Insights into the structure, distribution and function of the cardiac chloride channels

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Abstract

This review describes the properties and distribution of the three major types of chloride currents that have been studied in cardiac tissue. These include a cAMP- and protein kinase A (PKA)-dependent current, a calcium-activated current and a swelling-induced current. The study of cardiac anion currents is a less mature field than the study of cardiac cation currents. Consequently, less is known regarding the structure, molecular identity and physiological role of anion currents in comparison to cardiac cation currents. Where known, the available molecular and structural information is also discussed. Although there is no proven physiological role for cardiac chloride currents, the possible clinical electrophysiological roles of cardiac chloride currents are discussed.

Keywords: Experimental; heart; electrophysiology; Cl-channel; Stretch/m–e coupling; Signal transduction; Arrhythmias; supraventricular arrhythmia

1. Introduction

In the early 1960s, Carmeliet [1], and Hutter and Noble [2] demonstrated that replacement of extracellular chloride could affect maximum diastolic potential, action potential duration and membrane conductance of isolated cardiac tissues. Voltage clamp experiments subsequently suggested that a transient outward current was carried by chloride [3,4]. Although these results were consistent with the idea that chloride was the charge carrier for significant repolarizing current, the demonstration that the chloride substitutes that had been used could decrease potassium efflux and chelate calcium cast doubt on the interpretation [5–7]. The role of chloride currents in the heart was largely ignored after Kenyon and Gibbons [5,6] demonstrated that the major charge carrier for the transient outward current of sheep Purkinje fibers was potassium. Interest in the study of cardiac chloride currents has been revived with the discovery of several types of cardiac chloride currents since 1989 [8–13].

At least three distinct types of chloride currents have been detected in adult mammalian heart. These include: (1) a cAMP- and protein kinase A (PKA)-dependent current [8,9], (2) a calcium-activated current [10,14] and (3) a swelling-induced current [11–13]. The PKA-dependent chloride current is carried by a cardiac isoform of the cystic fibrosis transmembrane conductance regulator (CFTR; the protein that is defective in individuals with cystic fibrosis) [15–17].

It is possible that there are other types of chloride current in the heart [18]. Chloride currents that are basally activated [19] or induced by extracellular ATP [20,21], phorbol ester [22–26], depolarizing voltage steps [27,28] or angiotensin [29] have been described, but it is not clear if these represent new types of cardiac chloride currents or the regulation of known currents. At least a portion of the phorbol ester-activated current appears to be identical to the PKA-dependent current [22–24]. A large conductance chloride channel has been found in cultures of neonatal rat ventricle [30] but this channel is not found in cultured heart cells from rats older than 15 days [30]. The next few
sections of this review will discuss in detail the known properties of the three major types of chloride currents in adult heart.

2. Cardiac variant of CFTR

The cardiac variant of CFTR is the most studied cardiac chloride current. The interested reader is referred to additional reviews of the cardiac [31,32] and epithelial [33,34] forms of CFTR.

The observation that foreshadowed the discovery of a functionally significant chloride current in single guinea pig ventricle cells was that, under certain experimental conditions, isoproterenol caused the resting membrane to depolarize to a level that resulted in abnormal automatic activity [35]. The charge carrier for the depolarizing current was initially thought to be sodium [35], however, it was subsequently demonstrated to be chloride (i.e. the inward current was attributable to chloride efflux) [8,9,36]. The confusion regarding the charge carrier occurred as a result of the replacement ions used for sodium. Although tetramethylammonium, tetraethylammonium and Tris substitution inhibited $I_{\text{CFTR,cardiac}}$, sucrose or N-methyl-D-glucamine substitution for sodium had no effect [37,38]. The inhibition by certain sodium substitutes was ultimately attributed to effects on receptors. Tris and tetraethylammonium increased the EC$_50$ for isoproterenol to activate $I_{\text{CFTR,cardiac}}$ [37]. Tetramethylammonium inhibited $I_{\text{CFTR,cardiac}}$ by activating muscarinic receptors [39]. The resulting inhibition of adenylyl cyclase accounts for the decrease in $I_{\text{CFTR,cardiac}}$ when extracellular sodium is replaced by tetramethylammonium.

2.1. Activation of the cardiac variant of CFTR

PKA-dependent phosphorylation can completely account for the activation of $I_{\text{CFTR,cardiac}}$ by isoproterenol or forskolin. The current can be fully activated by intracellular dialysis with cAMP or by dialysis with the catalytic subunit of PKA [9]. $I_{\text{CFTR,cardiac}}$ can also be maximally activated by superfusion with 8-bromo-cAMP, forskolin or isobutylmethylxanthine (IBMX) [40]. Intracellular GTP is required for the activation of $I_{\text{CFTR,cardiac}}$ by surface receptors coupled to stimulatory GTP-binding proteins (G$_s$) [41], but available data do not support a role for phosphorylation-independent activation of the current [42].

Although it is known that the CFTR protein has at least four serines within its regulatory domain that are phosphorylated by PKA [43,44], two functionally distinct phosphorylation sites have been defined by their sensitivity to phosphatase inhibitors [45]. In the presence of saturating concentrations of either okadaic acid or microcystin, suggesting an essential role for type 1 or 2A phosphatase for complete deactivation of $I_{\text{CFTR,cardiac}}$ [45]. These data are consistent with a model where a single phosphorylation is sufficient to activate $I_{\text{CFTR,cardiac}}$ while a second phosphorylation is required for optimal current activation.

CFTR is known to contain two nucleotide binding domains that can bind ATP [33]. Activation requires ATP hydrolysis at one nucleotide binding domain [15,46,47]. The channel open time for fully phosphorylated $I_{\text{CFTR,cardiac}}$ activated in the presence of cytoplasmic ATP is prolonged by compounds that prevent ATP hydrolysis cycles, such as adenylylimidodiphosphate, beryllium and vanadate [46,47]. These data suggest that closing of the fully phosphorylated channels requires ATP hydrolysis at the second nucleotide binding site [46,47]. Adenylylimidodiphosphate does not prolong the open times of partially phosphorylated channels, suggesting that the phosphorylation state controls the interaction between nucleotides and the nucleotide binding domains [46].

2.2. Modulation of the cardiac variant of CFTR

Agonists that inhibit adenylyl cyclase attenuate $I_{\text{CFTR,cardiac}}$ that has been activated by isoproterenol, histamine or forskolin [8,40,48–50]. Inhibitory agents include muscarinic agonists [8,40,48,50] as well as $\alpha_1$-adrenoceptor agonists [49]. The inhibition of isoproterenol-stimulated current by muscarinic agonist has been shown to be uncompetitive [48]. An interesting phenomenon seen with muscarinic agonist is a rebound stimulation on washout of muscarinic agonist if submaximal concentrations of iso-CFTR, cardiac are used to induce $I_{\text{CFTR,cardiac}}$ [50,51]. The rebound is blocked by pertussis toxin and is independent of nitric oxide or cGMP formation [51].

