Role of cardiac chloride currents in changes in action potential characteristics and arrhythmias

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Abstract

Various types of Cl\textsuperscript{−} currents have been recorded in cardiac myocytes from different regions of the heart and in different species. With few exceptions, most of these currents are not active under basal conditions, but are activated under the influence of various agonists and by physical stress. These channels are distributed nonuniformly, depending on the cell type, tissue and region of the heart. Therefore, Cl\textsuperscript{−} current activation may influence membrane potential and impulse formation differently in different cells, and may play a role in arrhythmogenesis. Among these Cl\textsuperscript{−} currents, the protein kinase A-activated Cl\textsuperscript{−} current (\(I_{\text{Cl,PKA}}\)), the stretch- or swelling-activated Cl\textsuperscript{−} current (\(I_{\text{Cl,SWELL}}\)) and the Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} current (\(I_{\text{Cl,Ca}}\)) comprise the major anion currents that modify cardiac electrical activity. These currents exhibit outward-going rectification, or are predominantly activated at depolarized voltages and, thus, contribute significantly to shortening of the action potential duration but little to diastolic depolarization. The action potential shortening by Cl\textsuperscript{−} current activation may not only perpetuate reentry by shortening the refractory period in a reentry pathway, but may also prevent the development of early afterdepolarization and triggered activity caused by the prolongation of action potentials. \(I_{\text{Cl,PKA}}\) contributes to Cl\textsuperscript{−} replacement of extracellular Cl\textsuperscript{−} with an impermeable anion produced a marked prolongation of the duration of Cl\textsuperscript{−} currents on diastolic potentials is the presence of a predominantly opposing background K\textsuperscript{+} current, except at the nodal regions that lack these K\textsuperscript{+} channels, or under conditions of decreased K\textsuperscript{+} conductance. Therefore, the contribution of Cl\textsuperscript{−} currents to the genesis of arrhythmias may depend on their association with the conductance of other ions, especially that of K\textsuperscript{+}. © 1998 Elsevier Science B.V. All rights reserved.

1. Introduction

While the majority of ion currents in the heart are carried by univalent and divalent cations, activation of chloride currents can cause important changes in action potential characteristics. Previous studies have demonstrated that changes in extracellular chloride concentration caused little change in the resting membrane potential, but replacement of extracellular Cl\textsuperscript{−} with an impermeable anion produced a marked prolongation of the duration of action potential [1,2]. Voltage clamp studies in Purkinje fibers have indicated the dynamic nature of Cl\textsuperscript{−} currents [3,4], however, other studies have not supported these findings [5]. The advent of the patch–clamp technique allowed specific Cl\textsuperscript{−} currents to be isolated. A Cl\textsuperscript{−}-specific current, activated by catecholamines, has been described at the whole-cell [6–8] and single-channel level [9]. Molecular studies have provided a structural basis for this Cl\textsuperscript{−} current [10,11]. Since then, a number of other Cl\textsuperscript{−} currents have been described. Many of these Cl\textsuperscript{−} currents are activated by appropriate agonists [6–9,12–15] or physical

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stress [16–20]. The distribution of Cl− currents in the heart is not uniform (see, [21–23]). Therefore, activation of these Cl− currents may increase the dispersion of electrophysiological properties and provide the substrate for the occurrence of arrhythmias.

2. Presence of different types of Cl− currents in cardiac myocytes

Intracellular Cl− activity in cardiac cells is higher than that which would be expected from a passive distribution of Cl− [24,25]. This observation suggests that Cl− must be actively transported into cardiac cells and that the Cl− equilibrium potential (\(E_{Cl}\)) is more positive (usually between −60 and −40 mV) than the resting membrane potential [24–26]. Several different types of Cl− currents have been identified in myocytes from various regions of the heart, using patch-clamp techniques. The Cl− currents identified thus far include the protein kinase A-activated Cl− current \(I_{C_{1}-PKA}\) [6–9,12,27], the stretch- or swelling-activated Cl− current \(I_{C_{1}-SWELL}\) [16–20,27], the Ca2+-activated Cl− current \(I_{C_{1}-Ca}\) [13,28–30], the Cl− current activated by purinergic stimulation [15,31], the protein kinase C (PKC)-activated Cl− current [14,32,33] and the background-type Cl− current [34]. A Cl− current is also present in the sarcoplasmic reticulum (SR) membrane and this current is activated by protein kinase A (PKA)-dependent phosphorylation [35].

