ELECTRO-PHARMACO-PATHOPHYSIOLOGY OF CARDIAC ION CHANNELS AND IMPACT ON THE ELECTROCARDIOGRAM

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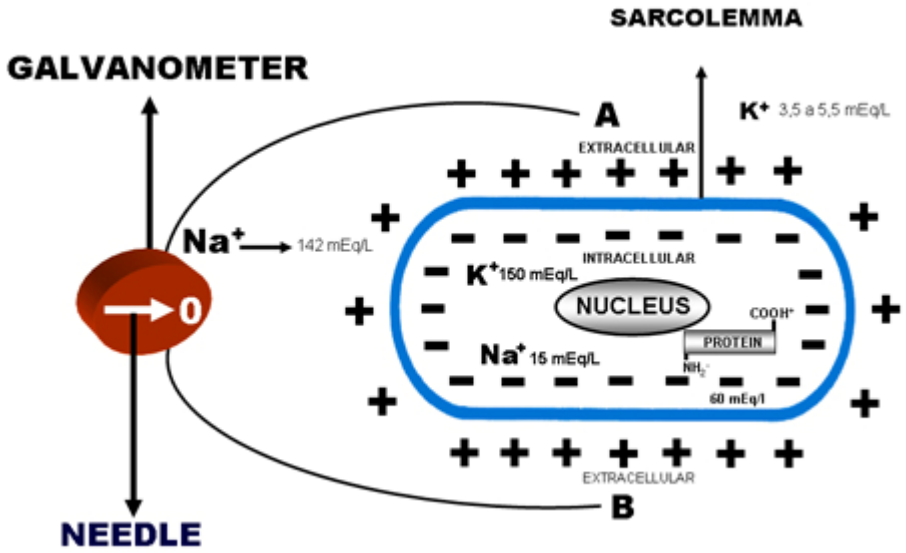
RESTING POTENTIAL, ELECTROLYTIC CONCENTRATION, FAST AND SLOW FIBERS, ACTION POTENTIAL PHASES, SARCOLEMMAL AND INTRACELLULAR CHANNELS

Introduction

If we place both electrodes or wires (A and B) from a galvanometer (***a device that records the difference in electric potential between two points***) in the exterior (extracellular milieu) of a cardiac cell in rest or polarized, we would see that the needle of the device does not move (it indicates zero), because both electrodes are sensing the same milieu (extracellular). That is to say, there is no difference in potential between both ends of the galvanometer electrodes: Figure 1.

FIGURE 1

ILLUSTRATION OF TRANSMEMBRANE RESTING OR DIASTOLIC POTENTIAL IN A CARDIAC CELL AND MEASUREMENT WITH GALVANOMETER



In rest, the extracellular milieu is predominantly positive in comparison to the intracellular one, as a consequence of positive charge (cations) predominance in the first in comparison to the intracellular one. What is the reason for the extracellular milieu to be predominantly positive in comparison to the intracellular? Reply: The reason lies in the greater concentration of proteins existing in the intracellular milieu in comparison to the extracellular one. Proteins have a double charge (positive or negative), and for this reason they are called amphoteric (***amphoteric is any substance that can behave either as an acid or as a base, depending on the reactive agent present. If in the presence of an acid, it behaves as a base; if in the presence of a base, it behaves as an acid***); therefore, in the intracellular pH negative charges are predominantly dissociated; i.e. proteins behave as anions (-) in the intracellular milieu.

Table 1 shows the normal concentrations of cations and anions in the extra and intracellular milieus. In this table, it is seen that the predominant cation within the cell is K^+ and in the extracellular milieu, Na^+.

TABLE 1

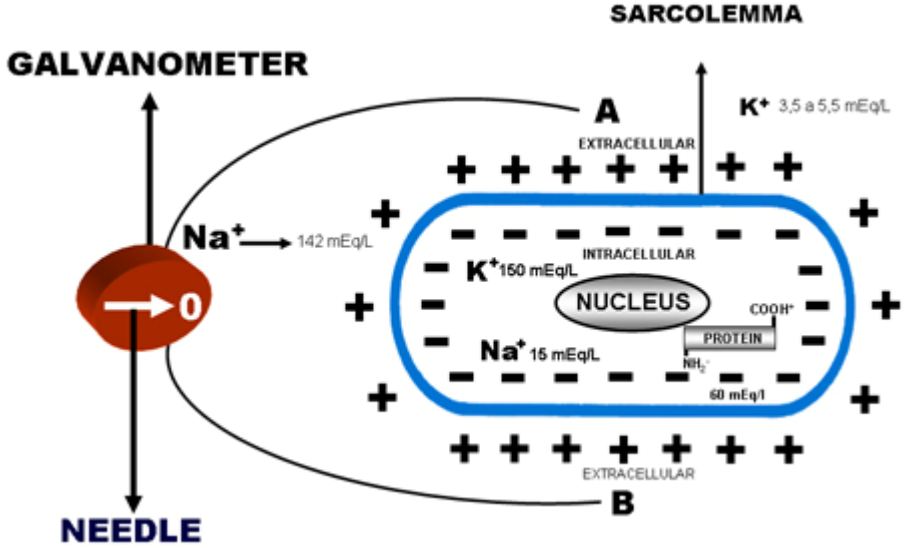
NORMAL ELECTROLYTIC CONCENTRATION IN THE EXTRA AND INTRACELLULAR MILIEUS

ELECTROLYTE	EXTRACELULLAR	INTRACELULLAR	Extracelullar/ intracellular E/I ratio
Na^+	135 to 145 mEq/L	10 mEq/L	14:1
K^+	3.5 to 5.5 mEq/L	155 mEq/l	1: 30
Ca^{2+}	2 mEq/L	10^{-4} ***Although intracellular Ca^{2+} concentration is 2 mM, most is attached or isolated in the mitochondria and the SR.***	$2 \times 10^{-4} = 1.$
Mg^{2+}	2 mEq/L	15 mEq/L	0.1333
Cl^-	95 to 110 mEq/L	20 to 30 mEq/L	4:1
$CO3H^+$	27 mEq/L	8 mEq/L	3.3
Proteins	2 mEq/L	90 mEq/l	0.022

If we place the ends of both electrodes (A and B) in the intracellular milieu, the galvanometer needle will remain in zero (Figure 2), because both electrodes are in the same milieu. Figure 2.

FIGURE 2

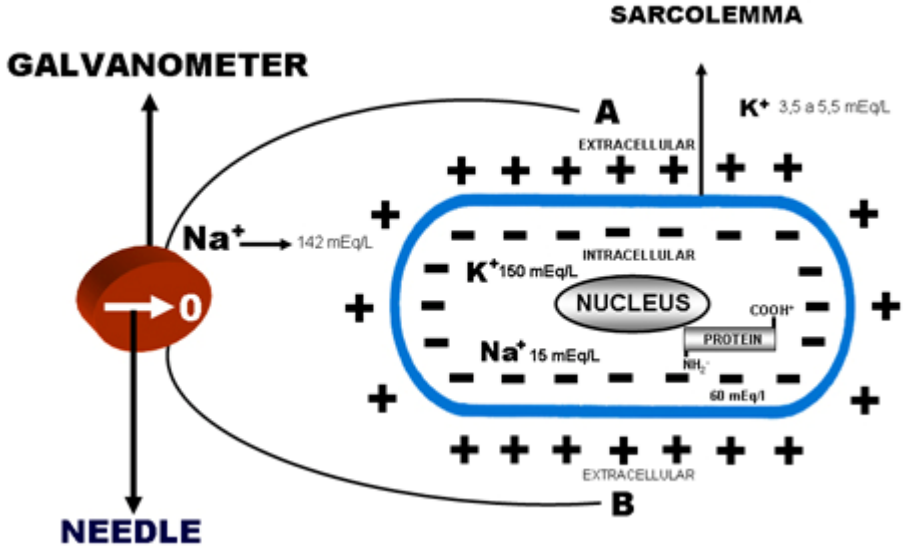
ILLUSTRATION OF TRANSMEMBRANE RESTING POTENTIAL IN CARDIAC CELLS AND MEASUREMENT WITH GALVANOMETER



Finally, if one of the ends is placed within the cell (wire B) and the other is in the extracellular milieu (wire A), we observe that the needle moves, indicating the difference in potentials between the extracellular (+) and intracellular (-) milieu, with a value of ≈ – 90 mV, indicating the existence of a difference in potentials of -90 mV. Figure 3.

FIGURE 3

ILLUSTRATION OF TRANSMEMBRANE DIASTOLIC OR RESTING POTENTIAL IN CARDIAC CELLS AND MEASUREMENT WITH GALVANOMETER



This value of -90 mV, represents the difference in **D**iastolic **T**ransmembrante **P**otential (**DTP**) or resting potential; i.e. the difference in potential existing during diastole between the extracellular milieu with predominantly positive charges (+) and the intracellular milieu with predominantly negative charges (-).

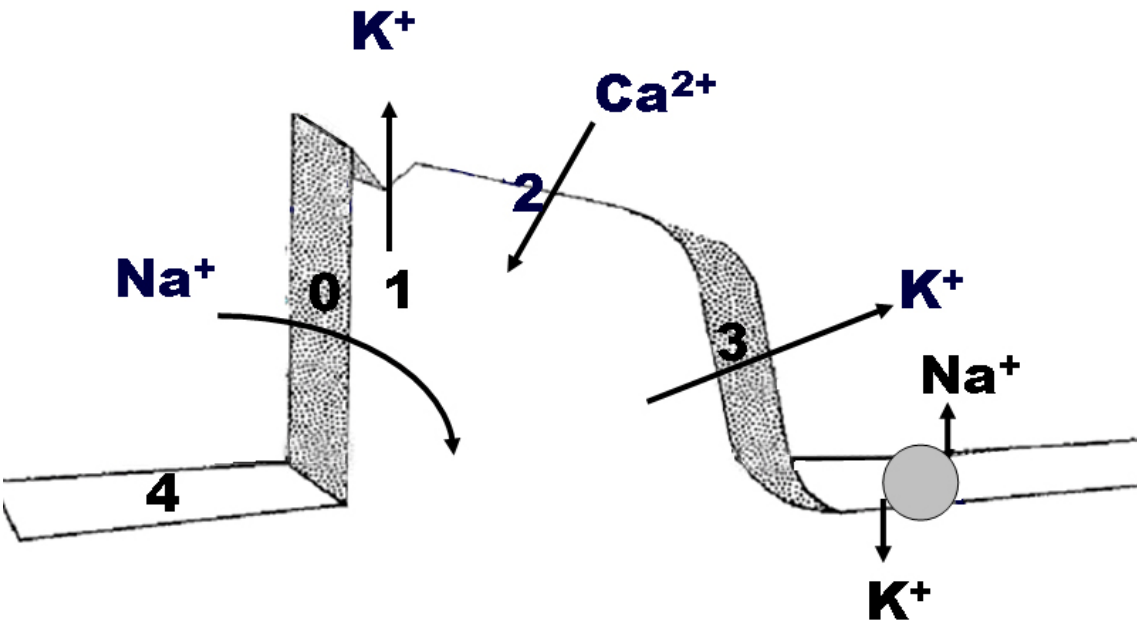
Considering the profile image of the action potential (AP) of cardiac cells or cardiac fibers are grouped into rapid and slow:

I) Rapid cells:

Present in automatic Purkinje cells and nonautomatic cells of the ventricular and atrial contractile myocardium ("**ordinary working muscle cells**"). In Figure 4, the characteristics of the AP profile of rapid fibers, and their main responsible ionic channels are outlined.

FIGURE 4

PROFILE OF RAPID FIBER AP AND MAIN ION CHANNELS



- Rapid inward Na^+ (in phase 0).
- Early inward K^+ by the I_{to} channel in phase 1.
- Slow inward Ca^{2+} in phase 2.
- End delayed inward K^+ in phase 3, made up by two or three slow delayed outward K+ channels: slow (I_{ks}), rapid (I_{kr}) and ultra-rapid (I_{kur}). The latter originates in the hKv1.5 channel (**1**). Kv1.5 channels conduct the delayed

ultra-rapid current of the rectifyier K^+ channel, I_{kur}. In human beings, Kv1.5 channels are highly expressed (are present) in the atria, but are scant (or absent) in the ventricles (**2**).

- Finally, the Na^+/K^+ ATP_{ase} pump acts in phase 4. This pump, with the energy consumption, places three Na^+ cations in the extracellular milieu, and introduces a K^+ ion. The pump is inhibited by digitalis (**3**).
- Pacemaker current, I_f or "**funny**" current: it operates exclusively in the initial portion of phase 4 (it only acts in a potential range from -60/-70 mV to -40 mV) and contributes 20% to determine the heart rate (HR) of P cells of the SA node (**4**), controlling diastolic depolarization and spontaneous activity of P, pacemaker cells. The molecular determinants of the I_f channel, belong to a family of channels activated in hyperpolarization known as HCN channels, made up by 4 isoforms (HCN1, HCN2, HCN3, HCN4), with HCN2 (chromosome 19p13.3) and HCN4 being the main ones in the heart (**Hyperpolarization-activated Cyclic Nucleotide-gated channels family (HCN)**). Based on the sequence of HCN channels, these are classified as members of a superfamily of **voltage-gated K^+ (Kv)** and CNG channels (**5;6**). A research showed that inhibiting the I_f current could be used to decrease the incidence of coronary artery disease (CAD) in a subset of patients with HR≥70 bpm (**7;8**). The mutations in HCN4 (chromosome 15q24-125.3) and CNBD (S672R) isoforms are associated to familial inherited bradycardia, as they cause an effect similar to parasympathetic stimulus, by reducing I_f channel activity (**9**). There are micro-domains of the membrane, rich in cholesterol and sphingolipids in cardiomyocytes, called caveolae. In caveolin-3 (CAV3), several channels have been located, such as L-type Ca^{2+}, I_{Na+} (Na(v)1.5), the I_f pacemaker channel (HCN4) or the Na^+/Ca^{2+} exchanger (NCX1) and others. Mutations in CAV3 may originate variant 9 of congenital Long QT Syndrome (LQT9) and other inherited arrhythmias. In acquired diseases that lead to congestive heart failure, CAV3 may be affected, originating arrhythmias (**10**). The fast Ca^{2+} T type current or T-type Ca^{2+} channel, transient I_{Ca-T} current or tiny conductance channel, voltage-dependent T-type calcium channel, and low-voltage-activated (T-type) calcium channels are

responsible for the entrance of Ca^{2+} in the final part of phase 4 in the SA Node, in the N region of the AV node and in the His-Purkinje System. The rapid type Ca^{2+} channel is blocked in a selective way by the Ca^{2+} antagonist mibefradil (**mibefradil-sensitive component**), and other drugs such as bepridil, flunarizine, and pimozide, which bind to the receptor channel in a concentration-dependent fashion, thus blocking the Ca^{2+} cation entrance. Mibefradil decreases HR, not affecting contractility (**11**). The great efficacy of bepridil to end with atrial fibrillation or flutter, is due in part to the block of this rapid type Ca^{2+} channel (**12**). ICa-T is not sensitive to dihydropyridine. ICa-T has its function increased with noradrenaline, the α adrenergic agonist phenylephrine, the ratio of extracellular ATP and endothelin-1 (ET-1).

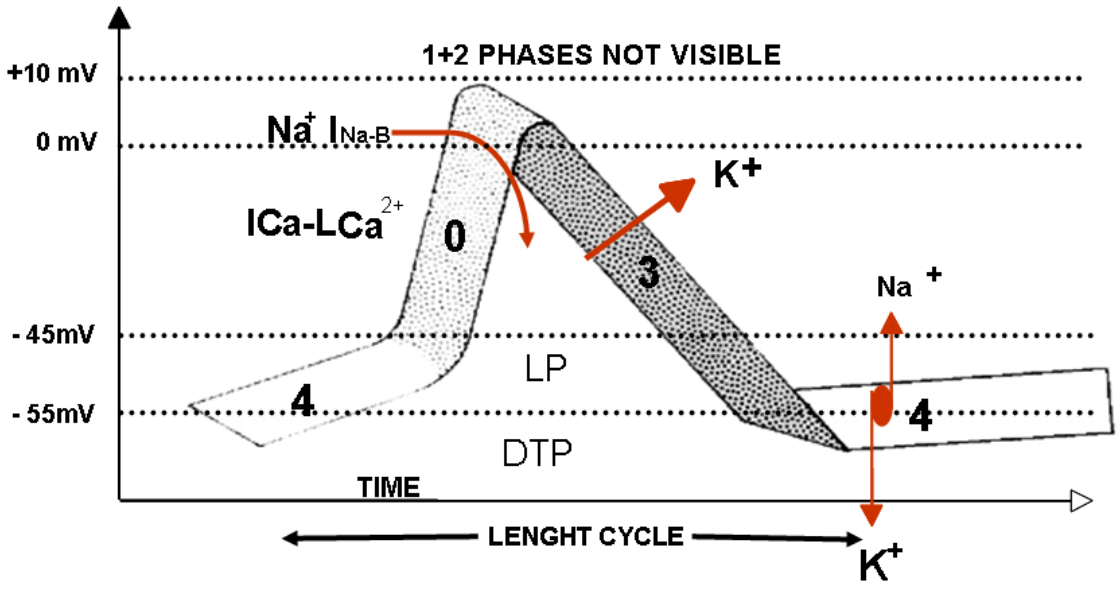
II) Slow cells:

Located within the limits of the SA Node of Keith and Flack, atrioventricular node, and in the mitro-tricuspid rings. They are characterized by presenting a not so negative resting potential (≈ -55 mV), a not so wide, slow Ca^{2+}-dependent phase 0, and with additional final entrance of Na^{+} through a channel independent from voltage, called $I_{Na}{}^{+}B$, and absence of identifiable phases 2 and 3.

