Dario DiFrancesco

Summary of scientific activity
(numbers of quotations refer to the list of publications)

A major contribution given by the author to the field of cardiac physiology is the discovery of the cardiac pacemaker ("funny") current in the sinoatrial node, the pacemaker region of the heart, and of its involvement in the mechanism underlying generation of pacemaker activity and control of heart rate by the autonomic nervous sytem.

As a postdoc in Cambridge in 1976, and then in Oxford in 1977-79, the author cooperated with the group led by Denis Noble on several projects on the properties of different ionic currents in Purkinje fibres and in amphibian atrial and sinus venosus tissues (1, 3, 7, 8). During this period he joined the laboratory of Wolfgang Trautwein in Homburg, where together with Akinori Noma applied the voltage clamp technique to small mammalian sino-atrial (SA) node preparations to study the properties of the ACh-activated current (5, 6). He brought back to Oxford the technique for voltage clamping SA node tissue, and there performed in collaboration with Hilary Brown and Susan Noble the first experiments demonstrating the existence of an ionic current, termed I_f (f for "funny"), which was activated on hyperpolarization and was carried unspecifically by Na+ and K+ ions (2). Because of its relevance to the generation of the diastolic depolarization phase of the action potential, and thus of pacemaker activity, this current was since referred to as the cardiac "pacemaker" current. As well as being involved in the generation of the diastolic depolarization, I_f was shown to have a main role in the cardiac rhythm acceleration induced by adrenaline (2, 4).

This first study of I_f paved the way to a yield of novel results on cardiac pacemaker mechanisms in the following years.

A major breakthrough on pacemaker mechanisms to which the discovery of I_f in the sinoatrial node contibuted was the re-interpretation of the I_{K2} current in Purkinje fibres. Spontaneous pacemaking in Purkinje fibres was thought to be generated by the decay of a K+ current, the I_{K2} current, which implied the very puzzling consideration that the pacemaking mechanisms in Purkinje fibres (K+ current decay) and in the sinoatrial node (I_f activation) were different processes.

In a set of experiments where the Purkinje I_{K2} was compared with the nodal I_f, and where it was shown that K+-depletion could distort the time-course of I_{K2} during voltage clamp, the author showed that the Purkinje fibre I_{K2} was, in fact, a mixed Na+ and K+ current activated on hyperpolarization like the nodal I_f (11, 12). Professor Edouard Corabouef of the University of Orsay, whom the author visited for several short periods in the early 80's defined the I_{K2} re-interpretation as "a little revolution" in the cardiac field. The inset below is a photographic copy exctracted from a report written by E. Coraboeuf in 1981 ("Avis sur la candidature de M. DiFrancesco a un post de chercheur", 26 janvier 1981) concerning the author's results:

> Il est, en particulier, en ce qui concerne l'analyse des mécanismes responsables de l'automatisme du tissu conducteur cardiaque (fibres de Purkinje), l'auteur d'une véritable petite révolution, comme on en enregistre dans la plupart des domaines tous les 20 ou 30 ans. Le fait qu'il vienne, en effet, de renverser un dogme établi et indiscuté depuis 1955, fait qu'aujourd'hui son nom est connu des électrophysiologistes cellulaires du monde entier.

In the early 80's the properties of I_f where described with greater detail in terms of ionic, kinetic and pharmacological properties (13, 14, 15). At the same time, other current components were investigated like the inward rectifier (16) and the fast Na+ current (18, 28) in multicellular and single-

cell preparations, and a theoretical model incorporating new experimental data was developed in collaboration with Professor Denis Noble of the University of Oxford (17).

Further progress in the understanding of the I_f properties came in 1986 with the recording of single f-channels (20). Several laboratories had failed to record single f-channel activity. This was achieved only thanks to a modification of the patch-clamp technique, which consisted of the use of two pipettes on the same cell, in particularly demanding experiments, and which allowed a much greater resolution of single-channel activity than that obtained by the traditional single-pipette technique. Such a modification was necessary due to the extremely small I_f single-channel conductance (1 pS). As of today, the 1986 experiments, and others performed on I_f by the author in a later investigation made with the inside-out patch recording protocol (39), represent the smallest direct single-channel recording ever made, a view shared by Erwin Neher, the Noble-Prize winning physiologist who developed the single-channel measuring technique. The difficulty of recording the activity of such a small single channel is proved by the fact that only in 2006, i.e. 20 years later, have similar recordings been made independently in another laboratory, that of Gary Yellen at Harvard (Dekker & Yellen, 2006, J. Gen. Physiol. 128, 561-7) on HCN channels, the molecular correlates of native funny channels.

