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Role of Cl^- Channels and Transporters in Cardiac Cell volume Homeostasis

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Abstract：Mammalian cells possess an ability to rapidly respond to changes in the difference between their external and internal osmolarity and adjust their cell volume to almost the original value. The regulation of cell volume is a basic property of all mammalian cells and is of particular importance in cardiac cells in heart where cell volume is associated with fatal outcomes. Cell volume homeostasis in th heart is an important new field, and the information about the mechanisms of detecting and transducing volume signals and their significance under physiological and pathophysiological conditions is increasing. Much less is known about the functional properties of Cl^- channels in cardiac pathophysiology in comparison to cation channels permeable to Na^+, K^+ and Ca^{2+}. Recent studies reveal that cardiac Cl^- channels contribute to the autonomous regulation of cell volume, and the cell volume homeostasis is an essential cellular function coupled to a variety of physiological processes in the heart, such as cell proliferation, differentiation, migration and apoptosis. This article presents some recent additions to the understanding of the role of cell volume homeostasis and Cl^- channels in the heart.

Key words：**Chloride channels, Heart disease, Signal transduction, Patch clamp, Cell volume homeostasis**

Introduction

Knowledge of the behavior of the volume–dependent channels/transporters pathophysiological conditions is important since a failure of cell volume homeostasis in the heart is connected directly with individual death. In addition, cell volume changes that occur during ischemia/reperfusion dramatically alter cardiac electrophysiology, and cell swelling has profound effects on cardiac excitability and cause the duration of action potentials to shorten.[1] The osmolarity of body fluid is tightly controlled under normal conditions, but osmotic changes can occur in pathological states such as ischemia, septic shock, and diabetic coma. Since persistent cell swelling or cell shrinkage during aniosmotic conditions results in cell death, the ability to regulate cell volume is important for the maintenance of cellular homeostasis.[1] Most cell membranes exhibit high water permeability which reflects the presence of aquaporins.[2] Water flux through the aquaporins is driven by a shift in extra – or intracellular osmolarity, and most cell types are able to counteract volume perturbations following the osmotic gradient (Fig. 1). Swollen cells release KCl and cell water, thereby reducing the cell volume towards the original value, the process of regulatory volume decrease (RVD).[3] In contrast, shrunken cells generally gain NaCl and cell water, thereby increasing cell volume towards the original value, the process of regulatory volume increase (RVI). Accumulated Na^+ is then exchanged for extracellular K^+ by the Na^+–K^+ pump.[4] Several transporter pathways have been reported to be responsible for the RVD–associated KCl loss and RVI–associated NaCl gain.[1)4)] RVD occurs through parallel activation of K^+ and Cl^- channels,

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and activation of electroneutral cotransporters such as the K^+–Cl^- cotransporter and K^+/H^+ and Cl^-/HCO_3^- exchanger, whereas RVI involves the activation of Na^+–K^+–$2Cl^-$ cotransporter, Na^+/H^+ exchanger, and nonselective cation channels.[4] The importance of each channel and transporter is dependent on types of cells. In cardiac cells, the coordinated action of K^+ and Cl^- channels is the principal mechanism for RVD. Therefore, this review focuses on the physiological regulation of cell volume and typical nature of cardiac chloride channels in heart.

Regulation of intracellular Cl^- concentration

Since direct measurements of intracellular Cl^- concentration using ion-selective microelectrodes have shown a range of 10–30 mM[5] in mammalian cardiac cells, the Cl^- equilibrium potential lies within the action potential range of membrane potentials (−50 to −30 mV). Cardiac Cl^- channels generate both inward and outward currents and cause both depolarization and repolarization during the action potential.[6] Therefore, the degree to which activation of Cl^- currents depolarizes the resting membrane or accelerates the repolarization of action potential depends on the Cl^- equilibrium potential and the magnitude of the Cl^- conductance relative to the total membrane conductance.[6] Both shortening the duration of the action potential and the depolarization of the resting membrane by activation of Cl^- channels may induce early afterdepolarization, while also playing a role in arrhythmogenesis under pathological conditions.[6] The maintenance of the intracellular concentration of Cl^-, has been attributed to not only Cl^- channels