Figure 5 shows the typical outline of a slow fiber, with the main operating channels.

FIGURE 5

PROFILE OF AP OF SLOW FIBER AND MAIN ION CHANNELS



The main differences between rapid and slow fibers are summarized in Table 2. Rapid fibers are Purkinje cells and muscle cells from the atria and ventricles, and slow fibers are constituted by P cells from the SA Node, nodal cells, atrioventricular or AV Node cells, and mitro-tricuspid rings.

TABLE 2

MAIN DIFFERENCES BETWEEN RAPID AND SLOW FIBERS

	RAPID FIBERS	**SLOW FIBERS**
Location:	Atrial and ventricular muscle, inter-nodal preferential pathways (of Bachmann, Wenckebach, and Thorel), His-Purkinje System (HPS).	SA node, AV node, and mitro-tricuspid rings.
Kinetics:	Rapid: activation and inactivation <1 ms.	<5 ms and inactivation between 3 - 80 ms.
Diastolic resting	-90 to -80 Mv.	-60 mV

potential:		
Activation or potential threshold	-70 to -55 mV.	-55 to -30 mV.
AP amplitude in mV:	100 to 130 mV.	At 35 to 70 mV.
Activation in ms:	<1 ms.	<5 ms.
Inactivation in ms:	<1 ms	3-80 ms.
Phase 0 blockers:	Tetrodotoxin (TTX) Anti-arrhythmic agents class I: IA, IB and IC.	Ca^{2+} antagonists, some divalent cations such as cadmium (Cd), manganese (Mn), cobalt (Co) and nickel (Ni).
Type of response to stimulus:	All-or-nothing type.	Dependent on the intensity of the stimulus applied.
Dromotropism:	0.3 to 3 or 5 M/s (Meters per second)	0.01 to 0.10 M/s.
Overshoot:	+20 mV.	It could be absent or up to +15 mV.
SA Node:	Absent.	Present.
Atrial	Present.	Absent.

myocardium		
Preferential interatrial pathways:	Present.	Absent.
AV node:	Absent.	Present.
His Purkinje system:	Present.	Absent.
Influence by β-adrenergic agents:	Null.	Significant.
Influence by cholinergic muscarinic agents:	Null.	It decreases in the atria and ventricles.

In the AP of rapid fibers, we can differentiate five successive, well defined phases, called 0, 1, 2, 3, and 4.

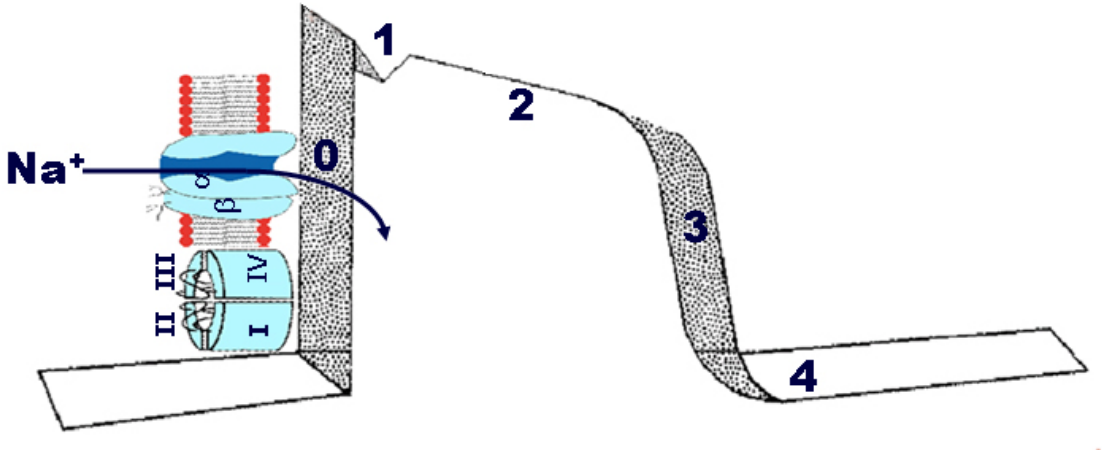
PHASE 0 (ZERO), RAPID OR ASCENDING DEPOLARIZATION

It corresponds to the rapid entrance of the cation sodium (Na^+). This phase is concomitant in surface ECG for the atria, to atrial depolarization (P wave) and for the ventricles to ventricular depolarization (QRS complex).

Figure 6 shows entrance of the Na^+ cation during phase 0 in a rapid fiber. When the channel opens, Na+ comes suddenly into the intracellular milieu, when it reaches the so-called threshold potential (TP), reversing the potential of the cell. Thus, phase 0 amplitude extends from ≈ -90 mV to +30 mV (120 mV).

FIGURE 6

IMAGE OF INWARD Na^+ IN PHASE 0 IN RAPID CELL AND AP REVERSION



In rapid fibers, trans-membrane diastolic resting potential is between ≈ -90 and -80 mV and activation or threshold potential (TP) between -70 mV and -55 mV.

Phase 0 in rapid fibers is very wide and fast, since it extends ≈ from -90 mV to +30 mV (median amplitude of 120 mV) and with activation and inactivation time <1 ms.

The greater the amplitude of phase 0, the greater the conduction velocity or fiber dromotropism (directly proportional).

The portion of phase 0 that extends from the resting potential up to the threshold potential (TP) is called the "base" of phase 0, and it occurs slowly; the portion that extends from the TP up to potential 0, occurs at a greater velocity of inward Na^+, being known as V_{max}. Finally, the portion of phase 0 that extends from potential 0 up to the reversion apex (≈ +30 mV) is called "overshoot" or Livaud's crista.

The response of the rapid fiber is of the all-or-nothing type, which means that when the stimulus reaches the TP, a sudden opening of the Na^+ channel occurs, with rapid cation inflow, "summoned" from inside the cells by a double electrical and osmotic gradient:

1) **Electrical:** because being positive (cation), it seeks the opposite (negative) intracellular milieu.

2) **Osmotic:** because there is a greater concentration of Na^+ in the extracellular milieu (142 mEq/L) than in the intracellular one (10 mEq/L): extracellular/intracellular ratio = 14:1 (**13**).

Rapid phase 0 can be blocked by class I antiarrhythmic agents (IA, IB, and IC) and by a toxin called Tetrodotoxin or TTX (anhydrotetrodotoxin 4-epitetrodotoxin, tetrodonic acid), found in several species of fish such as pufferfish, porcupine fish, ocean sunfish, and triggerfish. This toxin blocks the rapid Na^+ channels of contractile cells of cardiomyocytes (ordinary working muscle cells), by inhibiting contraction. Thus, people poisoned with TTX may die by cardiac muscle paralysis without affecting slow fiber AP. This mechanism was discovered by the Japanese researcher Toshio Narahashi, working at the Duke University in the early 60s. Currently, TTX is produced by certain bacteria, such as the pseudomonas tetraodonis and others.

Class I antiarrhythmic agents block Na^+ channels of rapid channels (**14**).

Class I antiarrhythmic agents have been divided in three categories, depending on the affinity of the drug with the Na+ channel by Vaughan-Williams, later modified by Harrison (**15;16**).

Class IA: they have intermediary binding and release kinetics with the Na^+ channel. They moderately reduce V_{max} and extend AP. The main representatives are: quinidine, procainamide, disopyramide, and ajmaline. Additionally, they have a significant anticholinergic effect. Class IA drugs that block both the rapid Na^+ channel and the I_{to} channel, such as quinidine and disopyramide, may normalize J point and ST segment elevation in right precordial leads in Brugada syndrome. On the contrary, those drugs of the same class IA, such as ajmaline and procainamide, that act exclusively on the rapid Na^+ channel without affecting the I_{to} channel, increase J point and ST segment elevation and may trigger fatal tachyarrhythmias in Brugada syndrome (**17**). On the other hand, quinidine is very efficient to prevent induction of ventricular fibrillation, sustained during the electrophysiologic study in patients with idiopathic ventricular fibrillation and Brugada syndrome. This efficacy is maintained in the long term; consequently, the therapy with quinidine guided by the electrophysiologic study represents a valuable alternative to cardioverter defibrillator in this population (**18**).

Class IB: they have rapid binding and release kinetics with the Na^+ channel, so they do not affect QRS duration and JT interval (from the J point up to the end of T wave). They mildly reduce V_{max}. They do not modify or shorten AP. The representatives are: mexiletine, tocainide, lidocaine; widely used for the acute management of ventricular tachyarrhythmias (**19**).

Class IC: they have slow binding kinetics with the Na^+ channel. They significantly reduce V_{max}, and consequently, conduction velocity: more intense negative dromotropic effect and no effect or decrease in AP duration (in the latter aspect, completely different from class IA). As a consequence of this slow kinetics, QRS complex and JT interval duration increase. They may extend refractoriness minimally. The representatives are propafenone, flecainide, encainide, moricizine, and lorcainide. Propafenone is the only one in this group with additional β_2 blocking effect, which offsets tachycardia by IC effect.

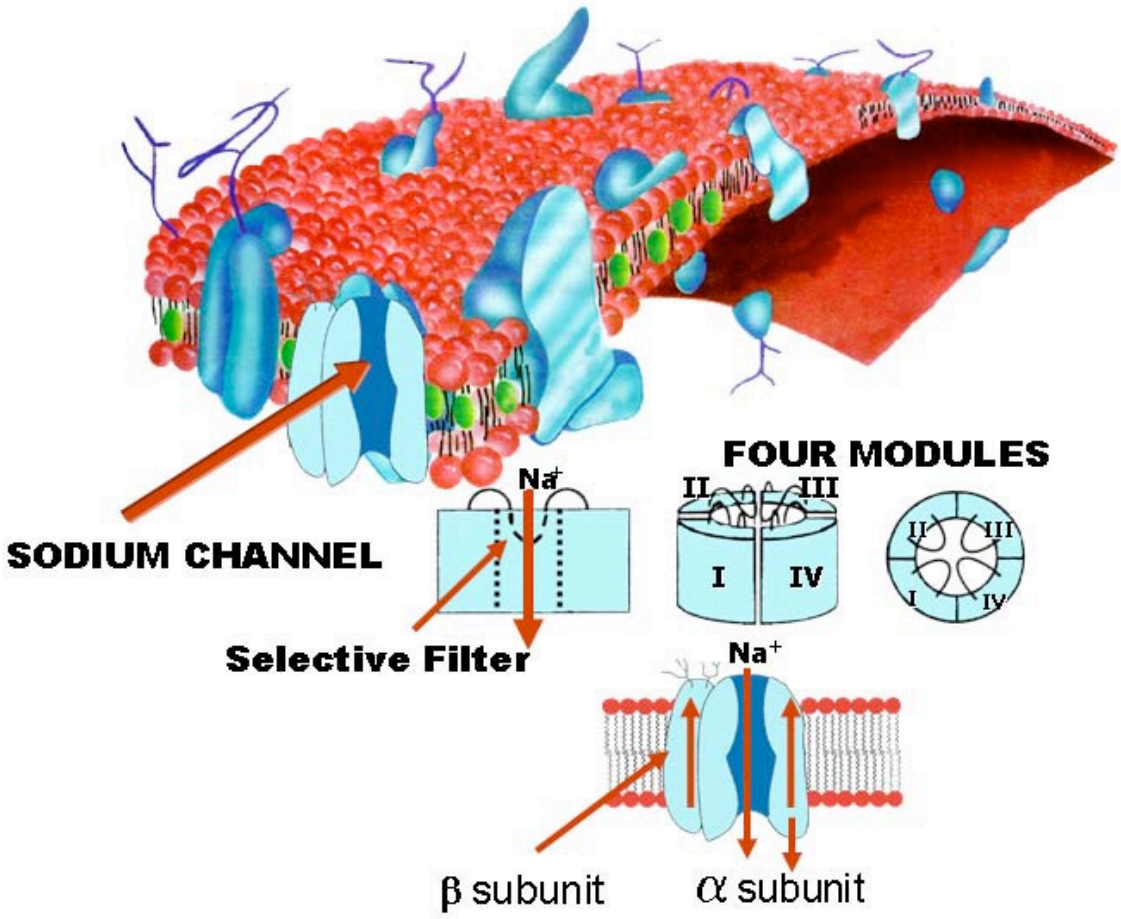
The sequence of figures 7, 8, 9, and 10 show the main characteristics of the Na^+ channel in phase 0 in the sarcolemma of cardiomyocytes and the structure of the Na^+ channel with the α and β subunits.

CONCEPTS ON THE STRUCTURE OF Na^+ CHANNEL

The Na^+ channel is a protein structure, made up by four modules that surround a central pore. It has a main unit, called α subunit and another two ancilliary surrounding ones, $\beta 1$ and $\beta 2$. The Na^+ channel determines the conduction velocity of the stimulus by the amplitude of its phase 0. Figure 7 shows the tetramodular structure of the α subunit of the Na^+ channel (**20;21**).

FIGURE 7

Na^+ CHANNEL STRUCTURE WITH ITS FOUR MODULES



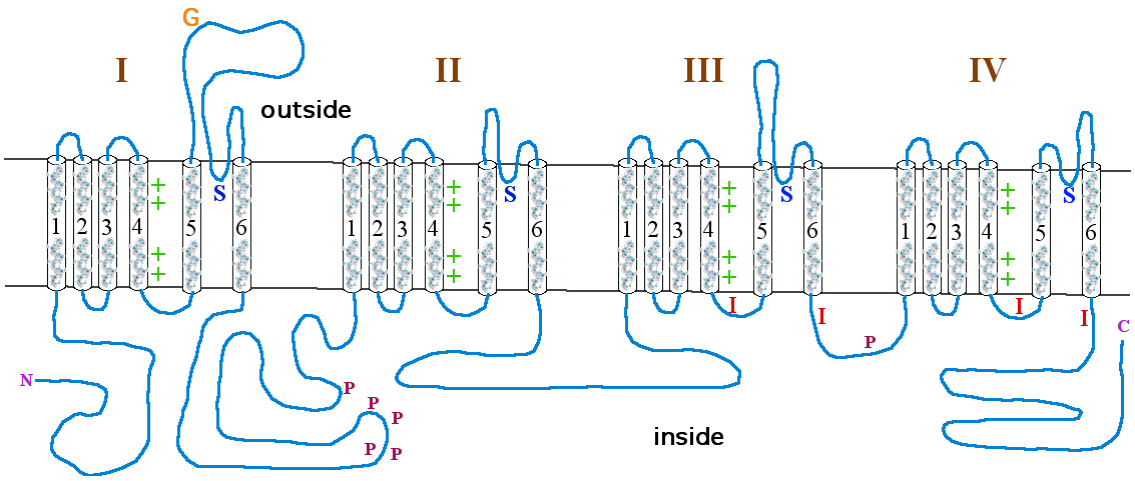
1) Main α subunit: made up by four modules or domains (I, II, III, and IV), arranged in a circle surrounding a central pore, determining conductance, impedance, and translocation properties of the Na^+ cation. Each of these domains contain 6 membrane regions called S1 through S6. Region S4 acts as a voltage sensor. The region between S5 and S6 in the IV domain may block the pore of the channel making up a loop called P loop, which is the most external one. This is the narrowest region of the pore, and it is responsible for its ion selectivity. The internal portion of the pore is made up by a combination of the S5 and S6 regions of the 4 domains. The region between domains III and IV connects the channel after prolonged activation and inactivation periods.

The main α subunit is affected by class I antiarrhythmic agents.

Figure 8 shows a diagram of the α subunit of the Na^+, voltage-sensitive channel, indicating the points of glycosylation, fosforilation, ion selectivity, and voltage sensors of positive loads in region S4.

FIGURE 8

OUTLINE OF α SUBUNIT OF VOLTAGE-SENSITIVE Na^+ CHANNEL



In SA node cells, phase 0 depends on slow inward Ca^{2+}; however, in the final portion of phase 0, a voltage-independent Na^+ channel, activates. The channel is called $I_{Na}B$.