These Cl− currents are not uniformly distributed in all cardiac myocytes and their distribution varies among different cell types, regions of the heart and different species. The most noteworthy example is seen in the distribution of \(I_{C_{1}-PKA}\) and \(I_{C_{1}-SWELL}\). The presence of \(I_{C_{1}-PKA}\) was first described in guinea pig ventricular myocytes as an isoprenaline- and forskolin-activated current [12], and this was later confirmed to be a Cl− current [6–9,27,36]. The current was also found in rabbit ventricular myocytes [37]. In both species, \(I_{C_{1}-PKA}\) was reported to be more abundant in epicardial than endocardial myocytes from the ventricle. It was considerably less abundant in atrial than in ventricular myocytes, and was absent in sino-atrial (S-A) node cells [27,36,37]. \(I_{C_{1}-PKA}\) was not found in canine or human atrial or ventricular myocytes [16,17,38–41].

Activation of \(I_{C_{1}-SWELL}\) was observed in the atrial myocytes of most of the species examined, including canine [16], rabbit [18], guinea pig [27] and human cells [39–41]. The current was also present in guinea pig [27,42], canine [17] and human [39] ventricular myocytes. \(I_{C_{1}-SWELL}\) was found to be activated by exposure to hypoosmotic solutions in new-born rat cardiac myocytes [19] and cultured chick heart cells [20]. In guinea pig hearts, fewer ventricular myocytes than atrial myocytes responded to swelling [27,42]. Furthermore, the proportion of cells exhibiting \(I_{C_{1}-SWELL}\) was demonstrated to be smaller than that showing \(I_{C_{1}-PKA}\) in ventricular myocytes [27]. Therefore, the cellular distribution of Cl− currents responsive to different stimuli appears to be species- and region-dependent.

3. Molecular biology of cardiac Cl− channels

Several studies have suggested that cardiac \(I_{C_{1}-PKA}\) channels correspond to a protein related to the cystic fibrosis transmembrane conductance regulator (CFTR) found in epithelial cells [22,23]. Molecular studies have revealed that \(I_{C_{1}-PKA}\) channels are the cardiac expression of an alternatively spliced form of CFTR, the CFTR\textsuperscript{cardiac} [10,11]. The distribution pattern of mRNA for CFTR\textsuperscript{cardiac} as evaluated by reverse transcription polymerase chain reaction (RT-PCR) and Northern blot analysis, showed a distribution pattern for \(I_{C_{1}-PKA}\) similar to that revealed by electrical measurements described in the previous section [10,11,36,43]: the mRNA was present in abundance in guinea pig and rabbit ventricles. It was scarce in the atrium of both species, and not present at all in dog heart. The mRNA of \(I_{C_{1}-PKA}\) was detected in human atrium and ventricle [44,45], while electrical measurements failed to record \(I_{C_{1}-PKA}\) currents [39,40]. Discrepant results between the current measurements and detection of mRNA may be explained by the following: (i) the presence of the channel in a very small percentage of cells, (ii) poor translation of the message, (iii) rapid turnover of the channel protein or (iv) failure of protein-trafficking preventing the channel from reaching the cell membrane [39]. A recent study in which both electrical and molecular methods were applied to guinea pig hearts has demonstrated a similar distribution pattern of \(I_{C_{1}-PKA}\) and mRNA [36], and a decreasing order of abundance from ventricular epicardium to ventricular endocardium and atrium.

\(I_{C_{1}-SWELL}\) channels identified in other tissues comprise members of the CIC family of genes [46,47]. Among them, the CIC-2 gene has a unique feature since the expressed current in Xenopus oocytes is activated by voltage changes and by external hypoosmosis [48,49]. The mRNA of CIC-2 was found in the atrium and ventricle of rabbit heart, and a homologue of the CIC-2 gene has been cloned [50]. The current through rabbit CIC-2 channels expressed in Xenopus oocytes exhibited halide-anion permeability in the order of Cl− > Br− > I−. The current was not activated by forskolin, but was activated by hypoosmotic solutions. It might play a role in cell-volume regulation of oocytes [51]. The characteristics of the expressed current, however, were not entirely consistent with the features of \(I_{C_{1}-SWELL}\) in native cardiac myocytes. Its physiological function remains to be clarified. Another member of the CIC family, the CIC-3 gene, has been cloned from guinea pig atrial and ventricular myocytes [52]. The expressed current of CIC-3 in NIH/3T3 cells results in a large chloride conductance with activation, which is strongly modulated by cell...
volume under basal conditions. In addition, the current exhibits many characteristics identical to $I_{\text{CL,SWELL}}$ found in native cardiac cells, such as an $I-V$ relationship, single-channel conductance and pharmacology (see Section 4).

