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REVIEW

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## Structural Basis and Molecular Mechanisms of Cl<sup>−</sup> Transmembrane Transport in Cardiomyocytes

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**Abstract**—The intracellular concentration of chloride anions ( $[Cl^-]_i$ ), the equilibrium potential for chloride anions ( $E_{Cl}$ ), and transmembrane chloride currents ( $I_{Cl}$ ) are significant factors influencing the electrophysiological properties of excitable tissues, including the myocardium. Several types of chloride conductance have been identified in the heart. In recent years, multiple transmembrane proteins demonstrating chloride conductance have been identified (CFTR, CIC, TMEM16, and LRRC8), and their expression in cardiac tissue has been confirmed. Accumulated data allow for establishing a molecular substrate for some chloride anion currents ( $I_{Cl,PKA}$ ,  $I_{Cl,ir}$ ,  $I_{Cl,vol}$ ,  $I_{Cl,swell}$ ,  $I_{Cl,Ca}$ , and  $I_{to2}$ ) detected in the heart. Furthermore, the molecular mechanisms regulating  $[Cl^-]_i$  and  $E_{Cl}$  through chloride cotransporters (KCC and NKCC1) and chloride-bicarbonate exchangers have been established. The variety of structures determining chloride transmembrane conductivity and the complexity of molecular mechanisms regulating chloride homeostasis underlie the complex effects of activation of chloride transporters in the pacemaker, conduction system, and working myocardium of the heart. This review discusses the structural and biophysical properties as well as molecular regulation of chloride transporter protein complexes identified in the myocardium. The review also covers the mechanisms by which chloride transmembrane transport influences the bioelectrical activity of cardiomyocytes.

**Keywords:** chloride channels, cation-chloride cotransporters, chloride-bicarbonate exchanger, chloride transport, chloride current, cardiac electrophysiology

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### 1. INTRODUCTION

Chloride is the predominant inorganic anion in biological systems. Since the mid-20th century, chloride has been known as one of the ions that forms the resting potential and determines the electrophysiological properties of excitable cells and tissues. More than half a century ago, it was discovered that chloride is an anion that regulates the volume and osmolarity of cells.

At the moment, it is obvious that the intracellular concentration of chloride anions ( $[Cl^-]_i$ ), the equilibrium potential for chloride anions ( $E_{Cl}$ ), and transmembrane chloride currents ( $I_{Cl}$ ) are factors that significantly affect the functioning of neuronal tissue, skeletal muscle cells, and smooth muscle cells of the vascular wall. For example, chloride homeostasis determines excitability, the magnitude of the resting potential, tone, contractility, synaptic transmission,

and the ability to respond to neurotransmitters in various types of tissue.

Nevertheless, the molecular structures mediating transmembrane anion chloride conductivity in various cell types and in cardiomyocytes in particular have remained unknown for a long time and, in some cases, have not been determined to this day. This fact underlies some ignoring of the role of chloride homeostasis in the physiology of certain cells and tissues, such as the myocardium. However, since the 1980s, there has been an intensive study of the role of chloride in the formation of the electrical activity of cardiomyocytes. It has been shown that chloride anions and transmembrane chloride currents determine the configuration of action potentials (AP) in various areas of the healthy heart, and disruption of homeostasis and transmembrane chloride transfer causes a change in normal electrical activity, which leads to cardiac pathologies and heart rhythm disturbances.

**Table 1.** Main characteristics of myocardial chloride ion channels

Channels	CFTR	CIC2	CaCC (TMEM16A)	VRAC (LRRC8)
Ligand-controllability	no	no	no	no
Electroneutrality selectivity	no	no	no	no
	Halide ions	Halide ions	Halide ions	Halide ions, organic osmolytes
Potential-controllability (gating)	—	+	at $[Ca^{2+}]_i < 1 \mu M$	—
Rectification	Goldman	inward	At $[Ca^{2+}]_i < 1 \mu M$ outward	Goldman
Calcium-sensitivity	—	—	+++	+
Mechanosensitivity	—	+	+	+++
Regulation by protein kinase A	+++	+	—	—
Regulation by protein kinase C	+	—	—	—

In recent years, a number of membrane proteins demonstrating chloride conductivity have been identified. A number of candidate molecules have also been identified that can potentially carry out transmembrane transport of chloride anions. The movement of chloride anions across the plasma membrane is carried out by proteins of three functional types: ion channels, exchangers, and cotransporters. This review examines chloride carriers with clearly defined electrophysiological properties and molecular nature.

## 2. TRANSMEMBRANE CHLORIDE CHANNELS AND TRANSPORTERS

Currently, the properties of chloride ion channels have been confirmed for five families (groups) of transmembrane macromolecules. The first family includes pentameric ligand-gated anion channels (e.g., glycine receptors or gamma-aminobutyric acid receptors of the A-type), belonging to the class of Cys-loop receptors. To date, there is no evidence of expression of ligand-gated chloride channels in the myocardium and/or cardiomyocyte membrane. The only representative of the second group is the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), an ABC-type transmembrane transporter that has chloride conductance. The next (third) family includes homodimeric “two-pore” voltage-gated chloride channels, designated CIC (Chloride Channels). However, of the nine types of chloride channels of the CIC family found in mammals, only four are “true” channels (CIC-1, -2, -Ka, and -Kb), while the rest are  $Cl^-/H^+$  exchangers (CIC-3, -4, -5, -6, -7). The fourth family includes voltage- and  $Ca^{2+}$ -dependent chloride channels, designated CaCC [1]. At present, it has been shown that the homodimeric “two-pore” proteins TMEM16A and TMEM16B have the properties of a  $Ca^{2+}$ -dependent chloride channel. Finally, the fifth family includes hexameric “pannexin-like” anion channels, volume-regulated

(VRAC), which are formed by proteins of the LRRC8 group (proteins containing repeats rich in leucine—Leucine Rich Repeats Containing (LRRC) protein). At present, there is very little information regarding the function, distribution, and regulation of VRAC channels consisting of LRRC8 monomers in the heart.

In addition to the above five families, there is a whole spectrum of proteins or macromolecular protein complexes that have features of chloride conductivity. Among such candidate structures, we should mention bestrophins (BEST1-4), TMEM206, proteins of the TWEETY family (TTYH1-3), and proteins of the SLCO2 family (SLCO2A1—transmembrane prostaglandin transporter). Bestrophins are pentameric  $Ca^{2+}$ -gated chloride channels [2] with a narrow range of sensitivity to intracellular  $Ca^{2+}$ . Bestrophins are predominantly expressed in the retina. Dimeric TWEETY proteins may also be  $Ca^{2+}$ -gated chloride channels [3]. TMEM206 is thought to be the major, pore-forming subunit of the channel conducting the pH-sensitive, acidification-activated chloride current (ASOR or PACC); in turn, SLCO2A1 may be the molecular basis of the chloride current and the ultrahigh-conductance channels designated “Maxi-Cl” [4]. The currently available data on the properties of the above four types of molecules are rather contradictory, and their expression in the myocardium has not been reliably established. Therefore, only those macromolecules for which chloride conductivity and expression in the heart have been confirmed and for which there is evidence indicating a functional role for these channels in the myocardium will be considered here (Table 1). The list of such channels includes CFTR, CIC-2, CaCC (TMEM16), and VRAC (LRRC8x).

### 2.1. Cystic Fibrosis Transmembrane Regulator—CFTR

CFTR channels belong to a large superfamily of ATP-binding cassette transporters, widely represented

in all living organisms [5]. ABC transporters ensure the removal of small organic molecules into the extracellular environment from the membrane and cytoplasm due to ATP hydrolysis. Proteins of this family have a rather conservative structure. The molecule of the canonical ABC transporter consists of four domains, two of which are transmembrane, and the other two are cytosolic or nucleotide-binding. Each of the two transmembrane domains contains six  $\alpha$ -helices. As a rule, in eukaryotes, all four domains of the ABC transporter are part of a single polypeptide chain. CFTR belongs to class C of ABC proteins, which are characterized by the linkage of four domains into a single polypeptide chain. Homologous parts of CFTR are linked to each other by a cytosolic regulatory chain, the R-domain [5] (Fig. 1a).

CFTR channels are expressed in large numbers in cardiomyocytes (Fig. 1a, Supplementary Information). CFTR channels are characterized by the so-called Goldman rectification. This means that the dependence of the current flowing through CFTR on the membrane potential (volt-ampere characteristic) is close to the Goldman (Fig. 2a). This type of volt-ampere characteristic indicates the absence of any gating mechanisms in the CFTR channel. When recording the electrical activity of single activated CFTR channels, typical discrete stepwise jumps in conductivity and discrete transitions between closed and open states are observed. Sufficiently long periods of CFTR in the open state (0.1–0.2 ms) are replaced by periods of the closed state (1–2 ms) [6, 7].

In order for the CFTR channel to acquire the ability to switch to the open state, firstly, phosphorylation of the regulatory R-chain by cAMP-dependent protein kinase (PKA) [8] and, secondly, binding of ATP to the nucleotide-binding domains are necessary [9]. Despite the fact that ATP is necessary for opening the CFTR channel, the intracellular concentration of ATP is not a regulator of channel activity since physiological regulation of channel conductance is carried out due to phosphorylation/dephosphorylation of the CFTR protein. It has been shown that phosphorylation of CFTR by protein kinase C (PKC) leads to opening of the CFTR channel [8]. According to some data, full activation of CFTR is possible only with phosphorylation of PKC in addition to PKA [10]. CFTR activity can also be influenced by AMP-dependent protein kinase (AMPK) [11], tyrosine kinases [12], and  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinases (CaMK) [6]. In turn, “classical” protein phosphatases, such as phosphatase 2A (PP2A), dephosphorylate CFTR, reducing its conductance and decreasing the channel’s open time [13]. Thus, CFTR is a typical channel whose conductance and current value depend on the level of phosphorylation. It is believed that most of the so-called PKA-dependent chloride current (ICl, PKA) is formed by CFTR channels.

