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Transient outward potassium current and Ca^{2+} homeostasis in the heart: beyond the action potential

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Abstract

The present review deals with Ca^{2+}-independent, K^+-carried transient outward current (I_{to}), an important determinant of the early repolarization phase of the myocardial action potential. The density of total I_{to} and of its fast and slow components ($I_{to,f}$ and $I_{to,s}$, respectively), as well as the expression of their molecular correlates (pore-forming protein isoforms Kv4.3/4.2 and Kv1.4, respectively), vary during postnatal development and aging across species and regions of the heart. Changes in I_{to} may also occur in disease conditions, which may affect the profile of cardiac repolarization and vulnerability to arrhythmias, and also influence excitation-contraction coupling. Decreased I_{to} density, observed in immature and aging myocardium, as well as during several types of cardiomyopathy and heart failure, may be associated with action potential prolongation, which favors Ca^{2+} influx during membrane depolarization and limits voltage-dependent Ca^{2+} efflux via the Na^+/Ca^{2+} exchanger. Both effects contribute to increasing sarcoplasmic reticulum (SR) Ca^{2+} content (the main source of contraction-activating Ca^{2+} in mammalian myocardium), which, in addition to the increased Ca^{2+} influx, should enhance the amount of Ca^{2+} released by the SR during systole. This change usually takes place under conditions in which SR function is depressed, and may be adaptive since it provides partial compensation for SR deficiency, although possibly at the cost of asynchronous SR Ca^{2+} release and greater propensity to triggered arrhythmias. Thus, I_{to} modulation appears to be an additional mechanism by which excitation-contraction coupling in myocardial cells is indirectly regulated.

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Introduction

The action potential (AP) is the triggering signal for contraction in striated muscle. Ion currents through sarcolemmal voltage- and ligand-dependent channels, as well as electrogenic ion transporters, determine the AP waveform. In a typical mammalian cardiac myocyte, 4 phases of the AP (Figure 1) can be identified (1,2):

Phase zero (upstroke): during this brief phase, the rapidly activating Na^+ current drives the membrane potential (E_m) from its diastolic level (~ -80 mV) to positive values (20-50 mV). The AP peak is limited mainly by Na^+ channel inactivation and decrease in the transsarcolemmal Na^+ electrochemical gradient.

Phase 1 (early repolarization): the rapid, transient outward current (I_{to}) is the predominant contributor to the partial membrane repolarization.

Phase 2 (plateau): this usually long phase may last up to a few hundred milliseconds, and is characterized by slow E_m variation, which is dependent on the delicate balance between depolarizing (mostly L-type Ca^{2+} current, $I_{Ca,L}$) and repolarizing (mainly mediated by delayed rectifier K^+ channels) currents.

Phase 3 (late repolarization): this phase relies on both a decrease in $I_{Ca,L}$ due to $I_{Ca,L}$ inactivation/deactivation, and activation of delayed rectifier K^+ channels. As repolarization proceeds, K^+ efflux through inwardly rectifying K^+ channels (I_{K1}) becomes greater (Figure 1C) due to relief of channel rectification and contributes to late restoration and stabilization of the diastolic E_m.

Recent investigation has provided evidence that the early repolarization phase may considerably influence the AP waveform. I_{to}, the main current responsible for this phase, has been shown to be carried by distinct ionic components: in addition to the Ca^{2+}-independent, voltage- and time-dependent K^+ current ($I_{to\text{-}1}$), a Ca^{2+}-dependent Cl^- current ($I_{Cl(Ca)}$ or $I_{to\text{-}2}$) was also identified. In this review, I will refer to the 4-aminopyridine-sensitive, Ca^{2+}-insensitive transient outward K^+ current ($I_{to\text{-}1}$) as I_{to}. More information on $I_{Cl(Ca)}$ can be found in a recent review (4).

I_{to}, in turn, is the net result of K^+ flux through at least two different types of channel associated with different isoforms of the pore-forming protein. The behavior of these channels differs especially in the time course of voltage-dependent inactivation and steady-state recovery from inactivation. Fast I_{to} ($I_{to,f}$) is mediated by channels that recover from inactivation in less than 100 ms, whereas for channels that carry slow I_{to} ($I_{to,s}$) recovery from inactivation takes a few seconds (5,6).

