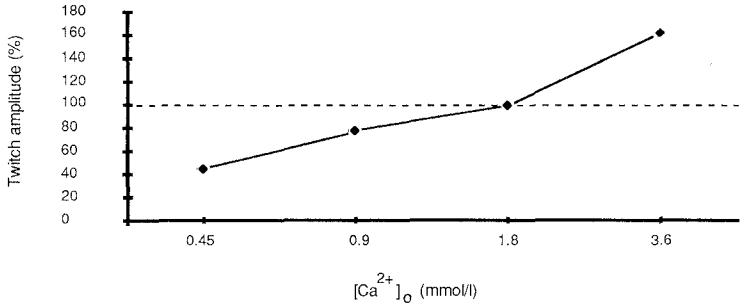

Figure 4. Twitch amplitude (represented as sarcomere shortening) of a single cardiac myocyte exposed to different concentrations of extracellular calcium. The values have been normalized with respect to that at the standard calcium concentration (1.8 mmol/l, dotted line). Stimulation frequency was 0.4 Hz.

mere shortening with time, a large amplitude of the first twitch after a rest period, or aftercontractions. In cases of severe Ca^{2+} overload the diastolic sarcomere length was found to be considerably shorter, though. Subsequent measurements showed a gradual development of irreversible contractures.

In conclusion, the contractile parameters investigated demonstrate that isolated adult myocytes exert a mechanical behavior similar to that of intact cardiac muscle. They represent a useful preparation for studying mechanical properties alone or in conjunction with electrical measurements.

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Currents through ionic channels in multicellular cardiac tissue and single heart cells

by D. Pelzer and W. Trautwein*

II. Physiologisches Institut, Medizinische Fakultät, Universität des Saarlandes, D-6650 Homburg/Saar (Federal Republic of Germany)

Summary. Ionic channels are elementary excitable elements in the cell membranes of heart and other tissues. They produce and transduce electrical signals. After decades of trouble with quantitative interpretation of voltage-clamp data from multicellular heart tissue, due to its morphological complexness and methodological limitations, cardiac electrophysiologists have developed new techniques for better control of membrane potential and of the ionic and metabolic environment on both sides of the plasma membrane, by the use of single heart cells. Direct recordings of the behavior of single ionic channels have become possible by using the patch-clamp technique, which was developed simultaneously. Biochemists have made excellent progress in purifying and characterizing ionic channel proteins, and there has been initial success in reconstituting some partially purified channels into lipid bilayers, where their function can be studied.
Key words. Cardiac tissue; single heart cell; voltage clamp; patch clamp; ionic channel current.

Introduction

Excitation and electrical signaling in nerve, muscle and other tissues involve the movement of ions through ionic channels. Na^+, K^+, Ca^{2+} and Cl^- ions seem to be responsible for almost all of the action. Each channel may be regarded as an excitable molecule as it is specifically responsive to some stimulus: a membrane potential change, a neurotransmitter or other chemical stimulus, a mechanical deformation, and so on. The channel's response, called 'gating', is the opening and closing of the macromolecular membrane-bound pore. The open channel has the important property of 'selective permeability', allowing some restricted class of small ions to flow passively down their electrochemical gradients at a very high rate ($> 10^6$ ions per second). These ionic fluxes generate electric currents across the membrane and therefore they have an instantaneous effect on membrane potential. Other voltage-sensitive channels in the membrane detect the potential change, and they in turn become excited. In this way, the electric response is made regenerative and self-propagating. However, the ultimate purpose of excitation is not to make electricity so that other channels will be excited and make electricity, except in the case of an electric organ. Electricity is the means to carry the signal to a point where a nonelectrical response is generated. As a broad generalization, most excitable cells translate their electricity into action by Ca^{2+} fluxes modulated by voltage-sensitive Ca channels or Ca^{2+}-permeable channels, either on the surface membrane or on an internal membrane. The ultimate response is then triggered by the internal Ca^{2+} ions which are an intracellular messenger capable of activating many cell functions. This is how the nervous system controls the contraction of a muscle fiber or the secretion of neurotransmitters, hormones, enzymes, and so on. Internal free Ca^{2+} also controls the gating of some channels and the activity of many enzymes. The foregoing has been recently reviewed by Hille[77] in his most useful monograph.