Phorbol esters are reported to induce $I_{\text{CFTR,cardiac}}$ in the absence of cAMP-elevating agents, by activating protein kinase C (PKC) [22–26]. The stimulatory effect of phorbol ester alone is not a universal finding [52,53]. The effect of phorbol ester on $I_{\text{CFTR,cardiac}}$ is reported to be dependent on PKA activity [53], similar to epithelial CFTR. It is likely that variation in basal PKA activity can explain the variable activation of $I_{\text{CFTR,cardiac}}$ by phorbol ester alone [53].

The tyrosine protein kinase inhibitor, genistein, has been observed to activate the epithelial form of CFTR [54–57]. The effect does not appear to be at the level of adenylyl cyclase because genistein does not elevate cAMP concentrations [58–60]. Three major mechanisms of action have been proposed. The first is a genistein-dependent phosphatase inhibition that occurs secondary to effects on tyrosine protein kinase [54,55]. Second is the modulation of channel gating by tyrosine phosphorylation [61]. The final proposal is a direct action of genistein on the CFTR channel, independent of tyrosine kinase inhibition [56,57]. Genistein can also stimulate $I_{\text{CFTR,cardiac}}$ [62–65].
balance of evidence supports the conclusion that the enhancing effect of genistein on $I_{CFTR,\text{cardiac}}$ is dependent on tyrosine protein kinase inhibition [62,63,65] (but see [64]). There are discrepancies regarding the details of the stimulatory action of genistein. Two reports concluded that activation of $I_{CFTR,\text{cardiac}}$ was independent of PKA activity [62,64]. In these studies, genistein induced $I_{CFTR,\text{cardiac}}$ in the absence of agents that elevate cAMP [62,64]. Moreover, the effect of genistein was not occluded when $I_{CFTR,\text{cardiac}}$ had been activated by 5 μM forskolin [62] or a combination of 0.8 μM isoproterenol, 50 μM forskolin and 10 μM IBMX [64]. One of these groups subsequently reconsidered and concluded that there was an essential role for PKA for the enhancing effect of genistein [65]. An effect of genistein that is prevented by the PKA inhibitor, H89, has been reported [63,65]. In the presence of intracellular okadaic acid and superfusion with 5 μM forskolin, the effect of genistein was occluded [65]. Genistein was found to have no effect in the absence of isoproterenol, but genistein enhanced the sensitivity for stimulation of $I_{CFTR,\text{cardiac}}$ by isoproterenol, and had no effect on the maximum current amplitude [63]. The authors of the latter study proposed that tyrosine kinase activity diminishes the responsiveness of the β-adrenoceptor/adenyllyl cyclase/PKA system [63]. This could have important implications for disease states in which tyrosine kinases are activated. Genistein does not slow the deactivation of $I_{CFTR,\text{cardiac}}$ after washout of isoproterenol or forskolin, suggesting that phosphatase inhibition is not contributing to the enhancement of $I_{CFTR,\text{cardiac}}$ [63,65]. The available data support the hypothesis that the enhancement of $I_{CFTR,\text{cardiac}}$ by genistein occurs distal to the β-adrenoceptor and could include effects on either PKA or the availability of PKA sites on the cardiac CFTR molecule.

### 2.3. Biophysical properties of the cardiac variant of CFTR

$I_{CFTR,\text{cardiac}}$ is time-independent when examined with voltage steps [8,9]. The current–voltage relationship is ohmic (linear) with 150 mM chloride on both sides of the membrane but exhibits outward rectification if the intracellular chloride concentration ($\text{Cl}^-$) is reduced [8,9]. Although the aforementioned observations would be predicted from constant field theory, outward rectification was still seen when $\text{Cl}^-$ and extracellular chloride ($\text{Cl}^-$) were both 40 mM and no inward rectification was seen when $\text{Cl}^-$ was 150 mM and $\text{Cl}^-$ was 40 mM [66]. The latter observations cannot be explained by constant field theory.

The selectivity sequence for $I_{CFTR,\text{cardiac}}$ was initially described as $\text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{I}^- > \text{isethionate} > \text{glutamate}$ [66]. Reevaluation has suggested that the selectivity sequence should have $\text{I}^- > \text{Cl}^-$ [67–69]. Studies of the epithelial form of CFTR suggest that $\text{I}^-$ can be either more or less permeant than $\text{Cl}^-$, depending on the protocol used [70]. The normal state of the channel is one with a higher permeability to $\text{I}^-$ than $\text{Cl}^-$, however, long-term exposure results in a shift to an altered permeability state with $\text{Cl}^- > \text{I}^-$ [70]. The conductance series for $I_{CFTR,\text{cardiac}}$ has been reported as $\text{NO}_3^- = \text{Cl}^- > \text{glutamate} > \text{Br}^- > \text{isethionate} > \text{I}^- > \text{aspartate}$ [68]. The low conductance measurements for iodide have been attributed to either $\text{I}^-$-dependent dephosphorylation of the channel [67] or to slow exit of $\text{I}^-$ from the channel after entering the pore [69].

Single channel measurements of $I_{CFTR,\text{cardiac}}$ were initially reported by Ebara and Ishihara [71] using the cell-attached configuration. A channel with a conductance of 13 pS for outward current was observed in a small percentage of patches [71]. The open probability was not affected by transmembrane potential [71]. Similar results have been obtained in inside-out [15] and outside-out [72] membrane patches from guinea pig ventricular myocytes. The unitary events closely resemble those observed for the epithelial CFTR current [15].

#### 2.4. Pharmacological properties of the cardiac variant of CFTR

$I_{CFTR,\text{cardiac}}$ has been reported to be inhibited by 1 mM anthracene-9-carboxylic acid (A9C), 200 μM niflumic acid, 50 μM 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), 500 μM diphenylamine-2-carboxylic acid (DPC), 1 mM clofibric acid, 250 μM gemfibrozil, 1 mM p-chlorophenoxy propionic acid and 100 μM glibenclamide [8,62,73–76]. All of these compounds, except glibenclamide, result in partial inhibition of $I_{CFTR,\text{cardiac}}$ at the indicated concentrations [8,62,73–76]. The inhibitory effect of A9C was not reproduced in some studies [68,77]. One study found no effect of A9C [68] whereas another study concluded that A9C blocks inward $I_{CFTR,\text{cardiac}}$ but enhances outward $I_{CFTR,\text{cardiac}}$ [77]. The stimulatory effect of A9C was attributed to an inhibition of an okadaic acid-insensitive phosphatase [77]. The EC$_{50}$ for stimulation of outward currents by A9C was 13 μM while the IC$_{50}$ for inhibition of inward currents was 940 μM [77]. Extracellular application of stilbene disulfonates, such as 4,4′-isothiocyanato-2,2′-disulfonic acid (DIDS) does not directly inhibit $I_{CFTR,\text{cardiac}}$ [68,74,78]. There may be an indirect effect of stilbene disulfonates in whole cell patch clamp experiments if intracellular calcium and/or pH are not well controlled [9,15]. Indanyloxyacetic acid 94 (IAA-94, 50 μM) was also ineffective [74].