Both experimental data and mathematical models have shown that I_{to} magnitude and composition (i.e., the relative contributions of $I_{to,f}$ and $I_{to,s}$ to total I_{to}) may markedly affect AP duration and shape (7-13). Expression of $I_{to,f}$ and $I_{to,s}$ channel proteins is highly regulated, and may vary with species, developmental stage, and region of the heart (6,11). For instance, $I_{to,f}$ is more prominent in ventricular epicardial myocytes than in endocardial myocytes. In the former, the AP waveform displays the so-called spike-and-dome configuration, with distinct phases 1 and 2, while in the latter AP duration tends to be greater and repolarization to the plateau level is slower and more gradual. Differences in $I_{to,f}$ density correlate with differences in the AP profile within and between ventricles (e.g., 5,11,14,15). The $I_{to,f}$ and AP duration gradients across and along the ven-

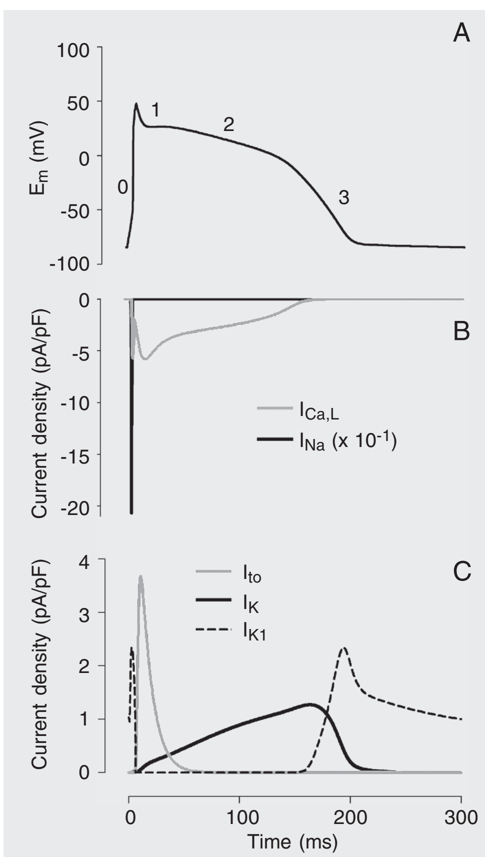

Figure 1. Action potential (AP) and ion currents in a rabbit ventricular myocyte. *A*, AP waveform, in which phases zero (upstroke), 1 (early repolarization), 2 (plateau), and 3 (late repolarization) are indicated. *B*, Voltage-dependent ion currents that contribute to membrane depolarization during the AP: Na^+ current (I_{Na}) and L-type Ca^{2+} current ($I_{Ca,L}$). Note: the actual I_{Na} density is 10-fold greater than that shown in the figure. *C*, Voltage-dependent K^+ currents that contribute to membrane repolarization during the AP: transient outward current (I_{to}), total delayed rectifier K^+ current (I_K) and inwardly rectifying K^+ current (I_{K1}). For clarity, I_K components (I_{Kr} and I_{Ks}) were combined, and currents carried by ion transporters (e.g., Na^+-Ca^{2+} exchanger, Na^+-K^+ ATPase), as well as $I_{Cl(Ca)}$, were omitted. Traces were obtained by simulation with the LabHEART 4.9.5 program (Ref. 3).

tricular wall have been proposed to contribute to the dispersion of ventricular refractoriness, which markedly influences ventricular repolarization path, direction and time course. It has been proposed that region-dependent changes in I_{to} reported in certain disease states (e.g., post-myocardial infarction, ventricular hypertrophy, heart failure) may underlie increased susceptibility to repolarization abnormalities and reentry arrhythmias (11,16,17). However, in this brief review, I will not deal with the influence of I_{to} on cardiac electric conduction (see Ref. 16 for further discussion), but its indirect effects, via the AP waveform, on Ca^{2+} homeostasis, which is of paramount importance for the development of adequate ventricular mechanical function and blood pumping.