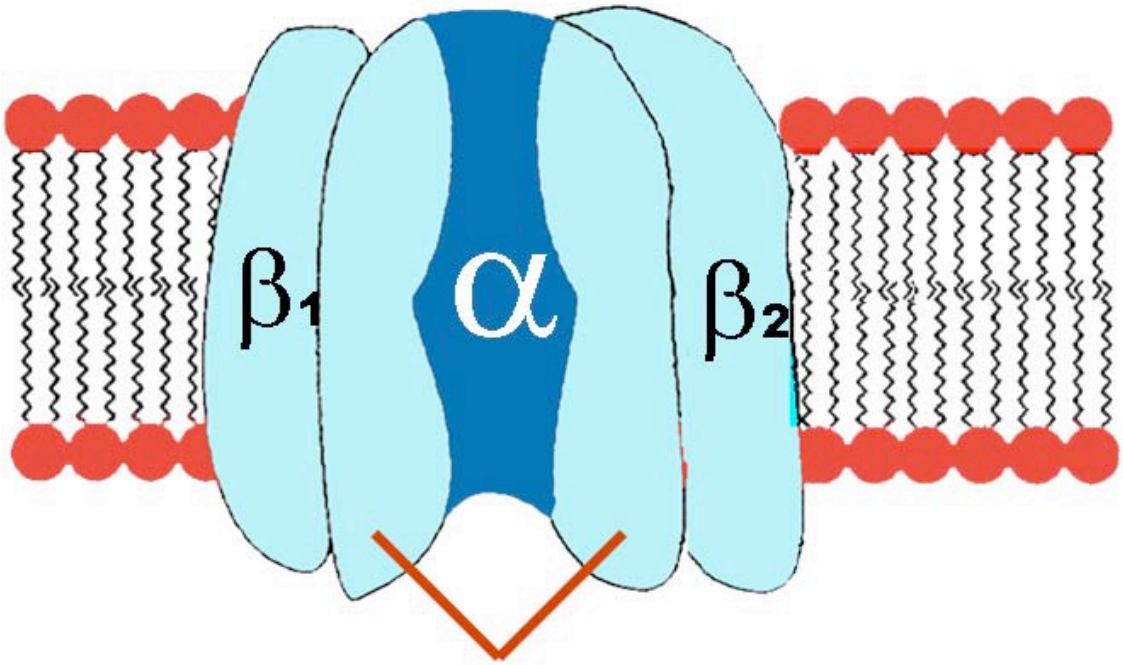
2) Ancilliary β1 subunit.

3) Ancillliary β2 subunit.

Figure 9 shows Na+ channel subunits: α is the main one, β1 and β2 are ancillliary.

FIGURE 9

IMAGE OF α, β1, AND β2 SUBUNITS OF THE Na^+ CHANNEL IN THE SARCOLEMMA



The Na^+ channel has three functional states, with two of them being the main ones: open (it allows for Na^+ passage) and closed (it prevents Na^+ passage), besides a state called inactive.

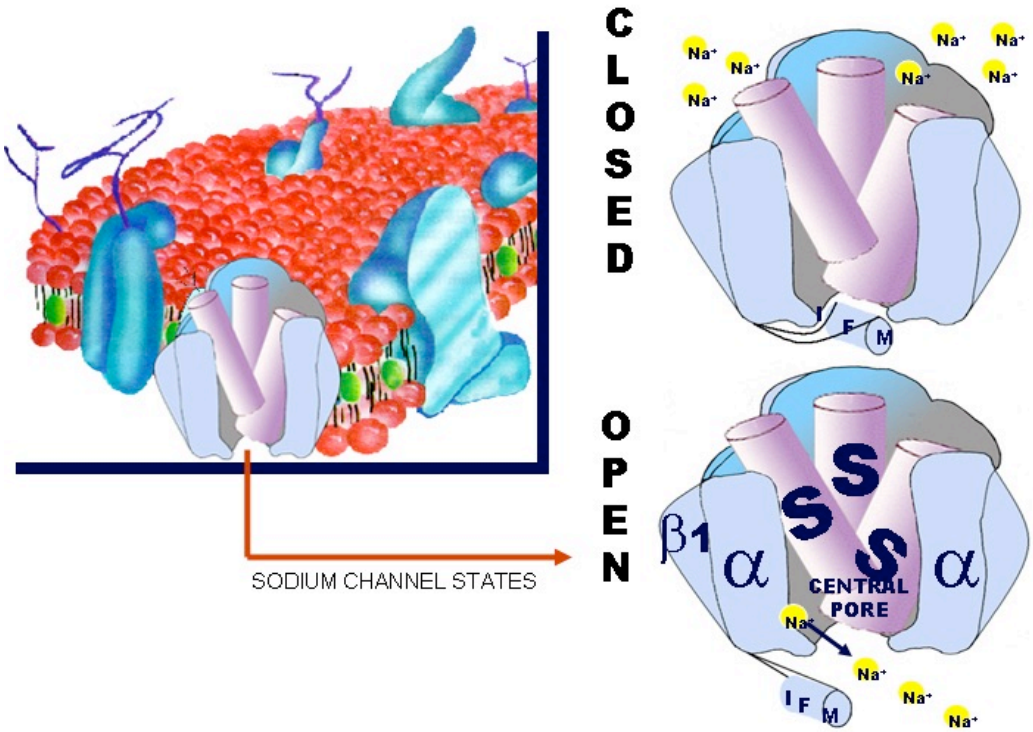
1) Open state: it allows for Na^+ passage.

2) Closed or resting state: during this resting functional state, the channel prevents the passage of Na^+, because a critical residue (Phe1489F) closes the intracellular mouth of the pore of the channel. This state corresponds to the transmembrane diastolic potential in rest, which in the rapid fiber is found in a value close to -90 mV. In this state, the so-called "gate m" is closed, and the "gate h" is open. The Na^+ channel is excitable even being closed. Na+ does not come inside rapidly until the TP is reached; i.e. from the resting potential up to the TP, the cation inflow is slow (base of phase 0).

Figure 10 shows the outline of the channel in its two main functional states: open and closed.

FIGURE 10

OUTLINE SHOWING THE Na^+ CHANNEL IN ITS TWO MAIN FUNCTIONAL STATES: OPEN AND CLOSED



3) Inactivated state: The channel is closed and not excitable; consequently, from this state, the channel cannot be opened. Voltage-dependent gate "h" closes when the cell starts to become positive internally. Na+ channel inactivation occurs exclusively through the open state.

Next, the 4 phases of **repolarization**, occur in succession: 1, 2, 3, and 4.

I) **PHASE 1: EARLY REPOLARIZATION**

II) **PHASE 2: PLATEAU OR DOME**

III) **PHASE 3: RAPID FINAL REPOLARIZATION**

IV) **PHASE 4: DIASTOLIC DEPOLARIZATION.**

PHASE 1 OR EARLY REPOLARIZATION PHASE

This phase, even being polyionic, occurs mainly by the early transient outflow of the K+ cation, or transient outward K^+ current, by a channel known as I_{to}, I_{to1}, $I_{to-fast}$, I_{to-f}, or I_{toA}. This channel is voltage-dependent (i.e. controlled by voltage), and its activation occurs in a range from -10 mV and +30 mV. It has a rapid activation and inactivation kinetics, and is blocked among others (sensitive to) 4-aminopyridine, and manifests at the end of ventricular depolarization, and the

onset of ventricular repolarization, which corresponds in surface ECG to the J point (from the word Junction), located between the end of the QRS complex and the onset of the ST segment.

CHANNELS OPERATING IN PHASE 1 AND SUBTYPES OF I_{to} CHANNEL

During phase 1, several operating channels are described: two I_{to} channels, known as I_{to1} and I_{to2}, and others like the time-independent chloride current, regulated by the cAMP/adenylate cyclase pathway (I_{ClcAMP}).

1) I_{to1}, I_A, $I_{to\text{-}fast}$, $I_{to\text{-}f}$ channel, transient outward K^+ current, channel sensitive or blocked by 4-aminopyridine, quinidine, and flecainide, voltage-activated, and modulated by neurotransmitters. It activates and inactivates rapidly. Table 3 shows the main I_{to1} channel, the main channel of phase 1.

2) $\boldsymbol{I_{to2}}$, I_{Cl}^-, Ca^{2+} or Cl^- channel activated by Ca^{2+}, slow transient K^+ outflow, resistant to 4-AP, transported by Cl^- anions: modulated by the percentage of intracellular Ca^{2+}. It has slower activation and inactivation. Its ion basis could be conditioned predominantly by outward Cl^-, by the so-called I_{Cl}^- channel. The channel is increased by adrenergic stimulation. Its molecular correlate is the Kv1.4 pore-forming protein isoform (**22**).

Table 3 shows the characteristics of I_{to1} and I_{to2} currents.

TABLE 3

I_{to1} channel of AP of ventricular myocardium						
Cation		**α subunit protein**	**GENE α subunit of gene**	**Phase of AP**	**CLONE**	**MOLECULAR STRUCTURE AND ASSEMBLY**
K^+	**Transient rapid outflow I_{to1} current**	**pore-forming protein isoforms** Kv4.3/4.2**K_V 4.2 and Kv4.3**	**KCND2/ KCND3**	**PHASE 1, notch**	K_y4.2/4.3	Single pore. 6 transmembrane domains. Tetramer.

		Probable clone.				
K^+	**Transient slow outflow I_{to2} current**	**Pore-forming protein isoforms** Kv1.4.	**KCNA4 KCNA7 KCNC4**	**PHASE 1**		Single pore. 6 transmembrane domains. Tetramer.

3) $I_{Cl\ AMPc}$ or time-independent chloride (Cl^-) channel, cAMP-activated Cl^- current. The channel is activated by the increase of intracellular concentration of AMPc. It is involved in cell volume, blood volume regulation, and regulation of osmolarity and type 1 response to cell chemical stimuli (**23**). The channel depolarizes slightly the resting potential, and significantly shortens AP duration, and antagonizes AP prolongation mediated by β–stimulation.

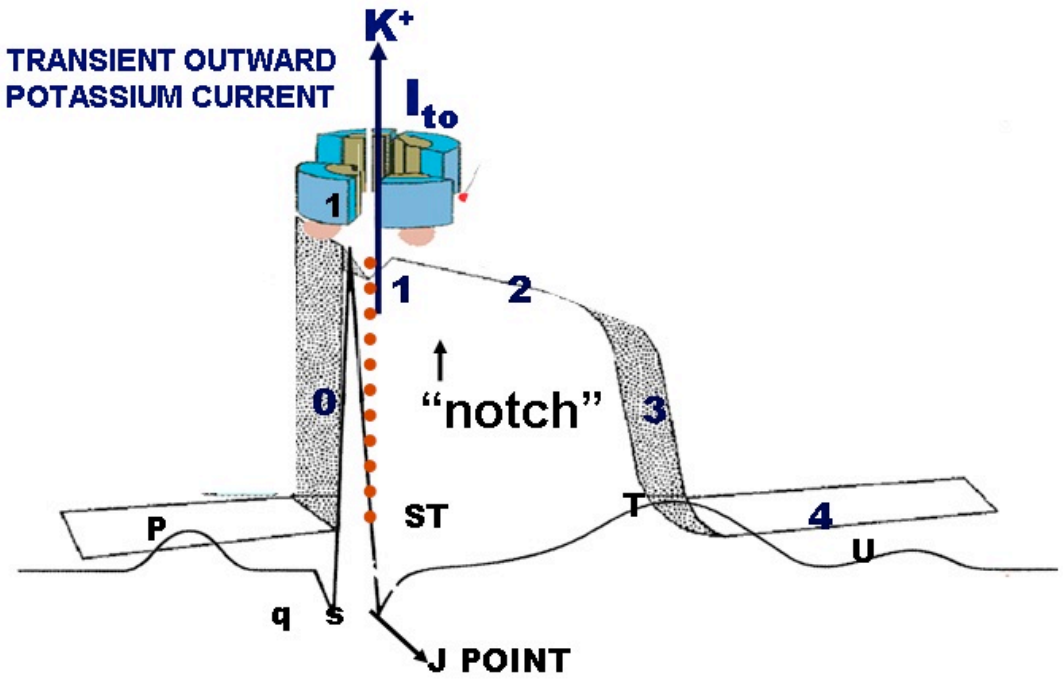
4) $I_{Cl\text{-}edema}$ or $I_{Cl^-\text{-}SWELL}$, swelling-activated chloride channel, swelling-activated, outward rectifying chloride channel. This channel belongs to the category of stretch-activated ion channels. It is inhibited by anthracene-9-carboxylic acid, tamoxifen, or natriuretic peptide precursor B (NPPB), and by diisothiocyanatostilbene-2.2'-disulphonic acid (DDSA) (**24**). It shortens AP and causes depolarization.

5) Inward Na^+ through Na^+/Ca^{2+} exchanger channel, operating in a reverse way.

Figure 11 shows the time of early transient outward K^+ and its correlation with surface ECG.

FIGURE 11

IMAGE OF EARLY K^+ OUTFLOW CHANNEL, I_{to1} IN PHASE 2 OF AP AND TIME CORRELATION WITH SURFACE ECG



In the initial transient or early phase of rapid repolarization (phase 1), inactivation of the I_{Na}^+ channel occurs (decline of inward Na^+ through Na^+/Ca^{2+} exchanger pump) and there is concomitant activation of several outward channels, mainly the I_{to1} and I_{to2} channel. The latter is known as chloride (Cl^-) channel, activated by 4-AP-resistant Ca^{2+} (I_{to2} or $I_{Cl}^- I_{Ca}^{2+}$).

The Cl^- channel also has an activation level close to -30 mV.

Ventricular wall thickness is made up by three functional layers, which have different AP profiles. In the ventricular epicardial and midmyocardium cells, the AP shows a significant notch, followed by an ascending slope known as spike-and-dome morphology, subsequent to the high I_{to} channel concentration in the epicardium and mesocardium.

In the depth of the middle layer, the so-called "M cells" can be found, which are a cell subpopulation with great conduction velocity and electrophysiologic features of their own, very significant in the pathophysiology of long and short QT syndromes, and probably in the genesis of U wave in ECG in these entities (**25**).

The I_{to} channel is in low concentrations or absent in the endocardial cells, a fact that explains the absence of notch in the AP of this region. This transmural

difference in the concentration of the I_{to} channel, is partly responsible for the transmural dispersion in repolarizaton, which conditions the Osborn wave of hypothermia (**26**), the J point and ST segment elevation in right precordial leads in Brugada syndrome, and other physiologic and pathologic situations in individuals with normal temperature (**27**). In brief, there is a concentration gradient for the I_{to1} channel in ventricular wall thickness (**28**).

The I_{to} channel is also present in atrial cells.

In Brugada syndrome, a channelopathy predominantly observed in the male gender (**29**) and in young adults (**30**), the I_{to} channel concentration is greater than in normal people in the epicardium of the right ventricular outflow tract, which leads to an increase in refractory periods dispersion, thus conditioning J point and ST segment elevation in the right precordial leads, followed by negative T wave (Brugada type 1 ECG pattern), creating an ideal substrate for developing reentry in phase 2, which in turn fosters the triggering of very fast polymorphic ventricular tachycardias with very short initial extrasystole coupling, which may degenerate into ventricular fibrillation and syncope or sudden cardiac death, mainly during night sleep.

Delpon et al (**31**), described a KCNE3 gene mutation, related to the voltage-dependent K^+ channel family, I_{ks}, which co-assembles with the KCND3 gene. The map of the genetic locus of KCND2 and KCND3 was determined in chromosomes 7q31 and 1p13.2, respectively. The beta subunit of KCND2 modulates outward K^+ channels in the human heart. Mutations in the KCNE3 could be the basis of Brugada syndrome and hypokalemic paralysis. From a population of 105 probands carriers of Brugada syndrome, in whom genetic tests were conducted, one had a missense mutation (R99H) in the KCNE3 (MiRP2) gene. Co-transfection of the R99H KCNE3 missense mutation, resulted in a significant increase of I_{to} channel intensity in comparison to the wild-type KCNE3+KCND3.

Using isolated tissue of the left atrial appendage from human hearts, the authors also showed that K(v)4.3 and KCNE3, could be co-immunoprecipitated. There is definitive evidence for the functional role of KCNE3 in the modulation of I_{to} channel in the human heart, suggesting that the mutation in KCNE3 could be in part, the basis of Brugada syndrome.

Experimentally in dogs, an I_{to} activator NS5806, increases in phase 1 the depth of the AP notch in the epicardium, but not in the endocardium, and emphasizes the J wave of ECG conducting –through reentry in phase 2- PVT/VF runs. The KCNE3 mutation leads to a gain of function of the I_{to} channel, which could explain variant 5 of Brugada syndrome, in which J point and ST segment elevation are evident in both right and left leads (**32**).

In diabetic patients, especially type 1, a greater tendency to sudden cardiac death and malignant arrhythmias is observed, and ECG alterations, partly because the I_{to} channel is more compromised. Such compromise occurs at three levels:

1) Recovery from inactivation, because this trigger exchanges the Kv4.x channel of rapid recovery, by Kv1.4 of slow recovery.
2) The responsibility of physiologic regulators: these display less responsiveness to sympathetic stimulus.
3) The functional expression of the channel, reducing the quantity of Kv4.2 and Kv4.3 proteins (**33**).

Changes in I_{to} concentration affect the AP profile, the vulnerability to arrhythmias appearance, and influence on excitation-contraction coupling.

A decrease in I_{to} channel density is observed in immature hearts and in elderly people, as well as in cardiomyopathies and heart failure. This lower density causes AP prolongation, fostering entrance and decreasing Ca^{2+} efflux by the Na^{+}/Ca^{2+} exchanger. Both facts favor increase of Ca2+ content in the sarcoplasmic reticulum (SR). This calcium accumulation is a trigger of arrhythmias.

II) PHASE 2, PLATEAU OR DOME

In surface ECG it corresponds to ST segment, and is also due to a polyionic mechanism; however, the main one is the Ca^{2+} cation, which enters slowly by the long lasting or L-type calcium $I_{Ca\text{-}L}$ current.

The plateau profile of phase 2 is maintained by the opposite forces of K^{+} and Cl^{-} outflow that oppose the forces of slow inward Ca^{2+} by the slow $I_{Ca\text{-}L}$ channel and by the Na^{+}/Ca^{2+} exchanger pump. Table 4 shows the main phase 2 channel.

