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Editorial

Cardiac sodium and calcium channels: a history of excitatory currents

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Abstract

The entire concept of ion channels as molecular machines to gate ion traffic across the membrane has only recently been generally recognized, even though ion channels are found in almost all cells. Na^+ and Ca^{2+} channels are the basis of excitability, and in the heart they generate the rhythm, coordinating and controling cardiac contraction. Dysfunction of ion channels results in arrhythmias that underlie 20–25% of deaths in the developed world, and some arrhythmias result directly from gene defects in ion channels. This is my personal perspective on the critical ideas and the pioneers in the field of cardiac Na^+ and Ca^{2+} channels. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

All electrophysiologists rejoiced over recognition of our field when the 1999 Lasker Prize was awarded to Bertil Hille, Clay Armstrong, and Rod MacKinnon for their cumulative contributions to voltage-gated ion channels, culminating in the first crystal structure for an ion channel pore [1]. This is a story of the discovery of Na^+ and Ca^{2+} channels, which play such crucial roles in the heart. Here I cite what have been for me landmark papers. I emphasize that this is not a systematic review; rather, it is a personal story based on my acquaintance with most of the field's major contributors. I hope this story will help new investigators in the field appreciate the pioneering achievements of a relatively small number of individuals who worked together to build this field. I credit their success to personal friendships, shared training experiences, and an amazing sharing of information worldwide.

Earl Sutherland was discovering cAMP as the second messenger for epinephrine, when he took me into his laboratory in 1952, and he triggered my fascination with the biology of cell membranes. Further intrigued with the electrocardiogram during my clinical training, I read with excitement the pioneering chapter by Walter Woodbury on the ionic basis of the cardiac action potential (AP) [2]. As background, it is important to explain that in the 1950s and 1960s biochemistry was believed to be the exclusive key to biomedical advances, and that physiology was no longer an active science. The concept of cell membranes had just received general acceptance, and only a few believed that ion channels existed. I almost abandoned this field as a beginning Assistant Professor of Medicine in St. Louis, because my Chairman informed me that ion channels were figments of my naive imagination and that cardiac electrophysiology had no future. Bertil Hille's outstanding book [3], the Lasker Awards, and NASPE are witnesses to how the world's view has changed.

1.1. Na^+ entry gated by membrane voltage generates the action potential

In the last century Bernstein proposed that the AP resulted from loss of selective membrane permeability. Helped by the rediscovery of the squid giant axon, Cole and Curtis [4] found that membrane conductance tran-

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siently increased many-fold during the AP. Alan Hodgkin, a graduate student at the famous Cambridge Physiological Laboratories, observed those experiments at the Marine Biological Laboratory at Woods Hole in the summer of 1938, while he was a visiting fellow at the Rockefeller Institute. The next question was: does the AP peak reach zero, as Bernstein predicted, or does the inside of the cell become positive? That next year back in Cambridge and at the Plymouth Marine Laboratories, Hodgkin and his undergraduate student Andrew Huxley thought they saw an overshoot [5]. World War II interrupted progress, sending Hodgkin to radar research. After the war Hodgkin and Bernard Katz, who escaped before the war from Nazi Germany, demonstrated a definite overshoot that paralleled the Nernst potential for Na^+—the 'Sodium Hypothesis' at last [6]!

1.2. Cole, Hodgkin, and the voltage clamp

During World War II Kenneth Cole was sent from New York to Chicago to join the Manhattan Project. By 1947 he and Marmont, working summers at Woods Hole, had begun voltage clamp studies with the squid giant axon. In 1948 Hodgkin traveled to the US so that his family could visit his wife's American parents after their long wartime separation. Hodgkin came to the University of Chicago to visit Cole, and two momentous events occurred: Cole shared his first records of voltage clamp and Gilbert Ling taught Hodgkin to make fine-tipped microelectrodes for intracellular recording [7,8]. Hodgkin and Huxley then continued their experiments at Plymouth (with a fortuitously large supply of squid) that stunned the electrophysiology community by demonstrating the ionic basis of the AP [9,10]. Amazingly, Hodgkin and Huxley never used the word 'channel' in their seminal papers that won them the 1963 Nobel Prize.