Myocardial Ca^{2+} cycling

Ca^{2+}-induced Ca^{2+} release (CICR) is the most accepted mechanism underlying excitation-contraction coupling (ECC) in the mammalian myocardium (18,19). Ca^{2+} influx through voltage-dependent, sarcolemmal $I_{Ca,L}$ during the AP is the main trigger for the release of a greater amount of Ca^{2+} by the sarcoplasmic reticulum (SR), which is the source of most Ca^{2+} that activates contraction. SR Ca^{2+} release causes an increase in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), which results in greater interaction of the ion with myofilament proteins and development of force and cell shortening. Thus, the AP is the signal for contraction development, and Ca^{2+} acts as the second messenger in the electro-mechanical coupling process. Contraction is limited by Ca^{2+} removal from the cytosol by several transporters, which promote $[Ca^{2+}]_i$ decline, Ca^{2+} dissociation from myofilaments and cell relaxation. The dominant transporter that ultimately determines relaxation is the SR Ca^{2+}-ATPase (SERCA), which is responsible for 70-90% of cytosolic Ca^{2+} clearance and repletion of the SR Ca^{2+} store. Ca^{2+} efflux is mainly mediated by the Na^+-Ca^{2+} exchanger (NCX), a sarcolemmal counter-transporter that is driven by the transsarcolemmal Na^+ and Ca^{2+} electrochemical gradients. NCX accounts for 7-30% of total cytosolic Ca^{2+} removal during relaxation, which is approximately equivalent to the amount of Ca^{2+} entering the cell via $I_{Ca,L}$ during excitation (1,20,21).

The Ca^{2+} transient amplitude is an important determinant of contraction amplitude and is greatly dependent on the amount of Ca^{2+} released by the SR during ECC. During each AP, the SR releases a fraction of its content, which increases with increasing trigger (i.e., $I_{Ca,L}$) amplitude and/or SR Ca^{2+} content (12,22,23). Moreover, ECC efficiency may be modulated by additional regulation of the SR Ca^{2+} channel activity by ions (e.g., Mg^{2+}) and proteins (e.g., Ca^{2+}-calmodulin-dependent protein kinase II, FK506-binding protein, sorcin) (1).

It is important to note that Ca^{2+} transport by the main influx ($I_{Ca,L}$) and efflux (NCX) pathways during the cardiac cycle is strongly influenced by E_m: the former because of the voltage dependence of $I_{Ca,L}$ activation and inactivation (and also because of the driving force for Ca^{2+} flux), and the latter because the direction and driving force for Ca^{2+} transport are determined by the difference between E_m and the exchanger reversal potential ($E_{NCX} = 3E_{Na} - 2E_{Ca}$, where E_{Na} and E_{Ca} are the Nernst equilibrium potentials for Na^+ and Ca^{2+}, respectively (24)). The consequences of the voltage dependence of $I_{Ca,L}$ and NCX operation for ECC, $[Ca^{2+}]_i$ and contraction amplitude are many. The amplitude and time course of the trigger Ca^{2+} signal were shown to markedly affect its ability to induce SR Ca^{2+} release (19) and the so-called fractional SR Ca^{2+} release, i.e., the fraction of the SR Ca^{2+} content that is released at a twitch (22). On the other hand, Ca^{2+} efflux by the NCX is thermodynamically favored by membrane repolarization. Changes in NCX function may influence SR

Ca^{2+} content and vulnerability to triggered arrhythmias (1). Thus, one can conclude that the AP does not represent simply an impulse to trigger ECC, but a complex input waveform that can modulate directly or indirectly ECC efficiency. On the other hand, Ca^{2+} cycling may conversely modulate the AP waveform, because of the effects of SR-released Ca^{2+} on membrane currents, for instance, inactivation of $I_{Ca,L}$, activation of Ca^{2+}-dependent Cl^- channels and NCX-mediated current (25).