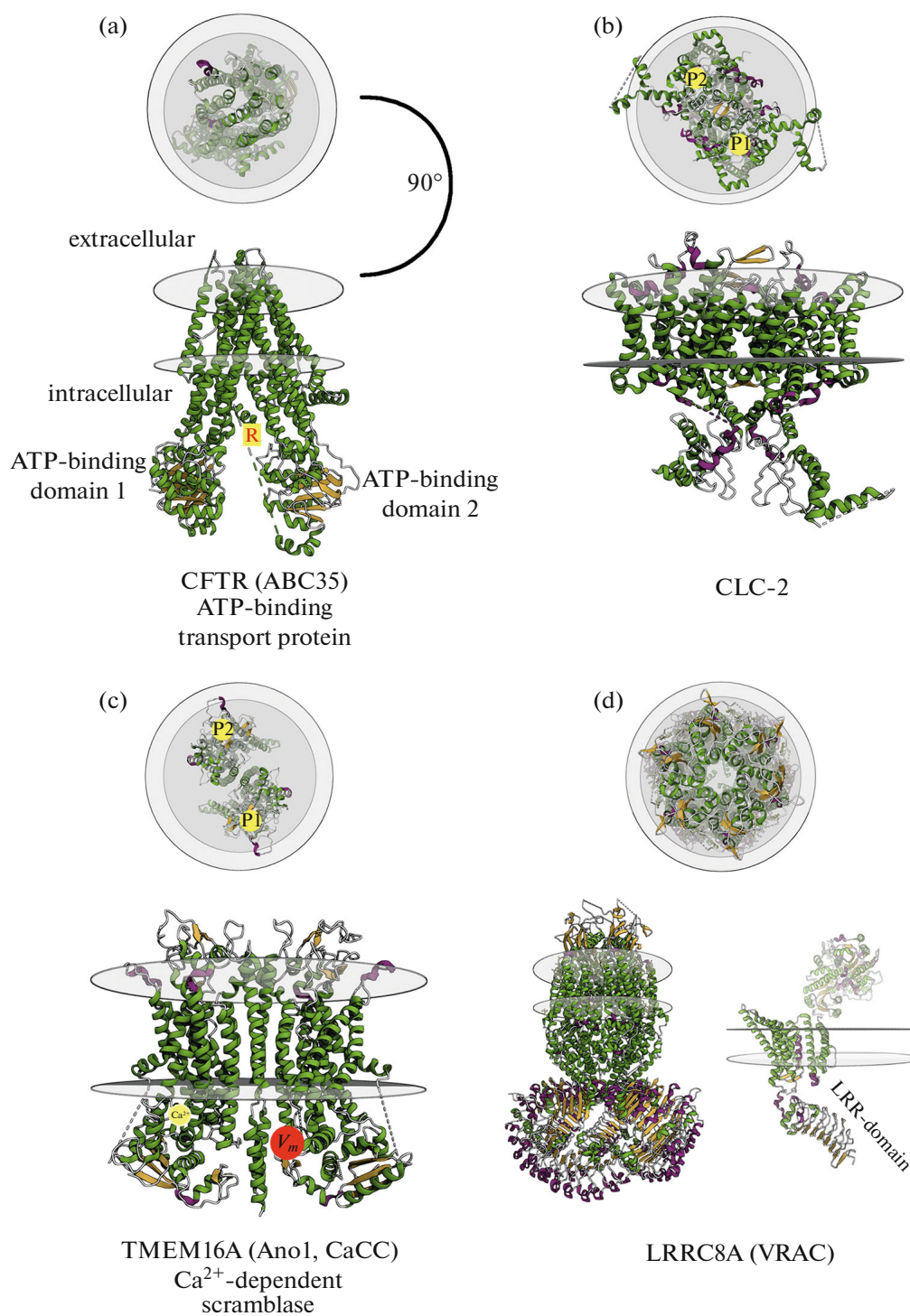
Since CFTR is an ABC transporter, a number of organic anions with a molecular weight of 200–1000 Da can block CFTR. As a rule, the degree of blocking depends on the membrane potential (MP) and is more pronounced at more negative MP values. Potential-dependence of the blocking effect is observed for such compounds as flufenamic acid, glibenclamide, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) [14], 3-(N-morpholino)propanesulfonic acid (MOPS), and anthracene-9-carboxylic acid [6].

## 2.2. CIC Family of Chloride Channels

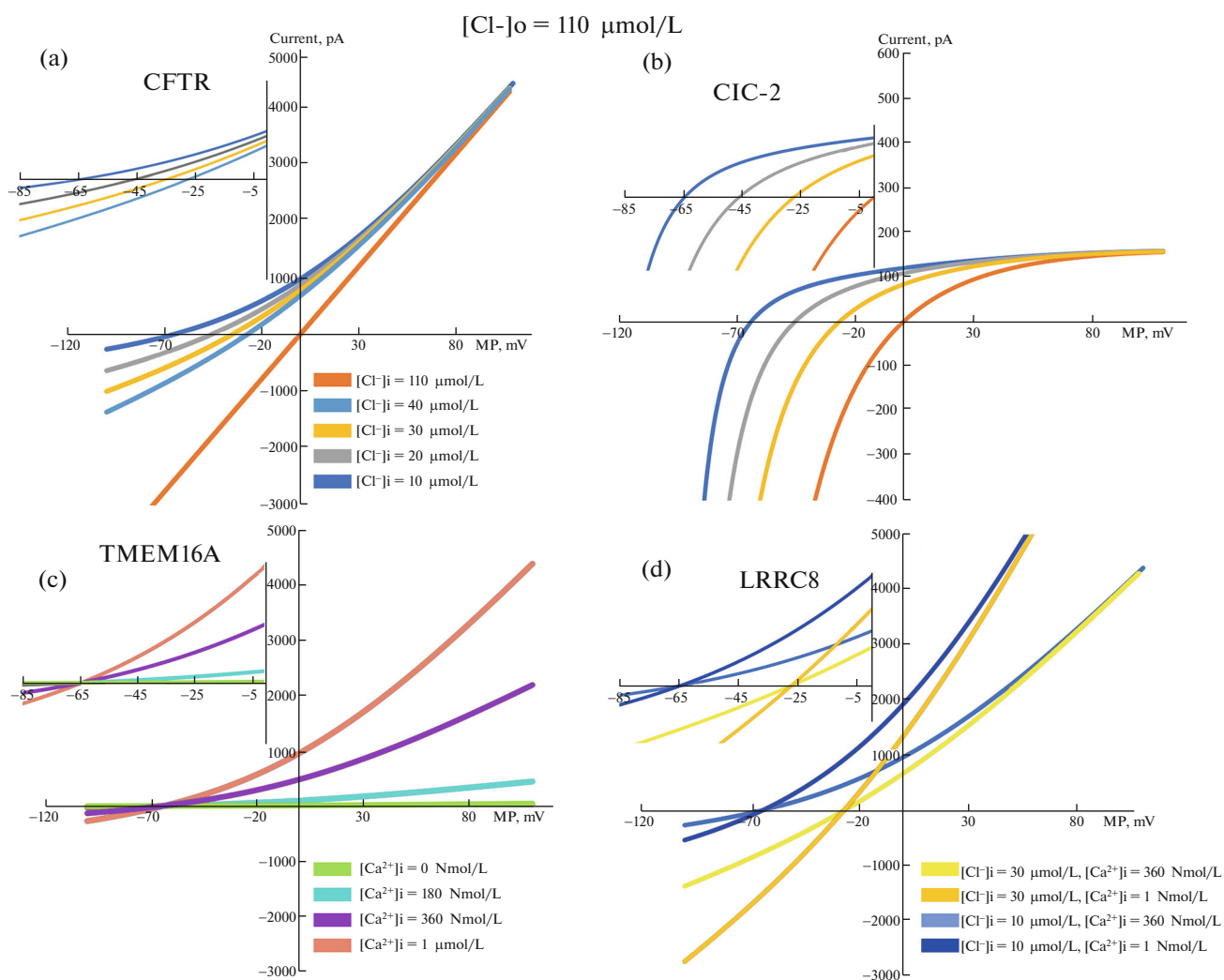
Transmembrane proteins of the CIC family are widely represented in all types of animals. In mammals, CIC are expressed in most cell types and participate in maintaining anion homeostasis, resting potential, and in regulating cell volume. Among the representatives of this family, two structurally similar but functionally different groups can be distinguished. The first group includes voltage-dependent chloride channels (CIC-1, -2, -Ka and -Kb, K—kidney). CIC-1 (CICN1) is the most studied chloride channel. Expression of CIC-1, like CIC-Ka/b, has not been shown in the heart. The second group (CIC-3, -4, -5, -6, -7) includes transmembrane proteins that are permeable not only to chloride ions, but also to protons, and are, in fact,  $\text{Cl}^-/\text{H}^+$  exchangers (antiporters), which are mainly localized in the membrane of intracellular organelles (endosomes, lysosomes, and vesicles).

All proteins of the CIC family consist of two identical subunits, although they can also form heterodimers [15]. Each of the subunits contains 18  $\alpha$ -helices, 17 of which are transmembrane. Some  $\alpha$ -helices are short and do not completely cross the membrane, which leads to the appearance of small loops inside the membrane that form intrachannel “pathways” for anions and protons. The C-terminal fragment of each subunit of CIC proteins contains two evolutionarily conserved regulatory CBS domains that bind cytoplasmic ATP, AMP, and other purine nucleotides.

It is important that the transmembrane transfer of chloride ions by each subunit of the CIC family of channels is kinetically independent since each subunit of the dimeric channel complex forms its own structurally complete ionic pathway (Fig. 1b). It should be noted that chloride ions can move inside the channel along two trajectories near the cytoplasmic boundary of the membrane, so the CIC ionic pathway is not a “pore” in the strict sense of this term. CIC channels have several types of gating mechanisms that determine their voltage dependence. It is assumed that there is a fast gating mechanism (“fast activation gate”) determining the individual state of each “monopore” as well as a slow gating mechanism affecting the accessibility of the pathway of both monomers simultaneously [16]. It has been suggested



**Fig. 1.** Structure of the major protein complexes that act as chloride anion channels in the myocardium. The upper panels show the channel from the extracellular side in the plane of the plasma membrane. In the protein structure,  $\alpha$ -helices are shown in green,  $\beta$ -sheets in yellow, and the 310-helix in purple. (a) Structure of the CFTR channel and its location in the plasma membrane with the location of the ATP-binding domains and the regulatory domain (R) indicated. (b) Structure of the CLC-2 channel and its location in the plasma membrane with channel pores formed by each of the channel subunits (P1 and P2) indicated. (c) Structure of the TMEM16A channel and its location in the plasma membrane with the channel pores formed by each of the channel subunits (P1 and P2), the calcium-binding pocket of the channel ( $\text{Ca}^{2+}$ ), and the voltage-sensing region of the channel ( $V_m$ ) indicated. (d) Structure of the LRRC8A channel and its location in the plasma membrane with the leucine-rich repeat-containing (LRR) domain indicated. Molecular structures were constructed using <https://www.rcsb.org/>.