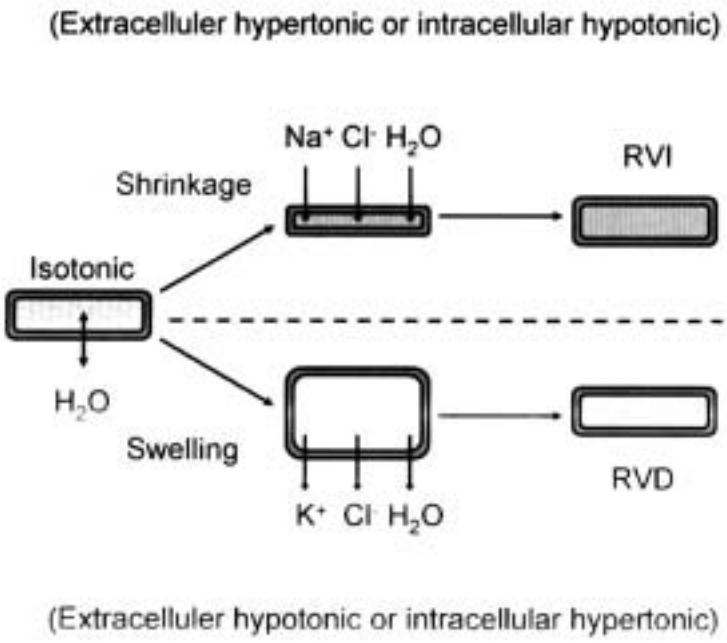

Fig. 1. Schema of cell volume homeostasis by ionic mechanisms for RVD and RVI, in cardiac cells.
Cell shrinkage, which is triggered by extracellular hypertonicity or intracellular hypotonicity, is counterbalanced by RVI, which induces the activation of Na^+–K^+–$2Cl^-$ cotransporter, resulting in the influx of chloride (with sodium and potassium) and water, thereby increasing cell volume.[3] Na^+/H^+, Cl^-/HCO_3^- and Cl^-/OH^- exchangers also have a supporting role in RVI. Cell swelling, which is triggered by extracellular hypotonicity or intracellular hypertonicity, is countered by RVD, which involves the cellular loss of chloride (and potassium) predominantly via the parallel activation of swelling-activated chloride channels such as VRAC, although K^+–Cl^- cotransporter and swelling-activated cation channels are also involved. Model based on Okada et al.[10)34)]

but also several electroneutral carriers and cotransporters (Fig. 2). These include the Cl^-/HCO_3^- exchanger,[7] Na^+–K^+–Cl^- cotransporter,[8] Na^+–Cl^- cotransporter[8] and K^+–Cl^- cotransporter.[9] In addition, these exchanger and transporters play a critical role in cell volume homeostasis. One important physiological role of these electroneutral carriers is to counter passive membrane Cl^- leak through the various types of sarcolemmal Cl^- channels.[10] The role of sarcolemmal Cl^- channels in maintaining intracellular concentration of Cl^- in cardiac cells is supported by the finding that β–adrenergic–induced activation of Cl^- channels in guinea pig ventricular cells decreases the cell volume under isotonic conditions.[11]

Molecular identity of cardiac Cl^- channels

Malfunctions in the ion channels, due to mutations in the genes encoding channel proteins, have been implicated in the pathogenesis of a growing number of diseases termed channelopathies.[12] One of the first identified channelopathies is cystic fibrosis, which represents a common hereditary disease in Caucasians.[12] This channelopathy is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) and is associated with defective chloride conductance, which leads to pulmonary and pancreatic insufficiency.[12] The CFTR is responsible for the Cl^- currents activated by protein kinase A(PKA), protein kinase C(PKC), and extracellular ATP in the heart,[10] therefore these currents can be unified as ICl.CFTR or CFTR currents.[13] The CFTR is composed of 1,480 amino acids, and hydropathy analysis predicts these are organized into two repeating motifs of six transmembrane spanning domains (M1–6, M7–12), two nucleotide binding domains, and one large regulatory domain that has numerous consensus phosphorylation sites for PKA and PKC.[10] The protein belongs to the ATP–binding cassette superfamily of transporters, which are structurally similar in terms of the organization of their transmembrane domains and nucleotide binding

