERCA pump returns Ca^{2+} to ER
3. Increase potassium out; chloride influx
4. Excess potassium outside diffuses away; increase Na$^+$/K$^+$-ATPase pump

VDCC voltage-dependent calcium channel; ER endoplasmic reticulum; I_{TO} transient outward current of voltage-gated K$^+$ channel; I_{Kr}, I_{Ks}, I_{Kur} are major repolarising currents of outwardly rectifying K$^+$ channels (I_K), which can be divided into ultrarapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) components; SERCA is a ER Ca^{2+} pump Sarco(Endo)plasmic Reticulum Ca^{2+}-ATPase.

Figure 1. Schematic illustration of an endocrine cell action potential. The major electrophysiological features of ion currents (I) are illustrated throughout the waveform phase 1-4. 1- K$^+$ channel closed (K_{ATP} in β-cell); Increase I_{Ca2+} (VDCC) from outside membrane; then more Ca^{2+} from ER run to cytoplasm. 2- Quick K$^+$ goes out (Ito) and relatively more K$^+$ leaves cell (I_{Kr}, I_{Ks}, I_{Kur}); Ca^{2+}-activated K$^+$ channel opens; less Ca^{2+} in, increase SERCA pump activity (Ca^{2+} returns to ER). 3- Increase I_{Kir} out and Cl$^-$ influx. 4-Excess K$^+$ outside diffuses away; increase Na$^+$/K$^+$-ATPase pump.
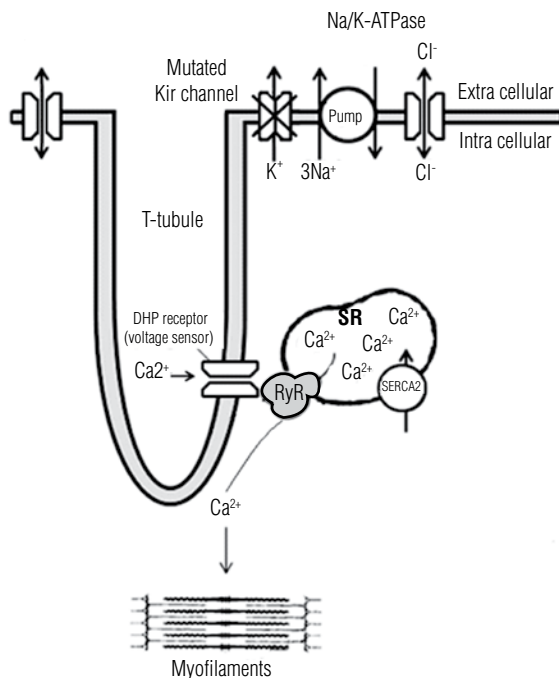
Figure 2. Excitation-contraction coupling of skeletal muscle. In TPP, loss of function of the Kir channel decreases outward potassium current leading to depolarization and loss of muscle excitability. DHP: dihydropyridine receptor; RyR: ryanodine receptor; SR: sarcoplasmic reticulum.

Pseudohypoaldosteronism (PHA) type 1

PHA type 1 is a rare salt-wasting disorder that was first described in 1958. It is associated with a decreased response to aldosterone. Symptoms include dehydration, hyponatremia, hypokalemia, metabolic acidosis and failure to thrive in the neonatal period (17). Plasma renin and aldosterone concentrations are grossly elevated due to a peripheral resistance to mineralocorticoids. Glomerular filtration, renal and adrenal functions, however, are normal. PHA is suspected when patients fail to respond to mineralocorticoid therapy (18).

This disease has two distinct forms with different physiologic and genetic characteristics: the renal form of PHA type 1 can be inherited either as an autosomal dominant (AD) trait or as a generalized autosomal recessive (AR) trait. Inactivating mutations in the mineralocorticoid receptor cause the autosomal dominant form of PHA type 1 (19), while the AR form is caused by inactivating mutations in α, β and γ subunits of the epithelial sodium channel (ENaC). The AR form is an ion channelopathy and is the more severe form of this disease.

ENaC is expressed in the apical plasma membrane of many epithelial tissues, and aldosterone resistance is seen in many tissues, including sweat glands, salivary glands, respiratory tract, colonic mucosa and kidneys.