**Fig. 2.** Current-voltage characteristics of the main chloride (anion) currents of myocardial cells at different concentrations of intracellular  $\text{Cl}^-$  ( $[\text{Cl}^-]_i$ ) and intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ). The insets show an enlarged portion of the current-voltage characteristics with the points of intersection of the curves with the X-axis, which corresponds to the equilibrium chloride potential. (a) PKA-dependent chloride current generated by the CFTR channel. (b) Anomalous rectifier chloride current generated by the CIC-2 channel. (c)  $\text{Ca}^{2+}$ -dependent chloride current generated by the TMEM16A channel. (d) Volume-regulated chloride current generated by the LRRC8 channel. The extracellular concentration of  $\text{Cl}^-$  ( $[\text{Cl}^-]_o$ ) is 110 mmol/L. Model curves were constructed using the Goldman–Hodgkin–Katz (GKH) equation for transmembrane ion current. Model curves for voltage-dependent and/or calcium-dependent ion currents were obtained by multiplying the GKH by the equilibrium probability of the channel being in the open state or a coefficient proportional to  $[\text{Ca}^{2+}]_i$ . The range limited by the curves for 10 and 40 mmol/L  $[\text{Cl}^-]_i$  corresponds to the possible values of ion current potentially achieved in cardiomyocytes of different parts of the heart.

that fast voltage-dependent activation (fast gating mechanism) is not associated with the presence of voltage-sensitive segments in the channel complex [17]. Indeed, CIC subunits and channel complexes lack a potential sensor with a canonical structure. Probably, the mechanisms of voltage-dependent activation and deactivation differ significantly for CIC channels of different types (CIC-1, -2, -Ka, and -Kb). This feature is expressed in the fact that the current-voltage characteristics of CIC-1, -2, -Ka, and -Kb have different shapes.

**2.2.1. CIC-2.** CIC-2 are potential-dependent chloride channels of the plasma membrane, which are expressed in most mammalian tissues [15]. Among all CIC, CIC-2 is highly expressed in the heart and directly in cardiomyocytes (Fig. 1b, Supplementary Information).

Like other channels of this family, CIC-2 are dimers with two pores [18]. CIC-2 are characterized by slow and fast gating mechanisms, which determine the potential dependence of the channel. It is assumed that the fast potential-dependent activation of CIC-2

is due to an electrosteric mechanism: under conditions when  $V_m < E_{Cl}$  (for example, during hyperpolarization), intracellular chloride ions enter the internal “gate” pore-forming regions of the molecule. Electrostatic repulsion of chloride ions and the polarized negatively charged glutamate residue leads to a change in the position of the latter as a result of the destruction of some and the formation of others of hydrogen bonds (“glutamate gates”), which makes the pore accessible for the movement of anions [16]. Under conditions where  $V_m > E_{Cl}$ , the electric field prevents intracellular  $Cl^-$  from entering the channel and overcoming the electrosteric glutamate gates. Thus, hyperpolarization ( $V_m < E_{Cl}$ ) leads to activation, and depolarization ( $V_m > E_{Cl}$ ) to deactivation of the channel. The electrosteric gating mechanism leads to the fact that the volt-ampere characteristic for CIC deviates from the “classical” curve with Goldman rectification (Fig. 2a) and takes the form of a curve with an inward (or “anomalous” or “incoming”) rectification (“inward rectifying,” “ir”; Fig. 2b).

Due to the peculiarities of the gating mechanism, the anion current through the CIC-2 channels is activated under native conditions by hyperpolarization with inward rectification ( $I_{Cl,ir}$ ). The amplitude and reversal potential ( $E_{Cl}$ ) of the  $I_{Cl,ir}$  current depend on the concentration of chloride ions on both sides of the membrane. An increase in  $[Cl^-]_i$  leads to a shift in  $E_{Cl}$  to less negative values and a corresponding shift in the MP value at which this channel can be activated and conduct a depolarizing current—inward in the direction of movement of positive charges but outward in the direction of movement of  $Cl^-$  anions (Fig. 2b). At low  $[Cl^-]_i$ , the current through the CIC-2 channels acquires a strong dependence on the extracellular concentration of chloride ( $[Cl^-]_o$ ).

The state of the CIC-2 channel strongly depends on the intracellular pH: a slight decrease in pH (acidification of the cytoplasm, an increase in  $[H^+]_i$ ) leads to easier activation of the channel, but the channel closes with a further decrease in pH [19]. The mechanism of conductivity increase with decreasing pH is that protons neutralize the negative charge of glutamate “glutamate gates,” facilitating the entry of intracellular chloride into the “vestibule” of the channel. In addition, CIC-2 channels can also be activated by intracellular hypervolemia [19]. CIC-2 channels can be a direct or indirect target of various types of protein kinases [20, 21]. A problem in studying the properties and role of CIC-2 is the lack of their low-molecular ligands with high affinity and selectivity [15].

### 2.3. $Ca^{2+}$ -Sensitive Chloride Channels—CaCC

As indicated above, structures with chloride conductivity, voltage dependence, and sensitivity to  $Ca^{2+}$  are designated as CaCC. The chloride ion current

demonstrating voltage- and  $Ca^{2+}$ -sensitivity formed by CaCC is usually designated as  $I_{Cl,Ca}$ . Previously, it was believed that the molecular basis of CaCC is homodimeric channel proteins, where each monomer consists of eight transmembrane segments ( $\alpha$ -helices) [1]. Such proteins were called anoctamines (Ano) [22–23]. However, recent studies [24–26] have established that each monomer of the pore-forming subunit of CaCC includes ten transmembrane  $\alpha$ -helices. Proteins of this type were previously designated TMEM16 as one of the families of weakly similar transmembrane proteins with unknown function combined into the TMEM group (TransMEMbrane Members). At the moment, there is no doubt that homodimers TMEM16A (Ano1) or TMEM16B (Ano2) are molecular substrates for CaCC and pore-forming subunits for the  $I_{Cl,Ca}$  current [27]. Each of the monomers of the homodimeric TMEM16 channel has a pore permeable to chloride ions. Thus, CaCC are “two-pore” channels (Fig. 1c). TMEM16A/B channels are widely expressed in cardiomyocytes of mice, dogs, [28], rabbits [29], and pigs [30]. The level of TMEM16 expression in the human myocardium, unlike other mammals, is low (Fig. 1c, Supplementary Information) [31].

The conductance of TMEM16A channels expressed in heterologous systems increases upon depolarization but decreases upon hyperpolarization, so that the  $I_{Cl,Ca}$  current exhibits the properties of an outward rectifier current (Fig. 2c) [32]. However, the main feature of CaCC/TMEM16A is the regulation of its conductance by cytosolic  $Ca^{2+}$  [33].

Each of the CaCC/TMEM16A channel dimers can bind two  $Ca^{2+}$  ions. The binding apparently occurs cooperatively [34–35]. However, no canonical  $Ca^{2+}$ -binding motifs have been identified in the TMEM16A structure. It is suggested that the amino acid sequence in the first intracellular loop forms a “calcium pocket” (Fig. 1c), characteristic, for example, of large-conductance  $Ca^{2+}$ -activated potassium channels (BK, Big Potassium) [1]. The negatively charged amino acid residues of the TMEM16A calcium pocket face the lumen of the conducting pore [36].

It is believed that the voltage-sensitivity of CaCC/TMEM16A is due to direct voltage-dependent  $Ca^{2+}$  binding by the negatively charged amino acid residues of the calcium pocket [24–25]: the more positive the potential, the more efficiently  $Ca^{2+}$  ions penetrate from the cytoplasm into the  $Ca^{2+}$ -binding pocket, neutralizing the negative electrostatic charge in the pore and making it accessible to chloride ions. In addition, the higher the  $[Ca^{2+}]_i$ , the more TMEM16A channels will be activated. Thus, voltage-sensitivity and  $[Ca^{2+}]_i$  dependence for CaCC/TMEM16A are related characteristics. An increase in  $[Ca^{2+}]_i$  leads to an increase in both the inward and outward components of the current through CaCC/TMEM16A (Fig. 2c).



However,  $I_{Cl,Ca}$  at low (basal,  $< 0.1 \mu M$ )  $[Ca^{2+}]_i$  corresponding to the resting state of the cell can also be voltage-dependently activated [37]. It is believed that the voltage dependence of CaCC/TMEM16A at zero or very low  $[Ca^{2+}]_i$  is due to either the presence of a separate “noncanonical”  $Ca^{2+}$ -independent potential sensor in the channel (Fig. 1c) or voltage-dependent protonation of amino acid residues of the  $Ca^{2+}$ -binding pocket and other cytoplasmic regions of the molecule [38]. Cytoplasmic acidification leads to an increase in the probability of TMEM16A remaining in the open state and an increase in  $I_{Cl,Ca}$  at all MP values [39]. Among other things, the TMEM16A conductivity and the  $I_{Cl,Ca}$  value depend on  $[Cl^-]_o$ . As a result, the mechanism of TMEM16A channel activation is extremely complex since it is determined by  $[Ca^{2+}]_i$ , membrane potential, intracellular pH, and  $[Cl^-]_o$ ; the CaCC/TMEM16A channel cannot, in the strict sense, be defined as voltage-gated. In turn, the presence of voltage sensitivity at zero  $[Ca^{2+}]_i$  does not allow us to consider the channel as ligand-gated.

It has been established that CaCC/TMEM16A activation occurs due to  $Ca^{2+}$  entering through voltage-dependent calcium channels, nonselective cation channels, and also during the release of  $Ca^{2+}$  from the sarcoplasmic reticulum [40]. TMEM16B is less sensitive to  $Ca^{2+}$  than TMEM16A, and the kinetics of  $Ca^{2+}$ -dependent activation and deactivation of TMEM16B are much faster than that of TMEM16A [22, 41]. After activation by calcium, TMEM16A/B does not show any inactivation of the channel over time.

CaCC/TMEM16A is a target of a number of intracellular signaling molecules and kinases [37]. The  $Ca^{2+}$ /calmodulin complex activates TMEM16A [42–43]. As with many other channels, phospholipids (phosphatidylinositol 4,5-bisphosphate) and cholesterol derivatives [44] have a significant effect on the ability of CaCC/TMEM16A to be activated [45]. Some studies have shown the sensitivity of TMEM16A to mechanical stimuli [46]. CaCC/TMEM16A has been shown to be activated by G-protein coupled receptors (GPCRs) [33, 35].