#### 2.5. Molecular identity and distribution of the cardiac variant of CFTR

The protein responsible for $I_{CFTR,\text{cardiac}}$ in rabbit and guinea pig ventricle is a splice variant of epithelial CFTR with exon 5 deleted [15–17,79]. Biophysical properties, such as time-independence of the whole cell current during voltage steps, single channel conductance, rectification...
properties and selectivity sequence, are nearly identical for epithelial [exon 5(+) and cardiac [exon 5(-)] CFTR [15,33,68–71]. Pharmacological properties of epithelial CFTR and cardiac CFTR are also similar [33,68,73,74,80]. Finally, antisense oligonucleotides directed against mRNA for rabbit cardiac CFTR have been shown to decrease IC\text{\textsubscript{CFTR, cardiac}} density in primary cultures of guinea pig ventricular myocytes [17].

CFTR is a member of the ATP-binding cassette superfamily of membrane transporters [33]. The molecule contains a motif that is repeated once. Each half of the molecule contains six transmembrane domains and a nucleotide-binding domain [33]. Between the motifs is a regulatory domain, which contains consensus phosphorylation sequences for PKA and PKC [33].

IC\text{\textsubscript{CFTR, cardiac}} can be found in guinea pig [8,9], rabbit [81,82], cat [23,83] and monkey [84], but not in dog [85], rat [86] or mouse [21] ventricle. Current density is greater in subepicardial than in subendocardial ventricular myocytes in both rabbit [82] and guinea pig [87]. Supraventricular cardiac cells are generally reported to lack IC\text{\textsubscript{CFTR, cardiac}} [11,13,16,18,82]. There are, however, reports suggesting that IC\text{\textsubscript{CFTR, cardiac}} can be found in ≥20% of guinea pig atrial myocytes [20,68,87].

In experimental animals, the detection of mRNA for cardiac CFTR usually corresponds with electrophysiological detection of IC\text{\textsubscript{CFTR, cardiac}}. Northern blots [15,79] and reverse transcriptase polymerase chain reaction (RT-PCR) [16] have been used to detect CFTR mRNA in guinea pig and rabbit ventricle. RT-PCR was unable to detect CFTR mRNA from the atrium of guinea pig, rabbit or dog, nor could message be found in dog ventricle [16]. Interestingly, a laboratory which found IC\text{\textsubscript{CFTR, cardiac}} electrophysiologically in guinea pig atrium could also find CFTR mRNA by RT-PCR [87]; both the electrical and molecular data conflict with a prior report [16]. Competitive RT-PCR has been used to show that CFTR mRNA is most abundant in guinea pig subepicardium, with decreasing abundance in subendocardium and atrium [87].

Since IC\text{\textsubscript{CFTR, cardiac}} is distributed in a species- and tissue-dependent manner, it is critical to determine if this current is an important contributor to human cardiac electrophysiology. Cardiac abnormalities are infrequently associated with cystic fibrosis and the cardiac manifestations that do occur are generally considered to occur secondary to pulmonary pathology. Hearts from individuals with cystic fibrosis are transplanted into individuals with heart failure with the assumption that the CF hearts are normal. However, it is controversial as to whether or not IC\text{\textsubscript{CFTR, cardiac}} is present in human heart.

The first published human data was from a Northern blot showing that CFTR mRNA was detectable in human atrium [79]. This observation was intriguing since IC\text{\textsubscript{CFTR, cardiac}} does not seem to be a significant current in the atrium of most experimental animals [16]. RT-PCR led to the detection of mRNA for cardiac CFTR in both human atrium and ventricle [84]. Intact human cardiac tissues were used for RNA isolation [84]. In contrast to the hearts of other animals, both the epithelial [exon 5(+)] and cardiac [exon 5(-)] forms of CFTR mRNA were detected in human heart.

Despite the detection of CFTR mRNA in human atrium, electrophysiological examination by three independent laboratories led to the conclusion that IC\text{\textsubscript{CFTR, cardiac}} was not an important current in human atrium [88–90]. All three of these studies examined PKA-dependent stimulation of the L-type calcium current as a positive control [88–90]. Forskolin enhanced the L-type calcium current in 100% of human myocytes in one of these studies [88], while the percentage of adenylate cyclase/PKA competent cells was not given in the other two reports. Pediatric human atrial cells have also been found to lack IC\text{\textsubscript{CFTR, cardiac}} [91]. A forskolin-stimulated chloride current could be detected if human atrial myocytes were swollen but it was concluded that this current was not IC\text{\textsubscript{CFTR, cardiac}} [88] (see Section 4.2). In contrast, Warth et al. [84] concluded that a forskolin-induced IC\text{\textsubscript{CFTR, cardiac}} is present in human atrial cells. Only eight of the 30 isolated human atrial myocytes examined had an intact adenyl cyclase/PKA pathway in the study by Warth et al. [84]. Of the eight cells with an intact adenyl cyclase/PKA pathway, five exhibited an increased steady-state conductance in response to forskolin [84]. The authors did not determine if the effect of forskolin subsided on washout [84]. With [Cl\textsuperscript{-}] =40 mM and [Cl\textsuperscript{-}] =150 mM, Warth et al. [84] recorded a nearly ohmic forskolin-induced current that was not blocked by DIDS (see Fig. 4 from Warth et al. [84]).

There is also a discrepancy between molecular and electrophysiological data on CFTR in human ventricle [84,88]. RT-PCR of mRNA isolated from intact human ventricle detects CFTR mRNA [84]. Both the epithelial and exon 5(-) forms were detected [84]. Warth et al. [84] have reported that IC\text{\textsubscript{CFTR, cardiac}} was detectable in simian ventricle. This should not be regarded as proof that human ventricle has IC\text{\textsubscript{CFTR, cardiac}} since there is a precedent for variable distribution of IC\text{\textsubscript{CFTR, cardiac}} within a zoological order (carnivora; cat, yes; dog, no). Electrical studies from cells isolated from failing human ventricle failed to detect forskolin-stimulated IC\text{\textsubscript{CFTR, cardiac}}, although forskolin increased the L-type calcium current in all cells examined [88]. In failing human ventricle cells, forskolin did increase a steady-state chloride current after cell swelling [88]. The possibility exists that CFTR is downregulated in diseased human ventricle. It will be important to determine if IC\text{\textsubscript{CFTR, cardiac}} can be found in nonfailing human ventricle.

Studies in which investigators exogenously expressed human epithelial exon 5(-) CFTR in HeLa or human embryonic kidney (HEK) cells may be relevant here [92,93]. CFTR-mediated halide efflux was not detected in cells transfected with human exon 5(-) CFTR. CFTR-dependent halide efflux was found if cells were infected with the full length human epithelial CFTR [exon 5(+)].
The exon 5(−) construct resulted in the production of CFTR protein but the protein failed to reach the cell membrane [92,93]. The processing defect is not observed when rabbit cardiac exon 5(−) CFTR is exogenously expressed in Xenopus oocytes [17]. However, human epithelial exon 5(−) CFTR fails to produce cAMP-stimulated Cl− currents when exogenously expressed in Xenopus oocytes (John Hanrahan, McGill University, personal communication). This suggests that there is a true species difference in the processing of exon 5(−) mRNAs.