Ionic channels are undoubtedly found in the membranes of all cells. Their known functions include establishing a resting membrane potential, shaping electrical signals, gating the flow of messenger Ca^{2+} ions, and controlling cell volume. The emphasis of this article is on Na, Ca and K channels underlying the action potentials of cardiac tissue. In the past, a quantitative interpretation of ionic mechanisms in heart was complicated by spatial electrical inhomogeneity due to the complex morphology of cardiac tissue, by local ion accumulation or depletion, and by inadequate tools for unequivocal separation of the various ionic current components. The advent of enzymatically isolated heart cells and tight-seal suction pipette and patch-clamp techniques nowadays allows the recording of ionic channel activity at the level of single cells and even single-channel molecules. It is possible now to study individual ionic channel species in isolation from other ionic pathways, with rapid and uniform control of membrane potential and external and internal solute compositions. In addition to progress in voltage clamping, new classes of drugs and biotoxins, interacting with ionic channels in a potent and highly specific manner, have aided our understanding of ionic channel structure and function. Nevertheless, all discussions of pores, filters, gates, and sensors remain abstract until the molecular structures of channel molecules are known and correlated to channel function. Thus, we will briefly consider molecular and functional approaches in the study of channel proteins.

Biophysical techniques for studying ionic channels in multicellular and unicellular cardiac preparations: capabilities and limitations of existing methods

Studies of the action potential established the important concepts of the ionic hypothesis. These ideas were proven and given a strong quantitative basis by an experimental procedure developed by Marmont[114], Cole[41], and Hodgkin, Huxley and Katz[79,80], and introduced to cardiac electrophysiology by Weidmann[188]. The method, known as the 'voltage clamp', has been the best technique for the study of ionic channels for about three decades. Any scheme used to voltage clamp active tissue is designed to allow, simultaneously, the accurate measurement of the cell membrane potential of that tissue and the passage of sufficient current across the membranes of the tissue, in such a way that the membrane potential can be controlled. In brief, effective or virtual electrodes should be placed on either side of the membrane throughout the preparation. Therefore, an ideal cardiac tissue for voltage clamping would be a single cell, large enough to allow the insertion of a chlorided silver wire down the middle. If the wire had zero resistance, then the space constant λ ($\lambda^2 = r_m/r_i$) would be infinite and the voltage distribution uniform.

Before single heart cells became available for biophysical approaches in the early 1980s (see Trube[181] for review), voltage clamping of cardiac tissue was performed on small bundles of cells orientated parallel to each other and interconnected by low-resistance junctions. Such bundles can be regarded as a functional syncytium. Since, however, electrodes, either real or virtual, can only be placed in localized regions of these multicellular cardiac preparations, bundles of cells must be treated as cables ($\lambda \ll \infty$), with the result that a voltage drop along the bundle must always be present. Attempts were made to achieve spatial uniformity of potential in short naturally occurring strands of atrial and ventricular muscle and Purkinje fibres, usually by using a small membrane area for current recording delimited by gaps, partitions, and barriers applied by the experimenter (single gap, double gap; see Beeler and McGuigan[7] for review). Certain multicellular preparations such as rabbit Purkinje fibres[40] and small pieces of bulk sinoatrial[142] and atrioventricular[144] nodal tissue approximated to a single cell. They were sufficiently small for the relatively low currents for clamping to be applied through a microelectrode[49]. In such simple cables, spatial variations of membrane potential measured by a second microelectrode, and the resulting spread of local circuit current, were minute. By eliminating the need for division of the extracellular compartment into pools by gaps of vaseline, sucrose, or air, shunt currents were avoided to a great extent, and a direct measurement of membrane currents was largely achieved. Even in these exceptionally favorable tissues, more than a millisecond was required to achieve voltage control. In all other preparations, the gap techniques of current application to multicellular heart tissue were in most cases the method of choice to allow for uniform planar spread of the current[62], where a double gap was theoretically worse than a single gap[7]. In conclusion, the methodology of clamping multicellular cardiac preparations was really an attempt to minimize cable complications. Unavoidable technical limitations often led to artifacts due to lack of voltage control (skewed current-voltage relations, current oscillations, 'inverted action potentials', and so on) and/or errors of measurement (E_{rev}) and interpretation (kinetics)[7]. Additionally, the narrow intercellular spaces have mostly resulted in local accumulation or depletion of ions, altering the ionic gradients that must be known or controlled if quantitative studies are to be made[4,113].