4. Functional modulations of cardiac Cl$^-$ currents and the effects of current activation on action potentials

4.1. PKA-activated Cl$^-$ current ($I_{\text{CL,PKA}}$)

This current is not activated under basal conditions but can be activated by the application of isoproterenol, norepinephrine, or forskolin, i.e., conditions under which the intracellular cAMP level is increased and the activity of PKA-dependent phosphorylase is stimulated. The current amplitude varies with membrane voltage, but not with time. The $I-V$ relation is linear with symmetrical Cl concentrations in intra- and extracellular solutions (about 140 mM), but shows an outward-going rectification with physiological (about 20 mM) intracellular Cl$^-$ concentrations [6–8,21–23]. $I_{\text{CL,PKA}}$ is usually insensitive to Cl$^-$ channel blockers [21–23,27]. In single-channel recordings, the Cl$^-$-channel current is activated by increased intracellular cAMP and possibly by PKA-dependent phosphorylation. Activation of the single-channel current is voltage-independent, and the $I-V$ relation exhibits outward-going rectification. The single-channel conductance is 14 pS under identical intra- and extracellular Cl$^-$ concentrations of 140 mM. The channel density is low, but the channel-open probability is high once it is activated; the success rate for recording single-channel activity is low, while the channel gives rise to a large macroscopic current [9,53].

$I_{\text{CL,PKA}}$ activated in the presence of catecholamines or β-adrenergic stimulation contributes to shortening of the duration of action potential and to depolarization of the resting membrane potential, but the effects on the two parameters are not equally expressed because of outward-going rectification of the current at physiological Cl$^-$ concentrations; the degree of resting potential depolarization induced by $I_{\text{CL,PKA}}$ is usually small (less than 5 mV), while a definite shortening of the duration of action potential can be observed [7,37,54,55]. $I_{\text{CL,PKA}}$ is modulated by various neurohormonal factors. The current is modulated by β-adrenergic and cholinergic stimulation with the same intracellular mechanisms as those modulating the L-type Ca$^{2+}$ and delayed outward K$^+$ currents [56–58]. The current is also activated by histamine, through the intracellular cAMP–PKA pathway and the G-protein-coupling mechanism [59–61]. $I_{\text{CL,PKA}}$ is inhibited by α-adrenergic stimulation [62,63] and by endothelin-1 [64]. These inhibitory influences may help to prolong action potential duration in the appropriate settings, but their functional role is not known.

4.2. Stretch- or swelling-activated Cl$^-$ current ($I_{\text{CL,SWELL}}$)

Stretching of the cell membrane by inflation and cell swelling induced by exposure to hypoosmotic solutions activates Cl$^-$ currents. The Cl$^-$ currents activated by both stimuli share nearly similar biophysical and pharmacological characteristics [16–20,27,38–42], although stretching and cell swelling may not necessarily be equivalent stimuli to the cell membrane. Since most previous studies have dealt with the Cl$^-$ currents activated by exposure to hypoosmotic solutions, this section will focus on $I_{\text{CL,SWELL}}$. The current through $I_{\text{CL,SWELL}}$ under basal or isotonic conditions is small, but can largely be activated with a delay of 2–3 min following exposure to a hypoosmotic solution. $I_{\text{CL,SWELL}}$ is a time-independent current when it is activated from depolarized holding potentials around −40 mV or by applying a ramp voltage protocol. The $I-V$ curve shows an outward-going rectification under physiological Cl$^-$ concentrations and the rectification becomes less prominent under symmetrical Cl$^-$ concentrations. Therefore, $I_{\text{CL,PKA}}$ and $I_{\text{CL,SWELL}}$ exhibit similar characteristics and appear to be indistinguishable on the basis of the current records once they are activated.