ENaC subunit genes are *SCNN1A*, *SCNN1B* and *SCNN1G* (18,20-22). Most reported mutations in these genes are frameshift or nonsense, leading to non-functioning proteins. Unlike the AD form, the AR condition does not spontaneously improve with age and, thus, requires lifelong therapy and treatment.

Liddle syndrome

Liddle syndrome was first described in 1963. It is an inherited autosomal dominant form of endocrine hypertension (23). Affected patients present with hypertension, hypokalemia and metabolic alkalosis (23). Hypertension frequently begins in childhood but can be asymptomatic and may not be detected until early adulthood. Plasma renin activity and aldosterone levels are low. This disease is caused by activating mutations of the epithelial sodium channel (ENaC), which leads to an endocrine hypertensive disorder due to increased sodium reabsorption and potassium wasting in the distal nephron.

Liddle syndrome is caused by mutations in *SCNN1B* or *SCNN1G* genes, which truncate the cytoplasmic carboxyl terminus of the β (*SCNN1B*) and γ (*SCNN1G*) subunits of the epithelial sodium channel (ENaC) (24). These mutations lead to ENaC gain-of-function through two mechanisms: enhancement of the channel activity by increasing the probability of opening and, most importantly, increasing the number of functioning channels by slowing degradation (25). Treatment involves a low-salt diet and the use of drugs, such as amiloride and triamterene, that directly inhibit epithelial sodium transport (23). It is important to point out that mineralocorticoid receptor antagonists, such as spironolactone, are ineffective.

Hypomagnesemia with secondary hypocalcemia

Familial hypomagnesemia with secondary hypocalcemia is an autosomal recessive disease that results in electrolyte abnormalities shortly after birth. Affected individuals show severe hypomagnesemia and secondary hypocalcemia, leading to seizures and muscle cramps (26,27). If left untreated, the disorder can result in neurological damage and death. Hypocalcemia is secondary to parathyroid failure as a consequence of magnesium deficiency (27).

Hypomagnesemia with secondary hypocalcemia is caused by mutations in the transient receptor potential cation channel, subfamily M, member 6 (TRPM6) (26,

27). TRPM6 is expressed in the intestinal mucosa and the kidney. It encodes for a protein that functions as both a protein kinase and a calcium and magnesium ion channel. Affected patients have abnormal intestinal absorption and renal excretion of magnesium (26).

Patients require life-long treatment with magnesium supplementation.

Bartter syndrome

Bartter syndrome (BS) is an inherited salt-losing renal tubulopathy characterized by hypokalemic metabolic alkalosis, normal to low blood pressure and elevated renin and aldosterone levels (28,29). Affected patients often present with polyhydramnios during pregnancy or polyuria and dehydration in early childhood. This disorder is caused by dysfunctional renal tubular electrolyte transporters in the thick ascending limb (TAL) of the nephron. There are five different types of Bartter syndrome that are correlated with predominantly autosomal recessive gene mutations. Urine samples in patients with Bartter syndrome show elevated levels of prostaglandins (e.g., PGE_2). Patients with BS types I, II or IV have isosthenuria or hyposthenuria. In addition to renal dysfunction, BS type IV patients experience sensorineural deafness. Patients with BS type I or II have hypermagnesiuria and hypercalciuria. BS type III is typically the mildest of the five BS types (29).

In BS type I, the affected gene, *SCL12A1,* encodes the apical furosemide-sensitive sodium potassium chloride cotransporter (NKCC2). NKCC2 arises on the TAL luminal membrane and reabsorbs sodium together with one potassium and two chloride ions (Figure 3). *SCL12A1* mutations result in active transtubular salt transport defects. BS type II results from mutation in the inwardly-rectifying potassium channel, subfamily J, member 1 (*KCNJ1*) gene (30). *KCNJ1* encodes the apical renal outer medullary K+ (ROMK1) channel, which is required for adequate recirculation of potassium into the luminal space. It also provides the driving force for paracellular absorption of calcium and magnesium. Sodium exits through NaK-ATPase and chloride exits via Cl- channel K+ proteins (ClC-Ka and ClC-Kb) on the basolateral side. These chloride channels require a functioning Barttin subunit for proper membrane localization. Mutations in *BSDN*, the gene that encodes Barttin, affect chloride channels and cause BS type IV. BS type III, also called classic Bartter's syndrome, is caused by ClC-Kb (*CLCNKB*) gene mutations (28,29,31).
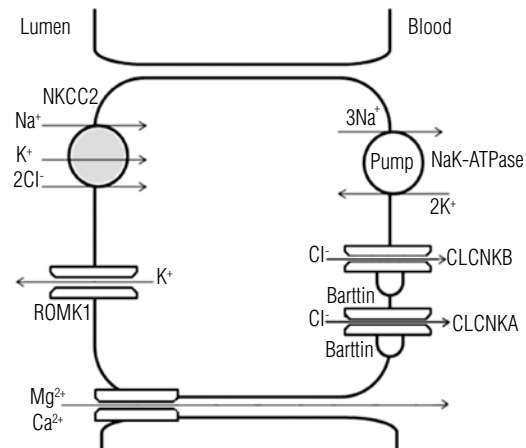