TABLE 4

Main channel during phase 2 of ventricular myocardium AP				
Cation	**Name of channel**	**α subunit protein**	**NAME OF GENE α subunit of gene**	**AP PHASE**
Ca^{2+}	$I_{Ca(L)}$ "L-type Ca^{2+} current", $Ca_n1.2$, α_{1C} CCTL	$Ca_n1.2$	CACNA1C	Phase 0 in slow fibers and pahse 2 in rapid fibers.

$I_{Ca(L)}$ or L-type Ca^{2+} currents are made up by four homologous domains (I through IV), and each of them in turn, has 6 transmembrane segments from S_1 through S_6, and pore nucleus. S_4 is the main voltage sensor and pore loop between S_5 and S_6. Besides, there is a long carboxylic tail.

$I_{Ca(L)}$ channels are blocked by Ca^{2+} antagonists or blockers. These drugs bind to regions $IIIS_5$, $IIIS_6$, and IVS_6 from the α subunit. These are chemically classified into three large groups: phenylalkylamines, benzothiazepines, and dihydropyridines.

1) **Phenylalkylamines (PAA):** Its main representative is verapamil. This drug reduces HR, decreases sinoatrial, atrioventricular and negative inotropic conduction, and reduces peripheral resistance.
2) **Benzothiazepines (BTZ):** Its main representative is diltiazem (**34**). In AV junction, verapamil is stronger than diltiazem in its negative dromotropic characteristic.
3) **1,4-dihydropyridines (DHP):** stronger vasodilators, that may induce reflexive tachycardia, with less effect on myocardial contractility and no properties on the conduction system. The group is constituted by:

nifedipine, nitrendipine, nisoldipine, isradipine, felodipine, amlodipine, lacidipine, and nicardipine.

Ca^{2+} channels are also blocked by certain divalent ions such as manganese (Mn), cobalt (Co), nickel (Ni), cadmium (Cd), and lantanium (La). Contrary to slow Ca^{2+} channels, they are opened in phase 2 by norepinephrine, isoproterenol and xanthine drugs that increase intracellular Ca^{2+} ratio.

$I_{Ca(L)}$ channel selectivity is 1000 times greater for divalent cations than for monovalent ones. This selectivity does not depend on the size, but it does on a glutamate ring in the pore called EEEE locus, in which each of the 4 P loops contributes with an E. Thus, there is a high affinity between divalent cations and the pore by the EEEE locus.

Another conditioning mechanism for phase 2 profile, is the Na^{+}/Ca^{2+} exchanger current. This cation exchanger current is processed by a mechanism called electrogenic, exchanging three Na^{+} molecules by one Ca^{2+}. This is the main mechanism of sarcolemmal Ca^{2+} withdrawal, essential for the relaxation of the cardiac muscle, causing a balance in inward and outward Ca^{2+}, controlling cardiac inotropism. The exchanging mechanism may work in both directions (outward and inward) and this operation depends on intracellular Ca^{2+} concentration and the potential threshold. The channel is voltage-dependent and activates on values close to -40 mV.

The Na^{+}/Ca^{2+} exchanger current has three different genes:

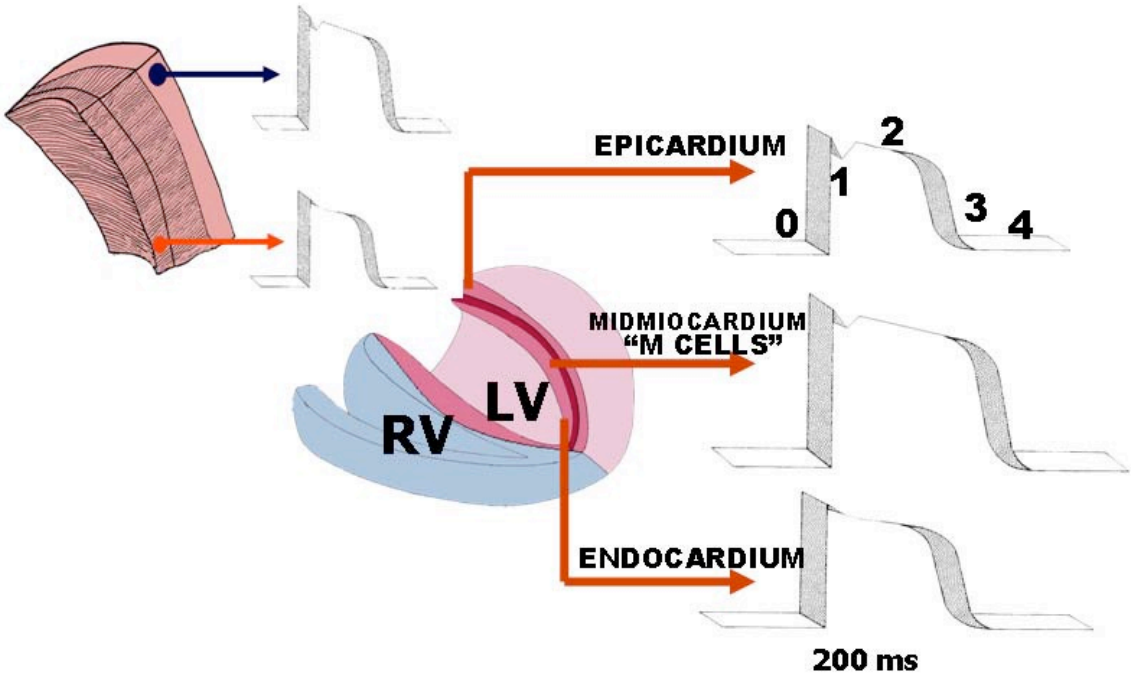
- NCX1: In the cardiac muscle, NCX1.1 is exclusively expressed. This is a molecule with 938 amino acids and a 110 kD mass. The Na^{+}/Ca^{2+} exchanger current has 9 transmembrane segments and a large cytoplasmic loop between transmembrane 5 and 6 segments.
- NCX2.
- NCX3.

Ca2+ velocity and amplitude may determine the formation of delayed afterdepolarizations (DADs) mainly by activation of the Na^{+}/Ca^{2+} exchanger current, inducing arrhythmias in the ventricle (**35**).

The arrhythmias caused by abnormal automaticity, such as accelerated idioventricular rhythm (AIVR), junctional tachycardia, atrial tachycardia, excluding those caused by digitalis intoxication, and VT during the first five days after acute infarction, are those where there is reduction in the Maximal

Diastolic Potential (MDP), corresponding to the end of phase 3 and onset of 4, and are always dependent on the slow Ca^{2+} current; therefore, cannot be suppressed by overdrive suppression. Arrhythmias triggered by early afterdepolarizations (EADs) are AP fluctuations that occur at the end of phases 2 and 3, causing triggered activity. Those of phase 2 respond to increase in inward Ca^{2+} by the slow $I_{Ca\text{-}L}$ current (**36**) or by late persistent Na^{+} inflow during the "plateau". The latter are those of congenital long QT syndrome, variant 3 (LQT3). ST prolongation observed in this variant, shortens with the use of pacemaker to a slightly greater HR, and with beta-adrenergic stimulation by different mechanisms (**37**). Those of the phase of rapid repolarization occurring during phase 3 of AP by reduction of outward K^{+} by the $I_{k\text{-}r}$ or $I_{k\text{-}s}$ channels, are typical of congenital long QT syndromes, variants 2 and 1, respectively. The latter differentiate from the first by displaying Ca^{2+} release from the Ca^{2+} release current from intracellular sarcolemma or ryanodine receptor (CRC). Additionally, $I_{Na^{+}\text{-}Ca^{2+}}$ exchanger current or cation exchanger current activation is observed by electrogenic mechanism (three Na^{+} molecules are exchanged by one Ca^{2+} molecules). They are characterized by occurring at low rates (bradycardia-dependent), faced with AP prolongation, and ending when repolarization has been fulfilled, and they occur in two AP levels: between 0 and -30 mV and between -60 to -70 mV. They are suppressed by rapid ventricular pacing. Figure 12 shows the different AP profiles of epicardial, midmyocardium, and endocardial cells, in ventricular wall thickness.

FIGURE 12
AP PROFILE OF VENTRICULAR CONTRACTILE CELLS IN EPI, MESO, AND ENDOCARDIAL CELLS IN WALL THICKNESS: HETEROGENEITY



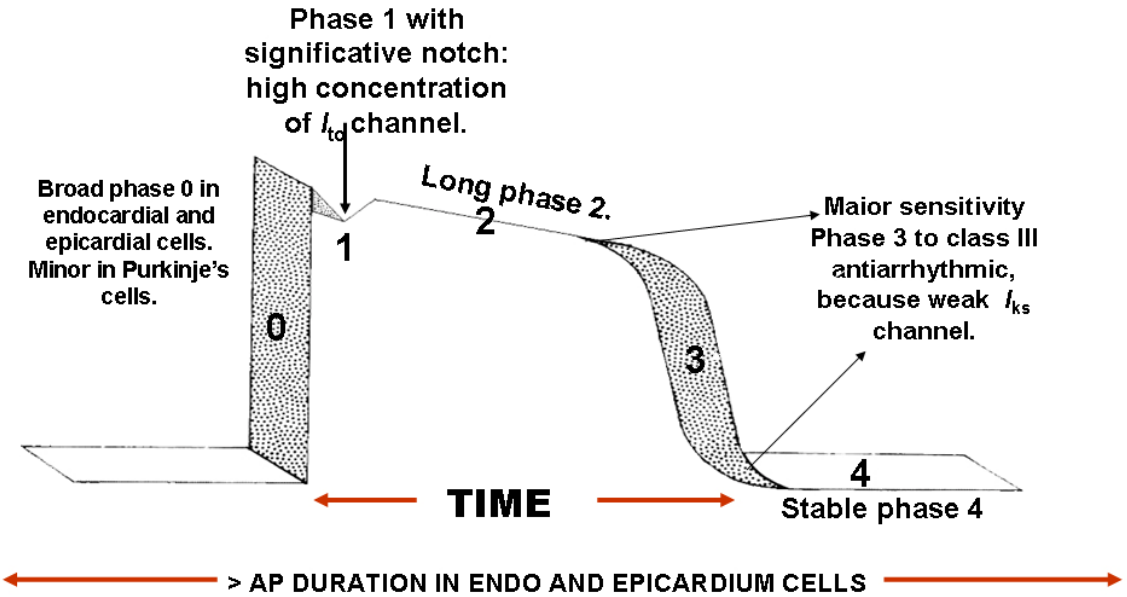
AP duration of the middle layer of ventricular wall thickness is much greater (800 ms) than those of epi and endocardial cells (300 ms) (midmyocardium > endocardium > epicardium). This greater duration is due to the presence in the myocardium of M cells. These cells are anatomopathologically indistinguishable and their particular spatial-dynamic modulation indicates that M cells become manifest only under the right circumstances (**38**). This proper electrophysiologic behavior is characterized by:

- Wide **phase 0** (greater than endo and epicardial cells); however, a little less wide than Purkinje cells.
- **Phase 1** with prominent notch: > I_{to} channel concentration.
- Prolonged **phase 2**, much greater than endo and epicardial cells: greater AP duration of midmyocardium.
- **Phase 3** much more sensitive to class II antiarrhythmic agents, because they have a weaker slow K^+ outflow channel I_{ks}.
- Stable **phase 4** (nonautomatic).

Figure 13 shows a diagram with the main AP features of M cells. M cells are a mixture between Purkinje and contractile cells.

FIGURE 13

M CELLS AP CHARACTERISTICS

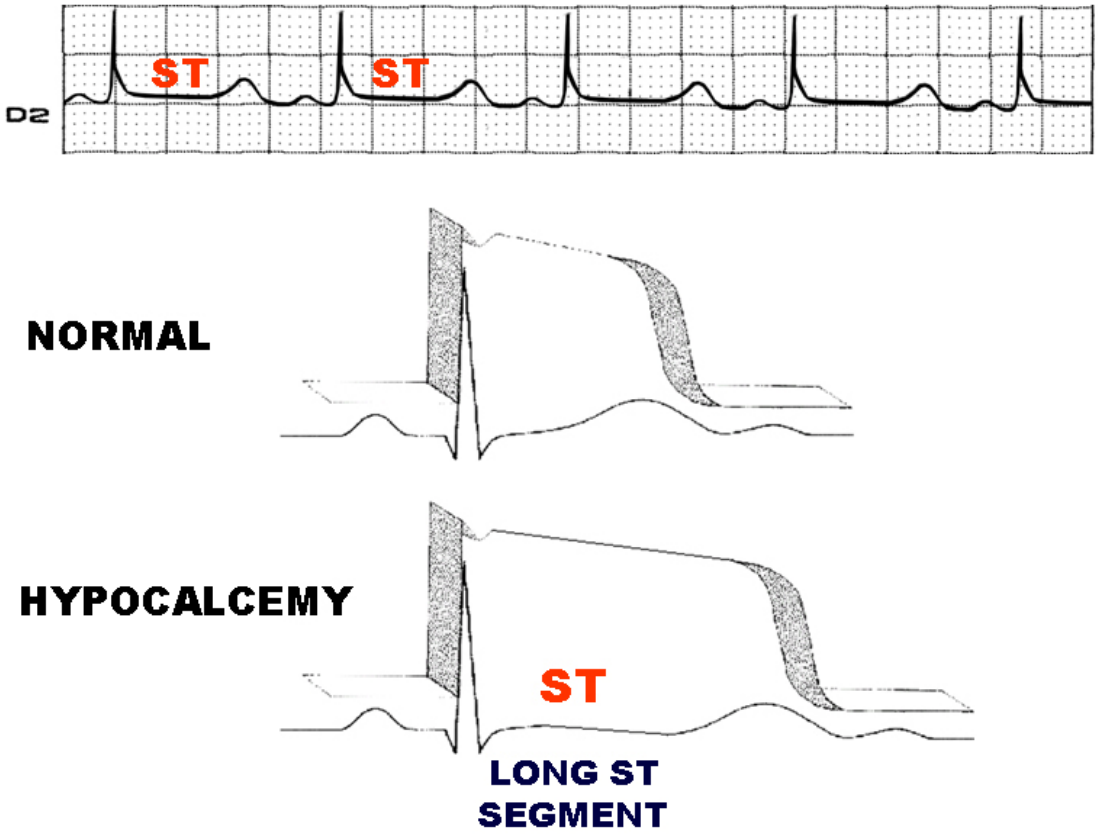


In hypocalcemia, usually ECG manifestations appear when ion calcium (Ca^{2+}) levels reach values below ≤7 mg/dl. The most significant electrocardiographic manifestation is QT interval prolongation at the expense of increase in ST segment duration with no T wave changes. Hypocalcemia can mimic long QT syndrome, variant 3 (LQT3) (**39**).

The upper part of Figure 14 shows an ECG strip with prolonged DII and increase in ST segment duration by hypocalcemia. In the lower part, a normal AP and ECG correlated to hypocalcemia are shown. There is AP phase 2 prolongation, and consequently, ST segment prolongation in ECG.

FIGURE 14

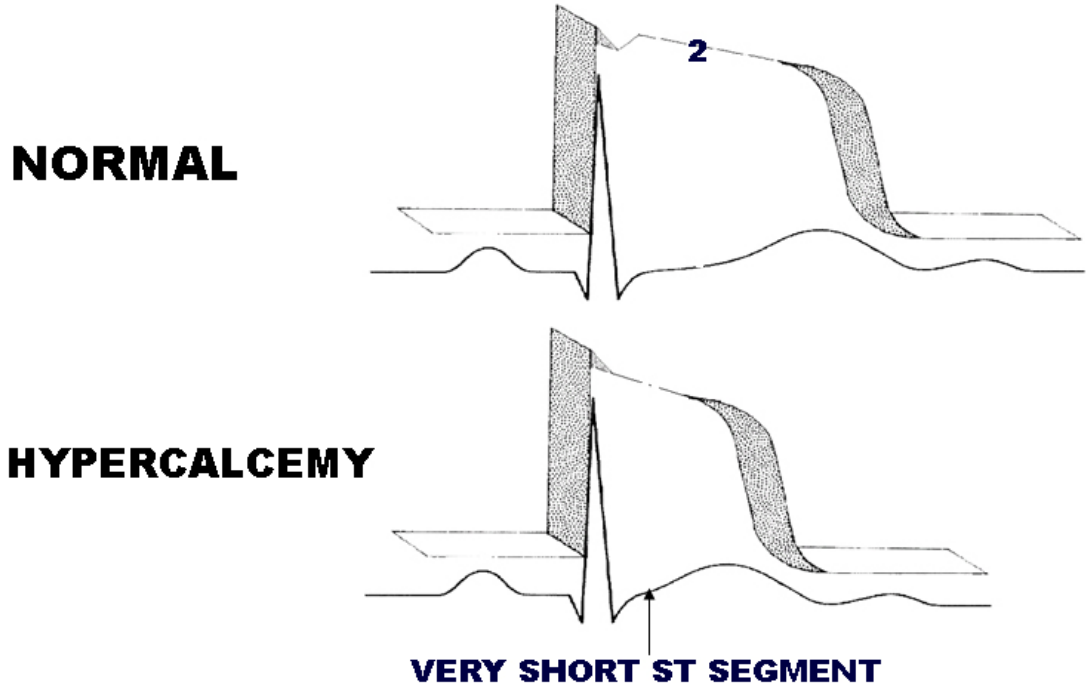
OUTLINE OF AP CHANGES AND ECG IN HYPOCALCEMIA



On the contrary, in hypercalcemia, ST segment shortening is observed, and consequenly QTc interval shortening. Figure 15 shows an outline comparing AP with surface ECG in normal conditions and in hypercalcemia.