After the war Silvio Weidmann left Switzerland to study excitability in *Nitella* with Torsten Teorell in Upsala, and then moved to Hodgkin's laboratory in Cambridge. Hodgkin suggested that Weidmann attempt to show that the cardiac AP was generated by mechanisms similar to those of squid axon. Using the fine-tipped micropipette method Hodgkin had brought back from Chicago, Weidmann recorded first the transmembrane action potentials of dog Purkinje strands with Edouard Coraboeuf [11], and reported their dependence on external Na^+ [12]. Back in Chicago Walter Woodbury learned the Ling fine-tipped micropipette method, and while visiting Salt lake City, he, his brother and Hans Hecht, chief of Cardiology, recorded frog ventricular APs [13]. Thus began cellular cardiac electrophysiology.

Weidmann had no way to change membrane potential rapidly, but used a feedback system from the oscilloscope vertical deflection plates to bias the membrane potential of Purkinje strands to different steady levels. He then switched the clamp off to test the maximal upstroke velocity as an index of Na^+ current, showing qualitatively that cardiac excitability resembled that of squid axon [14]. He also showed that membrane impedance increased progressively during the AP plateau and during diastolic depolarization in the Purkinje pacemaker cells [15] and that the Na^+ current was blocked by local anesthetic drugs [16].

In addition to Woodbury's laboratory in Seattle, another pioneering cardiac electrophysiology group in the United States at this time was in Brooklyn, led by Chandler Brooks and his colleague Brian Hoffmann [17], soon joined by Paul Cranefield. Using standard extracellular recording and the Ling microelectrodes they catalogued the behavior and special features of cardiac cells and their pharmacology [18]. They hosted Weidmann for an exciting year-long visit before he decided to return to Bern. The US hosted two more Europeans for an unlikely collaboration. Otto Hutter had come to the UK before the war on the last train for Jewish children allowed to leave Vienna. After the war he came to Steven Kuffler's laboratory in Philadelphia and joined Wolfgang Trautwein from Heidelberg, who had survived the Russian Front by becoming a medic. Together, they demonstrated the membrane action of sympathetic and parasympathetic neurotransmitters on the cardiac pacemaker [19].

1.3. A voltage clamp for cardiac cells

Edward Carmeliet, whose laboratory in Weidmann's group I inherited during my stay in Bern, found that micropipette injection of constant current gave almost uniform steady-state voltage distribution in very short Purkinje strands. Hutter (back in London) and Hans Hecht (on sabbatical before establishing the Chicago cardiac electrophysiology school) used this short Purkinje strand for early voltage clamp recordings.

Trautwein, back in Heidelberg, developed a voltage clamp with the short Purkinje strand, and recorded the first transient cardiac Na^+ current [20]. Then Hutter's student, Denis Noble, combined all of this information to show that a judicious modification of the Hodgkin–Huxley model for a single Na^+ current and a single delayed rectifier K^+ current (and some constant permeabilities) could reproduce most of the known behavior of the cardiac AP [21]. This first model of the cardiac action potential was wrong because it used only two voltage-dependent ionic currents, but it was a huge stimulus to progress.

In Bern, where we built our own amplifiers and pulled micropipettes by hand, I could not achieve sufficient voltage control to characterize the cardiac Na^+ current with the two-pipette voltage clamp in Purkinje strands because of their large capacity current [22]. A subsequent landmark critique of cardiac voltage clamp studies by Johnson and Lieberman [23] discouraged voltage clamp studies of cardiac inward currents for years. Then during a NIH physiology study section meeting Buzz Brown and I

discussed the method Kostyuk and colleagues in the Kiev Institute used to clamp and perfuse internally a single cell as the best approach for heart, and Brown with Trevor Powell soon reported good cardiac clamp studies [24,25]. The cardiac Na^+ current was kinetically similar to nerve but with significant kinetic and pharmacological differences, including antiarrhythmic drug affinities [26].