5. Differences between $I_{\text{CL,PKA}}$ and $I_{\text{CL,SWELL}}$

There are, however, certain differences in electrophysiological and pharmacological characteristics between $I_{\text{CL,PKA}}$ and $I_{\text{CL,SWELL}}$. While $I_{\text{CL,SWELL}}$ exhibits a higher conductance and permeability to I$^-$ relative to Cl$^-$, $I_{\text{CL,PKA}}$ exhibits a high permeability but markedly reduced conductance for I$^-$ compared to Cl$^-$ [21–23,27]. $I_{\text{CL,PKA}}$ is relatively insensitive to anion channel blockers such as stilbene disulfonates and tamoxifen [21,23,42,54], whereas $I_{\text{CL,SWELL}}$ is sensitive to these compounds [18,27]. $I_{\text{CL,SWELL}}$ shows a time-dependent decrease (inactivation) during depolarizing pulses at strongly positive voltages, when test pulses are applied from a negative holding potential of around −80 mV [65]. The current develops inactivation with a half maximal level at −25 mV. Repolarization to −80 mV elicits an inwardly developing tail current due to the removal of inactivation (see figures 1, 4 and 7 in Ref. [65]). $I_{\text{CL,PKA}}$ does not show such time-dependent changes or inactivation. Although $I_{\text{CL,PKA}}$ does not show inactivation in the absence of agonists, $I_{\text{CL,SWELL}}$ carries the background current under basal conditions without agonists in atrial myocytes [34,66]. Single-channel studies have disclosed that this current exhibits a larger single-channel conductance (60 pS under symmetrical Cl$^-$ conditions) than that of $I_{\text{CL,PKA}}$ [67]. $I_{\text{CL,PKA}}$ is activated by β-adrenoceptor stimulation via cAMP-dependent phosphorylation [6–8,21–23,43]; in contrast, $I_{\text{CL,SWELL}}$ does not require this phosphorylation process for activation [17,18]. However, once $I_{\text{CL,SWELL}}$ is activated by hypoosmotic solutions, it is
stimulated by isoproterenol and forskolin [16,39]. One exception, however, is the $I_{\text{Cl.SWELL}}$ in chick embryonic heart cells, which is inhibited by isoproterenol [20]. Another distinct characteristic of the current in chick heart is its dependence on elevation of $[\text{Ca}^{2+}]$, whereas its mammalian counterpart current appears to be calcium-independent [16–18]. α-Adrenergic stimulation has been shown to inhibit $I_{\text{Cl.SWELL}}$ [68].

6. Functional role of $I_{\text{Cl.SWELL}}$

Activation of $I_{\text{Cl.SWELL}}$ contributes to a mild depolarization of resting potential and shortening of action potential duration [69,70] (Fig. 1). It may also play a role in effecting a volume decrease after cell swelling [20,71]. Cell swelling induces not only activation of $I_{\text{Cl.SWELL}}$, but also functional alterations in other ion-transport pathways (see [42,72]). Electrical changes induced by stretching, therefore, may be modified by concomitant activation and/or suppression of other currents. Since $I_{\text{Cl.SWELL}}$ is predominantly found in atrial cells and S-A node cells [16,18,27,39–41], it may play a significant role in the electrical activity of the atrial tissue and in pacemaker function. If $I_{\text{Cl.SWELL}}$ acts as the stretch-activated channel and is present in S-A node cells [18], it may serve as an important mediator of mechanotransduction, similar to its function in various other tissues. Mechanical stretching or dilatation of the atrial myocardium has been shown to induce arrhythmias [73]. Another pathophysiological role for activated $I_{\text{Cl.SWELL}}$ may be seen during myocardial ischemia and reperfusion, since myocardial cells swell during ischemia and after reperfusion, and the wash-out of hyperosmotic extracellular fluid induces further cell swelling [74,75]. The extent of cell swelling during ischemia and reperfusion has not been correlated with the actual activation of $I_{\text{Cl.SWELL}}$ by electrical measurements. Enlargement of the cross-sectional area to over 110% of control by inflation results in the induction of $I_{\text{Cl.SWELL}}$ [18]. Since similar degrees of cell swelling can develop during ischemia and reperfusion, activation of $I_{\text{Cl.SWELL}}$ is likely to occur. Therefore, the current can be expected to contribute to membrane potential changes under these conditions (see below, the section on ‘substrates for arrhythmias’).