It has been shown that classical anion conductance blockers—DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid), NPPB (5-nitro-2-(3-phenylpropylamino) benzoic acid), and 9-AC (9-anthracene carboxylic acid) [1]—have a blocking effect on CaCC/TMEM16. A number of studies have shown that such compounds as T16inh-A0, CaCCinh-A01, benzobromarone, dichlorphen, and hexachlorophene can be TMEM16A blockers [47, 49]. N-((4-methoxy)-2-naphthyl)-5-nitroanthranilic acid (MONNA) is suggested to be a highly selective inhibitor of TMEM16A [50].

## 2.4. Volume-Regulated Chloride Channels (VRACs)—LRRC8x

The molecular substrate for the volume-regulated transmembrane anion conductance has not been identified for a long time. There are many variants of designation of putative “volume-sensitive” channels in the literature, such as VSOR (volume-sensitive outward rectifier channels), VSOAC (volume-sensitive anion/organic osmolyte channels), or VRAC (volume-regulated anion channels), which are homo- or heterohexamers formed by transmembrane proteins of the LRRC8 family.

LRRC8 heterohexamers can include monomers of five different types: LRRC8A...E [51]. All LRRC8s have a transmembrane pore-forming domain that consists of at least four transmembrane  $\alpha$ -helices and is highly homologous to the transmembrane domain of connexins and innexins. The phylogeny of LRRC8s is suggested to be related to pannexins and other gap junction proteins [52]. In addition to the transmembrane domain, each LRRC8 protein molecule has an extracellular domain and an intracellular, C-terminal leucine-rich repeat (LRR) domain with a characteristic tertiary structure (Fig. 1d) [53]. The biophysical properties of hexameric channels depend on their subunit composition [54–55].

LRRC8A, also known as Swell1, is an obligatory subunit of the VRAC/LRRC8 hexameric channel [56–57]. The functional channel is a heteromer comprising LRRC8A and any of the other monomers (LRRC8B, C, D, or E) [15]. Suppression of LRRC8A expression results in a decrease in the hypervolemia-activated chloride current ( $I_{Cl,vol}$ ), but elimination of any of the other subunits does not affect this current [57–58].

It has been established that VRAC/LRRC8 are expressed in all types of vertebrate cells, including cardiomyocytes (Fig. 1d, Supplementary Information). It has been shown that LRRC8C is expressed to the greatest extent in the heart [59]. Human cardiomyocytes express LRRC8A, LRRC8B, LRRC8C, and LRRC8E.

These channels are believed to play a key role in maintaining cell volume [51]. The conductance of the above channels increases in response to hypervolemia and (hypo)osmotic stress, which promotes the return of cells to their initial volume and initial osmolarity due to the regulated release of halogen ions and negatively charged organic osmolytes from the cytoplasm. It is believed that the restoration of cell volume is realized due to the passive transmembrane, outward movement of water molecules, which occurs following the activation of VRAC/LRRC8 [52].

The chloride anion current through VRAC/LRRC8 channels, designated as  $I_{Cl,vol}$  or  $I_{Cl,swell}$ , has an inward and outward component and demonstrates the properties of outward rectification

**Table 2.** Main characteristics of chloride cotransporters and exchangers in the myocardium

Transporters	Cotransporters		Exchangers	
	NKCC1	KCC1	$\text{HCO}_3^-/\text{Cl}^-$ -exchanger	
	SLC12A2	SLC12A4	SLC4A	SLC26A
Stoichiometry	$1\text{Na}^+ : 1\text{K}^+ : 2\text{Cl}^-$ (all ions in)	$1\text{K}^+ : 1\text{Cl}^-$ (all ions out)	$1\text{HCO}_3^-$ (out) : $1\text{Cl}^-$ (in)	$n\text{HCO}_3^-$ (out) : $1\text{Cl}^-$ (in) or $1\text{HCO}_3^-$ (out) : $2\text{Cl}^-$ (in) or $1\text{HCO}_3^-$ (out) : $1\text{Cl}^-$ (in)
Electroneutrality	+	+	+	+/-
Potential dependence	+	+	-	-
Mechanisensitivity	Activated by decreasing cell volume	Activated by increasing cell volume	-	-
Dependence on $[\text{Cl}^-]_i$	Increasing $[\text{Cl}^-]_i$ suppresses NKCC	Increasing $[\text{Cl}^-]_i$ suppresses KCC	-	-

close to the Goldman one (Fig. 2d).  $I_{\text{Cl,vol}}$  is characterized by inactivation over time, observed at positive MP values. The severity of this inactivation depends on the type of LRRC proteins forming the ion channel [51]. The anion selectivity of VRAC/LRRC8 is due to the high positive electrostatic charge of the channel conduction pathway.

VRAC/LRRC8 conductance depends on the concentration of intracellular ATP, cytoplasmic pH, and  $[\text{Ca}^{2+}]_i$  [61–62]. An increase in  $[\text{Ca}^{2+}]_i$  promotes an increase in VRAC conductance at all MP values (Fig. 2d). With a decrease in pH from 7.4 to 6, the conductance increases, but a further decrease in pH suppresses VRAC/LRRC8.

VRAC/LRRC8 are targets of GPCR-coupled kinases [63]. Phosphorylation by PKC $\alpha$  and PKC $\beta$  kinases enhances the activation of VRAC/LRRC8 channels by intracellular ATP [64]. VRAC/LRRC8 conductance is modulated by P2Y2-type purine receptors [65]. It is also known that inhibition of myosin light chain kinase suppresses conductivity, and inhibition of myosin light chain phosphatase increases VRAC/LRRC8 conductivity [51]. In addition, inhibition of the Rho/Rho kinase pathway leads to disruption of VRAC/LRRC8 activation up to their complete suppression of conductivity [66].

### 3. CHLORIDE TRANSPORTERS AND EXCHANGERS

Along with chloride channels, the heart is widely represented by chloride transmembrane ATP-independent transporters and exchangers that determine  $[\text{Cl}^-]_i$  and, accordingly,  $E_{\text{Cl}}$ , influencing the electrophysiological characteristics of the myocardium

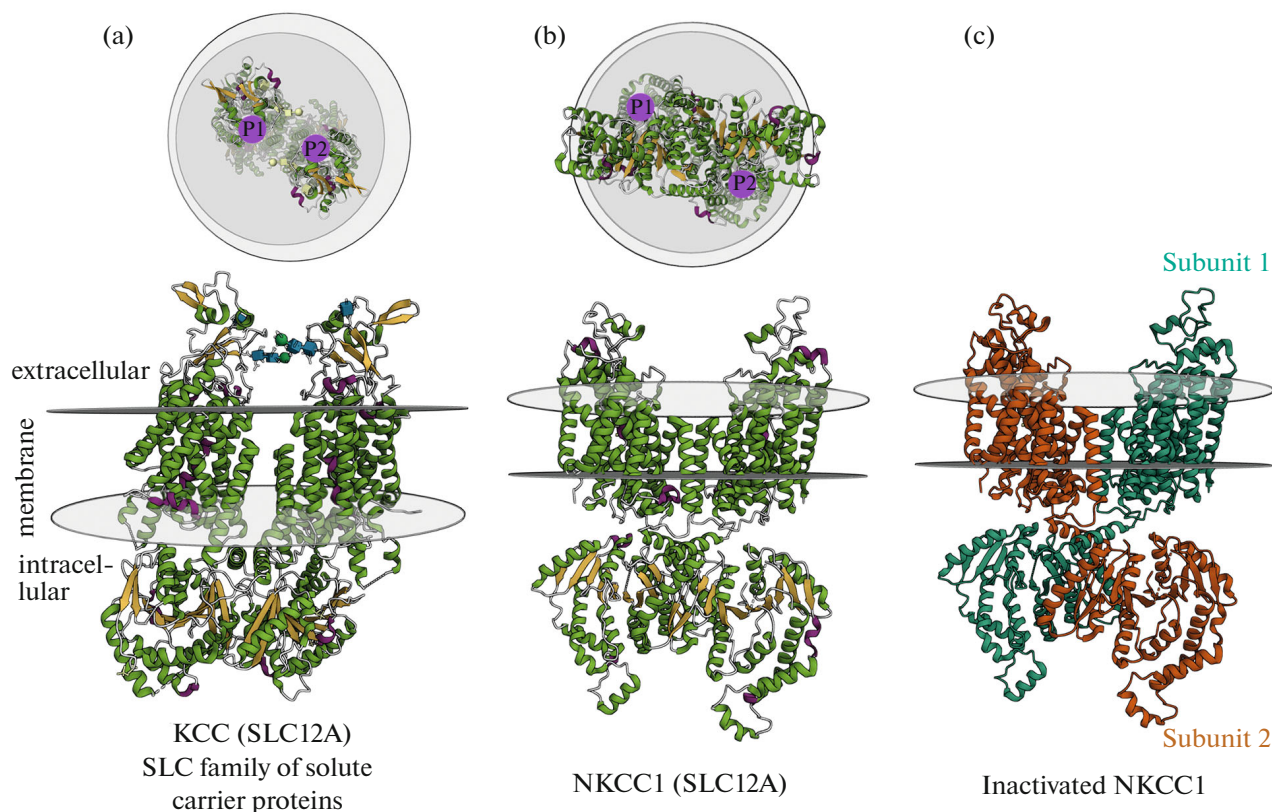
(Table 2). Currently, many chloride-cation and chloride-anionic transmembrane transporters are known, which can be both electroneutral and electrogenic. The key role among the chloride-cation cotransporters (or symporters) in the heart is played by NKCC and KCC, which belong to the large group of transmembrane molecules SLC12 (SoLute Carrier) [67]. Among the chloride-anion transporters in the heart, chloride-bicarbonate antiporters or otherwise  $\text{Cl}^-/\text{HCO}_3^-$ -exchangers [68], which are members of the SLC4 or SLC26 families [15], are important.

#### 3.1. Cation Chloride Cotransporters

Cation chloride cotransporters (CCC) provide transmembrane electroneutral symport of  $\text{Cl}^-$  and  $\text{K}^+$  or  $\text{Na}^+$ . The SLC12 family includes a single cotransporter SLC12A3, which transports one  $\text{Na}^+$  and one  $\text{Cl}^-$  (NCC), two cotransporters (SLC12A2—NKCC1 and SLC12A1—NKCC2), which perform simultaneous (unidirectional) transport of  $\text{Na}^+$ ,  $\text{K}^+$ , and two  $\text{Cl}^-$  (NKCC), and four cotransporters, which perform unidirectional transport of  $\text{K}^+$  and  $\text{Cl}^-$  (KCC, SLC12A4—KCC1, SLC12A5—KCC2, SLC12A6—KCC3, and SLC12A7—KCC4) [69]. The structure of all CCC (SLC12) is similar: the cotransporter monomer includes 12 transmembrane  $\alpha$ -helices and large intracellular N- and C-terminal domains [70] (Fig. 3). All cotransporters of this family, with the exception of KCC4, are dimers [67, 71].