Figure 3. Ion transepithelial transport in thick ascending limb cells (adapted from reference 29). NKCC2: sodium potassium chloride cotransporter; ClCKA: channel K+ protein A; ClCKB: channel K+ protein B; ROMK1: renal outer medullary K+.

BS type V, an autosomal dominant form of Bartter syndrome, is caused by a gain-of-function mutation in the extracellular basolateral calcium sensing receptor (CASR) gene (32). CASR is essential for regulating secretion of parathyroid hormone (PTH) but is also expressed in other tissues, such as the kidney. Activation of CASR inhibits ROMK1 activity, which leads to hypocalcemic hypercalciuria and low PTH levels.

Persistent hyperinsulinemic hypoglycemia of infancy (HHI)

Persistent hyperinsulinemic hypoglycemia of infancy (HHI), also named congenital hyperinsulinism, is the most common cause of nontransient hyperinsulinemic hypoglycemia in neonates and infants (33). Typical clinical signs, including hypoglycemia, lethargy, poor feeding and irritability, arise shortly after birth. Some newborns may show more severe symptoms including seizures and coma that, if not recognized and treated properly, can cause severe mental retardation and epilepsy (33). Diagnosis is based on the detection of non-ketotic hypoglycemia with low serum fatty acid levels, inappropriately high insulin levels and raised C-peptide levels. Glucose levels are also known to rise in response to glucagon administration in affected infants (4).

The most common causes of persistent HHI are inherited autosomal recessive mutations that inactivate *ABCC8* and *KCNJ11* genes (34,35). The pancreatic K_{ATP} channel is a functional complex of four sulfonylurea receptors 1 (SUR1) and four pore-forming inward rectifier potassium channel subunits (Kir 6.2). The K_{ATP}

channel is a critical regulator of beta-cell insulin secretion. *KCNJ11* encodes for Kir6.2 and *ABCC8* encodes for SUR1. *ABCC8* mutations are associated with 50–60% of persistent HHI cases, while *KCNJ11* mutations account for about 10%-15% of patients (5).

Inactivating mutations result in persistent β-cell membrane depolarization and insulin secretion, despite low plasma glucose levels. At the molecular level, these mutations cause multiple abnormalities in K_{ATP} channels turnover, regulation and open-state frequency (4,5).

Neonatal *diabetes mellitus*

Hyperglycemia during the first six months of life is defined as neonatal diabetes (NDM). NDM is associated with slowed intrauterine growth, failure to thrive, low birth weight, decreased adipose tissue and low or undetectable C-peptide levels. Most patients are markedly hyperglycemic and some present with ketoacidosis (36). This rare disorder has two forms: transient NDM (TNDM) and permanent NDM (PNDM). Unfortunately, there is no clinical distinction between these two forms at the time of presentation. In approximately 50%-60% of cases, TNDM resolves usually within three months (37).

Most TNDM cases (70%) are due to abnormalities on chromosome 6 (6q24), while PNDM is most commonly caused by genetic variations in K_{ATP} channel genes. Activating *KCNJ11* gene mutations (Kir6.2 subunit of the K_{ATP} channel) account for 31%-64% of cases of PNDM (37). Less commonly, KCNJ11 mutations are associated with TNDM or a multisystem disease, DEND syndrome (developmental delay, epilepsy and neonatal diabetes). Mutations in *ABCC8* gene (SUR subunit) are primarily associated with TNDM.