I_f single-channel measurements also allowed to establish the mode of action of adrenaline on I_f: it was shown that adrenaline activates I_f by increasing the probability of channel opening upon hyperpolarization, without increasing the single f-channel conductance (20).

A further advance in the knowledge of the properties of the pacemaker current in the SA node was obtained by studies of the neurotransmitter-induced I_f modulation. In 1987, the author and collaborators demonstrated the inhibitory action of acetylcholine on I_f (21, 22, 23). This finding was especially relevant because it modified the generally accepted view that cardiac rate slowing due to vagal stimulation occurred through activation of a K+ permeability (the ACh-activated K^+ current, $I_{K,ACh}$). Indeed, in a study where the activation of K+ permeability by ACh was compared with the ACh-induced I_f inhibition, the author and collaborators showed that the I_f inhibition, and not the K+-conductance activation, is responsible for the slowing of pacemaker rhythm at low ACh concentrations (25).

Further studies of the f-channel neurotransmitter-induced modulation then revealed that cAMP, the second-messenger controlling I_f, activates f-channels by direct binding to the channel protein, and not by a phosphorylation process mediated by the cAMP-dependent protein kinase (PKA) (31). Thus, f-channels behave quite differently from other channels (such as Ca2+ and delayed K+ channels) which are controlled by cAMP via phosphorylation. These studies were performed using inside-out macro-patch preparations, which allow test intracellular solutions to be perfused directly on the internal side of the channel. Single-channel measurements were also performed showing that, in agreement with the action of adrenaline previously reported, cAMP activates f-channels by increasing the probability of channel opening, and does not affect the single-channel conductance (39). This was the first evidence that funny channels had properties similar to what at the time was an apparently very distant family of channels, the cyclic nucleotide-gated (CNG) channels which mediate sensory transduction in the retina, olfactory neurons etca. Indeed several years later, in the late ‘90s, cloning of the HCN channels (the molecular components of funny channels) demonstrated that funny and CNG channels do belong to the same superfamily, fully confirming the original observation of direct f-channel activation by cAMP (31).

In parallel with studies aimed to a better understanding of the properties of I_f and its role in underlying generation and control of cardiac pacemaker activity, the author became involved in studies on the properties of a similar hyperpolarization-activated current (I_h-current) in cells other than

cardiac, namely neuronal cells. In the 80's and 90's, it had indeed become clear that funny channels have important roles in a variety of cellular mechanisms in different types on neurons. These include the control of excitability, the involvement in several aspects of sensory perception and transduction, the modulation of rhythmic firing and the involvement in the control of synaptic strength. Some of these aspects were investigated by the author (38, 41, 53, 55, 58).

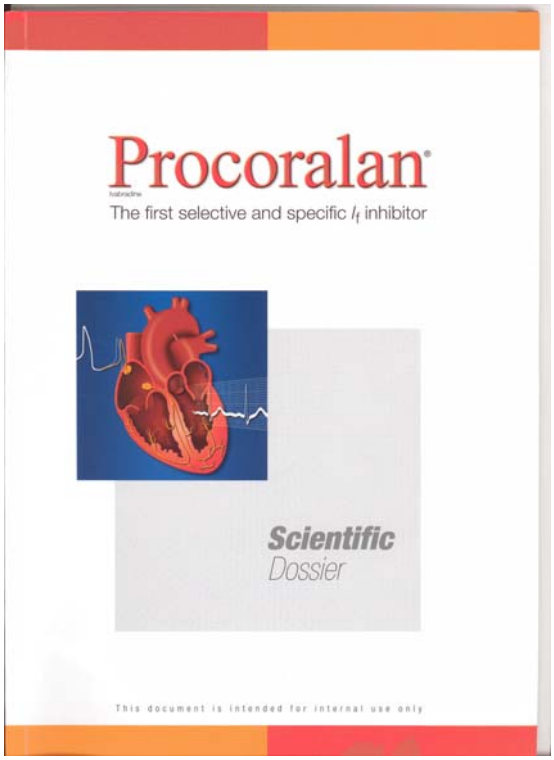
The author's lab was also involved in the cloning of isoforms of the HCN family of channels (hyperpolarization-activated, cyclic-nucleotide gated channels) and to the characterization of the properties of HCN clones and native f- channels, providing relevant contributions to the knowledge of the molecular basis for channel activity (66, 68, 70, 73, 75, 83, 84). In particular it was shown that the HCN4 isoform is, among the 4 HCN isoforms known, the most relevant in the pacemaker region of the sinoatrial node (77).