It is also possible that there are differences in the expression of cardiac exon 5(−) vs. epithelial exon 5(−) CFTR.

The resolution of whether or not human heart has a significant I_{CFTR, cardiac} is an important issue. There are obvious limitations to the approaches used to date. RT-PCR is a very sensitive technique that could conceivably amplify insignificant levels of mRNA. In cases in which the RNA is isolated from intact tissue, the cell type expressing the RNA cannot be determined. Isolation of cardiac myocytes for whole cell patch clamp studies employs proteolytic enzymes that could damage membrane proteins, including ion channels. The unequivocal resolution of this issue may depend on techniques such as immunocytochemistry on tissue slices. This should allow the determination of whether or not CFTR protein is present in human myocardial cells and whether it is localized in the cell membrane.

3. Cardiac calcium-activated chloride current

Although Kenyon and Gibbons [5,6] demonstrated in 1979 that much of what was previously believed to be a chloride current was, in fact, a 4-aminopyridine-sensitive potassium current, these authors also concluded that there was a small chloride-sensitive component for the transient (early) outward current [6]. For the next dozen years, several studies on cardiac transient outward current noted that there was a voltage-gated potassium current and a calcium-sensitive current, but all of these studies either failed to address the ionic nature of the calcium-sensitive component or assumed that it was carried by potassium [94–99]. In 1991, Zygmunt and Gibbons [10] showed that the 4-aminopyridine-insensitive component of the transient outward current of rabbit ventricle was a calcium-activated chloride current.

3.1. Activation mechanism of cardiac calcium-activated chloride current

The initial demonstrations of I_{Cl, Ca} showed a transient stilbenedisulfonate-sensitive current, which appeared as an outward notch superimposed on the early inactivation phase of the L-type calcium current [10,14]. I_{Cl, Ca} amplitude peaks at +20 to +40 mV and decreases at more positive voltages (e.g. Fig. 1). The decrease at more positive voltages was attributable to decreased calcium entry secondary to a decreased driving force and greater inactivation of the L-type calcium channel [10,14]. Any apparent voltage dependence that has been observed for I_{Cl, Ca} is attributable to the voltage-dependence of the L-type calcium current [10,14]. Buffering intracellular calcium with EGTA, blocking calcium currents with cadmium or nisoldipine, or preventing calcium release from the sarcoplasmic reticulum (SR) by prolonged exposure to ryanodine or caffeine prevent the activation of I_{Cl, Ca}[10,14]. Although prolonged exposure to caffeine prevented the activation of I_{Cl, Ca}, the rapid application of caffeine could acutely activate I_{Cl, Ca} [100]. Therefore, it appears that the normal activation of I_{Cl, Ca} involves calcium entry via L-type calcium channels and calcium release from the SR [10,14].

3.2. Biophysical properties and kinetics of the calcium-activated chloride current

It has been proposed that the intracellular calcium transient normally imparts a time-dependence to I_{Cl, Ca} when intracellular calcium homeostatic mechanisms are operating normally [10,14,101]. A steady-state I_{Cl, Ca} has been measured in rabbit ventricle in whole cell patch clamp experiments with 1 μM calcium in the recording electrode [102], and in dog ventricle cells exposed to the...
calcium ionophore A23187 with 30 mM 2,3-butanediol monoxime present to prevent hypercontracture [101]. Sustained elevation of cytoplasmic calcium has also been shown to result in the activation of time-independent single chloride channels [103].

Two components of $I_{\text{Cl,Ca}}$ have been observed in rabbit Purkinje cells; one that decays faster than the intracellular calcium transient and one that closely follows the time course of the intracellular calcium transient [100,104]. The slower component was only seen in cells with very large calcium transients [104]. It is not clear if the temporal dissociation observed for the rapidly decaying component is due to regulatory mechanisms such as voltage- or calcium-dependent inactivation of $I_{\text{Cl,Ca}}$ or if the signal measured with Indo-1 differs temporally from the calcium concentration near the sarcolemma [100,104]. Papp et al. [104] concluded that a nonlinear relationship between intracellular calcium and activation of $I_{\text{Cl,Ca}}$ was more likely than a true inactivation process [104]. These authors hypothesized that rabbit Purkinje cells have only a single type of calcium-activated chloride channel [104]. The two kinetic components of the current could be explained by spatial and temporal inhomogeneities in intracellular calcium [104]. Papp et al. [104] noted that the data could not exclude the possibility of two distinct calcium-activated chloride channels with different sensitivities to calcium.

$I_{\text{Cl,Ca}}$ has been reported to either be an outwardly rectifying current [10,14] or an ohmic current [100,102]. The rectification occurs with physiological intracellular chloride but is absent with symmetrical transmembrane chloride. The selectivity sequence for $I_{\text{Cl,Ca}}$ of rabbit ventricle was reported to be SCN$^-$ > I$^-$ > Br$^-$ > Cl$^-$ [102]. This partial sequence is consistent with Eisenman’s series 1 for a weak field strength site [105].

Single channel properties of an $I_{\text{Cl,Ca}}$ of dog ventricular myocytes have been reported. The channel has a conductance of 1 to 1.3 pS, is time-independent with a sustained increase in cytoplasmic calcium and exhibits a linear current–voltage relationship in symmetrical chloride [103]. Channel openings are prolonged as cytoplasmic calcium is increased above 1 μM [103]. The EC$_{50}$ for calcium was estimated to be 150 μM [103]. Rectification of the single channels could be described by the Goldman–Hodgkin–Katz equation, with asymptmetrical distribution of chloride across the membrane [103].

3.3. Pharmacological properties of the calcium-activated chloride current

$I_{\text{Cl,Ca}}$ was initially described as a transient outward current that was blocked by 100 μM DIDS or 2 mM SITS [10]. The stilbene disulfonates did not affect the amplitude or kinetics of the L-type calcium current at the concentrations that were used [10]. Although $I_{\text{Cl,Ca}}$ is generally reported as a DIDS-sensitive current, it should be noted that Sipido et al. [100] concluded that 100 μM DIDS only blocks 70% of the $I_{\text{Cl,Ca}}$ of rabbit Purkinje cells. The chloride channel blocker, niflumic acid (50 μM), has been shown to block $I_{\text{Cl,Ca}}$ [103]. Glibenclamide has also been reported to block $I_{\text{Cl,Ca}}$ with an IC$_{50}$ of approximately 65 μM [75]. No voltage dependence has been noted for any of the blockers.