In healthy individuals, the presence of glucose leads to intracellular ATP production, which results in K_{ATP} channel closure and membrane depolarization. This, in turn, leads to calcium influx and insulin secretion (5,36,37). Activating mutations in K_{ATP} channels decrease sensitivity to ATP inhibition, which causes them to remain open in the presence of glucose and reduce insulin secretion. Sulfonylureas can bind to the SUR1 subunit and closing the K_{ATP} channel and, thus, can be used to treat patients with ND activating K_{ATP} channel mutations (5).

Cystic fibrosis

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasians, with a frequency of about 1 in 2,500 live births. Clinical manifestations include pulmonary infection, pancreatic insufficiency, elevated chloride levels in sweat, infertility and pseudo-Bartter's syndrome (salt wasting with metabolic alkalosis) (38). Lung disease is the main cause of morbidity and mortality. Exocrine pancreatic insufficiency is present in 85%-90% of cases and is thought to result from reduced volume of pancreatic secretion with low concentrations of HCO3, which cause retention and premature activation of digestive proenzymes, which results in tissue destruction and fibrosis (38). This and other concurrent processes that are poorly understood may lead to β-cell death and result in *diabetes mellitus*. *Diabetes mellitus* is rare in the first decade of life and its prevalence increases with age. Insulin sensitivity is diminished in patients with CF due to a number of factors, including chronic infection and inflammation and systemic steroid use. There are conflicting data, however, with regard to the role of insulin resistance in the etiology of cystic fibrosis-related *diabetes mellitus* (39).

CF is caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) that produces a defective chloride channel in epithelial membranes (40). The disease phenotype varies according to the type of mutation and the presence of gene modifiers, such as transforming growth factor beta-1 and mannose-binding lectin 2 genes (41). Over 1,800 *CFTR* mutations have been described thus far (42). These mutations are divided into five different classes that represent a gradient of CFTR dysfunction, with class I indicating defective protein production and class V indicating production of reduced functioning CFTR protein. Class 1-3 mutations are the most common and are associated with pancreatic insufficiency, whereas patients with class 4 or 5 do not have pancreatic insufficiency. The most common CFTR mutation in patients with cystic fibrosis-related *diabetes mellitus* is Phe508del (39).

A concentration in sweat chloride greater than 60 mmol/L on repeated analysis is diagnostic. In cases of marginal sweat test results, CFTR genotyping is recommended (38); however, some suggest DNA testing in individuals with positive sweat test.

Neonatal screening for cystic fibrosis is carried out in some countries. It is unclear whether early diagnosis improves long-term outcomes, but there is evidence to suggest that there are nutritional benefits (43).

Dent's disease

Dent's disease is a X-linked recessive proximal renal tubular syndrome characterized by low-molecular-weight

proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease and progressive renal failure (44). Approximately 25% of affected males also have rickets or osteomalacia, with deforming bone disease in childhood (44,45). Interestingly, the occurrence of rickets varies between affected members of the same family as well as between different families with the same mutation. It is unknown whether this variability is due to modifying genes or environmental factors (45).

Sixty percent of Dent's disease cases are caused by mutations in the chloride channel gene, *CLCN5,* that encodes the chloride-proton antiporter, CLC-5 (46). CLC-5 is found primarily in the kidneys and is expressed with proton-ATPase in subapical endosomes of the tubular proximal cells where it facilitates acidification (47). Acidification is necessary to process proteins taken up by endosomes. Thus, loss of function CLC-5 mutations that impair acidification of vesicles in the endocytotic pathway are responsible for proteinuria seen with Dent's disease (47). In 15 percent of cases, the disease is associated with mutations in the *OCRL1* gene, that is also mutated in the oculocerebrorenal syndrome of Lowe (48). In some patients presenting with symptoms of Dent's disease, no *CLCN5* and *OCRL1* mutations were found, which suggests further genetic heterogeneity is involved.

Osteopetrosis

Mutations in the gene that encodes the chloride channel-7 protein (CLCN7) can cause autosomal dominant osteopetrosis type II (Albers-Schönberg disease) and autosomal recessive osteopetrosis type IV (infantile osteopetrosis) (49,50).

Infantile osteopetrosis is a rare autosomal recessive disorder in which failure to resorb bone and calcified metaphyseal cartilage causes near obliteration of the marrow spaces (49). About 15% of these patients have genetic defects on the osteoclast chloride channel (CLCN7), while 60% of the cases are due to mutations in the subunits of the vascular-type H+-adenosine triphosphatase (51).