1.4. Macroscopic current results from the ensemble of individual channels

A major technical discovery was that glass pipettes with smooth tips seal with high leak resistance (gigaseal) to cell membranes free of extracellular matrix, either isolating the enclosed small patch for single channel recordings or allowing rupture and low-resistance access to the single cell for diffusion of electrolyte and voltage control [27]. First, this showed us that each type of Na^+ channel has a unique single channel conductance [28]. Charles Stevens' laboratory showed that channels did not always open upon depolarization, but could enter an inactivated state from intermediate activated/closed states [29]. Harald Reuter from Bern, who was on sabbatical at Yale with Stevens and saw Erwin Neher's early single channel recordings, was the first to record single cardiac Na^+ channels [30]. They differed from nerve by having a higher probability of opening and a higher frequency of reopening [31,32]. But perhaps the most valuable contribution of the gigaseal method has been to allow high quality voltage clamp of small single high-input-resistance cells, allowing voltage control of individual cardiac cells. This led to an extraordinary growth in ion channel studies in general, including the heart. It also gave the 1991 Nobel Prize to Erwin Neher and Bert Sakmann, after the technique revealed certain mechanisms of disease.

1.5. Voltage-dependence of gating

Hodgkin and Huxley [10] suggested that voltage dependence of gating meant that charges on the transporting molecule (channel) must move in the membrane electric field. Clay Armstrong quit clinical medicine after his internship at Chicago and worked with Cole and Huxley before finally recording the gating currents of activation with Pancho Bezanilla [33], but they had difficulty recording any for inactivation. Indeed, Stevens' laboratory reported that apparent voltage-dependence of inactivation was derived from voltage-dependence of activation steps that preceded inactivation [34]. Early single channel studies of cardiac Na^+ channels, however, did find a controversial intrinsic voltage dependence of inactivation [32,35]. One good way to resolve this question was by recording gating currents in cardiac cells, which took several years to achieve [36,37]. During those early years, Dorothy Hanck, Mike Sheets and I worked at low temperature to slow the Na^+ currents. We followed the precedent of Hodgkin, whose low temperature nerve experiments from Cambridge were easily done because the Physiological Laboratories had no heating. In Chicago we simply did the experiments in the winter with the window open.

1.6. Certain marine toxins bind specifically to the Na^+ channel

Tetrodotoxin isolated from a Japanese culinary specialty, the puffer fish, blocks excitability in nerve and skeletal muscle, but not in heart. Toshio Narahashi brought tetrodotoxin with him from Japan when he moved to Duke, and with John Moore showed conclusively that tetrodotoxin blocks the Na^+ channel selectively and with high affinity [38]. Soon a second marine toxin, saxitoxin, showed even higher affinity. When the US biological warfare program was shut down in the early 1970s a large amount of stockpiled saxitoxin was made available for biological research, and with the development of radioactive tags we now had the very high affinity, specific biochemical marker needed for biochemical purification.

1.7. The Na^+ channel protein is cloned

Years of work by Rafter, Catterall, and colleagues [39,40] finally yielded enough protein sequence for Numa's laboratory in Kyoto to clone three brain Na^+ channel α-subunits of about 2000 amino acids [41,42], beginning the next great phase of study of this channel. It seems likely that if Numa had lived, he would have received the Nobel Prize for his extraordinary contribution cloning ion channels. Two laboratories simultaneously cloned the cardiac Na^+ channel isoform. Rogart and colleagues [43,44] in Chicago found a rat cardiac cDNA sequence that was nearly identical to the brainII clone and expressed cardiac-like Na^+ channels. During a 1989 visit by Bob Barchi to our laboratory in Chicago we shared our unpublished sequence, and he immediately confirmed by a phone call back to Penn that the denervated skeletal muscle channel had the same sequence [45]. Soon the human isoform was also cloned [46].