The advent of electrically and metabolically intact and Ca-tolerant single heart cells[81,91,92,157,171,176] greatly improved the technical situation for voltage clamping with two microelectrodes[93] or suction pipettes of various shapes and sizes[104,111]. In single cells, a voltage step can be completed within about 100 µs, which is at least an order of magnitude improvement over the time for multicellular preparations[65]. Their use furthermore opened up the possibility of control-

ling the ionic and metabolic environment on the outside and inside faces of the cell membrane in a quantitative manner[12]. Additionally, a new type of biophysical technique developed by Neher, Sakmann and colleagues[71] has revolutionized the study of ionic channels including those in cardiac cell membranes[161]. The method, known as the 'patch clamp', directly measures elementary currents through single ionic channels in a minute patch of membrane sealed to the tip of a glass pipette. Moreover, this technique is versatile. Four different configurations are commonly used, each of which allows one to expose any portion of the cell membrane to experimental solutions. All of them start with a clean pipette pressed against an intact cell to form a gigaohm seal between the pipette and the membrane it touches. The patch can be studied in place and single channels can be recorded in this cell-attached or on-cell mode. To be able to perfuse the patch membrane, Noma and coworkers[145,175] have developed a method of intrapipette dialysis. Or the patch may be pulled off the cell to form the inside-out configuration. In this excised patch recording mode, the cytoplasmatic side of the membrane faces the bath and can be easily exposed to a variety of test solutions. An on-cell patch may also be deliberately ruptured and the pipette may be used to record from the whole cell provided that the cell is small enough. This configuration additionally allows for a fast diffusional exchange between pipette and cell interior solutions which can be supported and quantified by intraelectrode dialysis. Again, the pipette may be pulled away to excise an outside-out patch. The methods and their applications are well described in an original paper[71] and in a book[165], and are summarized in a recent review[166]. The gigaseal method has also been modified to record reconstituted ionic channels in lipid bilayers formed at the tip of glass patch pipettes[61,72] or excised from giant proteoliposomes[121].

Almers and colleagues[1] have used the principle of the patch clamp to measure currents from large areas of membrane in intact tissue. Since seals to multicellular tissue are of less resistance, this method has been called 'loose' patch clamp. Leak currents are subtracted electronically and accurate recordings of net ionic currents through the patch are obtained. The advantage of this technique is that ionic currents may be sampled under more physiological conditions, but the use of this method in cardiac tissue has not yet been reported.

Ionic channels of cardiac cell membranes

The electrophysiology of the heart is notoriously complicated[130]. Table 1 gives an overview of the various ionic currents observed in multicellular cardiac preparations and single heart cells and of the respective channels identified in patches of cardiac cell membrane. The variety of channels is puzzling, a feature that heart shares with other excitable tissues[77].

The massive amount of information gleaned from the multiple ionic currents and channels in cardiac tissue and single heart cells using the various biophysical methods has led to numerous published papers. Major classical references are listed below (table 2), but the references used are only a small fraction of the important work in the area. Hence we must apologize in advance to many of our colleagues whose relevant work is not quoted directly.

The theoretical and experimental problems in the measurement of membrane currents under voltage-clamp conditions in multicellular preparations of the heart (diffusionally restricted extracellular spaces, spatial electrical inhomogeneity, interdiffusion of sucrose and physiological solutions into the adjacent compartments, spatial spread of current through the tissue from a current passing electrode) have obviously not prevented identification of most of the ionic current components. A thorough comparison shows that currents measured in single isolated cells, which are free of the multicellular limitations, are similar to those of multicellular preparations in ionic nature, drug sensitivity, and voltage dependence. These similarities are doubly reassuring, since they lend credence on the one hand to voltage-clamp data from tissue and on the other hand to the assumption that enzymatic dispersion of this tissue does not alter the electrical activity of the cell membrane. However, three major differences are worth noting: 1) currents due to extracellular accumulation or depletion of K^+ are absent in single cells, 2) inward Na and Ca currents have faster and more complex kinetics than those measured in multicellular preparations, and 3) currents measured with long clamp pulses are more outward in multicellular preparations at potentials positive to −20 mV[186].