Autosomal dominant osteopetrosis type II is characterized by generalized osteosclerosis with thickening of the vertebral endplates and pelvic bone. Clinical manifestations include fractures, osteoarthritis, skeletal deformities and cranial nerve involvemement (52).

The characterization of genetic defects in osteopetrosis opens up the possibility for gene replacement therapy as an alternative to hematopoietic stem cell transplantation in children with severe disease (53).

Nephrogenic *diabetes insipidus*

In nephrogenic *diabetes insipidus* (NDI), the distal nephron is insensitive to antidiuretic hormone (ADH), decreasing the ability of the kidney to concentrate urine. If fluid intake is not sufficient to compensate for the increased water loss, patients may experience dehydration and electrolyte imbalance. Acquired causes include drugs, especially lithium toxicity, renal disease, hypercalcemia and hypokalemia (54). Hereditary NDI is rare and is usually diagnosed soon after birth or in early childhood (55). In the majority of cases, NDI is caused by mutations in the vasopressin receptor (V2) gene and in the aquaporin-2 (AQP2) gene (56). However, since mutations in the above mentioned genes were not found in some affected families, other genes may be involved. With *AQP2* mutations, both recessive and dominant inheritance patterns have been reported (55).

AQP2 is expressed primarily in principal cells of the collecting ducts of renal tubules where regulated water reuptake is known to occur (57). Under the influence of ADH, the vesicles with AQP2 traffic to the luminal membrane, increasing the water permeability of the collecting duct and allowing water to be reabsorbed along the concentration gradient.

Elucidation of the mutations and mechanisms responsible for NDI is important for the development of therapeutic approaches, such as molecules to help direct AQP2 to the cell surface (58).

PERSPECTIVES: CHANNELS CONTROLLING TRH-INDUCED PROLACTIN SECRETION AND OVARY STEROIDOGENESIS

As with other neuroendocrine cells, hormone secretion from anterior pituitary lactotrophs is regulated by changes in their excitability. Thyroid releasing hormone (TRH) stimulates prolactin secretion through membrane depolarization. Membrane depolarization can be accompanied by an increase in the rate of action potential firing leading to an increase in intracellular Ca^{2+} content. In lactotroph cells, an inwardly rectifying K^+ (Kir) current contributes to the maintenance of the resting potential and is blocked by TRH. In these cells, K^+ current is typically carried by ether-à-go-go (erg) related K^+ channels. Erg channels are voltage-dependent K^+ channels that mediate Kir currents by shifting the membrane potential towards the K^+ equilibrium potential with small outward currents (59). TRH-induced reduction of the K^+ erg current is mediated by a G pro-

tein-coupled intracellular signal cascade. Elucidation of this pathway may allow the development of new drugs to target the erg K+ current (60) and, consequently, limit TRH-induced pathological hyperprolactinemia.

Another promising field has emerged from Kunz's study (10). The authors showed that the activity of Ca^{2+}-activated K^+ channel (BK_{Ca}), present in luteinized granulosa cells of the ovary, plays a prominent role in the cessation of Ca^{2+}-induced cellular responses by repolarizing the plasma membrane. These BK_{Ca} channels mediate intraovarian signaling and are regulated *in vitro* by oxytocin and acetylcholine. These findings suggest that there is an interaction between systemic hormones and the local neuroendocrine system in control of steroidogenesis.

CONCLUSION

There is growing interest in understanding ion channel diversity in endocrine cells. This interest stems, in part, from the role many of these ion channels play in hormone secretion (β-cells), mechanotransduction and acidification (chondrocytes), hormonal excess toxicity (neuromuscular junction), chemotransduction (ovarian and gonadotroph cells) and osmoregulation (neurohypophyseal cells). A few research groups, including ours, have focused on the molecular biology of endocrine ion channel disorders to help physicians make molecular diagnoses of rare endocrine channelopathies. It is likely that some ion channels in endocrine cells are multifunctional, serving a number of different physiological purposes involved, primarily, with metabolic regulation. In summary, ion channels play an important role in mediating the endocrine system by firing, controlling and hormonally tuning specialized cellular functions. This knowledge will help us understand the unique biology of excitable endocrine cells that can lead to the development and formulation of new therapeutic strategies to treat endocrine diseases.

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