FIGURE 15

OUTLINE OF AP CHANGES AND ECG IN HYPERCALCEMIA



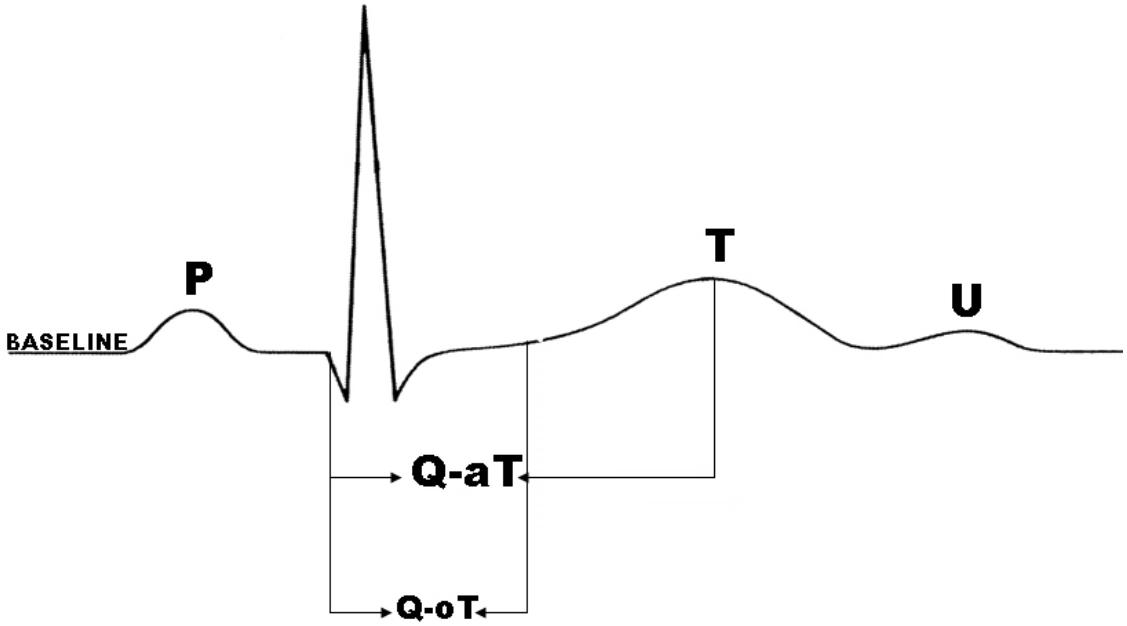
In hypercalcemia, Q-oTc interval shortening occurs. This the interval extends from Q wave onset until T wave onset, adjusted by heart rate.

Moreover, Q-aT interval decrease is observed: interval that extends between QRS onset until T wave apex. Values ≤270 ms are diagnostic for hypercalcemia. QoTc, QaTc sensibility to predict Ca^{2+} increase was 83% and 57% respectively, and specificity was 100% and 100% (**40**).

Figure 16 shows Q-oT and Q-aT intervals.

FIGURE 16

IMAGE OF Q-ot AND Q-aT INTERVALS

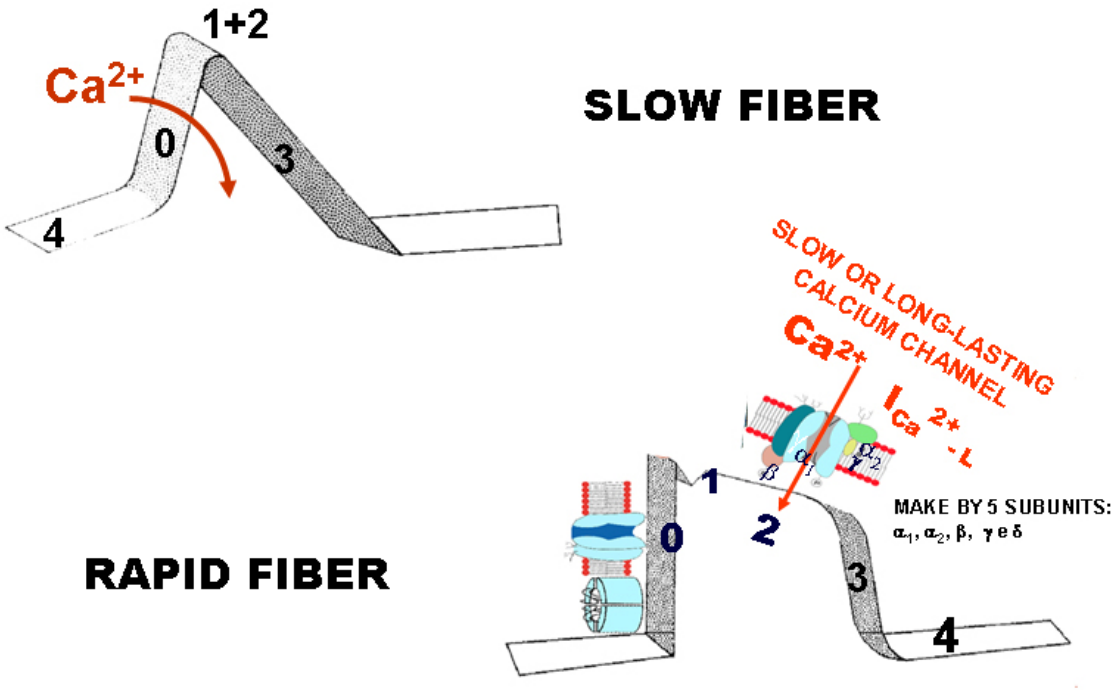


Antzelevitch et al (**41**) identified a mutation that affects the slow Ca^{2+} channel in the ß2 subunit, by mutation in the CACNB2b.Cavb2b gene, in chromosome 10p12.33 (OMIM number 600003). The patients affected show an ECG characterized by ST segment elevation in the right precordial leads and QTc interval, relatively short (≤370 ms). Ajmaline test triggers Brugada type 1 ECG pattern. We analyzed the ECG from the mentioned paper, and we observed that the Q-aT interval is very short, consequently this mutation displays a phenotype very similar to patients with hypercalcemia. This variant of Brugada syndrome is considered Brugada syndrome 4.

The Ca^{2+} $L_{(ICa-L)}$, L-type (slow or long-lasting) channel produces depolarization and phase 0 propagation in phase 0 of the slow fiber of the SA node and AV node, and contributes to the plateau or phase 2 in atrial and ventricular, and His-Purkinje system contractile cells. Figure 17 shows the characteristics of the slow Ca^{2+} channel of the sarcolemma in slow fibers, where it acts in phase 0 and in rapid fibers in phase 2.

FIGURE 17

SITE OF ACTION OF THE Ca^{2+} $I_{Ca\text{-}L}$, L-TYPE, SLOW OR LONG-LASTING CHANNELS IN SLOW AND RAPID CELLS



ICa-L currents are strongly influenced by neurotransmitters.

Another system acting in phase 2 or plateau is the $I_{Na+\text{-}Ca2+}$ channel or Na^+/Ca^{2+} exchanger current. Its characteristics are:

- They act by a mechanism called electrogenic, that consists of exchanging three Na+ molecules by a Ca2+ molecule.
- They act in phase 2 or plateau of rapid fibers.
- They may act in two directions, extracellular or intracellular.
- Its operation depends on the intracellular concentration of Ca^{2+} and the potential threshold.
- Its activation is voltage-dependent and it occurs at values close to -40 mV.

Finally, in phase 2, dome or plateau, the late $I_{Na}+$, steady-state, or Na^+ window current acts. In ischemic disease, the inhibition of this channel with ranolazine reduces recurring ischemia, Ca^{2+} overload, and electric and mechanic dysfunction (**42**). Local anesthetic agents and tetrodotoxin block this channel, shortening AP. Phase 2, AP plateau or dome, is prolonged in variant 3 of congenital long QT syndrome or LQT3 (OMIM: 600163). This variant affects the Na^+ channel by mutation in chromosome 3 (3p21-24) in the SCN5A gene, the

same one that affects Brugada syndrome (both entities are allelic). In surface ECG, it is characterized by a greater QT interval duration, at the expense of ST segment and delayed appearance of T wave, subsequent to AP phase prolongation by small delayed and persistent Na^+ inflow in phase 2, by delayed reopening, which explains QT interval prolongation.

Mexiletine, a class 1B antiarrhythmic agent, lidocaine-like, is much more efficient to shorten the QT interval of this LQT3 variant. In these patients, mexiletine significantly shortens QTc, thus preventing the appearance of torsades de pointes (TdP). The drug does not shorten the long QT of congenital long QT syndrome, that affects the K^+ channel (K^+ current HERG defect) or LQT2 (**43;44**).

Flecainide – a class IC antiarrhythmic agent- by causing delayed Na^+ inflow block in phase 2, seems promising for the oral management at low doses of LQT3, in patients with the DeltaKPQ mutation in the SCN5A (**45**).

III) PHASE 3, FINAL RAPID REPOLARIZATION

It corresponds in surface ECG, to T wave, and it responds to K^+ outflow by delayed opening of voltage-dependent delayed rectifier K^+ channels, made up by the following components:

1) A slow activation channel (I_{Ks}), that in fact activates from the end of phase 2;
2) A rapid activation channel (I_{Kr});
3) An ultra-rapid activation channel (I_{Kur});
4) Additionally, during phase 3, inactivation of the slow $Ca^{2+}{}_{Ca\text{-}L}$ channel occurs;
5) Increase of the $Na^+/K^+{}_{ATPase}$ pump activity, with increase of intracellular Na^+ concentration;
6) K^+ channel activation, I_{K1} (continued background inward steady-state K^+ current), which will remain activated during phase 4.

Table 5 shows the main delayed rectifier outward K^+ currents in phase 3.

TABLE 5

Delayed rectifier outward K^+ currents. The three are made up by a single pore, wiht 6 transmembrane domains (tetramers)

Cation	Name of the channel	α subunit protein	GENE Subunit of the gene	Phase of AP	Activation mechanism. De polarization.	Clone
K^+	I_{Ks} **Rectifier or slow delayed**	$K_V7.1$	KCNQ1	2,3	**Voltage-dependent. Depolarization.**	**K_yLQT 1**
K^+	I_{Kr} **Rectifier or rapid delayed**	$K_V11.1$ (HERG)	KCNH2	3	**Voltage-dependent. Depolarization.**	**HERG**
K^+	I_{K1} **Rectifier or rapid intense inward**	$K_{ir}2.1/2.2/2.3$	KCNJ2/ KCNJ12 / KCN4	3 e 4	**Voltage-dependent. Depolarization. Hyperpolarization.**	**Kir 2.1/2.2**

Note: Delayed rectifier, voltage-dependent, ultra-rapid activation K+ channel, or I_{Kur}, is expressed only in the atrial myocardium and during AP phase 2. The inactivation of I_{Kur} is ultra-slow, a fact that conditions it to determine AP duration in the atria. Its molecular structure and assembly is equal to the I_{tof} and I_{tos} channels, i.e. forming a single pore and six transmembrane domains (tetrameric). The clone of the I_{Kur} channel is $K_y1.5/3.1$.

I_{Kur} channels block could be the substrate for developing atrial fibrillation in healthy canine atria, allegedly due to shortening of AP duration (APD) and a relative refractory period (ERP) (**46**).

A mutation (KvSQT1 and the minK(1sK)) protein, that affects the alpha subunit of the slow delayed rectifier potasium channel (KvLQT1 or KCNQ1) (**47**), causing a decrease in I_{Ks} channel function, is responsible for the congenital long QT syndrome (LQTS), variant 1 or LQT1. (OMIM 192500). The initial identification of LQT1 occurred 1991 by Keating et al (**48**). These investigators identified the involvement of the short arm of chromosome 11 (11p15.5), proving for the first time the genetic origin of the congenital, inherited-familial long QT syndrome, LQTS, known as the Harvey RAS gene.

Variant 1 of LQTS or LQT1 is more prevalent, since it constitutes ≈60% of the total, and is characterized on ECG by displaying a long QT with broad-based and prolonged T wave, and moderate dependence of heart rate on the QT interval. This is the variety that benefits the most with ß–blockers. On the contrary, it is the one variant that worsens with ß-adrenergic stimulation. Thus, in LQT1 with normal QT interval (6%), known as concealed LQT1, epinephrine infusion causes QT interval prolongation (paradoxical response); while in controls and in LQT2 and LQT3, a tendency to QTc interval shortening was observed (**49**).

A mutation in the KCNQ1 gene, that affects the slow delayed rectifier outward K^+ channel (g919c substitution in the KCNQ1 gene, which encodes the K^+ I KvLQT1 channel), causes a gain of I_{Ks} function, which is responsible for variant 2 of congenital short QT syndrome (SQTS) (**50**).

Other works identify the HERG+MiRP1 mutation in chromosome 7, mutation 7p35-36. The kvLQT1 gene and the IsK(mink) protein associated to alpha subunit of the rapid delayed rectifier potassium channel (I_{kr}) (**51;52**) are responsible for long QT syndrome variant 2, or LQT2 (OMIM 152427), which constitutes 35% of the total. The LQT2 variant in ECG shows T waves with low amplitude or flattened, biphasic, bifid, and with notches.

Ramon Brugada et al (**53**), working at the Masonic Medical Research Laboratory, detected for the first time, a missense mutation (N588K), which affects the rectifier outward K^+ channel or I_{Kr}, causing a gain of function of the channel, which leads to congenital short QT syndrome, variant 1, or SQT1, controlled by the HERG gene (Human Ether-a-go-go-Related Gene) (KCNH2). This is the mirror image of congenital LQTS variant 2 or LQT2.

Finally, in phase 3 we also verify K^+ currents in the inward direction:

I) I_{k1}

II) I_{kAch} channel

III) K_{ATP}.

I) I_{k1} or rectifier inward K^+ current or barium-sensitive channel. Other names: inwardly rectifying K^+ current; continued background inward steady-state K^+ current; or inwardly rectifying Ba(2+)-sensitive current; Ba^{2+}-sensitive current. I_{k1} is responsible for maintaining the resting potential in the atria, ventricles, and

the His-Purkinje system. The I_{k1} channel acts in the final portion of atrial AP phase 3, and the onset of phase 4, in a range of voltage between the resting potential and -30 mV. The I_{k1} channel seems to be important in controlling the dynamics of the spiral waves responsible for ventricular tachycardia and fibrillation, besides contributing to the genesis and stability of these spiral waves, thus being a significant target in antiarrhythmic management (**54**).

Function: maintaining resting potential in the atria, AV node, and ventricular muscle. **Stimuli:** hyperpolarization (voltage-dependent). **I_{k1} channel blockers:** Ba^{2+}. This channel could be affected in the Andersen-Timothy syndrome (ATS1), a rare disorder characterized by periodical paralysis, cardiac arrhythmia, and different anomalies affecting the KCNJ2 gene, which encodes the alpha I_{k1} Kir2.1 subunit. In this entity, there is a loss of function in the I_{k1} channel or Ba^{2+}-sensitive current. This mutation causes a shift of the resting potential, causing depolarization (**55**). Kir2.x channels are critical components of the I_{k1} channel, native in cardiomyocytes of neonatal rats, and suppression of the I_{k1} channel is part of the pathogenia of Andersen's syndrome (**56**). A recent research pointed out that the I_{k1} channel regulates U wave voltage (**57**). In the ATS1 syndrome with specific genetic alteration, T-U wave pattern leads to a decrease in I_{k1}, due to mutation in the KCNJ2 gene. QTc is normal, which differentiates ECG from ATS1 from long QT syndrome, thus being inappropriate to call it LQT7 (**58**).

II) I_{kAch} channel or K^+ channel regulated by M2 muscarinic receptor in atrial and nodal cells, or G-protein-gated atrial K^+ channel. Heart rate partly depends on IkAch channel, acetylcholine-dependent K+ channel, or g-protein-gated atrial K+ channel activation. The activated muscarinic receptors stimulate the I_{kAch} channel, by G-protein βγ subunits. The I_{kAch} channel is encoded by the KCNJ3 and KCNK4 genes, being heteromultimetric and constituted by a single pore, 4 transmembrane domains, and two subunits (dimer) or components: GIRK1e CIR (**59**). The I_{kAch} channel acts in phase 4 of AP, and its activation depends on acetylcholine (vagal tone). Its clone is kir 3.1/3.4.

III) $I_{K\text{-}ATP}$, K_{ATP}, or rectifier inward K^+ current, activated by muscarinic (M2) receptors, and stimulation by purinergic I receptors, via G protein regulating sign transduction (GTP), or adenosine triphosphate-activated K^+ current (KATP) or ATP-sensitive K^+ channels. The stimulus occurs when ATP intracellular ratio

falls. This occurs in clinics, mainly in myocardial ischemia. The activation of this current causes AP shortening. Pinacidil, cromakalim, and nicorandil also open this channel. Sulfonylureas, such as glibenclamide, inhibit this channel. They are K^+ channels (symbolized by KCNJ11), which are expressed in the SA node, AV node, and atrial muscle. When activated, they cause a rectifier inward K^+ current, shorten AP, and cause hyperpolarization and chronotropic and negative dromotropic effects. The activation occurs in the following circumstances:

1) M2 muscarinic receptors stimulation;
2) Purinergic type I receptors stimulation via regulators of G protein signaling transduction (GTP);
3) Ischemia causing AP shortening during this state;
4) Intracellular AP concentration fall, a fact observed during heart failure with inotropic deficiency;
5) Effect of pinacidil, cromakalim, and nicorandil;
6) Idiopathic ventricular fibrillation.