CCC activity is under tight control by multiple regulatory factors, such as GPCR agonists. Moreover, the data obtained to date indicate a tissue-specific nature of CCC regulation by receptors and their intracellular





**Fig. 3.** Structure of chloride transmembrane cotransporters (symporters). The upper panels show the view of the transporters from the extracellular side in the plane of the plasma membrane. The  $\alpha$ -helices are shown in green,  $\beta$ -sheets in yellow, and the 310-helix in purple. (a) The structure of the SLC12A family potassium-chloride cation cotransporter (KCC) showing the pores formed by each subunit of the dimer (P1 and P2). (b) The structure of the SLC12A family potassium-sodium-chloride cation cotransporter (NKCC1) showing the pores formed by each subunit of the dimer (P1 and P2). (c) The relative positions of the subunits of the inactivated NKCC1 transporter. The molecular structures were constructed using <https://www.rcsb.org/>.

signaling cascades [72, 73]. For example, in isolated rat aortic smooth muscle cells, an increase in cAMP concentration upon stimulation of  $\beta$ -adrenergic receptors and adenylate cyclase (AC) causes suppression of NKCC1 activity, whereas an increase in  $[\text{Ca}^{2+}]_i$  increases its activity [74–75]. At the same time, an increase in the intracellular concentration of cGMP in smooth muscle cells does not affect NKCC1 but increases the activity of KCC [76].

Cotransporters of the CCC family have phosphorylation sites for protein kinases of various types [77]. Indeed, phosphorylation by serine-threonine kinases of the WNK family affects the activity of CCC [67]. Phosphorylation of NKCC1-2 and NCC by kinases of the WNK family leads to an increase in the activity of these transporters. In turn, phosphorylation of KCC 1–4 by these kinases leads to suppression of the activity of transporters [78]. Thus, the regulation of NKCC and KCC activity is reciprocal. Paradoxically, a number of studies have found that the “classical” serine-threonine kinases, PKA and PKC, do not affect NKCC and KCC [77].

Changes in cellular volume affect the activity of CCC. A decrease in cell volume (“compression”), a decrease in the tension of the cell membrane, for example, with an increase in the osmolarity of the external environment, leads to the activation of NKCC but suppresses KCC. An increase in NKCC conductivity under such conditions leads to the entry of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  into the cytoplasm, an increase in the concentration of intracellular osmolytes, and restoration of cell volume under the action of hyperosmotic shock.

Conversely, an increase in cell volume, for example, as a result of a decrease in the osmolarity of the extracellular environment, cell swelling, and membrane stretching leads to an increase in the conductivity of the KCC, as a result of which  $[\text{K}^+]_i$  and  $[\text{Cl}^-]_i$  decrease, and the effect of hypoosmotic shock on the cell is weakened and cell volume is restored [78, 79].

Thus, like the VRAC/LRRC8 channels, KCC and NKCC cotransporters participate in the regulation of volume, maintaining ionic and osmotic homeostasis of cells. The NKCC and KCC cotransporters are thus an important element of the cell volume regulation

system. KCC and NKCC work reciprocally—the action of NKCC is opposite to the effects of KCC. Due to the volume regulation, KCC and NKCC participate in the regulation of cell proliferation [78]. It should be noted that the activity of the CCC depends on  $[\text{Cl}^-]_i$ . For example, an increase in  $[\text{Cl}^-]_i$  suppresses NKCC but stimulates KCC [80].

The pharmacology of the CCC is well developed since these cotransporters are targets of “loop diuretics”—compounds used in the treatment of arterial hypertension. The best-known loop diuretic is furosemide, which, along with other targets, blocks the work of NKCC2 in the proximal convoluted tubules, suppressing sodium reabsorption and stimulating water excretion [81–82].

**3.1.1.  $\text{K}^+$  and  $\text{Cl}^-$  cotransporters—KCC.** KCC cotransporters mediate electroneutral  $\text{Na}^+$ -independent transport of  $\text{K}^+$  and  $\text{Cl}^-$  ions across the plasma membrane. KCC is expressed in virtually all tissues—epithelium, neurons, and other brain cells, kidneys, skeletal muscles, and heart (Fig. 2a, Supplementary Information) [83].

Transcripts of all four types of KCC transporters are present in the heart, but the presence of only KCC1, KCC3 and KCC4 proteins has been confirmed [79, 84]. Transcriptome analysis of single cells isolated from different parts of the human heart (left and right atrium, left and right ventricle, ventricular apex, interventricular septum) suggests [67] that KCC1 is the ubiquitously expressed transporter in almost all cell types of cardiac tissue, while KCC3 and KCC4 are expressed only in ventricular cardiomyocytes. Thus,  $\text{Cl}^-$  removal from cardiomyocytes is predominantly realized by the KCC1 isoform, as in most cells and tissues of the mammalian body. However, the KCC expression profile is species-specific. The level of KCC2 transcripts in the human heart is extremely low [85–86]. However, KCC2 is present in ventricular cardiomyocytes of the chicken heart, where it probably contributes to a decrease in  $[\text{Cl}^-]_i$  upon stimulation of  $\beta$ -adrenergic receptors [87].

**3.1.2. Cotransporters of  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$ —NKCC.** NKCC cotransporters mediate the electroneutral transfer of  $\text{Na}^+$ ,  $\text{K}^+$ , and two  $\text{Cl}^-$  ions across the plasma membrane. NKCC cotransporters are expressed ubiquitously [67].

NKCC1 is normally expressed in all areas of the heart, both at the mRNA and protein levels [84, 86]. To date, there is no evidence of expression of the “renal” isoform of NKCC2 (SLC12A1) in the heart [70]. As in the case of KCC,  $\text{Cl}^-$  transfer into cardiomyocytes is carried out by the ubiquitously expressed NKCC isoform, i.e., NKCC1 (Fig. 2b, Supplementary Information).

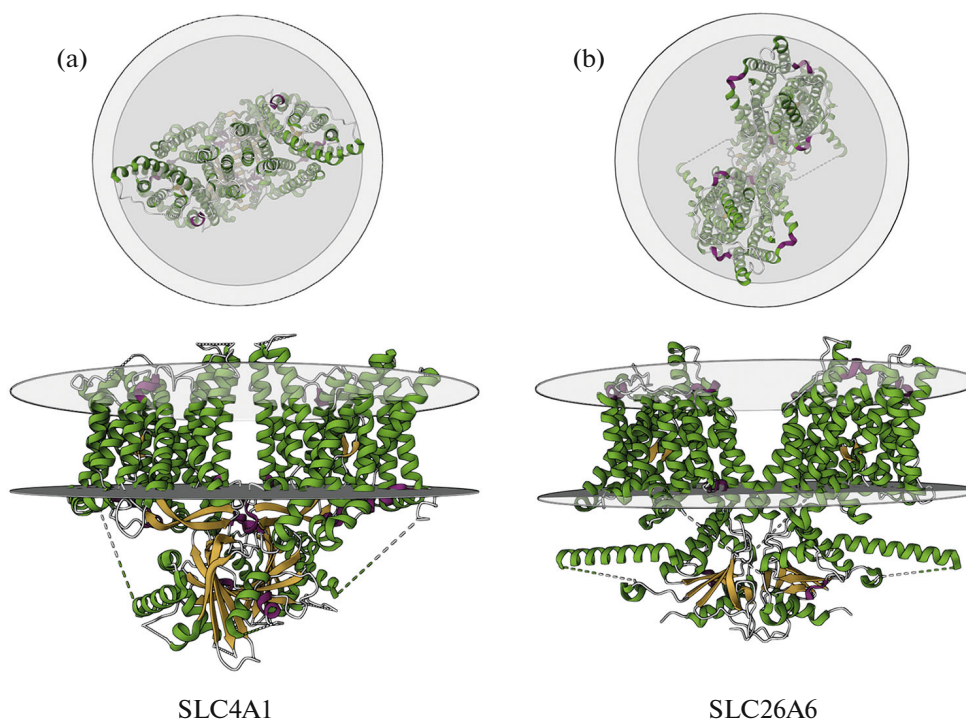
KCC and NKCC transporters are related not only functionally but also at the level of regulation of their gene expression: in KCC3 knockout mice, decreased NKCC1 expression is observed. Reciprocal attenuation of NKCC expression in KCC knockouts apparently contributes to a compensatory decrease in  $[\text{Cl}^-]_i$ . However, compensation is apparently not complete, since KCC knockouts exhibit a number of pathological changes in the cardiovascular system, including myocardial hypertrophy [88].

### 3.2. $\text{Cl}^-/\text{HCO}_3^-$ -Exchanger

The chloride-bicarbonate exchangers of the heart belong to two different families: SLC4A and SLC26A. The SLC4A family includes  $\text{Na}^+$ -dependent  $\text{Cl}^-/\text{HCO}_3^-$ -exchangers (NDCBE and NCBE) and  $\text{Na}^+$ -independent  $\text{Cl}^-/\text{HCO}_3^-$ -exchangers (AE1 (SLC4A1), AE2 (SLC4A2), and AE3 (SLC4A3)). The SLC26A family includes five different  $\text{Cl}^-/\text{HCO}_3^-$  exchangers (SLC26A3, pendrin, SLC26A6, SLC26A7, and SLC26A9). Expression of the electroneutral exchangers AE1–3, as well as SLC26A3 and SLC26A6 exchangers, has been shown in the heart to date [68, 89]. However, information on which exchanger is the main one in the heart is contradictory. It is likely that the expression profile of the above exchangers is species-specific, differs in certain parts of the heart, and changes during ontogenesis. For example, AE1 expression in the mouse heart is greatly reduced during prenatal ontogenesis [89].

The AE1–3 exchangers (SLC4A1–3) are homodimers, the monomer of which consists of three domains: the cytoplasmic N-terminal domain, the transmembrane domain, and the cytoplasmic C-terminal domain (Fig. 4a). The best known and studied  $\text{Cl}^-/\text{HCO}_3^-$ -exchanger is SLC4A1 (AE1, Band 3), a chloride-bicarbonate exchanger of the erythrocyte plasma membrane. In the mature myocardium, AE3 is hardly detected.