Recent developments concerning each type of net ionic current are summarized below. References are not included, since all information is extracted from those papers listed in table 2.

Inward currents

I_{Na} (fast and slow component): The recent progress in the analysis of cardiac I_{Na} is primarily due the advent of single heart cells and improved conditions for voltage clamping. Direct single-channel recordings yield a conductance of about 15 pS at 16–18 °C. The Na current changes its magnitude upon depolarization by increased probability of opening and by a somewhat longer mean open time. Inactivation results from a reduced opening probability. Single Na channel behavior averaged over many traces from cell-attached patches compares well with net I_{Na} measured in whole-cell recordings. Tetrodotoxin (TTX) blocks these channels from the extracellular face but not from the intracellular side. Na channels can be recorded in cell-attached as well as in excised membrane patches. After membrane detachment, there is a large shift in kinetics toward more negative potentials. The Na-channel density per surface area is 1–16 channels μm^{-2}. Whether the distribution of channels is random or localized is not yet known. Some studies have offered evidence, based on channel currents, kinetics, and/or response to TTX, that there may exist two populations of Na channels. There are also several strong indications that cardiac Na currents and channels are not accurately described by the original Hodgkin-Huxley mathematical formulation. Na channels may function in several different 'modes', each with a different inactivation rate.

I_{Ca} (L- and T-type channel current): L-type Ca channels respond to membrane depolarization in a steeply voltage-dependent manner (conductance about 25 pS with 50 mM $[Ba^{2+}]_o$, about 8 pS with 50 mM $[Ca^{2+}]_o$, about 0.5 pS with physiological $[Ca^{2+}]_o$). Channel open times and the probability of channel opening increase with increasing deplarization. The opening pathway (activation) is viewed as fast transit through two short-lived voltage-dependent closed states before the open state is entered. This view is consistent with the finding that Ca current activation follows a sigmoidal time course. Inactivation comes from a reduced probability of opening due to voltage-dependent slow gating transitions into at least one long-lived electrically silent channel state. Each channel inactivates monoexponentially with a different rate resulting in an incomplete multi- or non-exponential inactivation of macroscopic L-channel Ca current. Besides voltage, additional intracellular mechanisms ($[Ca^{2+}]_i$-dependent enzyme reaction(s)) may contribute to inactivation in the sense of a joint regulation. A large body of work has quantitatively shown that β-adrenergic stimulation primarily increases the availability of the channel to open on depolarization via cAMP-dependent phosphorylation of the channel protein. A recruitment of channels has never been

Table 1. Currents through ionic channels in cardiac tissue and single heart cells*

Current	Charge carrier	Activation mechanism	Tissue	Cell	Channel On cell	Excised	Function
Inward currents							
$I_{Na(fast)}$	Na^+	Voltage	+	+	+	+	AP-Upstroke
$I_{Na(slow)}$	Na^+	Voltage	+	+	+	+	?
$I_{Ca(L)}$	Ca^{2+}	Positive voltage	+	+	+	(+)	AP-Plateau; E.C.-coupl. impulse initiation and conduction (S–A & A–V)
$I_{Ca(T)}$	Ca^{2+}	Negative voltage	–	+	+	+	?
I_{TI}	Na^+ and K^+	$[Ca^{2+}]_i$	+	+	–	+	After-depolarization
I_f	Na^+ and K^+	Voltage	+	+	+	–	Impulse initiation (Purkinje fiber)
Outward currents							
I_{X1}	K^+ (+ Na^+)	Voltage	+	+	+	–	Repol.
I_{X2}	K^+ (+ Na^+)	Voltage	+	–	–	–	Repol.
I_{K1}	K^+	Voltage	+	+	+	+	Resting potential; repol.
$I_{K(ACh/Ado)}$	K^+	ACh Adenosine	+	+	+	+	Hyperpol. Inhibition
$I_{K(Na)}$	K^+	$[Na^+]_i$	–	+	–	+	Hyperpol.
$I_{K(ATP)}$	K^+	Lack of ATP	+	+	+	+	Repol.
$I_{K(Ca)}$	K^+	$[Ca^{2+}]_i$	+	–	–	–	Repol.
I_{bo} (I_{qr}?)	K^+	Voltage and $[Ca^{2+}]_i$	+	+	+	+	Early notch of AP (Purkinje fiber); repol. (rat ventricle)
I_{lo} (pos. dynamic I?)	K^+	Voltage	+	+	–	–	Early notch of AP (Purkinje fiber); repol. (rat ventricle)
I_{to} (pos. dynamic I?)	K^+ and Na^+	Voltage	–	+	+	–	Hyperpol.; latent pace-maker (A–V)
I_{Cl}	Cl^-	(Voltage?)	–	–	–	+	?