Haissaguerre et al (**60;61**) identified a variant of a missense mutation in exon 3 (NC-000012) of KCNJ8 gene, a subunit of the K_{ATP} 2;3 channel. Genomic DNA that sequences K_{ATP} channel genes, showed a missense variant in exon 3 (NC_000012) of the KCNJ8, a subunit of the K_{ATP} channel, providing a predisposition to dramatic changes in repolarization and ventricular vulnerability. From a multicenter cohort of 122 patients (90 men, ages from 37+/-12 years old), carriers of idiopathic ventricular fibrillation (IVF) and early repolarization pattern (ERP) in infero-lateral leads, Haissaguerre et al, selected those patients with more than three episodes of ventricular fibrillation, including those with electric storms (≥3 VF in 24 hs). Multiple recurrences of VF occurred in 27% of patients with ERP. Isoproterenol in acute cases and quinidine in chronic patients were effective. The latter is necessary when an ICD is implanted, as it decreases the number of shocks delivered by the device.

The so-called atypical Brugada syndrome is characterized by ST segment and J point elevation in the infero-lateral wall. The early repolarization pattern in the infero-lateral wall is not rare in Brugada syndrome (**62**). A high incidence of early repolarization is observed in infero-lateral leads in patients with IVF. ECG tracings show QRS-ST joint point elevation ≥0.1 mV in reference to the baseline

in infero-lateral wall leads and QRS complex notches. Between these patients with history of IVF, early repolarization prevalence is increased.

Bonakdar et al, described a patient carrier of Brugada syndrome, with frequent episodes of syncope. The patient showed alternating ST segment elevation in right precordial leads, and in the high lateral wall (**63**). K_{ATP} channels contain a subunit of the Kir6.0 type and sulfonylureas receptors (SUR) (**64**). By the position they hold within the cell, they are identified in three groups:

1) **Sarcolemmal, SarcK_{ATP}**. Made up by 8 protein subunits, with 4 of them being members of the family of the inwardly rectifying potassium channel Kir6.0, and the other 4 being sulfonylureas receptors (SUR1, SUR2A, and SUR2B) (**65**). Kir subunits have 2 transmembrane spans, and they form the pore of the channel. SUR subunits contain 3 additional transmembrane domains, and 2 nucleotide-binding domains in the cytoplasmic surface (**66**), with a critical role as sensors of the metabolic state. These SUR subunits are also sensitive to sulfonylureas, MgATP, and some other pharmacological opening channels. Although all sarcK_{ATP} are made up by 8 subunits in a 4:4 ratio, their composition varies with the type of tissue (**67**).

2) Mitochondrial (mitoK_{ATP}): initially identified in 1991 as a single channel, located in the internal portion of the mitochoncrial membrane (**68**). The molecular structure of the mitoK_{ATP} channels is less known than the one of sarcK_{ATP}. They are composed by Kir6.1 and Kir6.2 subunits, but no SUR1 or SUR2 (**69;70**). They have multiprotein complexes rich in succinate dehydrogenase, with activity similar to the K_{ATP} channel (**71**).

3) Nuclear K_{ATP} (nucK_{ATP}). The presence of nuclear KATP was confirmed by the discovery that isolated portions of the nuclear membrane have properties with kinetics and pharmacology similar to the sarcolemmal membrane K_{ATP} (**72**).

Cellular metabolism sensor and genetic expression regulation

Four genes have been identified as being members of the K_{ATP} family. The SUR and kir6.2 genes are located in chromosome 11p15.1; while kir6.1 and SUR2 genes are located in chromosome 12p12.1. The kir6.1 gene encodes the subunit that makes up the K_{ATP} channel pore, with a SUR subunit composed by the sur1 gene, or the selective SUR2 gene (SUR2A and SUR2B) (**73**). Changes in the transcription of these genes, and thus in the production of K_{ATP} channels,

are directly related with changes in the milieu metabolism. Thus, hyperglycemia causes kir6.2 decrease at mRNA level. This fact can be reversed by glycemia normalization (**74**). From this, in left ventricular tissue from rats, 1 hour of ischemia followed by 24 to 72 hs of reperfusion, increases kir6.2 transcription in this tissue (**75**).

Crawford et al (**76**) proposed that faced with hypoxia and ischemia, a low level of O_2 decreases the mitochondrial metabolic rate, slowing the Krebs cycle, rendering the organelle incapable of transferring electrons properly, and consequently decreasing the intracellular rate of NAD+NDAH. This lack activates phosphatidylinositol 3-kinase, which is the extracellular signal regulated by kinases. The phenomenon increases the regulation of c-jun transcription, creating a protein that binds to the sur2 promoter. In diabetic patients, K_{ATP} channels, very sensitive to hypoxia, cannot operate properly, leading to a loss of cellular capacity to adapt to an adverse oxidative condition (**77**).

In a condition of hypoxia in the cardiomyocytes, the greatest amount of energy comes from long chain fatty acids, and the equivalents of acetyl-CoA, inducing K_{ATP} channels opening, as long as free fatty acids stabilize the closed shape. This variation has been experimentally shown in transgenic rats. In the pancreas, unlike cardiomyocytes, the IK_{ATP} channels always remain open (**78;79**).

Mitochondrial K_{ATP} and aerobic metabolism regulation

In a condition of hypoxia, the mitochondria starts an overproduction of free radicals (**80**). In this situation, the mitoK_{ATP} channels open and close in an attempt to regulate the internal concentration of Ca^{2+} and the degree of edema of the membrane. This helps to restore the membrane potential properties with H^+ outflow to provide protons for ATP synthesis. Without the contribution of the K^+ channels, there would be a worsening of phosphate depletion of high energy, creating an unfavorable electrochemical transmembrane gradient (**81**). Sarcolemmal and nuclear K_{ATP} channels also contribute to adjustment to hypoxic metabolic stress. With the aim of saving energy, the sarcK_{ATP} channel opens, reducing AP duration as long as the nucK_{ATP} channel regulates the Ca^{2+}

concentration within the nucleus, with a protective effect in the expression of genes (**82**).

Cardiovascular K_{ATP} channels and protection from ischemia/lesion

Cardiac ischemia not always leads to immediate death; frequently, it leads to a slow death of cardiomyocytes by apoptosis, causing a permanent lesion on the cardiac muscle.

A form of ischemia initially described by Keith Reimer in 1986, is characterized by fast and nonlethal tissue compromise, with periods of 3-5 minutes of ischemia, occurring before major ischemic insult. This form of ischemia came to be known as ischemic pre-conditioning (IPC), which is partly dependent on K_{ATP} channel stimulation.

Both sarcK_{ATP} and mitoK_{ATP} channel are required for IPC to achieve its maximal effect. The selective block of mitoK_{ATP} with 5-hydroxydecanoid acid (5-HD) or with MCC-134 (**83**), completely inhibits the cardioprotection granted by IPC, and affects the genetic expression of the sarcK_{ATP} channel (**84**). Basal protection granted by the sarcK_{ATP} channel, is due to it preventing Ca^{2+} overload, and consequently preventing inotropic depression, saving energy sources (**85**). The absence of sarcK_{ATP} associated to weakening of the IPC benefit, makes cardiomyocytes to lose their capacity to distribute Ca^{2+}, decreasing sensitivity to the nervous sympathetic signal and predisposing to arrhythmias and sudden cardiac death (**86**). Likewise, sarcK_{ATP} regulates the tone of the vascular smooth muscle; and suppression of the kir6.2 or sur2 genes, leads to artery spasm and death (**87**).

Mutations in the sarcK_{ATP} channel particularly in the SUR2 subunit, lead to dilated cardiomyopathy, especially after ischemia/reperfusion (**88**).

The role of K_{ATP} channel in arrhythmogenesis is still a puzzle. An increase in the conductance of this channel should stabilize the membrane potential during ischemic insult, reducing the extension of the infarction area, and pacemaker ectopic activity. On the contrary, the channel opening and accelerating AP repolarization would enable induction of arrhythmias by reentry (**82**).

IV) PHASE 4 OF DIASTOLIC DEPOLARIZATION

It corresponds in surface ECG, to inconstant U wave. In it, energetic output occurs due to the action by the Na^+/K^+-A_{TPase} pump (**89**). The Na^+/K^+-A_{TPase} pump, acting in phase 4 by energetic output, reintroduces K^+ and "expels" Na^+. Note that intracellular K^+ concentration is much greater (150 mEq/L) than the extracellular one (5 mEq/L). On the contrary, Na^+ predominates in the extracellular milieu (142 mEq/L) than in the intracellular one (10 mEq/L).

Also in phase 4, the following channels act:

I) I_f channel or pacemaker channel in the initial part of phase 4. The I_f channel is a current activated by hyperpolarization, that acts on the SA node, AV node, and the His-Purkinje system in phase 4 of depolarization. It causes increase in the rate of impulses (pacemaker current), so it has a predominant role during more negative potentials or hyperpolarization (initial portion of phase 4). The I_f channel contributes only with 1/5 of the SA node pacemaker activity.

II) Fast T type Ca^{2+} channel, transient Ca^{2+} current, or tiny conductance Ca^{2+} current: it acts causing inward Ca2+ in the final portion of phase 4 in the SA node, N region of the AV node, and His-Purkinje system. Blocked selectively by the Ca^{2+} antagonist, mibefradil. Insensitive to dihydropiridinic agents. $I_{Ca\text{-}T}$ channel function is increased with noradrenaline, the α adrenergic agonist phenylephrine (**90**), increase of extracellular ATP and endothelin-1. Cardiomyocytes where $I_{Ca\text{-}T}$ is plentiful, are those with less extensive transverse T tubules. Table 6 shows the three main types of $I_{Ca\text{-}T}$.

TABLE 6

TYPE-T CALCIUM CHANNELS or $I_{Ca\text{-}T}$

ISOFORM	NAME OF GENE	NAME OF CHANNEL	TISSUE
α_{1G}	**CACNA1G**	**$Ca_n3.1$**	Neurons, Purkinje.
α_{1H}	**CACNA1H**	**$Ca_n3.2$**	Heart, kidneys, liver
α_{1I}	**CACNA1I**	**$Ca_n3.3$**	Neurons.

III) Acetylcholine-activated inward rectifying current ($I_{k(ACH)}$), which produces hyperpolarization and stimulated bradycardia. This channel is strongly inhibited by dronedarone, an analog to amiodarone in the SA node and the atrial tissue.

This also inhibits I_{k1}, L-Ca^{2+}, I_{kr} and to a lesser degree I_{ks}. This drug is an alpha and beta antagonist of adrenoreceptors, and unlike amiodarone, it has little effect on thyroid receptors (**91**). By the characteristics of phase 4, heart cells are classified into automatic and nonautomatic.

1) Automatic: They display unstable, ascending phase 4, or with diastolic, automatic, or rhythmic depolarization. This is the characteristic of cardiac cells, of spontaneously starting an impulse, in the absence of external stimulus. Phase 4 of diastolic depolarization originates by the inward current of the so-called pacemaker or I_f channel, that is the main mechanism by which the autonomic nervous system regulates automaticity. Thus, catecholamines open the I_f channel, increasing heart rate by making the phase 4 slope steeper. I_f, pacemaker subunit, or funny current is a current activated in hyperpolarization, present in the SA node, AV node, and His-Purkinje system cells, causing increase in shock rate of automatic or pacemaker cells (**92**).

Phase 4 of SA node is the one with greatest automaticity by a mechanism known as overdrive suppression; a phenomenon that consists of inhibiting subsidiary pacemakers by a faster pacemaker with spontaneous shocks.

2) Nonautomatic: characterized by presenting a stable phase 4; i.e. without spontanous ascending slope. This characteristic is mainly due to the presence of the rectifier inward I_{k1} channel, closed during depolarization. This channel, voltage-dependent and blocked by Ba^{2+}, is responsible for keeping the resting potential in atrial and ventricular muscle cells (ordinary working muscle cells).

Phase 4 corresponds to transmembrane resting potential or just membrane resting potential, which in the SA node cells is ≈ than -50 to -60 mV, in atrial muscle cells -80 to -90 mV, in AV node cells -55 to -70 mV, in Purkinje fibers -90 to -95 mV, and finally, in ventricular muscle cells -80 to -90 mV.

In brief, cardiac cells are divided into two groups from the point of view of rhythmicity, diastolic depolarization or automaticity. When phase 4 is horizontal (atrial and ventricular muscle cells), we say that cells are nonautomatic (it does not have the capacity to self-stimulate). When phase 4 is spontaneously ascending, as it happens with SA node, AV node, and His-Purkinje system cells, we say that cells are automatic or with diastolic depolarization.

Table 7 shows the main channels acting in phase 4.

TABLE 7

Main channels acting in phase 4				
Cation	**Channel**	**α subunit protein**	**Subunit of the gene**	**Phase / responsibility**
K^+	I_{K1}	K_{ir}2.1/2.2/2.3	KCNJ2KCNJ12KCNJ4	3,4
I_f pacemaker channel			HAC1	4. It contributes 20% of pacemaker function in the SA node.
Ca^{2+} T($I_{Ca\text{-}T}$); T-type Ca^{2+} channel		**Cav3.2**		Final portion of phase 4 in SA node, N region of AV node, and His-Purkinje system cells.
Na^+/ K^+ ATP_{ase} pump.				

CELL TYPES OF THE HEART ACCORDING TO AP AND ELECTRO-PHYSIO-PHARMACOLOGICAL BEHAVIOR

A) AUTOMATIC: WITH ASCENDING PHASE 4

(1A) Cells located in the SA node: with three varieties:

I) Pacemaker, P, or nodal cells: in turn, with two varieties:
   a. Spider-shaped cells
   b. Spindle-shaped cells.

II) Transitional or T cells

III) Atrial myocardial cells.

(2A) AV node cells

(3A) His-Purkinje system cells.

Note: SA node and AV node cells are slow and His-Purkinje system cells are fast.

B) NONAUTOMATIC: WITH STABLE PHASE 4

(1B) Atrial myocardial contractile cells

(2B) Ventricular myocardial contractile cells

1) Epicardial or subepicardial
2) Midmyocardium:
    a. Contractile
    b. M cells
3) Endocardial or subendocardial.

CHANNELS WITH INTRACELLULAR LOCATION OR FROM THE SARCOPLASMIC RETICULUM

To this date, we have analyzed only Ca^{2+} channels, located in the sarcolemma or the cell membrane, i.e. $I_{Ca\text{-}L}$ channel, L-type Ca^{2+} current, long-lasting $I_{Ca\text{-}L}$, slow response upstroke, and fast T type, transient $I_{Ca\text{-}T}$ or calcium fast channels. Intracellular or sarcoplasmic reticulum (SR) channels, in turn have four components, with the three first being of Ca^{2+}.

1) Calcium release channel (CRC), ryanodine receptor, or channel hyperphosphorylated by protein kinase (PKA) of the intracellular sarcoplasmic reticulum.

2) Ca^{2+} or Ca^{2+} +Mg^{2+} +ATP_{ase} uptake pump; SERCA, or protein known as cardiac calsequestrin (CASQ2). This pump has as its function to store or uptake Ca^{2+} within the SR. Investigators from the Department of Physiology, Dorothy M. Davis Heart and Lung Research Institute, in the State of Ohio University Medical Center, Columbus, Ohio (**93**), studying CASQ2 gene mutations caused by adenovirus, showed the relationship between the CASQ2 gene mutation and the predisposition to adrenergic-induced ventricular arrhythmias. The authors concluded that CASQ2 in the SR determines the magnitude and duration of Ca^{2+} release from each SR terminal cisternae, furnishing a local source of Ca^{2+} susceptible to be released due to its blocking effect on the ryanodine RyR2 Ca^{2+} channel, luminal dependent, which has the contrary effect of Ca2+ realease into cytosol. Moreover, the two CASQ2 and RyR2 mutations are responsible for increased diastolic events of Ca^{2+} release from the SR, and both exhibit the phenotype of catecholaminergic polymorphic ventricular tachycardia (CPVT).