6.1. $\text{Ca}^{2+}$-activated $\text{Cl}^-$ current ($I_{\text{Cl.Ca}}$)

The presence of $I_{\text{Cl.Ca}}$ has been demonstrated in Purkinje and ventricular myocytes of rabbit and dog hearts [13,28–30]. $I_{\text{Cl.Ca}}$ has a threshold at voltages of between 20–0 mV under conditions of physiological $[\text{Ca}^{2+}]$, and the current amplitude increases with membrane depolarization, reaching a peak at around +40 mV. Further depolarization decreases the current amplitude, that exhibits a bell-shaped $I$–$V$ curve. The current is rapidly activated to reach a peak within 10–20 ms upon depolarization and then declines in the following 100 ms at physiological temperatures. $I_{\text{Cl.Ca}}$ is activated by an increase in $[\text{Ca}^{2+}]$, associated with $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release from the SR. The current is inhibited either by blocking the $\text{Ca}^{2+}$ influx through the sarcolemma (the L-type $\text{Ca}^{2+}$ current and possibly the $\text{Na}^{+}$–$\text{Ca}^{2+}$ exchange mechanism) or by blocking $\text{Ca}^{2+}$ release from the SR. The activated current, therefore, follows the time course of the $\text{Ca}^{2+}$ transients [29]. The current is sensitive to stilbene disulfonates. The channel responsible for carrying $I_{\text{Cl.Ca}}$ appears to be a ligand-gated channel and $[\text{Ca}^{2+}]$, plays the role of a ligand [29,30]. Single-channel studies have revealed the existence of $\text{Cl}^-$ channels that are activated in a $[\text{Ca}^{2+}]$-dependent manner.
[76]. Channel activity exhibits time independence when 
$[\text{Ca}^{2+}]_o$ is held constant. The current is blocked by anion 
channel blockers, DIDS and niflumic acid, similar to the 
macroscopic currents. Because of these characteristics, the 
current mainly contributes to the rapid repolarization phase 
(phase 1) of the action potential. It also contributes to 
shortening of the action potential and alterations of action 
potential duration after a resumption of rapid stimulation 
following a long rest period or a slow heart rate [77,78].

### 6.2. Other types of Cl currents

The Cl$^-$ current activated by purinergic-receptor stimu-
lation (P$_2$) has characteristics similar to those of $I_{\text{Cl.SWELL}}$ 
[15,31]. It has not been clarified whether these two Cl$^-$ 
currents represent the same channels modulated by differ-
ent stimuli or whether they are different channel currents. 
Angiotensin II, which has multiple modulatory actions on 
the cardiovascular system, including inotropic and chronot-
ropic effects, and induction of myocyte hypertrophy [79] 
have been shown to activate a Cl$^-$ current [80,81].

The exact nature of the angiotensin II-activated Cl$^-$ current is 
not known, but its activation seems to require the presence of 
$[\text{Ca}^{2+}]_o > 10^{-7}$ M [81]. The angiotensin II-activated Cl$^-$ 
current appears to modulate action potential duration in 
rabbit ventricular myocytes. Clarification of the 
pathophysiological roles of these Cl$^-$ current activations, 
induced by different stimuli, requires further study.

### 7. Possible involvement of Cl$^-$ currents in 
arrhythmogenic mechanisms

#### 7.1. Automaticity

In the heart, an increased heart rate is seen in response 
to increased venous return, which might cause stretching of 
the atrial muscle and S-A node region. It was shown that 
stretching of isolated cardiac pacemaker tissue caused 
acceleration of the spontaneous rhythm [82,83]. In Pur-
kinje fibers, papillary muscles and atrial trabecular prepa-
rations, stretching was shown to induce membrane depo-
larization and synchronous pacemaker activity [84].

These membrane responses to mechanical stimuli may be me-
diated by changes in ionic currents. S-A node cells are 
likely to be influenced by activation of the time-independ-
ent Cl$^-$ current, since a substantial density of background 
current flows at pacemaker potentials and contributes to 
slow diastolic depolarization [85]. In addition, $I_{\text{Cl.SWELL}}$ in 
ventricular myocytes exhibits an inward tail current upon 
repolarization to the resting potential level from positive 
voltages [65]. This may also cause pacemaker depolariza-
tion in the ventricle when exposed to stretching and 
dilatation, as seen in congestive heart failure, and may 
form the basis for the development of arrhythmias. How-
ever, the actual contribution of $I_{\text{Cl.SWELL}}$ and stretch-acti-
vated anion channels has not been explored in relation to 
membrane potential changes, pacemaker activity and arr-
hythmogenesis under conditions where mechanical stimuli 
induce electrical changes in multicellular preparations.