+ Identified. – Not identified.
* Modified from Reuter[161].

Table 2. References of voltage-clamp studies on currents through cardiac ionic channels at the level of multicellular tissue, single cells and single channels (see bibliography)

Inward currents		
1) $I_{Na(fast)}$	Tissue	178, 159, 40, 39, 58
	Cell	112, 13, 9, 18, 161, 65
	Channel	19, 70, 177, 152, 161, 65
2) $I_{Na(slow)}$	Tissue	57, 3, 38, 59, 26, 68
	Cell	13, 63, 65
	Channel	19, 177, 152
3) $I_{Ca(L)}$	Tissue	178, 159, 44, 117, 160, 130, 180
	Cell	160, 183, 185, 76a, 130, 161, 180, 98, 73, 74, 76, 118, 184, 154a
	Channel	162, 34, 20, 160, 183, 185, 11, 17, 75, 161, 154, 180, 35, 76, 109, 118, 184, 157a, 154a
4) $I_{Ca(T)}$	Tissue	Not identified
	Cell	5, 122
	Channel	128
5) I_{TI}	Tissue	110, 101, 2, 23
	Cell	84, 85
	Channel	42
6) I_f	Tissue	189, 37, 52, 144a, 130, 53
	Cell	21, 125, 54
	Channel	14
Outward currents		
1) I_{X1}	Tissue	116, 132, 15, 16, 50, 51, 133, 149, 120, 55, 172
	Cell	82, 69
	Channel	36
2) I_{X2}	Tissue	132, 133, 172
	Cell and channel	Not identified
3) I_{K1}	Tissue	86, 24, 129, 116, 131, 132, 115, 89, 143, 120, 25
	Cell	145, 168, 83, 123
	Channel	6, 100, 145, 168, 169, 83, 107, 153
4) $I_{K(ACh/Ado)}$	Tissue	179, 67, 66, 148, 146, 27, 28, 29, 55, 151, 150, 30, 31, 32
	Cell	8, 167, 87, 108
	Channel	167, 175, 156, 108, 8a, 113a, 113b
5) $I_{K(Na)}$	Tissue	Not identified
	Cell	99
	Channel	99
6) $I_{K(ATP)}$	Tissue	126, 187, 43
	Cell	94, 147
	Channel	141, 96, 182, 97
7) $I_{K(Ca)}$	Tissue	88, 90
	Cell and channel	Not identified
8) Transient outward currents (I_{bo}, I_{qr}, I_{lo}, I_{to}, positive dynamic current)		
	Tissue	155, 158, 64, 78, 102, 103, 174, 173, 10, 45, 106
	Cell	95, 119, 124
	Channel	124, 22
9) I_{Cl}	Tissue and cell	Not identified
	Channel	47

observed. The decrease of Ca channel current by muscarinic inhibition seems to be mediated by inhibition of cAMP production via the GTP-binding protein N_i in mammalian heart, or by cAMP hydrolysis via a cGMP-stimulated cyclic nucleotide phosphodiesterase in frog heart. Phenylalkylamine or dihydropyridine $I_{Ca(L)}$ channel blockers acting from the intracellular face of the membrane promote prolonged periods of channel unavailability, while dihydropyridine Ca agonists induce long-term channel openings or increase the frequency of reopenings, depending on whether Ba^{2+} or Ca^{2+} ions are the charge carrier. Inorganic cations such as Mg^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+} and La^{3+}, as well as H^+, block L-type Ca channel currents. The mechanism of Ca channel 'run-down' or disappearance of Ca channels from dialyzed cells or excised patches is still unclear. Ca channels are multi-ion single-file pores with high conductances for monovalent cations in the absence of $[Ca]_o$ (85 pS for Na^+, 45 pS for Li^+). The high selectivity in the presence of micromolar $[Ca^{2+}]_o$ comes from high-affinity binding of Ca^{2+} to sites within the channel whereas current flow at millimolar $[Ca^{2+}]_o$ ensues from electrostatic repulsion between ions in doubly occupied channels. Ca-channel density is 3–5 channels per μm^2 membrane area and most of the channels may be localized in the T-tubular membrane.