Transient outward K^+ current (I_{to})

I_{to} is characteristic of neurons and cardiac muscle, and its voltage-dependent activation and inactivation kinetics is much faster than that of other cardiac K^+ currents. The channel is a macromolecular protein complex composed of pore-forming subunits α (which belong to the Kv gene subfamily), accessory ß subunits (several types of ß subunit have been identified) and other regulatory proteins, such as minimal K^+ channel subunit homologues, K^+ channel-associated proteins (possibly chaperone proteins) and K^+ channel-interacting proteins (KChIP, which belong to a family of neuronal Ca^{2+} binding proteins). KChIP2 is present in the heart, and its co-expression with Kv4.2, but not with Kv1.4, increases current amplitude, changes the biophysical channel properties and allows the channel to be regulated by protein kinase A. The α subunit presents 6 transmembrane domains, a K^+-selective pore region and a highly charged S4 domain, which is considered to be the region where the voltage sensor is located. The functional channel consists of the assembly of 4 α subunits. In rodent heart, it seems that the channel that mediates $I_{to,f}$ consists of Kv4.2 and/or 4.3 subunits co-assembled with KChIP2. A detailed description of the molecular aspects of the channels that mediate I_{to} can be found elsewhere (2,6,11,17).

Two types of α subunits may form channels with different kinetic properties. Kv4.2/4.3 expression correlates with $I_{to,f}$, which shows fast inactivation and recovery from steady-state inactivation (milliseconds). Kv4.2 appears to be the pore-forming subunit in rodent atria, whereas Kv4.3 mediates $I_{to,f}$ in canine and human ventricle. Kv1.4 is thought to form the channels that mediate $I_{to,s}$, which displays a longer time course, especially of recovery from steady-state inactivation (seconds) (5,6,11,17).

The expression of these rapidly activating K^+ channels is influenced by several factors.

Species

Kv4.2/4.3 and $I_{to,f}$ are strongly expressed in the ventricular myocardium of adult rodents, ferrets, dogs, and humans, with a lesser contribution of Kv1.4 and $I_{to,s}$ (6,12). $I_{to,f}$ has been considered to be responsible for the typically brief rodent ventricular AP (6,26). In other species (e.g., rabbit), $I_{to,s}$ and Kv1.4 are the dominant I_{to} component and channel isoform, respectively (6,12). In the guinea pig ventricle, I_{to} is absent, a fact that may contribute to the prolonged AP in this species (6).

Developmental stage

In several species, even those in which the heart presents large I_{to} expression during adulthood, I_{to} density is considerably small or absent during the fetal and neonatal period. In neonatal rodent ventricle, $I_{to,f}$ and $I_{to,s}$ (whose density is paralleled by Kv4.2/4.3 and Kv1.4 expression, respectively) show similar contributions to total $I_{to,}$ while in adults the former contributes over 90% (2,11,27,28). In the neonatal myocardium, the AP is longer than in the adult, and AP shortening with maturation coincides with an increase in myocardial I_{to} density and channel isoform switch (28). Rabbit ventricle also shows a developmental increase in I_{to}, but in this species the dominant component shifts from $I_{to,f}$ to $I_{to,s}$ (29). On the other hand, aging is associated with a decrease in I_{to} density and AP prolongation (30).

Region of the ventricle

I_{to} density (particularly $I_{to,f}$) is greater in epicardium *vs* endocardium, in the apex *vs* base, and in right *vs* left ventricle. This variation is considered to underlie the regional differences in the AP profile, as well as in the dispersion of repolarization (5,11,14,15). While in rodents this regional variation may rely on a gradient of Kv4.3 expression only, it has not yet been established whether the origin of this variation in canine and human ventricle depends on the expression of Kv4.3, KChIP2, or both (17,31,32).