Histamine is present in all regions of the mammalian 
heart [86] and the released histamine may play a major 
role in the development of arrhythmias associated with 
 systemic allergic reactions [87]. Histamine was shown to 
induce oscillatory activity and abnormal impulse formation 
in Purkinje fibers [88], and to activate $I_{\text{Cl.PKA}}$ in ventricular 
myocytes [59–61]. Action potentials were prolonged by 
small doses of histamine, whereas large doses caused 
diastolic potential instabilities and spontaneous arrhythmic 
bursts in ventricular myocytes [89]. These actions are 
assumed to be produced mainly by stimulation of the 
L-type Ca$^{2+}$ current [89,90], and the contribution of $I_{\text{Cl.PKA}}$ 
to these arrhythmic actions, if any, has not been explored.

#### 7.2. Early afterdepolarization (EAD) and triggered 
activity

When the L-type Ca$^{2+}$ current is augmented by β-
adrenergic stimulation, $I_{\text{Cl.PKA}}$ can be activated simul-
taneously. The activation of repolarizing current at po-
tentials that are positive to the reversal of $I_{\text{Cl.PKA}}$ is 
expected to prevent excessive action potential prolongation 
in the presence of β-agonists [55]. Therefore, the current 
may protect against the development of EAD and triggered 
activity due to excessive prolongation or delayed repolar-
ization of action potential caused by stimulated Ca$^{2+}$ 
current. $I_{\text{Cl.PKA}}$ contributes little to diastolic depolarization, 
since the outward-going rectification of $I_{\text{Cl.PKA}}$ induces a 
relatively small inward current at potentials that are 
negative to its reversal. The initial observation of the Cl$^-$ 
current-induced membrane depolarization and abnormal 
automatic activity in ventricular myocytes [12] may need 
to be interpreted cautiously, since the experiments were 
carried out with Cl$^-$ concentrations at which the reversal 
potential was more positive than the physiological level. 
When external Cl$^-$ concentrations were varied to shift the 
reversal potential of $I_{\text{Cl.PKA}}$, the degrees of depolar-
ization in resting potential upon activation of this current were low 
at physiological levels of reversal potential and became 
higher with positive shifts in reversal potential by either 
decreasing extracellular Cl$^-$ concentration or increasing 
the intracellular Cl$^-$ concentrations [91]. Another factor 
determining the depolarizing action of $I_{\text{Cl.PKA}}$ on resting 
potential and induction of abnormal impulse formation is 
the presence of background K$^+$ conductance ($I_{\text{K,1}}$) in 
ventricular tissues, which carries a dominant outward 
current at voltages between the resting and the reversal 
potentials of $I_{\text{Cl.PKA}}$. When the extracellular K$^+$ concentra-
tion was decreased to suppress $I_{\text{K,1}}$, the extent of resting 
potential depolarization became prominent compared to 
normal [K$^+$]o, and abnormal impulse formation due to
diastolic depolarization and EAD developed frequently [38,91]. Fig. 2 illustrates the appearance of abnormal impulses, including EAD, and the extent of depolarization in resting potentials following activation of $I_{Cl,PKA}$ at different levels of reversal potential for Cl$^-$ ($E_{Cl}$). In Fig. 2A, the application of isoproterenol to activate $I_{Cl,PKA}$ delayed repolarization, caused the appearance of EAD and triggered activity under conditions of reduced [K$^+$]$_o$ and $E_{Cl}$=0 mV. In Fig. 2B, the extent of $I_{Cl,PKA}$-induced depolarization at the resting potential became larger with a positive shift of $E_{Cl}$, as did the incidence of abnormal impulse formation in Fig. 2C. Therefore, activation of $I_{Cl,PKA}$ is potentially arrhythmogenic in the setting of hypokalemia and/or hypochloremia.

Atrial stretching produced EAD and triggered arrhythmias [73]. No changes in action potential were observed at a remote atrial site located away from the area of stretching, implying that the changes in action potential were mediated mainly by stretching. It remains to be confirmed, however, whether or not EAD induced by stretching is caused exclusively by activation of $I_{Cl,SWELL}$, since stretching has been shown to modulate other ionic transport pathways as well [72].