The novel T-type Ca channel activates and completely inactivates at potentials negative to those regulating the L-type Ca channel. Its kinetics are faster and its conductance and opening probabilty are lower (about 8 pS with isotonic $[Ba^{2+}]_o$ and < 0.2 at potentials of I_{max}, respectively). T channels conduct Ba^{2+} and Ca^{2+} ions equally well and function in detached membrane patches. Macroscopic T-channel current inactivation follows a monoexponential time course. T channels seem not to be modulated by cAMP-dependent phosphorylation and are largely insensitive to blocking by most inorganic blockers (except Ni^{2+} and Co^{2+}) and Ca antagonists or augmentation by Ca agonists. T-channel current is smaller and decays more quickly than L-channel current, so it probably contributes relatively little to Ca influx during the action potential plateau and contraction. Although their function is not precisely known, T channels may have significance for pacemaker depolarization and impulse initiation in the nodal regions, since these electrical phenomena critically depend on small inward currents at relatively negative potentials. In the ventricle, T-channel current may serve as trigger for Ca release from intracellular stores.

Transient inward current (I_{TI}): Transient inward or inward 'creep' currents are not mediated by voltage-gated Na, Ca, or K channels or by the electrogenic Na/K pump. I_{TI} in the presence of ouabain, or inward 'creep' currents induced either by Na loading or in response to an increase in the amplitude of I_{Ca}, are triggered by an elevation of $[Ca^{2+}]_i$. Since at least I_{TI} also occurs in the absence of electrogenic Na/Ca exchange, it may be composed of the Ca-activated nonspecific channel current seen in neonatal rat ventricle. Channel opening increases as a function of $[Ca^{2+}]_i$ above 0.5 μM; the single-channel conductance is about 35 pS. This channel does not exhibit significant voltage dependence but is temperature-sensitive. The function of this channel in the normal heart is unknown, but it can be arrhythmogenic under conditions where $[Ca^{2+}]_i$ is increased.

I_f (or I_h, former I_{K2}): The 'pacemaker' current has undergone major revisions in the last few years. It has been completely reinterpreted from a slowly inactivating pure outward K current to a slowly activating nonselective inward Na-K current. In some structures, such as Purkinje fibers, this current plays a role in pacemaker depolarization, whereas it may not contribute to pacemaking in others such as the S-A and/or A-V node. Single I_f channels are of small size (about −0.1 pA at −112 mV and about −0.05 pA at −72 mV with 70 mM $[K^+]_o$) and may not be uniformly distributed on the membrane surface.

Outward currents

I_{X1} and I_{X2}: The delayed rectifier I_{X1} identified in intact tissue and single cells, may be composed of a 62 pS K-selective channel which activates over the range −50 to +10 mV. This channel, as other rectifying K channels, may also carry some Na^+. The 62-pS channel is relatively sparse (1 per 50 μm^2). However, it is clearly different in conductance from the known delayed rectifier channels (15 pS) recorded in other tissues. I_{X2} has not yet been identified at the level of single cells and single channels.

I_{K1}: The inward rectifier channel is the most thoroughly studied heart cell K channel. I_{K1} was originally thought to be a quasi-instantaneous time-independent current, but the recent studies show time-dependent activation and inactivation. The single-channel conductance of I_{K1} is proportional to the square root of $[K^+]_o$. The density of I_{K1} channels is 10–100 times larger in the ventricle than in the S-A or A-V node. The sparsity of I_{K1} channels in nodal tissue may account for the low resting membrane potential and may play a role in making these cells the dominant pacemakers. Single K channel current measurements also confirm the existence of different I_{K1} channel properties in single atrial and ventricular myocytes. I_{K1} channels in both cell types have similar single-channel conductances (30–32 pS with $[K^+]_o = 145$ mM) but ventricular I_{K1} channels have significantly slower gating kinetics compared to atrial I_{K1} channels (ventricular I_{K1} channel mean open time = 223 ms; atrial I_{K1} channel mean open time = 1 ms at V_r −20 mV). These different properties of I_{K1} channels may contribute to the different action potential configurations of single atrial and ventricular cells.

