Ischemic, genetic and pharmacological origins of cardiac arrhythmias: The contribution of the Quebec Heart Institute

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B Drolet, C Simard, L Gailis, P Daleau. Ischemic, genetic and pharmacological origins of cardiac arrhythmias: The contribution of the Quebec Heart Institute. Can J Cardiol 2007;23(Suppl B):15B-22B.

Research in the field of basic electrophysiology at the Quebec Heart Institute (Laval Hospital, Quebec City, Quebec) has evolved since its beginning in the 1990s. Interests were focused on cardiac arrhythmias induced by drugs, allelic variants and metabolic factors produced during ischemia. The results have contributed to the creation of new standards in drug development, more specifically, testing all new drugs for their potential effects on cardiac potassium currents, which could produce life-threatening proarrhythmic effects. In a French-Canadian population, three heterozygous single nucleotide polymorphisms in *hK_v1.5*, a gene encoding for a major atrial repolarizing current, were found. These variants affect the expression level of the *hK_v1.5* channel and change the inactivation process in the presence of its accessory beta subunit. Because these effects could shorten atrial action potential, their presence was tested in postcoronary bypass patients and a higher prevalence was found in patients with postoperative atrial fibrillation. Finally, three potentially proarrhythmic factors characteristic of ischemia were identified: pH decrease; oxygen free radicals, which both increase the flow of K⁺ ions through human ether-a-go-go-related gene and *hK_v1.5*, producing a reduction in action potential duration, frequently leading to cardiac arrhythmias; and lysophosphatidylcholine, a metabolite involved in the production of cardiac arrhythmias early during ischemia that was shown to be a major cause of electrical uncoupling. Over the past decade, the Quebec Heart Institute has provided a significant amount of original data in the field of basic cardiac electrophysiology, specifically concerning arrhythmias originating from pharmacological agents, genetic background and cardiac ischemia.

Key Words: Arrhythmias; Drugs; Genetics; Ischemia

Research in the field of basic electrophysiology at the Quebec Heart Institute (Laval Hospital, Quebec City, Quebec) has evolved since its beginning in the 1990s. Our main interests were initially focused on drug-induced cardiac arrhythmias at both the pharmacodynamic and the pharmacokinetic levels. Our results have contributed to new standards in drug development, especially testing new drugs for their potential effects on cardiac potassium currents. This can prevent life-threatening proarrhythmic effects (ie, excessive QT prolongation and torsade de pointes). We were also interested in finding new allelic variants, which could be responsible for arrhythmias such as atrial fibrillation (AF). In addition, we searched for factors modifying potassium channels and gap

L'arythmie cardiaque et ses origines ischémiques, génétiques ou pharmacologiques : apport de l'Institut de cardiologie de Québec

La recherche dans le domaine de l'électrophysiologie fondamentale à l'Institut de cardiologie de Québec (hôpital Laval, Québec [Québec]) a évolué depuis sa fondation au cours des années 1990. On s'est principalement intéressé aux arythmies causées par les médicaments, les variants alléliques et les facteurs métaboliques produits durant les périodes d'ischémie. Les résultats ont permis d'établir de nouvelles normes dans la mise au point de nouveaux médicaments, plus précisément en ce qui concerne la recherche, pour tout nouveau médicament, d'éventuels effets sur les courants de potassium (K) dans le cœur, susceptibles de produire de l'activité proarythmique potentiellement mortelle. Au sein d'une population canadienne-française, trois polymorphismes d'un nucléotide simple, hétérozygote ont été découverts dans *hK_v1.5*, un gène codant d'un important courant de repolarisation auriculaire. Les variants agissent sur le degré d'expression du canal *hK_v1.5* et modifie le processus d'inactivation en présence de la sous-unité bêta accessoire. Comme ces effets peuvent raccourcir le potentiel d'action dans les oreillettes, leur présence a été évaluée chez des patients ayant subi un pontage coronarien, et une prévalence plus forte a été observée chez les patients ayant fait de la fibrillation auriculaire en phase postopératoire. Finalement, trois facteurs potentiellement proarythmiques, caractéristiques de l'ischémie ont été découverts, soit la diminution du pH; les radicaux exempts d'oxygène, qui ont pour effet, tous les deux, d'augmenter le flux d'ions K⁺ par l'intermédiaire d'un gène humain apparenté au gène éther-à-go-go et d'*hK_v1.5*, ce qui provoque une diminution de la durée du potentiel d'action et entraîne souvent, à son tour, de l'arythmie cardiaque; et la lysophosphatidylcholine, un métabolite mis en cause dans l'apparition d'arythmie cardiaque au début des épisodes d'ischémie, cause importante de dissociation électrique. Au cours des dix dernières années, l'Institut de cardiologie de Québec a produit une quantité appréciable de données en recherche nouvelle, dans le domaine de l'électrophysiologie cardiaque fondamentale, plus particulièrement en ce qui concerne l'origine des arythmies, qu'elle soit pharmacologique, génétique ou ischémique.

junction (GJ) function, which may mediate arrhythmias induced during cardiac ischemia.