Other contributions have concerned different channel types (45, 46, 52, 63, 65, 67, 69). Particularly relevant was the cloning and characterization of one of the smallest known ion channels, the first K^+ channel found in the genome of a virus (61).

More recent years have been characterized by clinically-relevant developments of the concept of funny channel-based pacemaking. There are three important applications: a pharmacological one, a genetic one and one involving the new generation of "biological" pacemakers.

1) Pharmacological application.

It is long known that cardiac diseases such as angina, ischaemic heart disease and heart failure take benefit from the slowing of heart rate. It is also known that high heart rate is associated with increased morbidity/mortality in some pathological conditions such as hypertension, myocardial infarction, diabetes and more. Classical agents prescribed so far to slow heart rate (β- blockers and Ca^{2+} antagonists) have unfortunately also potentially adverse side effects (such as for example reduced inotropism) that limit their use. Given the specific role of funny channels in the generation of spontaneous activity and heart rate control, they clearly represent a valid target for the development of drugs aimed to specifically control heart rate, without complicating side effects.



It is therefore not surprising that several drug companies have attempted for a long time to develop drugs specifically acting on I_f. One such drug (ivabradine, developed by Servier) has passed clinical testing and has reached the market with the commercial name of Procoralan (or Corlentor). The author's lab has contributed a number of publications on the mechanism of action of funny-channel blockers and more specifically ivabradine (72, 87, 101). Procoralan is presented by Servier as the "first selective and specific I_f inhibitor" and as such the molecule is prescribed against chronic *angina pectoris*.

The figure on the side is the first page of a booklet distributed by Servier to marketing agents which explains the basis of the heart rate-reducing action of ivabradine by a set of illustrations and diagrams. Much of the work of the author concerning the basic concept of pacemaking and I_f current, and its block by ivabradine is quoted in this booklet.

2) Genetic application

The hypothesis that I_f plays a role in generation of spontaneous activity and control of heart rate implies that functional defects of funny channels can affect the generation and maintenance of normal rhythm. In this case, one could expect to find rhythm disturbances which are attributable to mutated funny channels. The author's lab has therefore screened several cardiac patients with rhythm disturbances such as bradycardia, tachycardia, Sick Sinus Syndrome etca, and eventually found a large italian family with asymptomatic sinus bradycardia attibutable to a point-mutation of HCN4 (86). The mutation modifies the channel function in a way which reduces the inward current during diastole, thus slowing spontaneous frequency and generating bradycardia. This finding opens novel perspectives in the field of cardiac arrhythmias since it may reprent a specific case of a broader mechanism for rhythm disturbances based on constitutive alterations of funny channels.

3) Application to biological pacemakers

Several laboratories have recently developed new techniques to generate "biological pacemakers", i.e. autorhythmic cellular substrates able to induce or control pacemaker activity. The ultimate goal is to replace the electronic pacemaker used today with biological ones. Some of the protocols adopted rely upon the induction of pacemaker activity by overexpression of HCN channels via viral transfection or by transfer of cells engineered to overexpress HCN channels (such as mesenchymal stem cells). Another approach utilizes cells which are basically autorhythmic, such as Embryonic Stem (ES) cells differentiated towards myocytes expressing funny channels and able to pace spontaneously . The progress in this field is fast. The author's lab is engaged in characterizing a novel type of murine adult cardiac stem cell (named "mesoangioblasts") which is clonogenic and fast-replicating and has several features which suggest its potential usefulness in the development of autologous biological pacemakers.

In conclusion, the discovery of the funny current and of the mechanism of cardiac pacemaking and rate modulation contributed to by the author has represented an important step in the understanding of the cellular processes controlling such a fundamental function of the heart.

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Articles

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