$I_{K(ACh/Ado)}$: $I_{K(ACh/Ado)}$ is a unique current that is similar to, but different from, I_{K1}. Fluctuation analysis of ACh-activated currents in pacemaker tissue has shown that the increased K conductance is due to the opening of a separate class of K channels gated by muscarinic ACh receptors (m-AChRs). On the other hand, it has been suggested that m-AChRs may simply regulate the current flow through I_{K1} channels. Single-channel recordings show that the ACh-dependent K conductance increase in nodal cells is mediated by K channels which are different in their gating and conductance properties from the inward rectifying I_{K1} channels in atrial and ventricular cells. The resting K channels in nodal cells are, however, similar to those activated by ACh. The m-AChR complex increases the K channel open probability directly, rather than by a second messenger. Adenosine (Ado) also increases this K channel current in a dose-dependent manner. However, the maximum responses and the apparent dissociation constants are different for Ado and ACh activations of the current. Recently, it has been shown in the atrial cell membrane that Ado and m-ACh receptors link with the same population of K channels via GTP-binding proteins N_i and/or N_o. Whether the α or the β_γ subunits of G proteins are the mediators of the activation of K channels by m-AChR is still under debate.

$I_{K(Na)}$: In ventricular cells, a large conductance K channel ($\simeq$ 207 pS) is activated by $[Na^+]_i > 10$ mM. The channel is insensitive to $[Ca^{2+}]_i$ and voltage, and is inwardly rectifying. A prolonged failure of the Na/K pump would activate this channel, repolarize the cell and promote Ca extrusion via Na/Ca exchange.

$I_{K(ATP)}$: Hypoxia and/or glycolytic blockade result in an increased K conductance in cardiac tissue and single cells. Recently, two groups reported a K current that is blocked by intracellular ATP in the range 0.1–1 mM. Obviously, the channel functions to hyperpolarize the cell and to shorten the action potential when the cell is metabolically exhausted (normal $[ATP]_i$ = 3–4 mM). This channel is clearly distinct in conductance and kinetics from the I_{K1} channel. ADP blocks this channel less effectively; AMP has no effect.

$I_{K(Ca)}$: In analogy with neurones and smooth muscle, it has been suggested that $[Ca^{2+}]_i$ controls steady-state K conduc-

tance in cardiac Purkinje fibers. This notion has not yet been confirmed at the level of single cells and single channels.

Transient outward currents (positive dynamic current, I_{bo}, I_{qr}, I_{lo}, I_{to}): The magnitude and properties of K-sensitive transient outward currents (I_{to}) largely depend on tissue and animal species. Initially considered as Cl current, I_{to} is particularly found in Purkinje fibers where it underlies the rapid phase of repolarization between the upstroke and the plateau of the action potential. I_{to} also serves for the formation of a short action potential duration in rat ventricle, for the fast initial repolarization in cat myocardium, and for the rate-dependent changes of the action potential duration in rabbit papillary muscle. Recently, an I_{to} carried by K^+ and Na^+, has also been described in rabbit A-V-nodal cells. There, this current may hyperpolarize the cell shortly after depolarization. Since its amplitude is smaller in spontaneously active cells than in quiescent ones, I_{to} may contribute to the latent pacemaker activity of A-V-nodal cells. In some tissues, I_{to} is activated by voltage, wheras in others, I_{to} is triggered and/or modulated by $[Ca^{2+}]_i$. In cow Purkinje cells, a voltage-dependent K-selective channel of large conductance (120 pS) has been identified, whose frequency of opening is modulated by $[Ca^{2+}]_i$ in the range 0.01–1 μM. In sheep Purkinje cells, an I_{to} channel of smaller conductance (18 pS) is found which is not affected by $[Ca^{2+}]_i$. In its properties, this I_{to} channel seems similar to the one identified in rabbit A–V node.

I_{Cl}: An apparently Cl-sensitive channel of large conductance (400–450 pS with symmetrical 140 mM $[Cl^-]$) has been described in patches excised from cultured newborn rat ventricular cells. A similar channel has not yet been observed in cell-attached patches or in macroscopic current recordings from single heart cells or cardiac tissue.