3.4. Distribution of cardiac calcium-activated chloride current

$I_{\text{Cl,Ca}}$ has been found in rabbit ventricle [10], rabbit atrium [14], rabbit Purkinje cells [100], dog ventricle [101] and dog atrium [106]. Current density seems to be larger in atrium than in ventricle [10,14]. $I_{\text{Cl,Ca}}$ is not present in guinea pig ventricle [107]. Presently available data do not support the presence of $I_{\text{Cl,Ca}}$ in human atrial cells. Although a 4-aminopyridine-insensitive, caffeine-sensitive transient outward current has been observed in human atrial myocytes [97], this current does not appear to be $I_{\text{Cl,Ca}}$ [108]. A 4-aminopyridine-resistant transient outward current can be observed when human atrial cells are studied in potassium-containing solutions [97,108], but this current is due to voltage-dependent unblock of the effect of 4-aminopyridine on the voltage-gated potassium current, $I_{\text{TO,1}}$ [108]. The later study also demonstrated that human atrial $I_{\text{TO,1}}$ is sensitive to block by 10 mM caffeine [108]. A recent preliminary report also failed to find $I_{\text{Cl,Ca}}$ in human atrium and human ventricle during the rapid application of caffeine [109]. A calcium-activated non-specific cation current was reportedly present in human atrium but not human ventricle [109].

4. Cardiac swelling-induced chloride current

Swelling-induced chloride currents are found in many different cell types. Interested readers are referred to recent comprehensive reviews [110,111]. Cardiac swelling-induced chloride current ($I_{\text{Cl,swell}}$) was initially described in dog ventricle [12], dog atrium [11] and rabbit supraventricular cells [13]. Hypoosmotic bath solution or hypertonic solution in whole cell patch electrodes was used to induce swelling of dog ventricle cells and activate $I_{\text{Cl,swell}}$ [12]. Positive pressure inflation was used to activate $I_{\text{Cl,swell}}$ in rabbit supraventricular cells [13]. Dog atrial cells were noted to swell “spontaneously” when whole cell patch electrode filling solution and bath solution were osmotically matched [11]. Using osmotically matched intracellular and extracellular solutions for whole cell patch clamp experiments actually creates an osmotic stress across the cell membrane, which causes cell swelling [11,112]. There are high molecular weight (low mobility) osmolytes in the cytoplasm that do not equilibrate with the patch electrode filling solution, whereas all of the osmolytes within the patch electrode have a higher mobility and equilibrate more readily with the cytoplasm [112].
This results in the cell interior having more osmolytes than either the bath solution or the pipette filling solution [112]. The spontaneous development of $I_{C_l,swell}$ can complicate the interpretation of certain whole cell patch clamp experiments. A flawed argument that is often used when investigators are attempting to rule out “spontaneous” development of $I_{C_l,swell}$ is the time of cell dialysis. Although dog atrial cells’ $I_{C_l,swell}$ took 5 min or longer to “spontaneously” develop in one study [11], it should be noted that the time required for “spontaneous” swelling will depend on the efficiency of dialysis, which is a function of cell size, the size of the rupture of the cell membrane, and any pressure applied to the patch electrode. The starting condition of the cell is also important; it is possible that some isolated myocytes might be swollen before the start of whole cell patch clamp experiments. Finally, it should also be noted that, at least in the dog, isolated ventricular myocytes appear to resist “spontaneous” activation of $I_{C_l,swell}$ more than atrial myocytes [11].

A basally active outwardly rectifying chloride current was described by Duan et al. [19] who claimed discovery of a novel chloride current. In the report describing this current, the authors stated that “A role for cell swelling was excluded by the fact that the current was unaltered by exposure to hypertonic superfusate,...” (page H1970) [19]. Duan et al. [113] subsequently reported that “A basally Cl− current ($I_C$) was observed under isotonic conditions in 128 of 150 cells (85%), had the same dependency on [Cl−]o as $I_{C_l,swell}$, and was reduced by cell shrinkage induced by hypertonic superfusion...”. These authors concluded that the basalar chloride current and $I_{C_l,swell}$ were carried by the same channel and, in contrast to the original claim, the basal current was activated by the same stimulus as $I_{C_l,swell}$ (cell volume) [113].

The observation that rabbit atrium has an $I_{C_l,swell}$ that is basally operative is intriguing and raises the possibility that the volume setpoint for activation of $I_{C_l,swell}$ differs from that in other species studied to date. An alternative possibility is that the low ionic strength pipette solution used by Duan et al. [113] may have activated $I_{C_l,swell}$ in the absence of cell swelling. In bovine endothelial cells, reducing intracellular ionic strength pipette solution used by Duan et al. [113] Tyrosine protein kinases are also important for the activation of noncardiac $I_{C_l,swell}$ [120–122]. Studies in lymphocytes indicate that src family kinases are involved in activation of $I_{C_l,swell}$ [122]. The inhibition of cardiac $I_{C_l,swell}$ by herbimycin A is consistent with this idea [118]. Little else is known regarding the essential tyrosine protein kinase(s) in the heart or the protein(s) that are phosphorylated. This remains a significant area for future study.

A recent preliminary report suggests that swelling-induced inhibition of PKC is an important mechanism contributing to activation of cardiac $I_{C_l,swell}$ [123]. PKC down-regulation activates rabbit atrial $I_{C_l,swell}$ [113]. Acute PKC inhibition activated rabbit ventricular $I_{C_l,swell}$ [124]. In contrast, PKC activation stimulates $I_{C_l,swell}$ in dog atrial cells [125]. Furthermore down regulation of PKC by 24 hour exposure to PMA does not unmask a basal $I_{C_l,swell}$ in dog atrial cells or alter inflation-induced activation of $I_{C_l,swell}$ [125].

Swelling-induced inhibition of PKC activity achieved. A study using positive pressure inflation to produce sudden steps in cell size demonstrated that there is a true lag for $I_{C_l,swell}$ activation and that the activation rate is temperature-dependent [115]. These data are consistent with the hypothesis that cell swelling is coupled to current activation through signal transduction events.

Changes in resting calcium are not required for the activation of adult mammalian cardiac $I_{C_l,swell}$; activation occurs with 10 mM internal EGTA and when experiments are performed with nominally calcium free bath solution containing 3 μM ryanodine [12,13,116]. In contrast, calcium influx is required for activation of cultured embryonic chick heart $I_{C_l,swell}$ [117]. Blockers of PKA and PKC do not prevent activation of mammalian cardiac $I_{C_l,swell}$ [12,13]. There are, however, pharmacological data indicating that a tyrosine phosphorylation event is necessary for activation of $I_{C_l,swell}$ [118].

4.1. Activation mechanism of cardiac swelling-induced chloride current

As the name implies, $I_{C_l,swell}$ can be activated by an increase in cell size [11–13]. Returning cells to their control volume results in deactivation of $I_{C_l,swell}$ [11–13]. Studies of $I_{C_l,swell}$ in which a gradual increase in cell size was produced revealed a temporal dissociation between the increase in cell size and activation of $I_{C_l,swell}$ [12,13]. Because cell size was changed slowly, these studies could not rule out the possibility that current activation is immediately coupled to cell size once a threshold size is
may be important in rabbit but is unlikely to contribute to activation of $I_{\text{Cl,swell}}$ in dog atrium. It should be noted that there are no biochemical data available demonstrating that cell swelling inhibits PKC.