Disease

$I_{to,f}$ down-regulation associated with AP prolongation has been reported following myocardial infarction, in hypertension, diabetic cardiomyopathy, ventricular hypertrophy, and heart failure (7-9,32-35), although in some cases AP lengthening is not accompanied by changes in I_{to} density (36). The mechanisms responsible for these changes have not been ascertained, but it is possible that they involve increased activity of the sympathetic and renin-angiotensin-aldosterone systems, since norepinephrine (via α-adrenoceptors), angiotensin II and aldosterone may negatively regulate $I_{to,f}$-mediating channels (11,17,35,37,38). Although in most cases a decrease in $I_{to,f}$ is accompanied by Kv4.2/4.3 down-regulation, sometimes with an increase in $I_{to,s}$ and Kv1.4 up-regulation (9,32,33,38), the functional change may be also associated with direct modification of the biophysical properties of the current (e.g., by angiotensin II) (37). Because of the decrease in $I_{to,f}$ in the epicardium (where current density is greater) during disease, the transmural heterogeneity of AP duration is largely suppressed or even reversed, an event that may lead to repolarization abnormalities and possibly increase the predisposition to reentry arrhythmias (11,15).

How I_{to} can affect Ca^{2+} homeostasis

In the physiological context, I_{to} regional variability within the heart is thought to partially underlie regional differences in Ca^{2+} transient amplitude (15,39). The role of I_{to} magnitude and composition in cell Ca^{2+} homeostasis and contraction is mediated by I_{to} influence on AP waveform, especially the rate at which a plateau is attained, as well as its amplitude and duration. These AP features affect voltage-dependent Ca^{2+} transport pathways involved in ECC, in relaxation and in general regulation of cell Ca^{2+} load, such as $I_{Ca,L}$ and NCX (Figure 2).

Most of $I_{Ca,L}$ develops during the AP plateau, in the voltage range at which the current peak is nearly maximal (1). A decrease in I_{to} density results in AP prolongation, especially of the plateau phase. It is expected that a longer AP is associated with greater total Ca^{2+} influx, and this has been confirmed by experimental evidence (12, 21,40). Paucity of I_{to} and prolonged AP are observed in some conditions in which the SR function is depressed, such as immaturity and senescence, ventricular hypertrophy and heart failure. In these cases, increased Ca^{2+} influx may contribute not only to the contraction-activating cytosolic Ca^{2+} pool, but also to facilitating ECC by enhancement of the fractional SR Ca^{2+} release (22,23). It is tempting to speculate whether there is a negative relationship between I_{to} functional expression and the SR relative contribution to ECC. I_{to} density has been found to be higher in cardiomyocytes in which Ca^{2+} cycling between the SR and cytosol is more prominent, such as rodents and ferrets *vs* rabbits, and adults *vs* neonates (6,11,12,41). In adult rodents and ferrets, a short AP would allow large and short-lived $I_{Ca,L}$, better suited for triggering synchronized SR Ca^{2+} release than for providing Ca^{2+} for contraction and SR loading (see below).

During early postnatal development, SR contribution to Ca^{2+} cycling, although important, is smaller than in adult myocardium (1,41), probably due to structural SR underdevelopment and paucity of sarcolemma-SR specialized junctions, as well as to diminished sensitivity of the CICR mechanism (18). Because of the small volume and reduced Ca^{2+} buffering capacity in immature myocytes (42), a greater Ca^{2+} influx should have a considerable impact directly on cytosolic [Ca^{2+}] and/or indirectly via the induction of SR Ca^{2+} release, and thus on contractile activity. It has been recently shown that immature human myocytes (in which AP is long) stimulated with a waveform similar to the adult AP show depressed Ca^{2+} transients, which indicates that prolonged AP in developing myocytes is of paramount importance for proper Ca^{2+} cycling (43). Similar results were observed in myocytes from senescent animals (44), which present, as neonatal cells, reduced I_{to} density and prolonged AP (30).