The AE2 exchanger is expressed in most tissues. AE2 expression in the heart is quite low, and its presence in cardiomyocytes remains unconfirmed at the moment [68]. AE3 is considered a  $\text{Cl}^-/\text{HCO}_3^-$ -exchanger of excitable tissues since it is expressed only in the heart, brain, and retina (Fig. 2a, Supplementary Information) [90]. Several studies have shown that the level of AE3 (SLC4A3) mRNA in the heart is quite high [91]. Thus, AE3 is the main electroneutral chloride-bicarbonate exchanger in the heart and is localized mainly in the plasma membrane that forms T-tubules. Interestingly, in cardiomyocytes, in addition to the presence of AE3 in the plasma membrane, the presence of this exchanger in the membrane of the sarcoplasmic reticulum cisterns has been shown [92].



**Fig. 4.** Structure of chloride-bicarbonate transmembrane exchangers (antiporters). The upper panels show the extracellular side of the exchangers in the plane of the plasma membrane. The  $\alpha$ -helices in green,  $\beta$ -sheets in yellow, and the 310-helix in purple are shown in the protein structure. (a) The structure of the sodium-independent chloride-bicarbonate exchanger AE1 (SLC4A1) and its location in the plasma membrane. (b) The structure of the chloride-bicarbonate exchanger SLC26A6 and its location in the plasma membrane. Molecular structures were constructed using <https://www.rcsb.org/>.

Chloride-bicarbonate exchangers SLC26A are also dimers, each monomer of which includes ten to 14 transmembrane segments [93] (Fig. 4b). According to various literature data, SLC26A exchangers of cardiomyocytes (Fig. 2d, Supplementary Information) can be either electrogenic (with a stoichiometry of  $2 \text{ HCO}_3^-:1\text{Cl}^-$ ) or electroneutral [94]. Like AE3, SLC26A [89] is present in the T-tubule membrane and sarcoplasmic reticulum of cardiomyocytes [92]. Due to the ability to move anions either into or out of the cell, the main function of  $\text{Cl}^-/\text{HCO}_3^-$  exchangers is the regulation of cytoplasmic pH [95].

## CONCLUSIONS

Chloride channels of the CIC, TMEM16, CFTR, and LRRC8 families are the molecular substrate of chloride ion currents expressed in the heart. Due to the multiplicity and complexity of regulation, chloride conductivity has a complex effect on the electrical activity of the heart, moreover, it affects different areas of the heart differently. Nevertheless, chloride channels and chloride carriers are fundamental for the normal functioning of the pacemaker, conduction system, and contractile myocardium of the heart. Despite a whole range of established effects, the role of the balance of chloride and its conductivity in cardiac elec-

trophysiology remains largely unexplored. In particular, it is necessary to clarify the issue of the level of cytoplasmic  $\text{Cl}^-$  in cardiomyocytes of the cardiac conduction system. It is critically important to identify the expression profile of chloride ion channels in various types of cardiac pathologies, as this will allow us to establish new aspects of myocardial electrical remodeling and identify new targets for pharmacological therapy of cardiac arrhythmias.

## SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.3103/S0096392524600741>.

## ABBREVIATIONS AND NOTATION

$[\text{Ca}^{2+}]_i$	intracellular calcium concentration
$[\text{Cl}^-]_i$	intracellular chloride anion concentration
$[\text{Cl}^-]_o$	extracellular chloride anion concentration
MP	membrane potential
CaCC	calcium-dependent chloride channels

CCC	cation chloride cotransporter
CFTR	cystic fibrosis transmembrane conductance regulator
CIC	voltage-gated chloride channels
E <sub>Cl</sub>	equilibrium potential for chloride anions
GPCR	G-protein-coupled receptors
I <sub>Cl</sub>	transmembrane chloride current
I <sub>Cl,Ca</sub>	calcium-sensitive chloride ion current
I <sub>Cl,ir</sub>	hyperpolarization-activated chloride current with inward rectification
I <sub>Cl,PKA</sub>	PKA-dependent chloride current
KCC	potassium-chloride cotransporter
LRRC	proteins containing repeats rich in leucine (Leucine Rich Repeats Containing)
NKCC	potassium, sodium, and chloride cotransporter
PKA	protein kinase A
PKC	protein kinase C
VRAC	volume-regulated anion channels

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#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

#### CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

#### REFERENCES

- Duran, C., Thompson, C.H., Xiao, Q., and Hartzell, H.C., Chloride channels: often enigmatic, rarely predictable, *Annu. Rev. Physiol.*, 2009, vol. 72, pp. 95–121.
- Miller, A.N., Vaisey, G., and Long, S.B., Molecular mechanisms of gating in the calcium-activated chloride channel bestrophin, *Elife*, 2019, vol. 8, p. e43231.
- Li, B., Hoel, C.M., and Brohawn, S.G., Structures of twenty homolog proteins TTYH2 and TTYH3 reveal a Ca<sup>2+</sup>-dependent switch from intra- to intermembrane dimerization, *Nat. Commun.*, 2021, vol. 2, no. 1, p. 6913.
- Okada, Y., Sabirov, R.Z., Merzlyak, P.G., Numata, T., and Sato-Numata, K., Properties, structures, and physiological roles of three types of anion channels mo-

lecularly identified in the 2010's, *Front. Physiol.*, 2021, vol. 12, p. 805148.

- Locher, K.P., Mechanistic diversity in ATP-binding cassette (ABC) transporters, *Nat. Struct. Mol. Biol.*, 2016, vol. 23, no. 6, pp. 487–493.
- Csanády, L., Vergani, P., and Gadsby, D.C., Structure, gating, and regulation of the CFTR anion channel, *Physiol. Rev.*, 2019, vol. 99, no. 1, pp. 707–738.
- Tabcharani, J.A., Rommens, J.M., Hou, Y.X., Chang, X.B., Tsui, L.C., Riordan, J.R., and Hanrahan, J.W., Multi-ion pore behaviour in the CFTR chloride channel, *Nature*, 1993, vol. 366, no. 6450, pp. 79–82.
- Berger, H.A., Anderson, M.P., Gregory, R.J., Thompson, S., Howard, P.W., Maurer, R.A., Mulligan, R., Smith, A.E., and Welsh, M.J., Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel, *J. Clin. Invest.*, 1991, vol. 88, no. 4, pp. 1422–1431.
- Anderson, M.P., Berger, H.A., Rich, D.P., Gregory, R.J., Smith, A.E., and Welsh, M.J., Nucleoside triphosphates are required to open the CFTR chloride channel, *Cell*, 1991, vol. 67, no. 4, pp. 775–784.
- Jia, Y., Mathews, C.J., and Hanrahan, J.W., Phosphorylation by protein kinase C is required for acute activation of cystic fibrosis transmembrane conductance regulator by protein kinase A, *J. Biol. Chem.*, 1997, vol. 272, no. 8, pp. 4978–4984.
- Hallows, K.R., Raghuram, V., Kemp, B.E., Witters, L.A., and Foscett, J.K., Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase, *J. Clin. Invest.*, 2000, vol. 105, no. 12, pp. 1711–1721.
- Billet, A., Jia, Y., Jensen, T., Riordan, J.R., and Hanrahan, J.W., Regulation of the cystic fibrosis transmembrane conductance regulator anion channel by tyrosine phosphorylation, *FASEB J.*, 2015, vol. 29, no. 9, pp. 3945–3956.
- Luo, J., Pato, M.D., Riordan, J.R., and Hanrahan, J.W., Differential regulation of single CFTR channels by PP2C, PP2A, and other phosphatases, *Am. J. Physiol.: Cell Physiol.*, 1998, vol. 274, no. 5, pp. C1397–C1410.
- Uramoto, H., Takahashi, N., Dutta, A.K., Sabirov, R.Z., Ando-Akatsuka, Y., Morishima, S., and Okada, Y., Ischemia-induced enhancement of CFTR expression on the plasma membrane in neonatal rat ventricular myocytes, *Jpn. J. Physiol.*, 2003, vol. 53, no. 5, pp. 357–365.
- Jentsch, T.J. and Pusch, M., CLC chloride channels and transporters: Structure, function, physiology, and disease, *Physiol. Rev.*, 2018, vol. 98, no. 3, pp. 1493–1590.
- Okamoto, Y., Nagasawa, Y., Obara, Y., Ishii, K., Takagi, D., and Ono, K., Molecular identification of HSPA8 as an accessory protein of a hyperpolarization-activated chloride channel from rat pulmonary vein