4.2. Modulation of cardiac swelling-induced chloride current

Agents that elevate cytoplasmic cAMP can modulate cardiac $I_{\text{Cl,swell}}$ [11,88,126,127]. In dog atrial cells, application of isoproterenol or forskolin can result in a biphasic response with an initial stimulation of $I_{\text{Cl,swell}}$ followed by an inhibition [126]. Dual pathways exist for the modulation by cAMP: a PKA-dependent pathway and a kinase-independent pathway. PKA-dependent phosphorylation inhibits dog atrial or embryonic chick heart cell $I_{\text{Cl,swell}}$ [126,127]. When a peptide inhibitor of PKA (PKI 6-22 amide) causes a pronounced monophasic enhancement of dog atrial $I_{\text{Cl,swell}}$ [126] by a protein kinase-independent mechanism. Kinase-independent stimulation by cAMP is similar to the modulation of the hyperpolarization-activated current, $I_h$ [128]. The mechanism for the kinase-independent stimulation is not known. The data are consistent with a direct interaction of the cyclic nucleotide with the channel but cannot exclude a more indirect pathway [126].

As mentioned above, there are conflicting data on the modulation of $I_{\text{Cl,swell}}$ by PKC [113,124]. Activation of PKC inhibited $I_{\text{Cl,swell}}$ in rabbit cardiac myocytes [113,124]. Although PKC activation by $\alpha$-adrenceptors is generally mediated by the pertussis toxin-insensitive G-protein, $G_q$, stimulation of $\alpha$-adrenceptors inhibited $I_{\text{Cl,swell}}$ in a pertussis toxin-sensitive manner [113]. Phorbol esters stimulated $I_{\text{Cl,swell}}$ of dog atrial myocytes [125]. It is not clear if the apparent discrepancies are attributable to species differences in modulation or due to differences in the experimental conditions. It is possible that different PKC isoforms may have opposing actions on $I_{\text{Cl,swell}}$.

4.3. Biophysical properties of cardiac swelling-induced chloride current

$I_{\text{Cl,swell}}$ is time-independent within the physiological range of transmembrane potentials [12,13,116]. Inactivation of the current is observed at extreme positive potentials (e.g. $\geq +40$ mV) [129] (e.g. Fig. 2). This property is similar to voltage-dependent inactivation of epithelial $I_{\text{Cl,swell}}$ [112] but the degree of inactivation is much less for cardiac $I_{\text{Cl,swell}}$ and there is no cross-over of current traces [129]. The time-dependent inactivation at positive potentials is a useful biophysical signature that can be used to distinguish between $I_{\text{Cl,swell}}$ and $I_{\text{CFTR,cardiac}}$ [129].

Cardiac $I_{\text{Cl,swell}}$ exhibits outward rectification regardless of the intracellular chloride concentration [68,130]. This feature can also be used to distinguish $I_{\text{Cl,swell}}$ from $I_{\text{CFTR,cardiac}}$ if currents are recorded with 150 mM chloride on both sides of the membrane. Chloride selectivity of $I_{\text{Cl,swell}}$ has been demonstrated by monitoring shifts in reversal potential with partial replacement of intracellular or extracellular chloride by aspartate [12,13,116]. The selectivity sequence for cardiac $I_{\text{Cl,swell}}$ is $\text{SCN}^- > \Gamma > \text{NO}_3^- > \text{Br}^- > Cl^-$ [102,126]. The selectivity sequence for $I_{\text{Cl,swell}}$ is consistent with Eisenman’s series 1 for a weak field strength site [105]. There are discrepancies in the literature regarding the conductance series. In guinea pig atrial cells, the selectivity sequence and conductance series were identical [68] while the conductance series of $I_{\text{Cl,swell}}$ of dog atrium was $\text{NO}_3^- > \text{Cl}^- > \text{SCN}^- > \text{Br}^- > \text{F}^- > \text{aspartate} > \text{methanesulfonate}$ [130]. It is not clear if the discordant results are due to methodological differences or species differences in $I_{\text{Cl,swell}}$.

A 49 pS swelling-induced outwardly rectifying single channel was recorded in cell-attached patch clamp studies of rabbit atrial cells [131]. The properties of the swelling-induced single channel were indistinguishable from a basally active single channel that was observed in 5.7% of patches [132]. The single channel events were similar to the outwardly rectifying chloride channel of epithelial cells (ORCC) [131]. Open probability was independent of voltage [131]. Three open states with open time constants of approximately 1.7, 12 and 94 ms and four closed states were required to fit the data [131].
4.4. Pharmacological properties of cardiac swelling-induced chloride current

Initial descriptions of $I_{\text{Cl,swell}}$ indicated that A9C and stilbene disulfonates could inhibit the current [11–13]. Further examination revealed that the block by 1 mM A9C was partial, with approximately 50% block [68,116] (but see [133]). Moderate concentrations of stilbene disulfonates block $I_{\text{Cl,swell}}$ in a current-dependent manner with outward currents (chloride influx) being blocked more effectively than inward currents (e.g. 100 μM DIDS [116]). This behavior is consistent with direct block of the channel pore by external stilbene disulfonates, but the mechanism of block has not been conclusively demonstrated. Higher concentrations of stilbene disulfonates can result in complete block of inward as well as outward current (e.g. 1 mM DIDS [68]). Other compounds that are known to fully block $I_{\text{Cl,swell}}$ include 10 μM NPPB, 100 μM niflumic acid, 100 μM IAA-94 and 10–20 μM tamoxifen [68,116,130]. The effects of tamoxifen and IAA-94 are slow to develop, suggesting an indirect mechanism of action [68,116]. Of the full blockers that are available, none are totally specific. DIDS has been used to demonstrate that $I_{\text{Cl,swell}}$ can accelerate repolarization of the action potential of guinea pig ventricle cells [134]. Niflumic acid has been used to demonstrate that $I_{\text{Cl,swell}}$ can cause substantial depolarization of dog atrial cells [130]. Glibenclamide is reported to inhibit $I_{\text{Cl,swell}}$, but the details of the inhibition differ in two reports [75,135]. One study found a voltage-independent inhibition by glibenclamide with an IC$_{50}$ of 60 μM [135] while the other found a lower potency and preferential block at positive potentials [75]. Dideoxy forskolin (100 μM) has been shown to block $I_{\text{Cl,swell}}$ by 80% [116]. Tetrasulfonatotetramethoxy calixarene (300 nM), a compound that blocks the colonic epithelial ORCC, is ineffective at blocking dog atrial $I_{\text{Cl,swell}}$ [116,136].