2. Unique amino acid motifs form the selectivity filter, voltage sensors, and gating elements

The first major step for structure-function studies was a computer-based molecular model from the primary Na^+ channel amino acid sequence by Robert Guy and colleagues [47], proposing that the extracellular S5–S6 loops folded back into the protein to form the lining of the outer pore (P loops). Walter Stühmer and colleagues [48] then showed that mutation of one carboxyl in the four P loops dramatically reduced sensitivity of the brainII channel to block by the pore-binding guanidinium toxins [49]. They proposed an alignment that placed one residue from each

domain into a putative selectivity filter ring for both Na^+ (the DEKA motif) and Ca^{2+} (the EEEE motif) channels and showed that switching these motifs could reverse the selectivity functions [50]. Cardiac Na^+ currents are relatively resistant to tetrodotoxin, but the P loops differed from nerve by only two residues. Satin and colleagues [51] mutated a cysteine in the cardiac domain I just above the putative selectivity ring to a tyrosine or a phenylalanine, the residues found in skeletal or brain isoforms, and created a channel with 100-fold increase in tetrodotoxin sensitivity to block, while also reducing Cd^{2+} block. Simultaneously, Marbán and colleagues [52] swapped the tyrosine for a cysteine in the skeletal muscle isoform, reducing tetrodotoxin sensitivity and increasing Cd^{2+} sensitivity to block. These studies led to a detailed molecular model of the outer pore based on interactions with the guanidinium toxins [53].

Thus began the 'modern' era. The Na^+ channel is formed of four clockwise homologous domains, each formed by six transmembrane segments. The fourth segment (S4) of each domain contains multiple positively charged residues that have been shown to be the voltage sensor.

Although we all wanted three of the S4 segments to be activation sensors and one, an inactivation sensor (corresponding to the Hodgkin–Huxley m^3h gates), gating has turned out to be more complicated. The domains III–IV cytoplasmic linker contains a segment of three hydrophobic amino acids (Ile–Phe–Met) that form part of the inactivation gate and plug the inside mouth of the pore. As noted, the outer pore is formed by P loops. Three small proteins have been found to function as secondary subunits of the channel, although their roles in the heart is not yet resolved. Long QT syndrome and several rare familial kinds of lethal ventricular arrhythmias are caused by inherited mutations in the Na^+ channel [54], and silent variations in coding may predispose to arrhythmias in other cardiac diseases. How these arrhythmogenic mutations permit millions of normal heart beats, but then in the teen years or middle age, or in the presence of certain drugs, cause lethal arrhythmia is a major frontier for the next decades.

2.1. Some questions

It is not yet obvious why the heart has its own Na^+ channel isoform, if indeed it is the sole cardiac isoform. Are there unique properties of that isoform that make it specially suitable for cardiac function? How is its expression regulated in general and in specific regions of the heart? Will elegant modern structure–function studies lead to rational drug design and effective antiarrhythmics? How long before we have a crystal structure of this large molecule?

Perhaps the recently cloned prokaryotic Na^+ channel will be the key.

3. Calcium channels

3.1. Ca^{2+} can cross the membrane through channels

Challenging the Na^+ hypothesis, Fatt and Katz [55] found in 1953 that APs in crustacean muscle depended on external Ca^{2+}, rather than on Na^+. Hagiwara and Nakajima [56] confirmed that this was not some aberrant behavior of Na^+channels, but most likely Ca^{2+}-selective channels. In 1964 Harald Reuter and I were together in Bern arguing about a possible Ca^{2+} current, which he thought might explain the positive inotropic action of catecholamines. Between climbs in the Bernese Alps, we successfully recorded Ca^{2+}-dependent action potentials, and he subsequently presented compelling evidence for Ca^+ current (then called 'slow inward current') and its contribution to maintenance of the AP plateau [57,58]. These results triggered a large controversy. The challenging problem was how to identify and characterize accurately small currents in cells such as cardiac myocytes that have many overlapping currents. Many felt that the putative Ca^{2+} currents were artifacts of inadequate voltage control [23], because of the primitive voltage clamp methods available in heart muscle at that time [59], causing many frustrated investigators to abandon the voltage clamp.