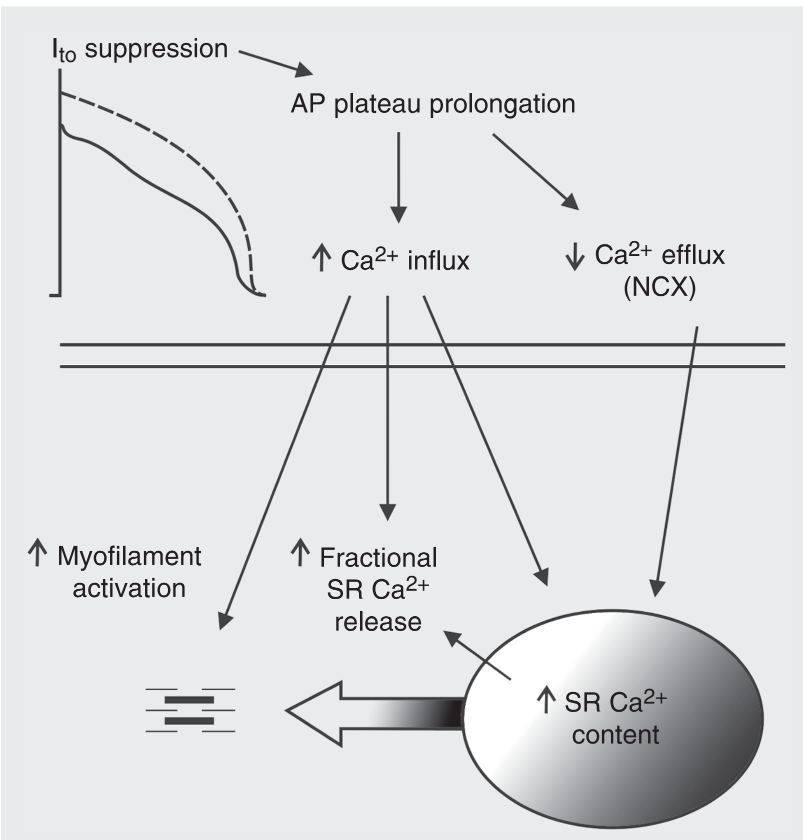

Figure 2. How lengthening of the action potential (AP) plateau by transient outward current (I_{to}) suppression may affect excitation-contraction coupling in mammalian cardiac myocytes. Prolonged depolarization increases Ca^{2+} influx mainly by voltage-dependent L-type Ca^{2+} channels, and decreases the driving force of Ca^{2+} extrusion via the sarcolemmal Na^+-Ca^{2+} exchanger (NCX). Both changes enhance Ca^{2+} availability for uptake by the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase, leading to an increase in SR Ca^{2+} content. Both increased Ca^{2+} influx and SR Ca^{2+} load synergistically increase the fractional SR Ca^{2+} release during systole, which causes greater myofilament activation and contraction. Increased Ca^{2+} influx can also permit a greater direct contribution to myofilament activation by Ca^{2+} originating from the extracellular medium.

In adults, enhanced cardiac workload due to increased hemodynamic load and/or decreased myocardial contractile function, such as in chronic ventricular hypertrophy induced by pressure overload, myocardial infarction and heart failure, are commonly associated with diminished total I_{to} or selectively $I_{to,f}$, and AP prolongation (7-9,35). These changes are accompanied by greater total Ca^{2+} influx during the long AP waveform, which may result in maintenance of Ca^{2+} transient amplitude, just as observed in immature and senescent ventricle. Interestingly, in these conditions the SR function is depressed, usually in association with SERCA down-regulation and diminished CICR sensitivity (1). One could thus interpret these findings from the viewpoint that I_{to} reduction may enable a presumably adaptive increase in Ca^{2+} influx during the long AP, so as to maintain Ca^{2+} cycling and contractile function compatible with survival.

Kassiri et al. (45) reported that I_{to} inhibition in cultured cardiac myocytes was effective in inducing myocyte hypertrophy by a Ca^{2+} influx-dependent mechanism. Expression and function of Kv4.2/4.3 channels are inhibited by signaling pathways normally activated during cardiac overload, possibly via phosphorylation by protein kinase C resulting from stimulation of angiotensin, α_1-