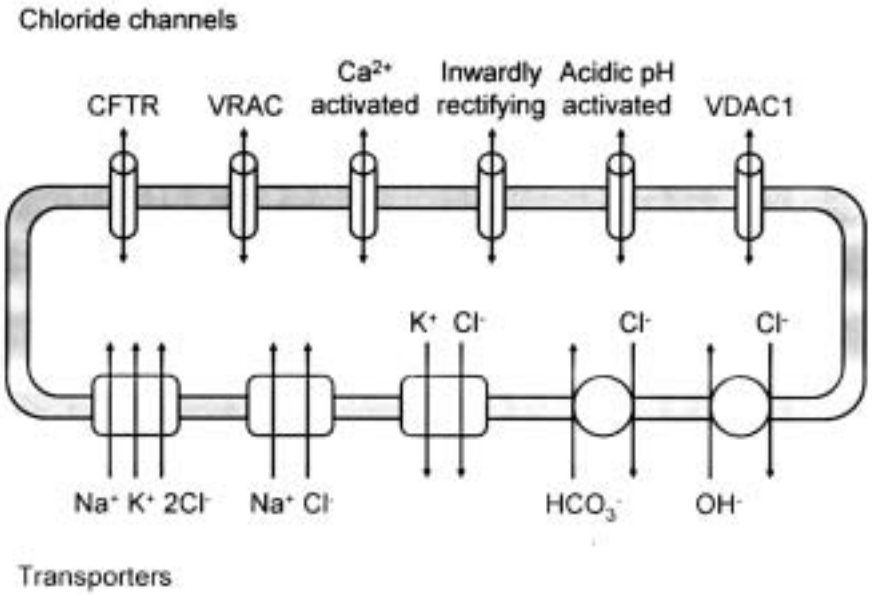

Fig. 2. Schematic representation of cardiac anion channels, transporters and exchangers in plasma membrane.
Upper : Cl^- channels that will be discussed in this review, including cystic fibrosis transmembrane conductance regulator(CFTR), volume–regulated anion channels (VRAC), Ca^{2+}–activated Cl^- channels, inwardly rectifying Cl^- channels (ClC–2?), acidic pH–activated Cl^- channels, and the voltage–dependent anion channel 1(VDAC1), which is predominantly expressed in the outer membrane of mitochondrion.
Lower : the most important families of Cl^- transporters, including Na^+–K^+–$2Cl^-$ cotransporter, Na^+–Cl^- cotransporter, K^+–Cl^- cotransporter, and Cl^-/HCO_3^- and Cl^-/OH^- exchangers. Several of these transporters are driven by the Na^+ gradient.[27]

domains.[10] Telemetry electrocardiograph recordings show no significant difference in electrocardiograph parameters between wild-type and CFTR knockout mice, which is consistent with the low basal activity of CFTR channels in the heart.[6] The activation of CFTR probably has a major physiological role to prevent excessive action potential duration prolongation and protect the heart against the development of early afterdepolarization and triggered activity caused by β-adrenergic stimulation of Ca^{2+} channels.[6] In addition, the targeted inactivation of the CFTR gene abolished the protective effects of ischemic preconditioning on the cardiac function and myocardium injury against sustained ischemia in an isolated mouse heart.[14]

Heterozygous mutations in the CLCN2 gene encoding the voltage-gated chloride channel ClC-2 have been identified in patients with idiopathic generalized epilepsy.[15] Yet the involvement of CLCN2 in epilepsy remains controversial.[16] Cardiac ClC-2 may be responsible for the cell swelling- or extracellular acidosis-activated inwardly rectifying Cl^- current ($I_{Cl.ir}$).[17-19] Intracellular dialysis of anti-ClC-2 antibody suppresses the activation of $I_{Cl.ir}$ in sinoatrial node cells[18] and atrial and ventricular cells ,[17] and reverses hypotonic induced decrease of the maximum diastolic potential, action potential amplitude and the cycle-length of sinoatrial node cells.[18] Telemetry electrocardiograph studies in conscious ClC-2 knockout mice show a decreased chronotropic response to acute exercise stress.[18] These results suggest that ClC-2 plays an important role in the regulation of cardiac pacemaker activity.

Unfortunately, the molecular identity of other Cl^- currents has not been determined. CLCA-1, Bestrophin and TMEM16 are molecular candidates for the Ca^{2+}-activated Cl^- current ($I_{Cl.Ca}$).[20-24] In addition, the voltage-dependent anion channel 1 (VDAC1), which is predominantly expressed in the outer membrane of mitochondrion, may also be expressed in the sarcolemmal membrane.[25] Extracellular acidosis-activated outwardly rectifying Cl^- currents ($I_{Cl.acid}$) are observed in cardiac myocytes[26] but the molecular candidate for $I_{Cl.acid}$ is not known. Though P-glycoprotein, the volume sensitive chloride conductance regulatory protein, phospholemman, and ClC-3 have been proposed as molecular candidates for the volume-regulated outwardly rectifying anion channel (VRAC), other studies report contradictory findings.[10][27] A mouse aortic banding model of myocardial hypertrophy demonstrated that targeted disruption of ClC-3 gene accelerated the development of myocardial hypertrophy and the discompensatory process, suggesting that activation of VRAC might be important in the adaptive remodeling of the heart during pressure overload.[6]