THE GENETIC BACKGROUND TO ARRHYTHMIA SUSCEPTIBILITY

In Canada, sudden cardiac death due to ventricular fibrillation and ventricular tachycardia kills over 40,000 people annually (1). These arrhythmias are most commonly associated with heart disease (2). They are also characteristic of the relatively rare congenital long QT syndrome (LQTS), an inherited group of conditions associated with prolongation of the QT interval on the surface electrocardiogram, syncope and sudden death (3). Genetic studies of LQTS have led to the hypothesis

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Received for publication March 28, 2007. Accepted June 3, 2007

that arrhythmias mostly represent abnormal ion channel function (4-6). Of increasing concern both to clinicians and to the pharmaceutical industry is the proarrhythmic effect of drugs targeted for both antiarrhythmic therapy and for noncardiovascular indications. As a consequence, drugs such as cisapride have been withdrawn from the Canadian market by regulatory authorities over the past decade. Moreover, many other potentially useful drugs have failed to gain regulatory approval for this same reason.

ACQUIRED LQTS: A COMMON AND INTRIGUING DISORDER

Drug-induced prolongation of repolarization as an antiarrhythmic mechanism was first described 35 years ago (Vaughan Williams class III action) (5), and is a property of several widely used antiarrhythmic agents including quinidine, amiodarone and sotalol. More recently, a range of methanesulfonanilide compounds (dofetilide, E4031 and MK499) have been reported to display very potent class III antiarrhythmic actions as a result of the human ether-a-go-go-related gene (hERG) or the rapid component of the delayed rectifier potassium current (I_{Kr}) channel blockade. However, analogous to loss-of-function allelic variants affecting potassium channels, clinical use of these compounds was also shown to increase the risk of arrhythmias and sudden cardiac death. Indeed, torsade de pointes was reported in 1% to 3% of patients treated with quinidine (6), 1% to 5% of patients treated with sotalol (7) and dofetilide (8), and up to 12.5% of patients treated with ibutilide (9). However, the real and intriguing health issue is that drug-induced LQTS has now been reported not only with antiarrhythmic drugs, but also with a wide variety of noncardiac drugs, from which, of course, prolongation of repolarization is entirely undesired (10). Indeed, our group at the Quebec Heart Institute has identified and characterized an important number of drugs with clinically relevant proarrhythmic properties. Among these are cisapride (Prepulsid, Janssen-Ortho, Canada) (withdrawn from the market by Health Canada and the Food and Drug Administration in 2000) (11), thioridazine (Mellaril, Novartis Pharma, Canada) (withdrawn from the market by Health Canada in 2005) (12), droperidol (Inapsine, Janssen-Ortho, Canada) (no longer marketed by Janssen-Ortho in Canada) (13), domperidone (Motilium, Janssen-Ortho, Canada) (no longer marketed by Janssen-Ortho in Canada) (14), risperidone (Risperdal, Janssen-Ortho, Canada) (15), pimozide (Orap, Pharmel Inc, Canada) (16), sildenafil (Viagra, Pfizer Canada) (17), venlafaxine (Effexor XR, Wyeth Canada) (18), erythromycin (ERYC, Pfizer Canada) (19), indapamide (Lozide, Servier Canada) (20), triamterene (various generics) (21), diphenhydramine (Benadryl, Pfizer Canada) (22) and vitamin K (various trademarks) (23). Most of these drugs (if not withdrawn) have seen their respective monographs modified to include appropriate warnings about QT interval prolongation and proarrhythmic potential. Interestingly, one of the most consistent findings is that although drug-induced LQTS could theoretically result from blockade of any of the outward potassium currents contributing to cardiac repolarization, virtually all of these drugs (except indapamide [20]) appear to do so by preferentially blocking I_{Kr} (24). Given that, one should wonder why all patients treated with these drugs do not suffer from arrhythmia. Therefore, this finding suggests that some individuals are especially predisposed to this adverse effect as a consequence of genetic and/or environmental factors.

CONGENITAL AND ACQUIRED LQTS: WHICH ONE PRECIPITATES THE OTHER?