adrenergic and endothelin-1 receptors (11, 37,46). Additionally, mineralocorticoid receptor activation has been implicated in early channel down-regulation following myocardial infarction (35). Thus, it has been speculated whether hypertrophy induction by activation of these pathways would partially involve enhanced Ca^{2+} influx resulting from I_{to} depression (45). Greater cell Ca^{2+} cycling, in addition to contributing to the preservation of cardiac mechanical function, might also play a role in excitation-transcription coupling, for instance via Ca^{2+}-calmodulin-dependent kinases and phosphatases (calcineurin), in the development of ventricular hypertrophy and remodeling, which may eventually deteriorate to heart failure (1,36,45). However, although there is strong evidence of the implication of Ca^{2+}-dependent biochemical pathways in hypertrophy development (see, e.g., 1,36,38), $I_{to,f}$ reduction may not be the only way by which cell Ca^{2+} cycling can be increased. A few days after aortic banding (before hypertrophy development), Ca^{2+} cycling is enhanced by augmented SR function, without signs of increased Ca^{2+} influx (47), whereas a few weeks later the observed AP prolongation may be associated with an increase in $I_{Ca,L}$ density, rather than a decrease in I_{to} (36). Also, Bodi et al. (38) observed that I_{to} suppression, reversal of Kv4.2/4.3 *vs* Kv1.4 dominance and AP prolongation in transgenic mice overexpressing $I_{Ca,L}$ occurred only several months after hypertrophy development. Thus, I_{to} suppression may also be a consequence, rather than a cause, of greater Ca^{2+} cycling. For instance, enhancement of Ca^{2+} transient amplitude by SERCA overexpression has been shown to cause Kv4.2/4.3 and KChIP2 down-regulation, a decrease in I_{to} density and AP lengthening, even in the absence of hypertrophy or heart failure (48). In summary, there is no compelling evidence supporting the hypothesis that I_{to} suppression is necessarily involved in hypertrophy signaling. Part of the discrepancies among studies might be due to the multiplicity of experimental hypertrophy models and of the signaling pathways involved.

Although greater Ca^{2+} influx during a long AP may help prevent a dramatic depression of Ca^{2+} cycling in disease conditions, this compensation may be only partial in heart failure, because: a) a decrease in SR Ca^{2+} content in this condition (49) probably limits the amount of released Ca^{2+}, even though influx is increased, and b) the slow AP phase 1 to phase 2 transition may slow down the $I_{Ca,L}$ time course. To investigate the latter aspect, Sah et al. (50) employed stimulating AP waveforms of similar total duration, but with different rates of phase 1 repolarization. They observed that when early repolarization is slowed, $I_{Ca,L}$ shows a decreased peak and slower kinetics, in spite of similar or greater total Ca^{2+} influx compared to that with fast early repolarization. Slow-developing $I_{Ca,L}$ results in asynchronous SR Ca^{2+} release, which is less efficient to rapidly increase $[Ca^{2+}]_i$ at systole (50,51). This alteration may become more accentuated, with progression to heart failure, when intrinsic reduction of $I_{Ca,L}$ amplitude may occur (51). One of the expected consequences would be slower and weaker contraction activation. Thus, an increase in Ca^{2+} influx due to I_{to} suppression and AP prolongation may provide a partial adaptation that is eventually offset by decreased ECC efficiency with maintenance and worsening of the disease condition.

Thus, the kinetic aspect involves an additional, subtler aspect of $I_{Ca,L}$ modulation by I_{to}, i.e., the timing and rate of early repolarization. Linz and Meyer (52) showed that, during the respective AP waveforms, $I_{Ca,L}$ is briefer and attains greater amplitude in rat (strong I_{to}) than in guinea pig myocyctes (where I_{to} and AP phase 1 are absent), even though the plateau is much shorter in the former. This difference was attributed to the ability of the large rodent I_{to} to rapidly drive E_m to a range at which the driving force for

$I_{Ca,L}$ is greater, before the channels rapidly inactivate and deactivate, causing $I_{Ca,L}$ to assume a quasi impulse-like waveform. Although total Ca^{2+} influx is lower, it develops much faster in the rat than in the guinea pig, which is consistent with an optimal triggering signal for SR Ca^{2+} release. On the other hand, comparing epicardial *vs* endocardial canine ventricular myocytes (both of which present much longer APs than observed in rodents), the greater I_{to} density in the former causes the AP to assume the spike-and-dome configuration, which apparently favors Ca^{2+} channel reopening during the plateau (see secondary $I_{Ca,L}$ peak in Figure 1B), resulting in greater Ca^{2+} influx (53). Thus, it appears that the fine-tuning of Ca^{2+} influx by the modulation of the AP waveform by I_{to} strongly depends on the type, density and behavior of the ion current profile present in a specific cell type and/or animal species.