Cell volume homeostasis

A. Changes of cell volume in aniosmotic conditions

RVD was initially observed following the osmotic swelling of cultured chick heart cells. The RVD during a 46% hypotonic challenge was typically from 142% to 120% of isotonic cell volume and was accompanied by a Cl^- current.[28] Another study in adult rabbit ventricular myocytes[29] showed that the RVD during 50% hypotonic challenge is observed from 141% to 131% of the control volume in some cells, whereas no RVI is observed. The time course of RVD during hypotonic challenge was examined in adult guinea-pig ventricular cells using a microscopic video-image analysis.[11] A video image of the cell was captured by a CCD camera. The number of pixels included within the area of video image was calculated every 6 sec using NIH-image public domain software (NIH, Bethesda, MD) and custom macros. As shown in Figure 3, the cell area was increased by exposure to 70% hypotonic solutions. It peaked about 4 min after the beginning of hypotonic challenge, and there was a spontaneous decrease thereafter. This spontaneous cell shrinkage was detected in almost all cells examined. When isotonic perfusion was resumed after hypotonic challenge, the cell area quickly decreased. The cell area in the reapplied isotonic perfusion was generally smaller than the control level, showing excessive cell shrinkage.[30] This undershooting of the cell area below the control level shows a loss of solute from the intracellular medium during RVD. Since the cell area exhibited no recovery to the control level during the isotonic perfusion up to 20 min, the ventricular cells did not exhibit secondary

RVI when the cells are returned to isotonic conditions after hypotonic cell swelling (post-RVD RVI). Similar results have been shown in mouse ventricular cells.[31] Therefore, it appears that both cultured chick embryo cardiocytes and adult ventricular myocytes can partially exhibit RVD, and that adult ventricular myocytes cannot exhibit RVI. In addition, a reduction of extracellular Cl^- during hypotonic perfusion enhances the RVD, which is suppressed by DIDS (a popular inhibitor of VRAC). These results strongly support the notion that VRAC mainly acts for RVD in heart.

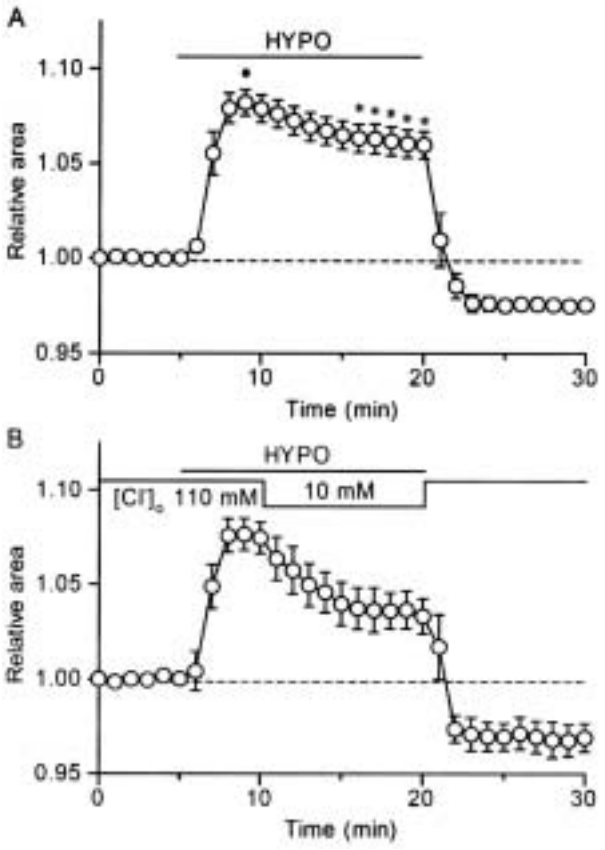

Fig. 3. Time course of the RVD observed when a guinea-pig ventricular cell is inflated by hypotonic bath solution.
Extracellular Cl^- dependent spontaneous cell shrinkage (RVD) is observed during application of hypotonic solution. The cells were initially bathed in normal Tyrode solution, and then hypotonic (70% of control) solution (HYPO) was applied during the period indicated by bar (A and B). Each point represents mean±SEM. In A, hypotonic solution was the standard one with 110 mM extracellular Cl^-, throughout (n=7). *: significantly smaller than the cell area at the maximum cell swelling (denoted by dot) with $p<0.05$. In B, extracellular Cl^- was lowered from 110 to 10 mM during application of hypotonic solution, as indicated by bar (n=4). Data from a previous report.[30]

B. Cell volume homeostasis and the Donnan equilibrium

Hypertonic shrinkage removes water from cells, and hypotonic swelling takes water into cells. Subsequently, several transporters that are activated by these cell volume changes transfer solute for maintenance of the original cell volume. In contrast, isotonic volume change is also reported in cardiac cells. It is important to distinguish between aniosmotic and isotonic cell volume change. Aniosmotic challenge removes only water from cells, whereas isotonic shrinkage and swelling results from loss and gain of osmotically active molecules, respectively.