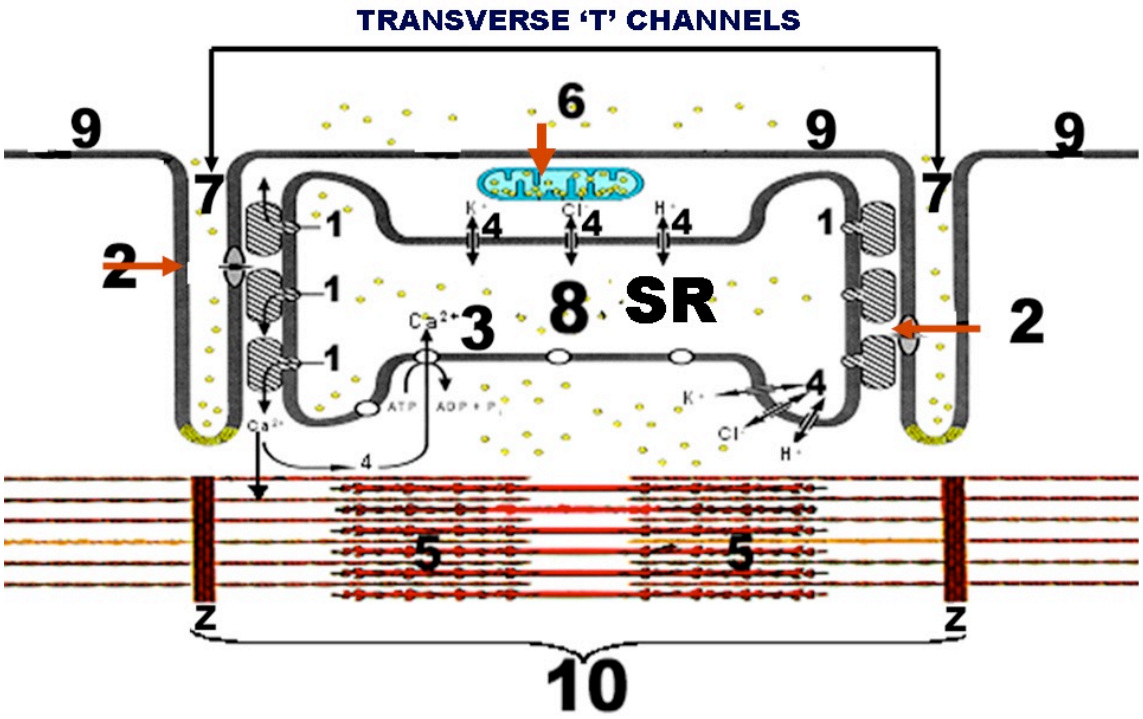
3) Inositol 1,4,5-triphosphate receptor (IP_3R), channel hyperphosphorylated by protein kinase (PKA) of the sarcoplasmic reticulum or inositol triphosphate IP3. This forms from phosphatidylinositol-biphosphate, which by action of the enzyme phospholipase C, becomes inositol triphosphate or IP3. In turn, this enzyme could be activated by angiotensin II (**94**), and α adrenergic stimulation. It is located in Purkinje cells, intercalated discs, conduction system, and the smooth muscle.

4) Channels or pathways permeable to monovalent ions of: H^+, Cl^-, K^+, and Na^+.

In Figure 18, the components of the compound intracellular activation system are shown.

FIGURE 18

COMPONENTS OF THE CELLULAR ACTIVATION SYSTEM NECESSARY FOR CARDIAC CONTRACTION



1- Figure 18: Ca2+ release channel, ryanodine receptor, or channel hyperphosphorylated by protein kinase (PKA) from the intracellular sarcoplasmic reticulum.

This channel, located and adhered to the sarcoplasmic reticulum (SR) membrane, is an intracellular structure key in muscle contraction and the relaxation process, due to its capacity for rapid release and uptake of the Ca^{2+}

ion from the myoplasm, by means of CRC or ryanodine receptor. Each channel is a large and complex protein, 30S, made up by four polypeptidic subunits, strongly associated to Mr ~560,000 with a four-leaf clover morphology (quatrefoil or tetrameric), that contour a single, cation-selective, hydrophilic pore, with conductance for divalent ions from 100 to 150 pS, with 50 mM Ca^{2+}, and for monovalent ions of ~750 pS with 250 mM K^{+}, which is found in the SR membrane, and fulfills its role by releasing the cation from the SR lumen into cytosol (efflux). It is found very near the sarcolemmal $I_{Ca\text{-}L}$ type channels, as this is voltage- and time-dependent. Each $I_{Ca\text{-}L}$ type channel can be blocked by ryanodine, a toxin derived from an alkaloid plant with nanomolar affinity. For this reason, it is also known as ryanodine receptor (RyR2). The substances that stimulate this channel improve contractility, and those that block it, worsen contractility. It seems to be the most important channel in insufficient hearts, since it undergoes a dramatic increase in phosphorylation (hyperphosphorylation) in patients with terminal heart failure, which would provide another basis for the use of β-blockers in this condition. Studies of cardiac hypertrophy in animal models suggest that a destabilization in the interaction between the N-terminal and the central domain (RyR2), becomes a factor for hypertrophy (**95**). The disruption of the interaction between the central interdomain and the N-terminal within the RyR2 is a cause of cardiac hypertrophy (**96**).

Patients carriers of familial catecholaminergic polymorphic ventricular tachycardia may have a SR missense mutation in the CRC, in the type 2 ryanodine receptor (RyR2) where three mutations were observed (P2328S, Q4201R, V4653F). These mutations were not found in nonaffected members of the same family, and in 100 normal controls. Inheritance could be autosomal dominant (mutations in ryanodine receptor RyR2) or recessive, associated to homozygous mutation in the gene that encodes the isoform calsequestrin (CASQ2), usually of high penetrance (**97**). This rare entity, with early clinical onset, and mean mortality rate of 30% up to 30 years old, was mapped in chromosome 1 (1q42-q43 and 1p11.13.3). Up to this moment, the following genetico-familial forms of catecholaminergic polymorphic ventricular tachycardia (CPVT) have been described (30% of cases), and they are shown in Table 8. The first mutation additionally to events induced by strain-stress, may present

sinus dysfunction, atrial fibrillation, atrial arrest, AV dysfunction, and dilated cardiomyopathy (**98**). Characterized by bidirectional TV and polymorphic VT salvos related to exercise; i.e. catecholamine-dependent and with no evidence of structural heart disease, manifest by recurring runs of syncope by unknown cause, occurring during or after exercise or emotions, usually in the pediatric or young age group. It may cause sudden death. Sudden infant death syndrome causes a great social impact because its occurrence is so unexpected (**99**). CPVT is a significant cause of syncope and sudden cardiac death induced by stress and emotions in children and young people (**100**).

TABLE 8

GENETIC OF CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA(CPVT)

Variant	Chromosomal Locus	Gene	Protein	Inheritance	Phenotype
CPVT1	1q42-q43	RYR2[1]	Cardiac RyR	AD	CPVT, IVF
CPVT2	1p11.13.3	CASQ2	Calsequestrin	AR	CPVT
CPVT3?	7P14-22	Unknown	?	AD	CPVT, QT prolong.
CPVT-Related Phenotypes					
LQT4	4q25-26	ANK2		AD	IVF, QT atypical prolongation, Stress induced BVT
ATS	17q23.1-q24.2	KCNJ2		AD	U waves, BVT Periodic paralysis Facial dysmorphisms
CPVT/ARVD	1q42-43	RYR2		AD	Stress induced VT, Sinus node dysfunction ARVD

AD= AUTOSOMAL DOMINANT; AR = AUTOSOMAL RECESSIVE; IVF= idiopathyc ventricular fibrillation; DCM = Dilated cardiomyopathy;

NOTE: Arrhythmogenic right ventricular dysplasia type 2 (ARVD2, OMIM 600996) is an autosomal dominant cardiomyopathy, characterized by partial right ventricular degeneration, electrical instability, and tendency to sudden

cardiac death with mutation mapped in the RYR2 gene, in chromosome 1q42—q43 (**101**).

2- The sarcolemmal membrane slow inflow Ca^{2+} channel acting in phase of AP and in phase 0 of slow fibers, L-type, slow response upstroke, or long-lasting $I_{Ca}{}^{2+}$-L channel, is illustrated with number 2 in Figure 18.

3- In the sarcoplasmic reticulum system membrane in Figure 18, the SERCA or Ca^{2+}-ATP_{ase} uptake pump, sarcoplasmic Ca^{2+}-(ATP_{ase} reticulum SERCA), Sarco/Endoplasmic Reticulum Ca^{2+}-ATP_{ase}, calcium ATP_{ase} type P-ATP_{ase} or Ca2+ ATPase of cardiac sarcoplasmic reticulum is illustrated with number 3 (**102**).

Function: SERCA is an enzymatic pump, located in the intracellular milieu in the sarcoplasmic reticulum. It is an enzymatic $Ca^{2+}ATP_{ase}$ complex, the function of which is to transfer Ca^{2+} from the cellular cytosol into the SR by ATP hydrolysis during muscle relaxation. There are three major domains in the cytoplasmic surface of SERCA: the domain where phosphorylation occurs, the binding domain with a nucleotide with catalytic function, and the domain called “actuator”, involved in transmitting composition changes. Moreover, SERCA1 is thermogenic in some adipocytes (**103**).

Regulation: the enzymatic complex, SERCA, is inhibited by a protein called phospholamban, to which is very close and highly associated.

Another protein related to SERCA is calsequestrin. It binds to Ca^{2+} within the SR, contributing to reducing free Ca^{2+} cation concentration within the SR. For this reason, it is considered to help SERCA by making it reduce free Ca^{2+} within the SR, and thus serving as an assitant to SERCA. The protein calsequestrin 2 (CASQ2) is for Ca^{2+} storage in the SR. An autosomal recessive disease was identified in a family of Bedouins with a missense mutation, that consists of exchanging aspartic acid by histidine in calsequestrin 2 (CASQ2), thus originating a tendency to appearance of catecholaminergic polymorphic ventricular tachycardia with a deleterious effect for Ca^{2+} cation storage. Viatchenko-Karpinski et al, studying CASQ2 with mutations caused by adenovirus, proved at cellular level, the relationship between the mutation in CASQ2 and a predisposition to adrenergic-induced ventricular arrhythmias, observed in patients carriers of this defect in CASQ2. The concentration of Ca^{2+} within the SR is much greater than in the intracellular millieu. The rate with

which SERCA shifts Ca^{2+} through the membrane of SR could be controlled by phospholamban (PLB/PLN) under β–adrenergic stimulation. When PLB is associated to SERCA, the rate of Ca^{2+} movement is lower, and when both dissociate, the movement of Ca^{2+} increases.

Paralogs: They are genes related to duplication within a genome. Unlike the so-called orthologs, that keep the same function over the course of evolution, paralogs evolve into new functions, even if related to the original function. There are 3 major paralogs, SERCA1-3, which express at several levels in different types of cells. ATP2A1–SERCA1; ATP2A2–SERCA2; ATP2A3-SERCA3. There are also additional post-translational isoforms (in biology, translation is defined as the process by which messenger RNA affects the sequence of amino acids during protein synthesis) of SERCA2 and 3, which serve to reintroduce the possibility of a specific cell type of Ca^{2+} reuptake, that may also respond with an increase of global complexity of the Ca^{2+} signaling mechanism.

4- Monovalent H^+, Cl^-, and K^+ ion channels are illustrated with number 4 in Figure 18.

5- Actin thin and myosin thick filaments are illustrated with number 5 in Figure 18: both integrate during muscle contraction and relaxation.

6- Mitochondrias are illustrated with number 6 of Figure 18. They provide energy for contraction through M ATP. Mitochondrias are considered as cell “power stations”.

7- T-tubular system: this system is illustrated with number 7 of Figure 18. Its basic function is transmitting the sarcolemmal electrical signal into the cell, penetrating within it through Z lines.

8- Sarcoplasmic reticulum (SR): the intracellular cisternae that uptakes (Ca^{2+} pump) and releases Ca^{2+} (RyR2), which has an essential function in the contraction/relaxation mechanism, is illustrated with number 8 in Figure 18.

9- The sarcolemmal membrane is illusted with number 9 in Figure 18. This structure has a role in controlling ion gradients, has ion channels (AP), enables cell integrity, and has receptors for drugs and hormones.

10- Sarcomere: The anatomo-functional unit of the muscle is illustrated with number 10 of Figure 18. It is the distance between two Z lines.

INTRACELLULAR CHANNELS INTEGRATED IN THE SARCOPLASMIC RETICULUM MEMBRANE

I) Ca2+ release channel, ryanodine receptor hyperphosphorylated by protein kinase A (PKA) from the intracellular sarcoplasmic reticulum, or calcium release channel (CRC).

II) SERCA or Ca2+-ATPase uptake pump, sarcoplasmic Ca2+-(ATPase reticulum

SERCA), Sarco/Endoplasmic Reticulum Ca2+-ATPase, calcium ATPase type PATPase

or Ca2+ ATPase of cardiac sarcoplasmic reticulum.

Function: SERCA is an enzymatic pump located in the intracellular milieu in the sarcoplasmic reticulum. It is a Ca2+-ATPase enzyme, the function of which consists of transferring Ca2+ from the cytosol of the cell into the SR by ATP hydrolysis during muscle relaxation. There are three major domains in the cytoplasmic surface of SERCA: the domain where phosphorylation occurs, the binding domain with a nucleotide with catalytic function, and the domain called “acting”, involved in transmitting shape changes. Moreover, SERCA1 is thermogenic in some adipocytes (103).

Regulation: the enzymatic complex, SERCA, is inhibited by a protein called phospholamban, from which it is very close and highly associated. When phospholamban is phosphorylated by protain kinase A (PKA), it loses its capacity to inhibit the SR SERCA pump. Thus, PKA activation such as by action of beta-agonist epinephrine may increase myocyte relaxation rate. Additionally, since SERCA is more active, the next AP will cause an increase in Ca2+ release, leading to an increase of the positive inotropic effect. When phospholamban is not phosphorylated, such as it occurs when PKA is inactive, it may interact inhibiting SERCA. The total effect of phospholamban is accelerating the muscle relaxation rate and increasing contractility, that increases in this way, the heart rate and the systolic volume respectively (104). Another protein is calsequestrin, which binds to Ca2+ within the SR, contributing

to reducing free Ca2+ cation concentration within the SR, and for this reason it is

considered to help SERCA by making this reduce free Ca2+ within the SR, and

thus serving as an assistant to SERCA. The protein calsequestrin 2 (CASQ2) is for Ca2+ storage in the SR. An autosomal recessive disease was recently identified in a family of Bedouins with a missense mutation, that consists of exchanging aspartic acid by histidine in calsequestrin 2 (CASQ2), thus originating a tendency to appearance of catecholaminergic polymorphic ventricular tachycardia with a deleterious effect for Ca2+ cation storage.
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Viatchenko-Karpinski et al, studying CASQ2 with mutations caused by adenovirus, proved at cellular level, the relationship between the mutation in CASQ2 and a predisposition to adrenergic-induced ventricular arrhythmias, observed in patients carriers of this defect in CASQ2. The concentration of Ca2+
within the SR is much greater than in the intracellular millieu. The rate with which SERCA shifts Ca2+ through the membrane of SR could be controlled by phospholamban (PLB/PLN) under β–adrenergic stimulation. When PLB is associated to SERCA, the rate of Ca2+ movement is lower, and when both dissociate, the movement of Ca2+ increases.
Paralogs: They are genes related to duplication within a genome. Unlike the so-called orthologs, that keep the same function over the course of evolution, paralogs evolve into new functions, even if related to the original function. There are 3 major paralogs, SERCA1-3, which express at several levels in different types of cells. ATP2A1–SERCA1; ATP2A2–SERCA2; ATP2A3-SERCA3. There are also additional post-translational isoforms (in biology, translation is defined as the process by which messenger RNA affects the sequence of amino acids during protein synthesis) of SERCA2 and 3, which serve to reintroduce the possibility of a specific cell type of Ca2+ reuptake, that may also respond with an
increase of global complexity of the Ca2+ signaling mechanism.
III) IP3 or inositol triphosphate receptor, 1,4,5-triphosphate (IP3R) receptor channel, inositol triphosphate receptor (IP3R), inositol triphosphate receptor Ca2+ release channels (105).
This is another channel with glycoprotein structure of PM 315,000, and made up by 2749 amino acids, Ca2+-selective integrated to the SR membrane, belonging

to a family of Ca2+ release channels, with a high degree of homology with the ryanodine receptor. Made by 3 paralogs that may form homo-oligomers or hetero-oligomers. The one with greatest expression, called IP3R-1, is spread in all types of tissue, and in all stages of development of life. IP3 receptor has 4 binding sites with 9 different exons. These combinations enable transcription to modulate its pharmacological activity.

Function: Ca2+ release within the cytoplasm (cytosol) from the SR as a response to several stimuli. Its main trigger is InsP3, inositol triphosphate, or IP3. This is known as the second cellular messenger, made up from phosphatidylinositol-biphosphate, by action of the enzyme phospholipase C,

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which turns it into inositol triphosphate or IP3. In turn, this enzyme could be activated by angiotensin II, and α adrenergic stimulation. It is located in Purkinje cells, intercalated discs, conduction system, and the smooth muscle. IP3 regulates several physiologic functions, such as gene transcription, secretion, learning, and memory.

IV) Monovalent ion channels: H+, Cl-, K+, and Na+.

GAP JUNCTIONS

Intercalated discs are the sites of the membrane where cardiomyocytes connect. Adherens or desmosomes, and gap junctions are located in intercalated discs, and ensure the mechanical coupling, thus enabling cardiac electrical impulse to spread.

Arrhythmogenic right ventricular dysplasia (ARVD) is an entity that affects these structures, and consequently the mechanical coupling with electric organic defficiency, and a tendency to fatal arrhythmias occurrence (**106**). In boxer dogs, one of the animal models of ARVD, severe mechanical and electrical modifications were observed in cell-to-cell interaction, with a significant reduction in gap junctions density, a factor that promotes the appearance of malignant ventricular arrhythmias. This model may help in the advancement of our understanding of molecular basis, pathophysiology, and potential therapeutic approach in patients carriers of ARVD (**107**).