- cardiomyocytes, *J. Biol. Chem.*, 2019, vol. 294, no. 44, pp. 16049–16061.
17. De Jesús-Pérez, J.J., Castro-Chong, A., Shieh, R.C., and Hernández-Carballo, C.Y., Gating the glutamate gate of CLC-2 chloride channel by pore occupancy, *J. Gen. Physiol.*, 2016, vol. 147, pp. 576–613.
  18. Weinreich, F. and Jentsch, T.J., Pores formed by single subunits in mixed dimers of different CLC chloride channels, *J. Biol. Chem.*, 2001, vol. 276, no. 4, pp. 2347–2353.
  19. Arreola, J., Begenisich, T., and Melvin, J.E., Conformation-dependent regulation of inward rectifier chloride channel gating by extracellular protons, *J. Physiol.*, 2002, vol. 541, no. 1, pp. 103–112.
  20. Palmada, M., Dieter, M., Boehmer, C., Waldegger, S., and Lang, F., Serum and glucocorticoid inducible kinases functionally regulate CLC-2 channels, *Biochem. Biophys. Res. Commun.*, 2004, vol. 321, no. 4, pp. 1001–1006.
  21. Park, K., Begenisich, T., and Melvin, J.E., Protein kinase A activation phosphorylates the rat CLC-2 Cl<sup>-</sup> channel but does not change activity, *J. Membr. Biol.*, 2001, vol. 182, no. 1, pp. 31–37.
  22. Caputo, A., Caci, E., Ferrera, L., Pedemonte, N., Barsanti, C., Sondo, E., Pfeffer, U., Ravazzolo, R., Zegarra-Moran, O., and Galiotta, L.J.V., TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity, *Science*, 2008, vol. 322, no. 5901, pp. 590–594.
  23. Yannoukakos, D., Stuart-Tilley, A., Fernandez, H.A., Fey, P., Duyk, G., and Alper, S.L., Molecular cloning, expression, and chromosomal localization of two isoforms of the AE3 anion exchanger from human heart, *Circ. Res.*, 1994, vol. 75, no. 4, pp. 603–614.
  24. Dang, S., Feng, S., Tien, J., et al., Cryo-EM structures of the TMEM16A calcium-activated chloride channel, *Nature*, 2017, vol. 552, no. 7685, pp. 426–429.
  25. Paulino, C., Kalienkova, V., Lam, A.K.M., Neldner, Y., and Dutzler, R., Activation mechanism of the calcium-activated chloride channel TMEM16A revealed by cryo-EM, *Nature*, 2017, vol. 552, no. 7685, pp. 421–425.
  26. Paulino, C., Neldner, Y., Lam, A.K.M., Kalienkova, V., Brunner, J.D., Schenck, S., and Dutzler, R., Structural basis for anion conduction in the calcium-activated chloride channel TMEM16A, *eLife*, 2017, vol. 6, p. e26232.
  27. Hartzell, H.C., Yu, K., Xiao, Q., Chien, L.T., and Qu, Z., Anoctamin/TMEM16 family members are Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels, *J. Physiol.*, 2009, vol. 587, pt. 10, pp. 2127–2139.
  28. Horváth, B., Vácz, K., Hegyi, B., Gönczi, M., Dienes, B., Kistamás, K., Bányász, T., and Magyar, J., Sarcolemmal Ca<sup>2+</sup>-entry through L-type Ca<sup>2+</sup> channels controls the profile of Ca<sup>2+</sup>-activated Cl<sup>-</sup> current in canine ventricular myocytes, *J. Mol. Cell Cardiol.*, 2016, vol. 97, pp. 125–139.
  29. Sipido, K.R., Callewaert, G., and Carmeliet, E., [Ca<sup>2+</sup>]<sub>i</sub> transients and [Ca<sup>2+</sup>]<sub>i</sub>-dependent chloride current in single Purkinje cells from rabbit heart, *J. Physiol.*, 1993, vol. 468, pp. 641–667.
  30. Li, G.R., Sun, H., To, J., Tse, H.F., and Lau, C.P., Demonstration of calcium-activated transient outward chloride current and delayed rectifier potassium currents in Swine atrial myocytes, *J. Mol. Cell Cardiol.*, 2004, vol. 36, no. 4, pp. 495–504.
  31. El Chemaly, A., Norez, C., Magaud, C., Bescond, J., Chatelier, A., Fares, N., Findlay, I., Jayle, C., Becq, F., Faivre, J.F., and Bois, P., ANO1 contributes to Angiotensin-II-activated Ca<sup>2+</sup>-dependent Cl<sup>-</sup> current in human atrial fibroblasts, *J. Mol. Cell Cardiol.*, 2014, vol. 68, pp. 12–19.
  32. Berg, J., Yang, H., and Jan, L.Y., Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels at a glance, *J. Cell Sci.*, 2012, vol. 125, pt. 6, pp. 1367–1371.
  33. Pedemonte, N. and Galiotta, L.J.V., Structure and function of tmem16 proteins (anoctamins), *Physiol. Rev.*, 2014, vol. 94, no. 2, pp. 419–459.
  34. Kuruma, A. and Hartzell, H.C., Bimodal control of a Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel by different Ca<sup>2+</sup> signals, *J. Gen. Physiol.*, 2000, vol. 115, no. 1, pp. 59–80.
  35. Yang, Y.D., Cho, H., Koo, J.Y., Tak, M.H., Cho, Y., Shim, W.S., Park, S.P., Lee, J., Lee, B., Kim, B.M., Raouf, R., Shin, Y.K., and Oh, U., TMEM16A confers receptor-activated calcium-dependent chloride conductance, *Nature*, 2008, vol. 455, no. 7217, pp. 1210–1215.
  36. Brunner, J.D., Lim, N.K., Schenck, S., Duerst, A., and Dutzler, R., X-ray structure of a calcium-activated TMEM16 lipid scramblase, *Nature*, 2014, vol. 516, no. 7530, pp. 207–212.
  37. Schulte, G., International Union of Basic and Clinical Pharmacology. LXXX. The class Frizzled receptors, *Pharmacol. Rev.*, 2010, vol. 62, no. 4, pp. 632–667.
  38. Ma, K., Wang, H., Yu, J., Wei, M., and Xiao, Q., New insights on the regulation of Ca<sup>2+</sup>-activated chloride channel TMEM16A, *J. Cell Physiol.*, 2017, vol. 232, no. 4, pp. 707–716.
  39. Chun, H., Cho, H., Choi, J., Lee, J., Kim, S.M., Kim, H., and Oh, U., Protons inhibit anoctamin 1 by competing with calcium, *Cell Calcium*, 2015, vol. 58, no. 5, pp. 431–441.
  40. Ferrera, L., Caputo, A., and Galiotta, L.J.V., TMEM16A protein: A new identity for Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channels, *Physiol.*, 2010, vol. 25, no. 6, pp. 357–363.
  41. Pifferi, S., Dibattista, M., and Menini, A., TMEM16B induces chloride currents activated by calcium in mammalian cells, *Pflügers Arch., Eur. J. Physiol.*, 2009, vol. 458, no. 6, pp. 1023–1038.



42. Jung, J., Nam, J.H., Park, H.W., Oh, U., Yoon, J.H., and Lee, M.G., Dynamic modulation of ANO1/TMEM16A  $\text{HCO}_3^-$  permeability by  $\text{Ca}^{2+}$ /calmodulin, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, vol. 110, no. 1, pp. 360–365.
43. Vocke, K., Dauner, K., Hahn, A., Ulbrich, A., Broecker, J., Keller, S., Frings, S., and Mohrlen, F., Calmodulin-dependent activation and inactivation of anoctamin calcium-gated chloride channels, *J. Gen. Physiol.*, 2013, vol. 142, no. 4, pp. 381–404.
44. Cipriani, G., Serboiu, C.S., Gherghiceanu, M., Simonetta Fausone-Pellegrini, M., and Vannucchi, M.G., NK receptors, Substance P, Ano1 expression and ultrastructural features of the muscle coat in Cav-1<sup>-/-</sup> mouse ileum, *J. Cell Mol. Med.*, 2011, vol. 15, no. 11, pp. 2411–2420.
45. Pritchard, H., Leblanc, N., Albert, A.P., and Greenwood, I.A., Inhibitory role of phosphatidylinositol 4,5-bisphosphate on TMEM16A-encoded calcium-activated chloride channels in rat pulmonary artery, *Br. J. Pharmacol.*, 2014, vol. 171, no. 18, pp. 4311–4321.
46. Bulley, S., Neeb, Z.P., Burris, S.K., Bannister, J.P., Thomas-Gatewood, C.M., Jangsangthong, W., and Jaggar, J.H., TMEM16A/ANO1 channels contribute to the myogenic response in cerebral arteries, *Circ. Res.*, 2012, vol. 111, no. 8, pp. 1027–1036.
47. Balderas, E., Ateaga-Tlecuitl, R., Rivera, M., Gomora, J.C., and Darszon, A., Niflumic acid blocks native and recombinant T-type channels, *J. Cell Physiol.*, 2012, vol. 227, no. 6, pp. 2542–2555.
48. De La Fuente, R., Namkung, W., Mills, A., and Verkman, A.S., Small-molecule screen identifies inhibitors of a human intestinal calcium-activated chloride channel, *Mol. Pharmacol.*, 2008, vol. 73, no. 3, pp. 758–768.
49. Huang, F., Zhang, H., Wu M., et al., Calcium-activated chloride channel TMEM16A modulates mucin secretion and airway smooth muscle contraction, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, vol. 109, no. 40, pp. 16354–16359.
50. Oh, S.J., Hwang, S.J., Jung, J., Yu, K., Kim, J., Choi, J.Y., Hartzell, H.C., Roh, E.J., and Lee, C.J., MONNA, a potent and selective blocker for transmembrane protein with unknown function 16/anoctamin-1, *Mol. Pharmacol.*, 2013, vol. 84, no. 5, pp. 726–735.
51. Ghoul, M.R., Fiocco, T.A., and Binder, D.K., Structure-function relationships of the LRRC8 subunits and subdomains of the volume-regulated anion channel (VRAC), *Front. Cell Neurosci.*, 2022, vol. 16, p. 962714.
52. Lutter, D., Ullrich, F., Lueck, J.C., Kempa, S., and Jentsch, T.J., Selective transport of neurotransmitters and modulators by distinct volume-regulated LRRC8 anion channels, *J. Cell Sci.*, 2017, vol. 130, no. 6, pp. 1122–1133.
53. Jentsch, T.J., VRACs and other ion channels and transporters in the regulation of cell volume and beyond, *Nat. Rev. Mol. Cell Biol.*, 2016, vol. 17, no. 5, pp. 293–307.
54. Deneka, D., Sawicka, M., Lam, A.K.M., Paulino, C., and Dutzler, R., Structure of a volume-regulated anion channel of the LRRC8 family, *Nature*, 2018, vol. 558, no. 7709, pp. 254–259.
55. Kasuya, G., Nakane, T., Yokoyama, T., Jia, Y., Inoue, M., Watanabe, K., Nakamura, R., Nishizawa, T., Kusakizako, T., Tsutsumi, A., Yanagisawa, H., Dohmae, N., Hattori, M., Ichijo, H., Yan, Z., Kikkawa, M., Shirouzu, M., Ishitani, R., and Nureki, O., Cryo-EM structures of the human volume-regulated anion channel LRRC8, *Nat. Struct. Mol. Biol.*, 2018, vol. 25, no. 9, pp. 797–804.
56. Qiu, Z., Dubin, A.E., Mathur, J., Tu, B., Reddy, K., Miraglia, L.J., Reinhardt, J., Orth, A.P., and Papatoulian, A., SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel, *Cell*, 2014, vol. 157, no. 2, pp. 447–458.
57. Voss, F.K., Ullrich, F., Munch, J., Lazarow, K., Lutte, D., Mah, N., Andrade-Navarro, M.A., von Kries, J.P., Stauber, T., and Jentsch, T.J., Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC, *Science*, 2014, vol. 344, no. 6184, pp. 634–638.
58. Okada, T., Islam, M.R., Tsiferova, N.A., Okada, Y., and Sabirov, R.Z., Specific and essential but not sufficient roles of LRRC8A in the activity of volume-sensitive outwardly rectifying anion channel (VSOR), *Channels*, 2017, vol. 11, no. 2, pp. 109–120.
59. Pervaiz, S., Kopp, A., von Kleist, L., and Stauber, T., Absolute protein amounts and relative abundance of volume-regulated anion channel (VRAC) LRRC8 subunits in cells and tissues revealed by quantitative immunoblotting, *Int. J. Mol. Sci.*, 2019, vol. 20, no. 23, p. 5879.
60. Egorov, Y.V., Lang, D., Tyan, L., Turner, D., Lim, E., Piro, Z.D., Hernandez, J.J., Lodin, R., Wang, R., Schmuck, E.G., Raval, A.N., Ralphe, C.J., Kamp, T.J., Rosenshtraukh, L.V., and Glukhov, A.V., Caveolae-mediated activation of mechanosensitive chloride channels in pulmonary veins triggers atrial arrhythmogenesis, *J. Am. Heart Assoc.*, 2019, vol. 8, no. 20, p. e012748.
61. Centeio, R., Ousingsawat, J., Schreiber, R., and Kunzelmann, K.,  $\text{Ca}^{2+}$  dependence of volume-regulated VRAC/LRRC8 and TMEM16A  $\text{Cl}^-$  channels, *Front. Cell Dev. Biol.*, 2020, vol. 8, p. 596879.
62. Liu, Y., Zhang, H., Men, H., Du, Y., Xiao, Z., Zhang, F., Huang, D., Du, X., Gamper, N., and Zhang, H., Volume-regulated  $\text{Cl}^-$  current: Contributions of distinct  $\text{Cl}^-$  channels and localized  $\text{Ca}^{2+}$  signals, *Am. J. Physiol.: Cell Physiol.*, 2019, vol. 317, no. 3, pp. C466–C480.
63. Nilius, B., Eggermont, J., Voets, T., Buyse, G., Manolopoulos, V., and Droogmans, G., Properties of volume-regulated anion channels in mammalian cells, *Prog. Biophys. Mol. Biol.*, 1997, vol. 68, no. 1, pp. 69–119.