4.5. Molecular identity and distribution of cardiac swelling-induced chloride current

Cardiac $I_{\text{Cl,swell}}$ appears to be evolutionarily conserved; i.e. an $I_{\text{Cl,swell}}$ has been detected in every cardiac species and tissue in which the current has been looked for. This suggests a physiological role for cardiac $I_{\text{Cl,swell}}$. Since genes that lack a significant role could be deleted from the genome without adverse effect on survival of the species. The current is not found in every cell examined, however. $I_{\text{Cl,swell}}$ appears to be larger and more frequently detected in the atrium than the ventricle [68,88]. Although the gene product responsible for cardiac $I_{\text{Cl,swell}}$ has not been unambiguously identified, a number of different gene products have been proposed to be responsible for swelling-induced chloride currents in a variety of cell types. Two gene products commonly mentioned include Cln [137] and P-glycoprotein [138], the product of the multi-drug resistance gene. Cln appears to be a stimulatory modulator of an endogenous swelling-induced chloride current in Xenopus oocytes [139]. However, some still propose that Cln is responsible for the endogenous $I_{\text{Cl,swell}}$ in NIH 3T3 fibroblasts [137]. Antisense oligonucleotides against Cln inhibit $I_{\text{Cl,swell}}$ in NIH 3T3 cells [137]. It is unlikely that Cln underlies cardiac $I_{\text{Cl,swell}}$. However. Extracellular cAMP (at mM concentrations) blocks $I_{\text{Cl,swell}}$ in NIH 3T3 cells or chloride currents in Xenopus oocytes injected with Cln mRNA by >50% [137,140], while dog atrial $I_{\text{Cl,swell}}$ is hardly affected [116]. The pharmacology for block of P-glycoprotein and cardiac $I_{\text{Cl,swell}}$ are similar and P-glycoprotein is expressed in the heart [116,141]. Despite this correspondence, there is no convincing evidence linking P-glycoprotein and cardiac $I_{\text{Cl,swell}}$. In non-cardiac cells, $I_{\text{Cl,swell}}$ and P-glycoprotein have been clearly dissociated, although some forms of P-glycoprotein may speed the activation of $I_{\text{Cl,swell}}$ [110,142]. CIC-2, a member of a voltage-gated chloride channel family, has been shown to be activated by osmotic swelling [143,144], but a current with strong inward rectification and a selectivity sequence of Cl$^-$/I$^-$ results when CIC-2 is exogenously expressed. These properties are the opposite to those of native cardiac $I_{\text{Cl,swell}}$.

Recent reports suggest that another member of the CIC gene family, CIC-3, may encode for rabbit atrial $I_{\text{Cl,swell}}$ [123,145]. Expression of CIC-3, cloned from guinea pig heart, in NIH 3T3 cells resulted in a basally active chloride current that was regulated by cell volume. Pharmacological properties, rectification and ionic selectivity of the current produced by exogenous expression of CIC-3 were consistent with native cardiac $I_{\text{Cl,swell}}$. Single channel properties of exogenously expressed CIC-3 were similar to those observed for basally active, swelling-stimulated chloride currents in native rabbit atrial cells [131,145]. The rabbit appears to differ from other mammalian species in two important ways. First, rabbit atrial cells are reported to have a basally active $I_{\text{Cl,swell}}$ [113] (but see Hagiwara et al. [13]), whereas dog, guinea pig and human atrial $I_{\text{Cl,swell}}$ is only observed after cell volume increases are produced [11,68,88]. Second, activation of PKC inhibits rabbit cardiac $I_{\text{Cl,swell}}$ [113,124], while phorbol ester enhances dog atrial $I_{\text{Cl,swell}}$[125]. CIC-3 seems to be an excellent candidate for rabbit and guinea pig cardiac $I_{\text{Cl,swell}}$. It remains to be determined if CIC-3 is in fact a gene encoding rabbit cardiac $I_{\text{Cl,swell}}$ and whether the same gene encodes cardiac $I_{\text{Cl,swell}}$ in other mammalian species. The unequivocal identification of a gene encoding a cardiac $I_{\text{Cl,swell}}$ would be a great boon to the field.

5. Do cardiac chloride currents have clinical physiological and pathophysiological significance?

A current can only be clinically relevant if it is expressed in human heart at a density sufficient to affect
the transmembrane potential. The only cardiac chloride current that has been unequivocally demonstrated to be present in human heart is $I_{\text{Cl,swell}}$ [88–90]. Besides possibly contributing to cardiac electrical activity, $I_{\text{Cl,swell}}$ may also be important in cell volume regulation [133,146]. $I_{\text{Cl,swell}}$ was not detected in human atrium [108]. There is a preliminary confirmation of this negative result in human atrium with the additional finding that $I_{\text{Cl,swell}}$ is also not detectable in human ventricle [109]. It is still not resolved if $I_{\text{CFTR,cardiac}}$ is important in human heart [84,88–90] (Table 1).

The reversal potential for chloride ($E_{\text{Cl}}$) in the heart has been estimated to be between −40 and −60 mV from studies using ion-sensitive electrodes (reviewed [147]). Chloride is actively taken up into cardiac tissue. The mechanisms for chloride uptake include chloride/bicarbonate exchange [148], which is the major uptake mechanism in sheep Purkinje fibers, and furosemide-sensitive sodium-dependent uptake [149,150], which is more important in the ventricle. Little is known regarding intracellular chloride homeostasis in the atrium. Based on measuring total chloride and correcting for the extracellular content, rat atrial $E_{\text{Cl}}$ has been estimated to be −47 mV [151]. Intracellular chloride activity may change under pathologi-

<table>
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<th>Table 1 Summary of cardiac chloride current properties</th>
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<tr>
<td>$I_{\text{CFTR,cardiac}}$</td>
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<tr>
<td>Activation</td>
</tr>
<tr>
<td>Glibenclamide [73,75]</td>
</tr>
<tr>
<td>NPPB, partial [74]</td>
</tr>
<tr>
<td>DPC, partial [74]</td>
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<tr>
<td>A9C* [8,68,77]</td>
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<tr>
<td>Current–voltage relationship</td>
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<tr>
<td>Selectivity sequence</td>
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<tr>
<td>Br &gt; Cl &gt; I &gt; Fl [66]</td>
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<tr>
<td>Conductance</td>
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<tr>
<td>Species distribution</td>
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<tr>
<td>Ventricle</td>
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<tr>
<td>guinea pig [8,9]</td>
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<tr>
<td>rabbit [81]</td>
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<tr>
<td>human* [84,88]</td>
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* Outward currents blocked more readily than inward currents.

* Indicates conflicting data in the literature.

* Confirmation required.
eral conditions. Nuclear magnetic resonance spectroscopy studies have suggested that intracellular chloride activity rises dramatically during ischemia [152].

The possible roles of cardiac chloride currents in shaping the cardiac action potential and in arrhythmogenesis have recently been reviewed [153] and will only be described briefly here. Since $E_{\text{Cl}}$ is positive to the resting potential of cardiac myocytes and negative to plateau potentials, it is possible for chloride currents to depolarize the resting potential and to accelerate repolarization of the action potential. Both chloride current-dependent depolarization [35,40,130] and action potential shortening [8,40,134,154] have been experimentally documented. If the intracellular chloride concentration is elevated, $I_{\text{CFTR, cardiac}}$ activation can produce abnormal automatic activity and early after-depolarizations [155,156].

Calcium-activated chloride currents have been proposed to contribute to action potential plateau amplitude [154] and to transient inward currents under conditions of intracellular calcium overload [101,157].