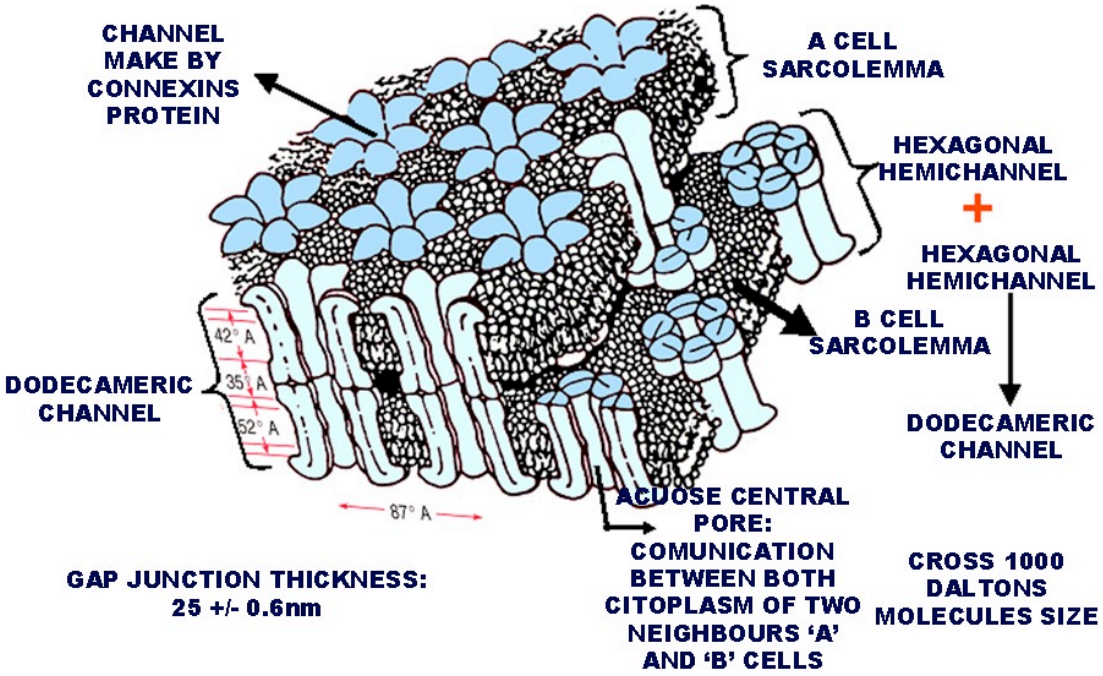
Gap junctions are electrical points of continuity between cardiac cells and between smooth muscle fibers.

These structures are protein channels with low resistance, dodecameric (12 structures), constituted by hexagonal hemichannels, arranged around a central watery pore with a 9 to 11 nm diameter, and located in the sarcolemma of neighbor cells. This pore enables the passage of molecules of up to 1000 daltons, and provides access to the cytoplasm of the two neighbor cells.

Figure 19 shows a dodecameric structure of gap junctions, composed by 2 hexagonal hemichannels that surround a central watery pore, which enables the passage of small molecules. These structures are made up by proteins called connexins (**108**).

FIGURE 19

IMAGE OF GAP JUNCTION



Which are the functions of gap junctions?

1) Enabling the electrical binding between two adjacent cells, thus AP spreads more easily from fiber to fiber;
2) Cardiac gap junction channels are crucial for conducting electrical impulse between cardiomyocytes;
3) Structurally, they may be constituted by connexin 40 (Cx40), connexin 43 (Cx43), and connexin 45 (Cx45). A fourth isoform, Cx37, expresses in the endothelium;

4) Enabling a greater conduction velocity in the site where they are. Because they are located in the longitudinal direction of the fiber, conduction velocity is two to three times greater in the longitudinal direction than in the transversal direction (anisotropic conduction). This longitudinal arrangement of gap junctions explains why dromotropic disorders and blocks occur more frequently in the longitudinal direction;
5) Providing a biochemical coupling by enabling cell-to-cell movement of small molecules such as high-energy phosphates (energetic support, growth control, and embryogenesis), e.g. ATP. These are small molecules that may go through, because gap junctions enable the passage of elements of up to 1000 daltons.
6) Suppression of tumor genes (Cx43, Cx32, and Cx36);
7) Adhesive function, independent from dromotropic properties.

The proteins that make up the gap junctions are known as connexins. The most abundant connexin is found in the heart, and is connexin 43, and to a lesser degree, connexin 40 (Cx40) and 45 (Cx45) (**109**).

In the ventricles, there is a large amount of connexin 43 and 45, and a very small amount of connexin 40. SA and AV nodes only have connexins 40 and 45, and in the atria, there is a large amount of three types, however, connexin 40 (Cx40) and the largest gap-junction protein in atrial muscle tissue. Cx40 expressed abnormally increases the vulnerability to occurrence of atrial fibrillation, which is triggered by alteration in the genetic formation of thoracic veins (**110**).

Connexin 43 is the main decisive factor between the electrical properties of the cardiac muscle (**111**). Closure of gap junctions at the level of this connexin causes negative dromotropism.

Purkinje cells have a greater concentration of gap junctions in comparison to bundle cells, which explains why the septal fascicle of the His bundle left branch (AF) activates the left middle surface earlier than the anterior fascicle and posterior fascicle (PF). This Purkinje cell has very prominent and abundant gap junctions, with a rapid termino-terminal and side-to-side transmission. The termino-terminal one is mainly constituted by connexin 43.

The entities that hamper gap junction conduction have arrhythmogenic potential. On the contrary, drugs that open these structures could potentially be

used as another management strategy for arrhythmias. Peptide ZP123 increases conductance in gap junctions, significantly decreasing their closure during acidosis. This property of decreasing intracellular binding in these conditions, shows the antiarrhythmic potential of the drug in conditions of acidosis.

Gap junctions are properly developed in Purkinje cells and in cells with which bundle fibers bind, and ventricular myocardial cells; they are very prominent and abundant, with fast termino-terminal and side-to-side transmission. The first one is mainly made up by connexin 45.

Note: Purkinje cells usually make up groups of three, yielding an aspect of Y. This arrangement is the anatomical basis for the main mechanism of arrhythmias: anatomical reentry.

These cells are located in the His bundle, Purkinje branches and arborizations, with less density in the baseline region of the ventricles, and tip of papillary muscles. Additionally, they are observed in a low amount in the preferential pathways or interatrial bundles.

TABLE 9

SUMMARY OF SARCOLEMMAL AND INTRACELLULAR ION CHANNELS

A) Sarcolemma:

1) Rapid Na^+ channel: voltage-gated Na^+ current, sensitive to tetrodotoxin (TTX).

2) $I_{Na\text{-}B}$: inward Na^+ current by a voltage-independent channel, in the final part of AP phase 0 of SA node cells.

3) Transient outward K^+ current, I_{to}, I_{to1}, $I_{to\text{-}fast}$, $I_{to\text{-}f}$, I_{toA}, 4-aminopyridine-sensitive outward K^+ current.

4) I_{to2}, IC^{-l}, Ca^{2+}, or Cl^- channel activated by Ca^{2+}, transient outward K^+ resistant to 4-AP, transported by Cl^- anions, and modulated by the percentage of intracellular Ca^{2+}.

5) I_{CLcAMP} or time-independent chloride (Cl^-) channel, regulated by cyclic AMP pathway.

6) $I_{Cl\text{-}edema}$ or outward Cl^- channel activated (rectified) by edema, swelling-activated chloride channel, $I_{Cl\text{-}SWELL}$, swelling-activated, outward rectifying chloride channel. This channel belongs to the category of stretch-activated ion

channels. The $I_{Cl\text{-}edema}$ channel is inhibited by anthracene-9-carboxylic acid, tamoxifen, and natriuretic peptide precursor B (NPPB) and diisothiocyanatostilbene-2,2'-disulphonic acid (DDS) (**112**). It shortens AP and causes depolarization.

7) Long-lasting, L-type Ca^{2+} currents. Slow inward Ca^{2+} channels, that operate in phase 2 of rapid fiber and phase 0 of slow fiber in the SA node and AV node. Blocked by dihydropyridine Ca^{2+} antagonists, phenylalkylamines, benzothiazepines, and by divalent ions such as manganese (Mn), cobalt (Co), nickel (Ni), and lanthanum (La).

8) Delayed inward Na^+ channel in phase 2; late I_{Na}. In ischemic disease, the inhibition of this channel by ranolazine, reduces recurrent ischemia, Ca^{2+} overload, and electrical and mechanical dysfunction (**113**).

9) K+ rectifier channels, delayed rectifier channels.

(9-1) Slow activation channel (I_{Ks})

(9-2) Rapid activation channel (I_{Kr})

(9-3) Ultra-rapid activation channel (I_{Kur}).

10) K^+ channel, I_{K1}, inwardly rectifying K^+ current, continued background inward steady-state K^+ current, inwardly rectifying Ba(2+)-sensitive current, Ba^{2+}-sensitive current, continued backward inward steady-state K^+ current, or inward K^+ rectifying I_{K1}. I_{K1} is responsible for maintaining the resting potential of the atria, ventricles, and His-Purkinje system. I_{K1} acts in the final portion of phase 3 and onset of phase 4 of atrial AP, in a range of voltage between resting potential and -30 mV.

11) Rectifier inward K^+ current, $I_{K(Ach)}$, $I_{K(Ado)}$, acetylcholine-activated K^+ currents: activated by stimulation of parasympathetic M2 muscarinic receptors with release of acetylcholine and by stimulation of type I purinergic receptors via regulators of G protein signaling (GTP) transduction. The current also opens by action of stimulation to the adenosine receptor (adenosine triphosphate (ATP)-sensitive K^+ channels). The drug levosimendan belongs to the category of “calcium sensitizer”, which increase the sensitivity of the heart to Ca2+ without increasing the percentage of intracellular Ca^{2+} by binding to troponin C in a calcium-dependent way (**114**). The drug also has a vasodilator effect by opening the channels in the smooth muscle of vessels. Both effects in combination lead to an increase of contraction force, decreasing pre-load and

after-load, besides a cardioprotective effect, acting on mitochondrial (ATP)-sensitive K^+ channels. They express in the SA node, AV node, and atrial muscle, where they cause hyperpolarization and AP shortening (**115**).

12) Pacemaker current or I_f activates by hyperpolarization of the membrane permeable to K^+ and to Na^+, being 4 times more permeable than the former. They are modulated by cyclic nucleotides, called HCN. Four types of kinetics are known, dependent on temperature, and called:

- HCN1: activates more rapidly between 30 and 300 ms. Activation potentials range between -70 mV and -140 mV.
- HCN2: intermediary activation kinetics between 200 and 500 ms.
- HCN3: intermediary activation kinetics between 200 and 500 ms.
- HCN4: slow activation kinetics between 300 ms and several seconds. Activation potentials range between -75 mV and -110 m V.

13) Rapid Ca^{2+} channel, $I_{Ca\text{-}T}$, fast T type, transient current, or tiny conductance. It causes Ca^{2+} inflow in the final part of phase 4 in the SA node, N region of the AV node, and His-Purkinje system. Selectively blocked by the Ca^{2+} antagonist, mibefradil. Insensitive to dihydropyridine. They increase their function with noradrenaline, the α-adrenergic agonist phenylephrine, percentage of extracellular ATP, and endothelin 1.

14) $I_{Na^+}/_{Ca^{2+}}$ exchanger or sodium/calcium counter transport system. This is an electrogenic exchanger current of cations in opposite directions: three outward Na^+ ions exchanged by one inward Ca^{2+} ion. This current contributes to depolarization, extends AP phase 2, and contributes to depolarization during diastole. Its function consists of placing the Ca^{2+} that entered in phase 2 in the extracellular milieu, and thus being mainly useful in conditions of intracellular Ca^{2+} overload. More rarely, this exchange could be the opposite, i.e. inflow of Na^+ through the Na^+/Ca^{2+} exchanger current, operating in a reverse way.

15) Na^+/K^+ATP_{ase} pump: it acts in phase 4 with energetic output. It places three Na^+ cations in the extracellular milieu, and introduces one K^+ ion. This pump is inhibited by digitalis.

16) $Ca^{2+}ATP_{ase}$: it removes cytosolic Ca^{2+}, placing it in the extracellular milieu by energetic output, transforming ATP into ADP + Pi.

17) Na^+-K^+-$2Cl^-$: co-transporting channel, blocked by amiloride. This is another electroneutral protein of ion exchange (**116**).

B) Intracytoplasmatic

18) SERCA or Ca^{2+}-ATP_{ase} or Ca^{2+} Mg^{2+} ATP_{ase} uptake pump; sarcoplasmic Ca^{2+}-ATP_{ase} reticulum SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATP_{ase}, calcium ATP_{ase} type P-ATP_{ase}, or Ca^{2+} ATP_{ase} of cardiac sarcoplasmic reticulum. It transfers Ca2+ from cytosol from the cell into the SR by hydrolysis of ATP during muscle relaxation.

19) IP3, inositol triphosphate or IP3 receptor, 1,4,5-triphosphate (IP3R) receptor channel, inositol triphosphate receptor (IP3R), inositol triphosphate receptor Ca^{2+} release channel.

20) Monovalent ion channels: H^+, Cl^-, K^+, Na^+ adhered to the sarcoplasmic reticulum membrane.

21) Na+/H+: electroneutral protein of cation exchange of intracellular H^+ by extracellular Na^+. Cardiomyocytes express the isoform NHE1. Its inhibition causes acidification within the cell. Inhibited by the derivatives of benzylguanidine.

C) Sarcolemmal and intracellular inward K^+ currents

22) I_{K1}, continued background inward steady-state K^+ current, rectifier inward K^+ current in a range of voltage between resting potential and -30 mV. It acts in the final portion of phase 3 of the AP from the atria, AV node, His-Purkinje system, and ventricular myocardium cells. **Function:** maintaining resting potential in the atria, AV node, and ventricular muscle. **Stimuli:** Hyperpolarization. **Channel blockers:** Ba^{2+}, I_{KArch}.

23) $K\text{-}_{ATP}$ or $I_{K\text{-}ATP}$, adenosine triphosphate-activated K^+ current (K_{ATP}), ATP-sensitive K(+) channels. Rectifier inward K^+ current, activated by muscarinic M2 receptors and stimulation of purinergic I receptors via regulators of G protein (GTP) signaling transduction. The stimulus occurs when ATP intracellular percentage falls. This happens in clinics, mainly in myocardial ischemia. The activation of this current causes AP shortening. Pinacidil, cromakalim, and nicorandil also open this channel. Sulfonylureas, such as glibenclamide, inhibit

this channel. According to the position they hold within the cell, three groups are identified:

1) Sarcolemmal, SarcK_{ATP};

2) Mitochondrial, MitoK_{ATP};

3) Nuclear K_{ATP}, nucK_{ATP}.

Conclusion:

The diastolic potential of cardiac fibers in rest is maintained by a different anionic and cationic concentration between the intracellular and extracellular milieu. The predominance of negative charges inside is due to a great amount of amphoteric proteins, which in intracellular pH dissociate predominantly their negative charges.

By the characteristics of its phase 0, cardiomyocytes are classified into rapid (sodium-dependent), located in atrial and ventricular contractile cells, in internodal bundles and th eHis-Purkinje system; and slow (calcium-dependent) in the SA node, AV node, and mitro-tricuspid rings.

The action potential of cardiomyocytes have five successive phases: phase 0 of depolarization (QRS), phase 1 of rapid repolarization (J point), phase 2 (ST segment) mainly dependent on slow calcium inflow, phase 3 of rapid repolarization (T wave) and phase 4, which could be stable (nonautomatic cells), or spontaneously ascending (automatic), coinciding with U wave.

The sarcolemma is equipped with numerous transmembrane Na^+ channels of initial outward K^+ in phase 1, $I_{Ca(L)}$ (phase 2), slow (I_{Ks}), rapid (I_{Kr}), and ultra-rapid (I_{Kur}) delayed rectifier channels of phase 3. Finally, the pacemaker (I_f) channel and the rapid Ca^{2+} channel ($I_{Ca\text{-}T}$), the $Na^+/K^+{}_{ATPase}$ pump with energetic output, and the I_{K1} channel or rectifier inward current act initially in phases 3 and 4. The I_{kAch} channel of phase 4 depends on acetylcholine. K_{ATP} or rectifier inward K^+ current is activated by muscarinic M2 I receptors.

There are intracellular channels located in the sarcoplasmic reticulum and mytochondria, such as the Ca^{2+} release channel, ryanodine receptor (RyR2), Ca^{2+} or Ca^{2+} + Mg^{2+} +ATP_{ase} uptake pump, SERCA, or cardiac calsequestrin (CASQ2), or inositol 1,4,5-triphosphate receptor (IP_3R), and permeable pathways for monovalent ions: H^+, Cl^-, K^+, and Na^+.

Finally, there are structures called gap junctions, with low resistance, dodecameric, located in the sarcolemma of two neighbor cells, which enable the passage of molecules and provides access to the cytoplasm of two neighbor cells, enabling a better propagation of impulse.

The numerous ion channels may present genetic mutations that will originate the different channelopathies, such as long and short QT syndromes, Brugada syndrome, and familial catecholaminergic polymorphic ventricular tachycardia. Different drugs act on these channels, modifying their electrophysiologic and electropharmacological properties.

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