In a simple system in which there is a single membrane-permeable cation such as K^+ and a single membrane-permeable anion such as Cl^-, it is possible for these ions to be in equilibrium such that there is no net flux across the membrane of either ion. At this equilibrium, the membrane potential equals both the equilibrium potential for K^+ and that for Cl^- and leads to the Donnan equilibrium. However, the animal cells contain 10-20 mM of membrane-impermeable macromolecular anions. If the Donnan equilibrium is still to be satisfied, then the sum of intracellular K^+ and Cl^- must exceed that of extracellular K^+ and Cl^-, in which case the total solute concentration inside the cell exceeds the external concentration. Because the cell membrane is permeable to water, this difference leads to water uptake by the cell. Therefore, animal cells must resist the tendency to take in water, and maintain a constant volume by actively Na^+-K^+ pump out of their intracellular milieu, effectively making Na^+ an extracellular impermeable ion.[1] This mechanism is called the "double-Donnan equilibrium" or "pump-leak steady-state" concept.[4]

C. Cell volume homeostasis and Na^+-K^+ pump

Blockade of the Na^+-K^+ pump in many tissues leads directly to cell swelling. Surprisingly, the blockade of the Na^+-K^+ pump with ouabain does not affect the isotonic cell volume in adult rabbit ventricular myocytes.[8] More recently, Takeuchi et al.[32] found that when Cl^- conductance via the

CFTR channels is activated with isoproterenol during the ouabain treatment, the cells show a marked swelling 52 min after drug application. They revealed that the gradual membrane depolarization induced by the Na^+–K^+ pump block activates the window current of the L–type Ca^{2+} current, which increases the intracellular Ca^{2+} concentration, and then the activation of Ca^{2+}–dependent cation conductance induces the jump of membrane potential, and the rapid accumulation of intracellular Na^+ accompanied by the Cl^- influx via the CFTR, resulting in the cell swelling. Therefore, the cell swelling induced by the Na^+–K^+ pump block depends on the magnitude of membrane Cl^- conductance.

D. Cell volume homeostasis and electroneutral cotransporters

Although the driving force for Na^+–K^+–$2Cl^-$ cotransporter and Na^+–Cl^- cotransporter (Cl^- influx) and K^+–Cl^- cotransporter (Cl^- efflux) are highly dependent on the respective Na^+ and K^+ gradients maintained by the Na^+–K^+ pump, the parallel operation of these transporters is believed to result in net accumulation of intracellular Cl^-.[10] The intracellular Cl^- concentration influences cell volume in several types of mammalian cells including cardiac cells. Drewnowska and Baumgarten[8] investigated the sensitivity of single cell volume to blockade of Na^+–K^+–$2Cl^-$ cotransporter and Na^+–Cl^- cotransporter in isotonic extracellular solutions in adult rabbit ventricular myocytes. The cell volume was decreased by 29% in cells exposed to bumetanide (a blocker of the Na^+–K^+–$2Cl^-$ cotransporter) and chlorothiazide (a blocker of Na^+–Cl^- cotransporter). Similar results have also been shown in guinea–pig ventricular cells.[33] In addition, a decrease of cardiac cell volume is occurs with administration of furosemide (an inhibitor of both Na^+–K^+–$2Cl^-$ and K^+–Cl^- cotransporter), and reduction of extracellular concentrations of Na^+ or Cl^-.[33] The Na^+–K^+–$2Cl^-$ cotransporter may contribute to reducing extracellular Na^+ concentration induced volume decrease because the volume decrease is suppressed by bumatenide and furosemide. In contrast, the reduction of the extracellular Cl^- concentration induced volume decrease is suppressed by furosemide but not by bumetanide, suggesting involvement of the K^+–Cl^- cotransporter.[33]

Volume–Regulated Outwardly Rectifying Anion Channel

A. Electrophysiological characteristics of VRAC current

The cardiac VRAC is a functionally important membrane anion channels.[10) 34)] VRAC is a channel protein of volume–regulated outwardly rectifying Cl^- current, which is sometimes referred to as VRAC current. The VRAC current was initially described in T lymphocytes,[35)] and is ubiquitously expressed in mammalian cells.[10)] In most cells, an increase in cell volume activates VRAC, which contributes to the regulation of cell volume, and the cell volume homeostasis is an essential cellular function coupled to a variety of physiological processes, such as cell proliferation, differentiation, migration and apoptosis, in most animal cell types.[34)36)] In particular, the VRAC current is believed to contribute to arrhythmogenesis in myocardial injury, cardiac ischemic preconditioning and the adaptive remodeling of the heart during myocardial hypertrophy and heart failure.[37)]

The cardiac VRAC current initially was identified in canine ventricular cells[38)] and is expressed throughout the heart in many species.[39)] The VRAC current is found in >90% of guinea pig atrial but only ~30% of ventricular cells exposed to extracellular hypotonic challenge.[40)] and isotonic inflation by positive pressure consistently elicits a VRAC current in canine atrial but not ventricular cells.[41)] These disparities in the phenotypic expression of VRAC current may reflect methodological issues or species differences. Oz and Sorota[42)] observed the VRAC current in almost all cells from human atria and ventricles. The VRAC current is found in >90% of mouse atrial and ventricular cells challenged by hypotonic bath solution.[43)–45)]