Another problem for study of Ca^{2+} channels was the existence of Na/Ca exchange (See Sheu and Blaustein [60] for historical review). Building on the earlier work of Wilbrandt and Koller [61] and Lüttgau and Niedergerke [62], Reuter and Seitz [63] found that radioactively labeled Ca^{2+} flux in heart was coupled to Na^+. Similar studies in squid axon by Mordy Blaustein and colleagues [64] led to the first suggestion that Na/Ca exchange was the link between inhibition of the Na,K pump and intracellular Ca^{2+} in the mechanism of digitalis action. Because of this transport system, simple ion substitution methods to separate Na^+ and Ca^{2+} currents in voltage clamp were confusing. After a prolonged debate over stoichiometry, it was eventually concluded that the exchange generates depolarizing membrane current during Ca^{2+} efflux [65], simulating a Ca^{2+} channel current and generating arrhythmogenic delayed afterdepolarizations. The positive contraction (Bowditch) staircase turned out to be partly because the Na^+ current during APs loads the cell with Na^+, and via Na/Ca exchange allows the cell to be loaded with Ca^{2+} [66,67].

3.2. Ca^{2+} channels are essential for contraction

In spite of confusion over Na/Ca exchange, it was obvious that Ca^{2+} channels could play an important role in contraction and in adrenergic modulation of contraction. Inward Ca^{2+} current not only adds positive charge to the cytoplasm and depolarizes the membrane, but it also

introduces a multipotent cytoplasmic second messenger. Internal Ca^{2+} concentration is normally so low that the small currents needed for electrical signaling can raise the concentration to levels that activate many enzymatic cascades, relating Ca^{2+} current to a bewildering, ever increasing, array of cellular events, from protein phosphorylation to transcriptional regulation. Cardiac contraction depended on membrane depolarization similar to that of Ca^{2+} channel current [68–70], and Ca^{2+} currents were soon shown to be the trigger and modulator of contraction [71,72]. Although the Ca^{2+} entering via channels can contribute directly to troponin-actin-myosin interaction, its major effect is to trigger Ca^{2+} release from the sarcoplasmic reticulum (Ca-triggered Ca release) [73]. Fulfilling his original goal, Reuter showed that Ca^{2+} current is increased by β-adrenergic agonists [58], producing a positive inotropic effect and establishing the important concept that ion channels are major mediators of hormone action. One of Richard Tsien s early studies at Yale after leaving Denis Noble's Oxford laboratory showed that this increase in current can be produced by injection of cAMP into the myocytes [74].

3.3. Sino-atrial and atrio-ventricular nodal action potentials

It is difficult to exaggerate our misunderstanding of the basis of slow SA and AV nodal conduction before discovery of Ca^{2+} channels. Gradually, it became apparent that conduction was slow because of the low density and slow kinetics of Ca^{2+} channels, not because of some peculiar tissue geometry, with especially superb contributions by Hiroshi Irisawa, Aki Noma, and colleagues in Japan [75,76]. I failed such experiments in the 1960s and greatly enjoyed seeing Irisawa's skillful dissection of the central SA node when he visited Chicago in the late 1970s. The AV node was equally mysterious until the Ca^{2+} channel mechanism became apparent [77], and Wit and Cranefield [78] showed that Ca^{2+} channel blockers interfere with AV nodal reentry.

3.4. Single Ca^{2+} channel recordings define subtypes and action of hormones

In the summer of 1981 Reuter assembled in Bern an all-star team of Erwin Neher, David Colqhoun, Charles Stevens, Richard Tsien, and Gary Yellen to record single Ca^{2+} channel currents in cardiac myocytes. This was finally achieved, after much frustration, by using Ba^{2+} as the charge carrier [79]. Tsien's laboratory demonstrated three types of Ca^{2+} channels and named them L (long-lasting current, or large conductance), T (transient current, or tiny conductance), and N (non-L, non-T-type) [80], with L-type and T-type found in heart. The L-type was further characterized by its interaction with dihydropyridines. Although both types are activated by depolarization, T-type also has voltage-dependent inactivation, while L-type has little of that and mainly is closed by intracellular Ca^{2+} [81], probably by formation of a Ca–calmodulin-channel complex. Cardiac T-type Ca^{2+} currents have different pharmacological properties and contribute to pacemaker function.