Future directions

An important and different approach in the study of ionic channels is their biochemical and genetic characterization. Channel proteins have been isolated and purified[33], and their primary structure has been deduced from their cDNA sequence[134, 135, 137–140, 175a]. The functional state of these channels has been assessed by reconstitution in artificial lipid bilayers[46, 60, 61, 72, 105, 121, 127, 163] and/or by expression in Xenopus oocytes[48, 136, 164]. Experiments are under way to study the effects of altered gene sequences on gating and selectivity properties of ionic channels[170].

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* To whom correspondence and reprint requests should be addressed.

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Sodium channels in cardiac Purkinje cells

by H. A. Fozzard, D. A. Hanck, J. C. Makielski, B. E. Scanley and M. F. Sheets

Cardiac Electrophysiology Labs, Departments of Medicine and the Pharmacological & Physiological Sciences, The University of Chicago, Chicago (Illinois 60637, USA)

Summary. Sodium (Na^+) currents are responsible for excitation and conduction in most cardiac cells, but their study has been hampered by the lack of a satisfactory method for voltage clamp. We report a new method for low resistance access to single freshly isolated canine cardiac Purkinje cells that permits good control of voltage and intracellular ionic solutions. The series resistance was usually less than 3Ω cm^2, similar to that of the squid giant axon. Cardiac Na^+ currents resemble those of nerve. However, Na^+ current decay is multiexponential. The basis for this was further studied with cell-attached patch clamp recording of single Na^+ channel properties. A prominent characteristic of the single channels was their ability to reopen after closure. There was also a long opening state that may be the basis for a small very slowly decaying Na^+ current. This rare long opening state may contribute to the Na^+ current during the action potential plateau.
Key words. Cardiac Purkinje cells; Na^+ channels; voltage clamp; single channel recording.

Introduction

Silvio Weidmann and his colleagues demonstrated in a series of papers the importance of sodium (Na^+) ions for excitation and conduction in cardiac Purkinje fibers[14, 16, 38]. Detailed studies of Na^+ current (I_{Na}) have been made in the giant axon of the squid, with good spacial voltage control[23] intracellular perfusion[3] and identification of channel gating currents[2]. However, equivalent studies were not possible in cardiac cells because of their small size and complex geometry. Such detailed study in cardiac cells is important and necessary, since it is clear that the cardiac Na^+ channel is a separate molecule, different from that of nerve and skeletal muscle[26, 29].

The first step toward systematic study of cardiac I_{Na} was the development of the two-micropipette voltage clamp in a shortened Purkinje strand by Deck et al.[15] and Hecht et al.[22]. This method was used by Dudel and Rudel[17] in their systematic study of I_{Na} in cooled Purkinje fibers. An alternative sucrose-gap voltage clamp method was used for voltage clamp studies in ventricular muscle by Beeler and Reuter[5]. While in retrospect we know that these investigators had relatively poor voltage control, they nevertheless were able to make valuable measurements. The data they obtained were then used as the justification for modeling I_{Na} in heart muscle in a modified Hodgkin-Huxley form[6, 28, 37].

In a continuing effort to improve the quality of the voltage clamp, Colatsky and Tsien[13] were able to control I_{Na} in small, shortened rabbit Purkinje strands in which the current was reduced by low temperatures and low Na^+ solutions. Ebihara and Johnson[18] used very small cultured aggregates of cardiac cells in a similar way. Several valuable studies on the mechanism of action of antiarrhythmic drugs on the Na^+ channel were forthcoming[4], but these studies on multicellular preparations were inevitably less reliable than desired.

Cell culture methods have provided us the means to isolate single cardiac cells. Normally cardiac cells are wrapped in connective tissue and are electrically coupled by large-conductance gap junctions. Isolation of single cells for physiological studies has been achieved by use of gentle enzyme treatment to digest away the connective tissue and supportive ionic environments to promote cell survival while the disrupted gap junctions close. Brown et al.[8] first took advantage of these single cells and used large-bore sealed pipettes to perform voltage clamp studies of cardiac I_{Na}.

Recently, a new and dramatic way of studying Na^+ current has become available with the development of the patch clamp method for recording of single channel openings[21]. For most of us who are accustomed to thinking of currents in