The effect of chloride current activation on cardiac rhythm and excitability will depend on the setting in which chloride current activation occurs. For example, if the resting potential were far from threshold and sodium channels were fully available, then a slight chloride current-induced depolarization could increase conduction velocity. Further depolarization into a range where sodium channels are only partially available would slow conduction. The magnitude of chloride current-dependent depolarization will depend on intracellular chloride activity [40,85], resting potassium conductance [156] and the rectification properties of the chloride current(s) that is/are involved. The enhanced repolarization that chloride currents can cause could be beneficial by preventing excessively long action potentials that could result in early after-depolarization. However, in the presence of conditions that favor reentry, action potential shortening could be deleterious by stabilizing reentrant arrhythmias.

Blockers of chloride currents can hyperpolarize the resting membrane and prolong the action potential duration [130,134,154,158]. In the setting of a reentrant arrhythmia, this would serve two useful purposes. First, the class III action potential would prolong the wavelength of the cycle. Hyperpolarizing the resting membrane could increase conduction velocity by promoting the recovery of sodium channels from inactivation. Both of these actions would tend to destabilize reentrant circuits. Hyperpolarization of the resting membrane could also lead to a reduction in abnormal automatic activity. Chloride current blockers may even prove to be somewhat selective for diseased parts of the heart since conditions that are known to activate chloride currents are associated with certain pathological states.

In light of the limited success of the available cation-channel-blocking antiarrhythmic agents, it is worth considering anion channels as targets for antiarrhythmic interven-

ions. However, chloride currents serve important roles in many organs throughout the body and are particularly important in transepithelial fluid movement. Therefore, the use of chloride channel blockers as antiarrhythmic agents will be dependent on finding unique epitopes on cardiac chloride channels.

It is possible that any of the chloride currents described above could contribute to electrophysiological changes in the setting of ischemia. In ischemia, there is cell swelling, enhanced catecholamine release and elevation of intracellular calcium (reviewed [159]). At present, the proposal that pathological conditions augment the significance of cardiac chloride currents remains an untested hypothesis. Nevertheless, an ischemia-induced chloride current offers a potential explanation for several observations in the literature.

Substitution of chloride by nitrate significantly reduced the incidence of ventricular fibrillation during periods of ischemia and also during reperfusion of isolated perfused rat hearts [160,161]. Nitrate has a higher permeability than chloride through swelling-induced and protein kinase A-regulated channels [13,66]. The effect of acute nitrate substitution for chloride would be a hyperpolarizing shift in the reversal potential for anion currents. This would cause a decrease in depolarizing anion current at the resting potential and an increase in the repolarizing current during the action potential plateau. Decreased inward current at rest could provide protection against the development of abnormal automatic activity and also increase conduction velocity by enhancing recovery of sodium channels from inactivation. Increased outward current during the plateau could reduce the incidence of triggered activity that results from delayed after-depolarizations. The effect of a series of chloride substitutes was independent of effects on intracellular pH [161], suggesting that the protective effect did not depend on changes in chloride–bicarbonate exchange, but correlated well with relative membrane permeability [161]. In the ischemic rat heart model, substitution of extracellular chloride with anions that were less membrane permeable than chloride resulted in an exacerbation of arrhythmias [161]. There are caveats for the interpretation of anion substitution studies: (1) predicting the actual effect of anion substitution on the reversal potential for anion currents will be complex and will depend upon the relative permeability of the test anion and the intracellular anion concentrations; (2) cytoplasmic application [162] or prolonged extracellular exposure to chloride substitutes [7] can have effects on potassium conductance; (3) anion replacement can affect extracellular calcium activity [5] and (4) anion substitution can affect signal transduction mechanisms [67,163].

Studies with chloride channel blockers also support a role for chloride channels in the setting of ischemia and reperfusion. A9C and SITS have been shown to protect against contractile dysfunction after ischemia and reperfu-
sion in globally ischemic guinea pig hearts [76]. Both drugs also antagonized ischemia-induced action potential shortening [76]. These data should be cautiously interpreted because of known nonspecific actions of stilbene disulfonates [164] and A9C [77]. Stilbene disulfonates can affect intracellular pH through effects on Cl⁻/HCO₃⁻ exchange and have been shown to block certain potassium currents [164]. A dihydropyridine that blocked isoproterenol-induced chloride currents but not calcium currents, AHC-52, was also found to improve recovery of contractile function after ischemia and reperfusion [165]. In chronically diseased human heart, one finds depolarized cells with resting potentials of ≈−50 mV. The resting potential of these cells is significantly more positive than E₉ and unresponsive to changes in extracellular potassium between 2 and 10 mM [166–168]. The inward current responsible for the depolarization of the resting membrane of diseased human heart has not been identified. Neither substitution of extracellular sodium or chloride, nor calcium channel blockade were capable of altering the resting potential [166–168]. However, decreasing Cl⁻ would shift E₉ to more positive potentials but would not cause a large change in the inward current through a chloride channel if the channel exhibited strong outward rectification. In support of this statement, it should be noted that extracellular chloride substitution experiments were part of the basis for the erroneous conclusion by Egan et al.[35], i.e. that the isoproterenol-dependent depolarization of guinea pig ventricle cells was not due to a chloride current. Therefore, the possibility that inward current due to anion efflux may contribute to the depolarized resting potential found in cells from diseased human hearts has not been excluded.

Chloride current activation may not necessarily be limited to the setting of ischemia. Consistent with the observation that there may be common signaling events associated with hypertrophy and activation of I_{Cl,swell} [118], a basal chloride current is activated in a rat aortic banding model of cardiac hypertrophy [169]. The authors discounted the possibility that the current they observed was I_{Cl,swell}, based on stability of the current over time after establishing the whole cell configuration, but further evidence is required to formally rule out I_{Cl,swell}.

A recent report indicates that ventricular I_{Cl,swell} is persistently activated under basal conditions in a dog rapid pacing model of congestive heart failure [133]. In myocytes from failed hearts, basal I_{Cl,swell} could be turned off by shrinking the cells with hypertonic bath solution [133]. The maximum I_{Cl,swell} density was increased by 30% in myocytes from failed hearts [133].

Finally, distention of cardiac chambers may enhance cardiac chloride currents. Stretch-induced increases in the spontaneous rate of isolated rabbit sinoatrial node strips have been found to be inhibited by stilbene disulfonates [170]. The data did not allow the authors to discriminate between stretch-induced increases in I_{Cl,h} or I_{Cl,swell} [170]. Distention of rabbit atria has been shown to result in a gradual decrease in the refractory period [171]. The time course for the decrease in refractory period after an abrupt increase in the hydrostatic pressure load on the atria [171] is consistent with the rate of activation of I_{Cl,swell} [115].

Although the study of cardiac chloride currents has been restricted mainly to patch clamp experiments in isolated myocytes, there is some experimental evidence on isolated tissues and organs that hint that chloride currents may be significant contributors to cardiac electrical activity. It is important to remember that it is not known if the activation of chloride currents would be protective or detrimental to the heart. Whether chloride current activation favors arrhythmias or contributes to the maintenance of normal rhythm will probably be critically dependent on the setting. Determining the effect of chloride currents in intact tissues and the in situ heart and whether chloride currents make substantive contributions to cardiac electrophysiology in humans remain key areas for future research.

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