An important side effect of I_{to} down-regulation and AP prolongation present in disease states may be the greater vulnerability to arrhythmias. In addition to reentry-predisposing changes in refractoriness dispersion (16), triggered activity is often associated with increased AP duration (1). Prolonged depolarization may result in diminished Ca^{2+} efflux via NCX (due to a decreased driving force), which, in combination with greater Ca^{2+} influx, leads to a greater cell and SR Ca^{2+} load. Although this might cause a further increase in Ca^{2+} transient and contraction amplitude because SR Ca^{2+} content greatly influences the fractional SR Ca^{2+} release (22,23), it may also facilitate arrhythmogenesis. SR Ca^{2+} overload is often accompanied by enhanced diastolic SR Ca^{2+} release (1,54) that results in the generation of a depolarizing, inward membrane current by electrogenic efflux of the leaked Ca^{2+} via NCX (stoichiometry of 1 Ca^{2+}:3 Na^+) (24). If of sufficient magnitude, this current can drive E_m to the excitation threshold and give rise to triggered arrhythmias. In the particular case of heart failure, arrhythmogenesis by this mechanism would be additionally favored by NCX up-regulation and E_m instability due to decreased I_{K1} density (1).

Finally, it is also worthwhile to consider the relationship between AP duration and cycle length. This relation seems to stem from multifactorial mechanisms: I_{to} may be one of the underlying mechanisms in some species, in addition to modulation of $I_{Ca,L}$ and other Ca^{2+}-dependent currents by SR-released Ca^{2+}, and a rate-dependent increase in density of delayed-rectifier K^+ currents (e.g., 25,55). In most large mammals, including man, which present marked Kv4.2/4.3-dependent I_{to}, AP duration is decreased or little affected by increasing rate, especially in epicardial cells (16,56,57). This response might be important to allow adequate relaxation and ventricular filling during the shortened diastole. However, in rabbit and hamster myocardium, in which total I_{to} density is lower and $I_{to,s}$ is the dominant component, the AP is lengthened with increasing rate (12,56,58). This might be due, at least in part, to the slow $I_{to,s}$ recovery from inactivation, which would decrease channel availability at short intervals (2,55,56). A positive relationship between AP duration and rate may limit ventricular function and predispose the heart to triggered arrhythmias at high rates. As pointed out earlier, $I_{to,f}$ down-regulation has been described in canine and human hypertrophied and failing ventricle. However, this event *per se* does not seem to affect the AP duration-rate relation, since the AP is prolonged at long, but not short cycle lengths (16,25). Recent results from computer simulations predicted that the positive rate-AP duration relationship relies on the presence of $I_{to,s}$, rather than on a decrease in $I_{to,f}$, since a negative relation is still present if I_{to} is totally suppressed (12). Interestingly, our prediction is in agreement with experimental data that show a negative AP duration-rate curve in the guinea pig ventricle (in which I_{to} is absent (55,59)) which is not changed by chronic ventricular hypertrophy induced by pressure overload (59). The finding that diabetic cardiomyopathy is associated with a frankly positive AP duration-rate relationship in the rat is intriguing, in contrast with the weak rate dependence observed in controls (60). However, information on $I_{to,s}$ or Kv1.4 expression in this condition is lacking, and thus a possible link between $I_{to,s}$ dominance and positive AP duration-rate still requires experimental confirmation.

Changes in I_{to} density and profile caused by pathological cardiovascular conditions may be overall adaptive, as they contribute to increase Ca^{2+} influx and may confer some protection against reentry due to prolonged refractoriness, although excessive Ca^{2+} loading, in conjunction with a decrease in membrane electrical stability, may favor the appearance of triggered arrhythmias. However, the fact that these changes are superimposed on the naturally occurring regional heterogeneity of this current (and its components) in the adult ventricle makes it difficult to predict their net effect on vulnerability to arrhythmia. This also complicates the development of pharmacological and gene therapy strategies targeted to I_{to} channels. Hopefully, this might be overcome with novel information to be gathered in the coming years.

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