In general electrophysiological experiments, a VRAC current is induced by increased cell volume (Fig. 4A and B). The macroscopic current exhibits outwardly rectifying activity with both a physiologic[38)] and symmetric[40)] Cl^- gradient, and shows time–independent activation over the physiologic voltage range, but is partially inactivated at strongly positive potentials.[46)47)] The VRAC current in normal mammalian myocytes is not depend-

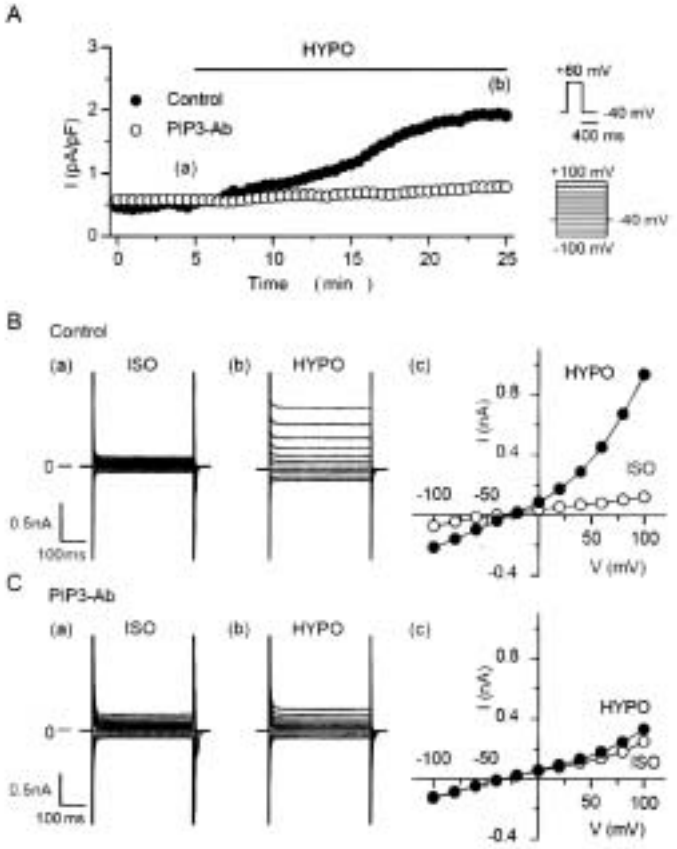

Fig. 4. Inhibition of VRAC currents in mouse ventricular cells dialyzed with anti-PIP3 antibody.
A, time course of activation of VRAC currents at +60 mV in cells dialyzed with control (●) and anti PIP3 antibody (PIP3-Ab)-containing (○) pipette solutions. The cells were initially bathed in isotonic solution(ISO)and then in hypotonic solution(HYPO)for the period indicated by bar. B and C, current traces in response to various voltage steps(a and b)and the corresponding current-voltage relationships (c), obtained in control cells (B) and cells loaded with PIP3-Ab (C). Current data shown in a and b were obtained at the time points indicated by a and b in A. The voltage pulse protocol is the same as shown in the right in A. Modified from Yamamoto et al.[44]

ent on Na^+, K^+ or Ca^{2+}.[39] The unitary current exhibits Cl^- selectivity, volume sensitivity, outward rectification, an intermediate single-channel conductance (~38 pS at +100 mV), large open probability (Po ~0.75 at +100 mV), and DIDS sensitivity.[48]

The Cl^- equilibrium potential is between −65 and −35 mV under normal physiological conditions.[5][10] Therefore, membrane Cl^- channels have the unique ability, in comparison to cation channels, to contribute both inward as well as outward current during the cardiac action potentia.[16][10][37] Consequently, activation of the VRAC current should decrease the action potential duration and thereby the effective refractory period and should depolarize the membrane potential,[39] and may contribute to the development of stretch-induced membrane depolarizations and arrhythmias.[49]