7.3. Delayed afterdepolarization (DAD) and triggered activity

When [Ca$^{2+}$]$_i$ is substantially increased above the physiological resting level of around $10^{-7}$ M, $I_{Cl,Ca}$ carries a significant amount of current. The current amplitude and time course depend on the [Ca$^{2+}$]$_i$ and voltage (Fig. 3) [30]. This result suggests that $I_{Cl,Ca}$ can contribute to the arrhythmogenic transient inward current ($I_{TI}$) observed in Ca$^{2+}$-overloaded cardiac preparations [92–94]. $I_{TI}$

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Fig. 2. Effects of [Cl$^-$] and Cl$^-$-gradient dependence on action potentials, depolarization of resting potentials and appearance of abnormal automatic activities. (A) isoproterenol-induced changes in action potentials in the presence of a [Cl$^-$] of 140 mM. Dotted lines at the top of the records indicate the duration of isoproterenol application. The cell was stimulated at 0.5 Hz, except during the recording of (d) and (e). The numbers at the top left indicate [Cl$^-$]/[Cl$^-$]$_o$ in mM. [K$^+$]$_o$=3 mM. (B) Cl$^-$-gradient-dependence of isoproterenol-induced membrane depolarization in resting potential ($dV$) and (C) indicates the percentage incidence (%) of abnormal automatic activities in examined preparations. Pooled data with [K$^+$]$_o$ of 2 and 3 mM were divided into three groups based on the reversal potential for Cl$^-$ ($E_{Cl}$). The groups $'-'80'$ consisted of cases with $E_{Cl}$ of between $-80$ and $-90$ mV; $'-'50'$ comprised cases with an $E_{Cl}$ of between $-43$ and $-53$ mV and $'0'$ represents cases with $E_{Cl}$ of between $-10$ and 0 mV. The error bars in (a) denote the standard error of the mean. Note that the depolarization is greater and the incidence of abnormal activities is higher in the group with $E_{Cl}$=0 mV, compared to other groups. (From reference [91], by permission of Academic Press).
produces delayed afterdepolarizations (DADs) and induces triggered activity, which is assumed to be an important mechanism for abnormal impulse formation. $I_{\text{TI}}$ is believed to be carried by an electrogenic $\text{Na}^+–\text{Ca}^{2+}$ exchange mechanism and by the current through a nonselective cation channel [95]. Both have been demonstrated to carry membrane currents in cardiac cells [96–99]. Between the two, the $\text{Na}^+–\text{Ca}^{2+}$ exchange mechanism appears to be the major component of $I_{\text{TI}}$ in the presence of normal extracellular Na concentrations. While earlier results in Purkinje fibers intoxicated with cardiotonic steroids demonstrated a clear reversal potential near 0 mV [95], the tail currents in other studies were predominantly inward and did not reverse near 0 mV [100–102], which would be expected for a reversal potential of a nonselective cation channel. Under conditions where $\text{Na}^+–\text{Ca}^{2+}$ exchange was eliminated, however, $I_{\text{TI}}$ did show a reversal at around 0 mV and the nonselective cation channel could contribute to the formation of this arrhythmogenic current [103–105]. Two components of $[\text{Ca}^{2+}]_i$-activated $\text{Cl}^-$ currents, fast and slow, have been demonstrated in single rabbit Purkinje cells during large $[\text{Ca}^{2+}]_i$ transients [106]. While the fast component can be seen under physiological conditions and contributes to normal excitation–contraction coupling, the slow component is only observed with a large $[\text{Ca}^{2+}]_i$ transient. The authors [106] postulated that the slow component contributed to $I_{\text{TI}}$ under $\text{Ca}^{2+}$-overloaded conditions, carrying both outward and inward currents at potentials that were positive and negative to $E_{\text{Cl}}$, respectively, with almost equivalent amplitudes. In multicellular Purkinje fibers, a slow $I_{\text{Cl,CA}}$ under $\text{Ca}^{2+}$-overloaded conditions producing $I_{\text{TI}}$ has also been observed [105,107]. Another study [108] has shown that $I_{\text{Cl,CA}}$ can form an inward $I_{\text{TI}}$ current induced by isoproterenol and high $[\text{Ca}^{2+}]_i$ at voltages negative to $E_{\text{Cl}}$, and the current is inhibited by 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfic acid (SITS) (Fig. 4). Thus, $I_{\text{Cl,CA}}$ contributes to the formation of arrhythmogenic $I_{\text{TI}}$, but the extent of the contribution of $I_{\text{Cl,CA}}$ to $I_{\text{TI}}$ relative to two other factors, namely the $\text{Na}^+–\text{Ca}^{2+}$ exchange mechanism and nonselective cation channel, remains to be evaluated.