64. Rudkouskaya, A., Chernoguz, A., Haskew-Layton, R.E., and Mongin, A.A., Two conventional protein kinase C isoforms,  $\alpha$  and  $\beta$ I, are involved in the ATP-induced activation of volume-regulated anion channel and glutamate release in cultured astrocytes, *J. Neurochem.*, 2008, vol. 105, no. 6, pp. 2260–2270.
65. Fisher, S.K., Cheema, T.A., Foster, D.J., and Heacock, A.M., Volume-dependent osmolyte efflux from neural tissues: regulation by G-protein-coupled receptors, *J. Neurochem.*, 2008, vol. 106, no. 5, pp. 1998–2014.
66. Nilius, B., Voets, T., Prenen, J., Barth, H., Aktories, K., Kaibuchi, K., Droogmans, G., and Eggermont, J., Role of Rho and Rho kinase in the activation of volume-regulated anion channels in bovine endothelial cells, *J. Physiol.*, 1999, vol. 516, pt. 1, pp. 67–74.
67. Modi, A.D., Khan, A.N., Cheng, W.Y.E., and Modi, D.M., KCCs, NKCCs, and NCC: Potential targets for cardiovascular therapeutics? A comprehensive review of cell and region specific expression and function, *Acta Histochem.*, 2023, vol. 125, no. 4, p. 152045.
68. Wang, H.S., Critical role of bicarbonate and bicarbonate transporters in cardiac function, *World J. Biol. Chem.*, 2014, vol. 5, no. 3, p. 334.
69. Meor Azlan, N.F. and Zhang, J., Role of the cation-chloride-cotransporters in cardiovascular disease, *Cells*, 2020, vol. 9, no. 10, p. 2293.
70. Hartmann, A.M. and Nothwang, H.G., Molecular and evolutionary insights into the structural organization of cation chloride cotransporters, *Front. Cell Neurosci.*, 2015, vol. 8, p. 470.
71. Nan, J., Yuan, Y., Yang, X., Shan, Z., Liu, H., Wei, F., Zhang, W., and Zhang, Y., Cryo-EM structure of the human sodium-chloride cotransporter NCC, *Sci. Adv.*, 2022, vol. 8, no. 45, p. eadd7176.
72. Lang, F. and Voelkl, J., Therapeutic potential of serum and glucocorticoid inducible kinase inhibition, *Expert Opin. Invest. Drugs*, 2013, vol. 22, no. 6, pp. 701–714.
73. Orlov, S.N., Tremblay, J., and Hamet, P., Cell volume in vascular smooth muscle is regulated by bumetanide-sensitive ion transport, *Am. J. Physiol.: Cell Physiol.*, 1996, vol. 270, no. 5, pp. C1388–C1397.
74. Smith, J.B. and Smith, L.,  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  cotransport in cultured vascular smooth muscle cells: Stimulation by angiotensin II and calcium ionophores, inhibition by cyclic AMP and calmodulin antagonists, *J. Membr. Biol.*, 1987, vol. 99, no. 1, pp. 51–63.
75. Adragna, N.C., White, R.E., Orlov, S.N., and Lauf, P.K., K-Cl cotransport in vascular smooth muscle and erythrocytes: possible implication in vasodilation, *Am. J. Physiol.: Cell Physiol.*, 2000, vol. 278, no. 2, pp. C381–C390.
76. Kahle, K.T., Rinehart, J., Ring, A., Gimenez, I., Gamba, G., Hebert, S.C., and Lifton, R.P., WNK protein kinases modulate cellular  $\text{Cl}^-$  flux by altering the phosphorylation state of the Na-K-Cl and K-Cl cotransporters, *Physiology* (Bethesda), 2006, vol. 21, pp. 326–335.
77. Orlov, S.N., Koltsova, S.V., Kapilevich, L.V., Dulin, N.O., and Gusakova, S.V., Cation-chloride cotransporters: Regulation, physiological significance, and role in pathogenesis of arterial hypertension, *Biochemistry* (Moscow), 2014, vol. 79, no. 13, pp. 1546–1561.
78. Mount, D.B., Mercado, A., Song, L., Xu, J., George, A.L., Jr. Delpire, E., and Gamba, G., Cloning and characterization of KCC3 and KCC4, new members of the cation-chloride cotransporter gene family, *J. Biol. Chem.*, 1999, vol. 274, no. 23, pp. 16355–16362.
79. Lytle, C. and McManus, T., Coordinate modulation of Na-K-2Cl cotransport and K-Cl cotransport by cell volume and chloride, *Am. J. Physiol.: Cell Physiol.*, 2002, vol. 283, no. 5, pp. C1422–C1431.
80. Delpire, E., Advances in the development of novel compounds targeting cation-chloride cotransporter physiology, *Am. J. Physiol.: Cell Physiol.*, 2021, vol. 320, no. 3, pp. C324–C340.
81. Gagnon, M., Bergeron, M.J., Lavertu, G., et al., Chloride extrusion enhancers as novel therapeutics for neurological diseases, *Nat. Med.*, 2013, vol. 19, no. 11, pp. 1524–1528.
82. Garneau, A.P., Marcoux, A.A., Slimani, S., Tremblay, L.E., Frenette-Cotton, R., Mac-Way, F., and Isenring, P., Physiological roles and molecular mechanisms of  $\text{K}^+/\text{Cl}^-$  cotransport in the mammalian kidney and cardiovascular system: where are we?, *J. Physiol.*, 2019, vol. 597, no. 6, pp. 1451–1465.
83. Uhlen, M., Uhlén, M., Fagerberg, L., et al., Proteomics. Tissue-based map of the human proteome, *Science*, 2015, vol. 347, no. 6220, p. 1260419.
84. Karlsson, M., Zhang, C., Méar, L., et al., A single-cell type transcriptomics map of human tissues, *Sci. Adv.*, 2021, vol. 7, no. 31, p. eabh2169.
85. Litviňuková, M., Talavera-López, C., Maatz, H., et al., Cells of the adult human heart, *Nature*, 2020, vol. 588, no. 7838, pp. 466–472.
86. Ludwig, A., Rivera, C., and Uvarov, P., A noninvasive optical approach for assessing chloride extrusion activity of the K-Cl cotransporter KCC2 in neuronal cells, *BMC Neurosci.*, 2017, vol. 18, no. 1, p. 23.
87. Garneau, A.P., Marcoux, A.A., Noel, M., Frenette-Cotton, R., Drolet, M.C., Couet, J., Larivière, R., and Isenring, P., Ablation of potassium-chloride cotransporter type 3 (Kcc3) in mouse causes multiple cardiovascular defects and isosmotic polyuria, *PLoS One*, 2016, vol. 11, no. 5, p. e0154398.
88. Alvarez, B.V., Kieller, D.M., Quon, A.L., Markovich, D., and Casey, J.R., Slc26a6: A cardiac chloride-hydroxyl exchanger and predominant chloride-bicarbonate exchanger of the mouse heart, *J. Physiol.*, 2004, vol. 561, pt. 3, pp. 721–734.
89. Bonar, P.T. and Casey, J.R., Plasma membrane  $\text{Cl}^-/\text{HCO}_3^-$  exchangers: Structure, mechanism and physiology, *Channels*, 2008, vol. 2, no. 5, pp. 337–345.



90. Kudrycki, K.E., Newman, P.R., and Shull, G.E., cDNA cloning and tissue distribution of mRNAs for two proteins that are related to the band 3  $\text{Cl}^-/\text{HCO}_3^-$  exchanger, *J. Biol. Chem.*, 1990, vol. 265, no. 1, pp. 462–471.
91. Alvarez, B.V., Kieller, D.M., Quon, A.L., Robertson, M., and Casey, J.R. Cardiac hypertrophy in anion exchanger 1-null mutant mice with severe hemolytic anemia, *Am. J. Physiol.: Heart Circ. Physiol.*, 2007, vol. 292, no. 3, pp. H1301–H1312.
92. Lohi, H., Lamprecht, G., Markovich, D., Heil, A., Kujala, M., Seidler, U., and Kere, J., Isoforms of SLC26A6 mediate anion transport and have functional PDZ interaction domains, *Am. J. Physiol.: Cell Physiol.*, 2003, vol. 284, no. 3, pp. C769–C779.
93. Chernova, M.N., Jiang, L., Friedman, D.J., Darman, R.B., Lohi, H., Kere, J., Vandorpe, D.H., and Alper, S.L., Functional comparison of mouse *slc26a6* anion exchanger with human SLC26A6 polypeptide variants: Differences in anion selectivity, regulation, and electrogenicity, *J. Biol. Chem.*, 2005, vol. 280, no. 9, pp. 8564–8580.
94. Cingolani, H.E., Chiappe, G.E., Ennis, I.L., Morgan, P.G., Alvarez, B.V., Casey, J.R., Dulce, R.A., Perez, N.G., and Camili6n de Hurtado, M.C., Influence of  $\text{Na}^+$ -independent  $\text{Cl}^-/\text{HCO}_3^-$  exchange on the slow force response to myocardial stretch, *Circ. Res.*, 2003, vol. 93, no. 11, pp. 1082–1088.

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