B. Pharmacological properties of the VRAC current

Highly selective and potent Cl^- channel blockers have not yet been identified.[10][27] The most selective and potent blocker of cardiac VRAC current

appears to be tamoxifen, an antiestrogen, which fully blocks the VRAC current at 10 μM.[40)50)] Most Cl^- currents are insensitive to this reagent. However tamoxifen also blocks several cation channels including the Ca^{2+} current,[51)] BK K^+ current,[52)] delayed rectifier K^+ current and Na^+ current,[53)] and gap junctions,[54)] in the heart. The ethacrynic-acid derivative 4-(2-butyl-6,7-dichlor-2-cyclo-pentyl-indan-1-on-5-yl) oxybutyric acid (DCPIB) is also one of the most selective compounds.[55)] Treatment with 10 μM DCPIB almost completely inhibits the VRAC current, but is not sensitive to various cloned chloride channels after heterologous expression in Xenopus oocytes or on native cation and CFTR currents in guinea-pig cardiomyoctes. Stilbene derivatives, such as SITS (4-acetamido-4'-isothiocyanostelbene-2,2'-disulfonic acid), DIDS (4,4'-diisothiocyanostilbene-2, 2'-disulfonic acids) and DNDS (4, 4'-dinitrostilbene-2, 2'-disulfonic acid), are also popular tools to identify the VRAC current, because these stilbene derivatives are voltage-dependent with greater inhibition of outward than inward VRAC current.[47)56)] DIDS (100 μM) does not inhibit the CFTR current,[10)] whereas the same concentration of DIDS inhibits several Cl^- channels and Cl^- transporters. Phloretin is a known blocker of the glucose uniport and anion antiport, and also blocks the VRAC current at low concentrations (below 100 μM) and the CFTR current at higher concentrations, whereas phloretin does not inhibit $I_{Cl.Ca}$, in epithelial cells.[57)] In addition, niflumic acid, NPPB (5-nitro-2-(3-phenylpropylamino) benozic acid), IAA-94 (indanyloxyacetic acid-94), 9AC (9-anthracene carboxylic acid), deoxyforskolin, DPC (diphehylamine-2-carboxylic acid) and glibenclamide are reported to be inhibitors of the VRAC current.[28)56)58)59)]

Regulation of VRAC current

A. Protein kinases

The activation of the VRAC current is delayed 30-90s after swelling, and it is inferred that signaling cascades participate in its activation.[38)40)60)] VRAC can be also activated under isotonic conditions by reducing intracellular ionic strength, by intracellular GTPγS, or by application of shear stress in endothelial cells,[27)] but this has yet to be examined in cardiac cells. In most cells, activation of the VRAC current does not appear to require phosphorylation, since channels can be activated in the absence of cytoplasmic Ca^{2+} and Mg^{2+} and in the presence of nonhydrolyzable analogs of ATP.[4)] However, the role of PKA phosphorylation of VRAC in the heart is controversial. Although phosphorylation by protein kinases does not appear to play a direct role in channel activation, they may modulate channel activity by direct phosphorylation of the channel or some accessory protein that regulates channel activity.[10)] Another potentially important regulatory mechanism of the VRAC current is phosphorylation by tyrosine protein kinase. The VRAC current in canine atrial cells may be regulated by tyrosine protein kinase.[61)] However, whether the substrate for tyrosine phosphorylation is the channel itself or another regulatory protein is not known. ClC-3, a molecular candidate for the VRAC, is consistently inhibited by PKC activation and activated by PKC inhibition,[10)] whereas the effect of PKC in the native VRAC current is under consideration.

B. Integrin

It is still not known whether VRAC is regulated by membrane tension or shape or by mechanical stretch alone. Baumgarten and his coworkers showed that direct mechanical activation of the VRAC current in rabbit ventricular myocytes can be accomplished by selectively stretching β1-integrins with mAb-coated magnetic beads.[62)] The binding of beads without stretching does not provoke a current, and the stretch-induced Cl^- current is blocked by tamoxifen, a selective blocker of VRAC,[63)] and activated via Src, a focal adhesion kinase, and the stretch activates angiotensin II type 1(AT1) receptor signaling cascade which involves trans-activation of epidermal growth factor receptor kinase, phosphatidylinositol 3-kinase (PI3K), NADPH oxidase, superoxide anion, and ultimately H_2O_2. Similar results were shown in the hypotonic activated VRAC current in rabbit ventricular cells.[64)]

C. Phosphatidylinositides

Phosphoinositides (PIs), including phosphatidylinositol 3,4,5-trisphosphate (PIP3) and phospha-

tidylinositol 4, 5–bisphosphate (PIP2), serve as important second messengers in many intracellular signaling cascades. The phosphoinositides directly and/or indirectly bind several types of membrane transporters and channels.[65][66] Interestingly, cell swelling is accompanied by activation of PI3K which are enzymes that convert PIP 2 to PIP3,[67] and that PI3K activation enhances cell survival and antagonizes apoptosis in many cell types including cardiomyocytes.[68] In addition, recent studies in various types of cells have shown that the inhibition of PI3K attenuates the VRAC current.[69]–[73]