7.4. Reentry

Reentry is wont to occur when slow conduction and a short refractory period are present in the setting of ordered reentry, or when disparity of the refractory period between contiguous areas of the heart is increased, as in the case of random reentry. Activation of $I_{\text{CL,PKA}}$ or $I_{\text{CL,SWELL}}$ may accelerate the development of reentry, due to shortening of action potential duration and refractoriness, and due to a decrease in conduction velocity caused by a slight depolarization of diastolic potential leading to inactivation of the $\text{Na}^+$ current [6,7,54,55,69,70]. Nonuniform distribution of $I_{\text{CL,PKA}}$ between epicardial and endocardial myocardium [27,36,37] may have a nonuniform influence on repolarization in different layers of the ventricular wall. Stretching of the epicardial and endocardial wall, or of different regions of the atrium and ventricle may not be equal, depending on the basal and diseased states of the heart muscle. In addition, the proportion of cells responding to a hypotonic solution is different in different regions [27,42]. Therefore, activation of $I_{\text{CL,SWELL}}$ is not uniform among different regions of the heart, and their influences on repolarization are heterogeneous. These factors promote
8. Effects of Cl⁻ channel currents on conditions causing a predisposition to arrhythmias

8.1. Acute ischemia and ischemia/reperfusion

Since various types of arrhythmias develop during ischemia and reperfusion [109], few studies have focused on the contribution of different types of Cl⁻ currents to the genesis of these arrhythmias. In rat and rabbit hearts, substitution of extracellular Cl⁻ by NO₃⁻ was shown to prevent ischemia- and reperfusion-induced ventricular fibrillation [110], not as a result of hemodynamic changes but by widening the QT interval. Subsequent studies demonstrated that ventricular fibrillation was prevented when the membrane permeability of the Cl⁻ surrogate was greater than that of Cl⁻ (NO₃⁻, Br⁻, I⁻), while the least permeant anion, methysulphate, was proarrhythmic during ischemia. Arrhythmia suppression was accompanied by significant widening of the QT interval that developed during ischemia [111]. The authors speculated that increased anion permeability would delay the final repolarization phase, causing prolongation of action potential duration and the refractory period. However, they excluded I Cl,PKA as a possible reason for widening of the QT interval because of the opposite effects of anion substitution, and could not specify the anion current responsible for the action potential prolongation. Endogenous catecholamine release occurring during early ischemia could activate I Cl,PKA, which has been shown to contribute to action potential shortening [112]. It was also shown that the use of the chloride-channel blockers, anthracene-9-carboxylic acid (9-AC) and SITS, significantly inhibited ischemia-induced shortening of action potential duration and exerted protective effects against ischemia–reperfusion damage in arterially perfused guinea pig ventricular preparations [113]. While protection of the action potential shortening during early ischemia, by inhibition of Cl⁻ currents, is expected to prevent the development of reentry, the mechanism by which Cl⁻ manipulation decreases anion permeability and inhibits the Cl⁻ currents, protecting against reperfusion arrhythmias and damage, is not known. Further studies are warranted to clarify these protective mechanisms, with special attention being given to the types and roles of Cl⁻ currents involved, and the development of specific pharmacological tools to prevent these arrhythmias.

8.2. Hypertrophy

It is of interest to note that cardiac hypertrophy induced by pressure overload in rat induced the development of a Cl⁻ current component [114]. This current can partially balance the effect of decreased density in the transient outward K⁺ current (I ox) [115], preventing the excessive action potential prolongation seen in hypertrophy [116,117]. The authors speculate that this Cl⁻ current

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Fig. 4. Isoproterenol and high extracellular Ca²⁺ activate a transient inward current in the absence of Na⁺ and K⁺. Na⁺-free standard external and pipette solutions were modified to K⁺-free solutions. (A) With 3.6 mM CaCl₂ in the external solution, depolarizing steps failed to elicit inward currents at -80 mV. (B) In the presence of isoproterenol and 5 mM CaCl₂, oscillating inward currents were noted. (C) Oscillating inward currents nearly disappeared when the potential was equal to E Cl. (D) Currents in a second mid-myocardial cell after the addition of 1 μM isoproterenol and 5 mM CaCl₂. The cell was held at -80 mV and stepped to 0 mV for 150 ms. The protocol was repeated after 1 mM SITS was added to the external solution. SITS blocked oscillating inward currents and reduced inward shifts in holding current due to high intracellular Ca²⁺. (Reproduced from reference [108], by permission of American Physiological Society).
component might represent a compensatory mechanism or an endogenous antiarrhythmic adaptation to reduce the possibility of early afterdepolarization and triggered activity that would otherwise arise when action potentials are prolonged [118].

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