3.5. Clinically important drugs modify gating in these channels

Gating behavior of L-type channels were shown by Tsien, Peter Hess, and colleagues [82] to have at least three gating modes: mode 0, very low probability of opening; mode 1, increased probability of short openings; and mode 2, long openings, and also a mode with long bursts of openings. Switch from the basal mode to those with greater opening probability is accomplished by protein kinase A-dependent phosphorylation [83–85], and reversed by exposure to the dihydropyridine drugs [82]. These 'Ca antagonist' drugs were a huge step for both ion channel study and cardiovascular therapy [86]. Surprisingly, Reuter showed that optical isomers of dihydropyridines bind to the same site within the channel pore and can either inhibit Ca^{2+} current or enhance it [87], implying that their action is not a physical block of the channel but rather an effect on gating. Ca^{2+} channel gating currents were recorded by Bruce Bean's laboratory [36], and this technique could answer many important questions.

3.6. Early after-depolarizations (EAD) and arrhythmia

One type of 'triggered activity' of heart muscle is EAD, one or a series of AP's originating from an AP plateau or early during repolarization [88]. Na^+ channels are largely inactivated during the plateau, but Ca^{2+} channels can recover as the membrane repolarizes and as intracellular Ca^{2+} is pumped back into the sarcoplasmic reticulum. Craig January showed conclusively that Ca^{2+} channels are the excitatory current responsible for EAD [89]. The conditions favoring this reexcitation of Ca^{2+} current are a long AP (plateau), enhancement of Ca^{2+} channel opening by catecholamines, and reduced repolarizing K^+ currents. This is almost certainly the mechanism for polymorphic ventricular tachycardia.

3.7. Ca^{2+} channels are cloned

Cloning of the Ca^{2+} channel followed the pattern already noted for Na^+ channels. Skeletal muscle T-tubules have a high concentration of L-type channels, and these were purified by Curtis and Catterall [90], using tritiated nitrendipine. This allowed Numa's laboratory to clone the large, pore-forming α_1-subunit [91]. Eventually, the Numa laboratory cloned the cardiac isoform, now called α_{1C} or $Ca_v1.2$ [92]. Subsequently the Ca^{2+} channel was shown to

include several subunits crucial to gating, drug interaction, and cell processing/targeting. The α_1 subunit topology greatly resembles that of the Na^+ channel α subunit, with 24 transmembrane segments organized into four homologous domains, each containing the S4 segment characteristic of voltage-gated channels. Finding the characteristic S4 pattern of positive charges every third residue in the Ca^{2+} channel, confirmed the Numa suggestion that this was likely to be the voltage sensor, and it soon was apparent that all channels with strong voltage-dependent gating have this structure. The channel's P loops were also homologous with the selectivity filter of the Na^+ channel, showing one glutamate residue from each P loop [50].

3.8. Some questions

The remarkable ability of L-type Ca^{2+} channels to alter their kinetics is poorly understood at the molecular level, and the relationship of modes to the channel's gating currents and to modulatory phosphorylation remains challenging. No cardiac disease due to cardiac Ca^{2+} channel mutation has yet been identified, although there are many examples from muscle and brain. The roles of L- and T-type Ca^{2+} channels in cardiac hypertrophy and heart failure remain confusing, and we are not sure if the T-type channel will be a useful drug target. We have a paradox that a major mechanism of arrhythmogenesis is EAD but treatment with Ca^{2+} channel blockers has marginal benefit. The mechanism of Ca^{2+} channel blockers is unresolved. These remain fascinating, but still mysterious channels.

4. Summary

Ion channels have emerged as a major biomedical field because they are the key mediators of electrical signals in nerve, muscle, and endocrine cells, and are modulated by hormones. Their dysfunction is responsible for massive disability and death, so they are prime drug targets. From a tiny group of inspired investigators, this area has grown into a huge field, profiting from the great progress in biological and physical sciences and contributing unique insights to many diverse areas. Na^+ and Ca^{2+} channels are now ready for use in the development of effective and safe therapeutic agents to control arrhythmias and other diseases of excitability.

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