Furthermore, there is a reduction of the cardiac VRAC current and a lack of RVD in ventricular myocytes derived from type 1 diabetic mice, and intracellular loading of the diabetic myocytes with PIP3, but not PIP2, reverses the attenuation of VRAC current.[44] In normal mice, intracellular dialysis of anti–PIP3 antibody[44] strongly inhibits the activation of the VRAC current (Fig. 4A and C). Similar results were shown by intracellular application of LY294002 (a PI3K inhibitor) or anti –PIP2 antibody. PIP3, but not PIP2, restores the VRAC current suppressed by LY294002 or anti–PIP 2 antibody, whereas intracellular PIP3 or PIP2 influences neither the basal background current in isotonic solution nor the VRAC current in hypotonic solution.[45] These results suggest that two distinct steps are required for VRAC activation (Fig. 5). First, PIP3(or related compounds) can change from the membrane stretch–insensitive to –sensitive VRAC. Next, the VRAC in the latter can be opened in hypotonic solutions. Alternatively, the mechanical stretch might change from the closed to pre–opened VRAC, and then PIP3(or related compounds)might open the channel. Furthermore, the question arises as to how PIP3 can be involved in such downstream reactions. In this re-

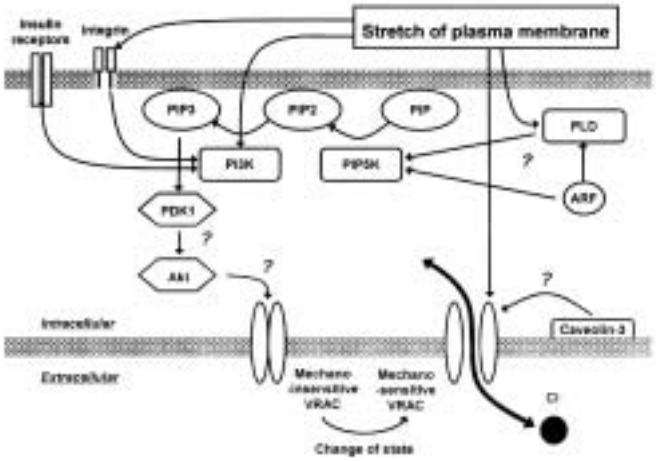

Fig. 5. Proposed scheme for regulation of VRAC by phospholipid components in mouse ventricular cells.
Phosphoinositide 3–kinase (PI3K) is activated by several pathways, including direct mechanical stretch, integrin and insulin receptors. Mechanical stretch may also activate phospholipase D (PLD) with cooperation of ADP–ribosylation factor (ARF), causing PIP2 generation via phosphatidylinositol 4–phosphate 5–kinase (PIP5K) activation. The PI3K activation converts PIP2 to PIP3 on the inner leaflet of the plasma membrane, which helps to recruit the phosphoinositide–dependent protein kinase–1 (PDK1) via its pleckstrin–homology (PH) domain. PDK1, in turn, phosphorylates and activates the serine/threonine kinase Akt, another protein anchored to PIP3 at the plasma membrane by virtue of a PH domain. Probably, this PIP3 downstream signaling can change from the mechano–insensitive to –sensitive VRAC, which can open in hypotonic solutions. Thus the activation of VRAC requires both the mechanical stretch of plasma membrane and PIP3–dependent signalings.

gard, it is noteworthy that most of these signaling proteins such as GDP–GTP exchanger factors for Rac or serum– and glucocorticoid–regulated protein kinase structurally include pleckstrin–homology or phox–homology domains, and that PIP3 binds to these domains to transduce intracellular signals.[74] It is therefore possible that similar PIP 3–dependent processes are involved in the activation of the VRAC current in mouse cardiac cells.

Conclusion

Recent studies using physiological and pharmacological techniques indicate that Cl^- channels contribute to cardiac electrical activity and cell volume homeostasis in cardiac myocytes. The coupling of mechanical stretch and electrical activity in the heart has been termed mechanoelectrical feedback and is believed to play a role in arrhythmias observed with pathological conditions including congestive heart failure and left ventricular hypertrophy. Since VRAC is elicited by mechanical stretch, this current may contribute to the mechanoelectrical feedback. Furthermore, it has been suggested that VRAC modulates arrhythmogenesis, myocardial injury, ischemic preconditioning, and apoptosis, and that remodeling of CFTR channels may contribute to myocardial hypertrophy and heart failure. Given these findings, cardiac Cl^- channels represents an important novel target for therapeutic approaches. Despite these developments, a lot of questions remain unanswered for the assessment of cell volume homeostasis in healthy and diseased hearts. Although transgenic mice are invaluable experiment models for understanding the function of cardiac Cl^- channels, the molecular basis for most Cl^- channels remains unresolved. In addition, the further understanding of the signal cascades that regulate the cardiac Cl^- channels is important for the development of therapeutic approaches for the treatment of heart diseases. Due to these continuing challenges, both the Cl^- channels and cell volume homeostasis in the heart are certain to remain a focus of research for years to come.

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