The demonstration of incomplete penetrance in congenital LQTS led to the speculation that carriers of silent or latent ion channel allelic variants, even with no signs of the typical phenotype, may be more susceptible to arrhythmias should they be exposed to triggering factors. Therefore, molecular screening of the LQTS genes in patients with drug-induced prolongation of the QT interval and torsade de pointes was carried out, and cases of silent or latent allelic variants have been reported (25-34). Although quite interesting from a mechanistic perspective, these reports were disappointing from an epidemiological point of view. Indeed, variants in ion channel genes associated with an increased risk for proarrhythmia were identified in only a minority of patients (4% to 15%) with drug-induced torsade de pointes (10).

OTHER RISK FACTORS

Numerous factors that increase the risk of torsade de pointes among patients treated with QT-prolonging drugs have been identified. These include female sex (35), heart disease (left ventricular hypertrophy or dysfunction, heart failure and myocardial infarction) (10), hypokalemia (which not only reduces baseline I_{Kr} (36), but also increases sensitivity of the current to drug-blocking effects [37]), high drug levels (due to excessive dosage or impaired elimination) and drug interactions (most often involving inhibition of P450 hepatic metabolizing enzymes) (10). Indeed, we identified the P450 enzymes involved in the biotransformation of domperidone, a potent I_{Kr} blocker (38), which led to a warning of potential life-threatening drug interactions. Moreover, we conducted a clinical study evaluating a drug interaction between cisapride and simvastatin, two drugs metabolized by the same P450 enzyme, namely, CYP3A4 (39). We showed an elevation of cisapride concentration (and consequently of the proarrhythmic risk) when used in combination with simvastatin (Zocor, Merck & Co Inc, USA).

Other less commonly identified risk factors for torsades de pointes include bradycardia (40), diuretic use (even in the absence of hypokalemia) (10), hypomagnesemia (41) and prolonged baseline QT interval (including patients with unrecognized congenital LQTS) (42-44). Interestingly, only a minority of subjects treated with QT-prolonging drugs develop drug-induced proarrhythmia. Therefore, interplay of multiple precipitating factors is probably needed to induce proarrhythmia.

REPOLARIZATION RESERVE

In the normal human heart, potassium currents (such as I_{Kr} and the slowly activating cardiac potassium current [I_{Ks}]) contribute to preserve a stable repolarization process and a sufficiently large 'repolarization reserve'. Multiple ion currents contribute to normal repolarization. This physiological redundancy precludes torsade de pointes. However, patients may have multiple risk factors that contribute to the reduction of repolarizing currents. Therefore, whenever repolarizing currents are genetically reduced, such as in the presence of allelic variants affecting cardiac ion channels, the superimposition of common environmental factors such as hypokalemia or I_{Kr} -blocking drugs may result in failure of repolarization and torsade de pointes (41). Classic examples of this concept have been described. Napolitano et al (25) reported a case of proarrhythmia induced by the I_{Kr} -blocking drug cisapride in a patient presenting with a silent mutation on *KCNQ1*, encoding for the

alpha subunit of I_{Ks} . Another *KCNQ1* mutation has been reported by Kubota et al (26) in a patient presenting with torsade de pointes induced by hypokalemia, well known to reduce I_{Kr} (36). Furthermore, a mutation on hERG, the alpha subunit of I_{Kr} , was reported in a patient with quinidine-induced torsade de pointes (29). Another report showed that the I_{Kr} -blocker dofetilide was identified as the culprit drug for torsade de pointes in patients with a mutation on *KCNQ1* (28). These reports support the idea that altering the repolarization reserve simultaneously on two or more fronts increases the likelihood of failure of repolarization and ventricular arrhythmias. We have also demonstrated this concept by showing that indapamide (an I_{Ks} blocker) potentiates the action potential-prolonging effect of d-sotalol (an I_{Kr} blocker) in dogs (45), as well as in guinea pigs, when dofetilide is used as an I_{Kr} blocker (46).

ARRHYTHMIAS DURING MYOCARDIAL ISCHEMIA

Cardiac ischemia causes life-threatening cardiac arrhythmias by decreasing conduction velocity and shortening action potential duration (APD) (47). Cardiac arrhythmias are likely to arise from a combination of disturbances both to the active ionic properties of plasma membrane channels, as well as to the electrical and metabolic GJ-mediated coupling between cells. The Cellular and Molecular Pharmacology Laboratory of the Quebec Heart Institute is primarily interested in the study of two categories of ion channels involved in the cardiac electrical activity: potassium channels and GJs. Both channel types play a major role in the fine-tuning of the APD and conduction properties.

In the heart, ischemia is known to profoundly disturb the cell environment. Increased levels of H^+ , oxygen free radicals and specific lipid metabolites such as arachidonic acid, nonesterified free fatty acids, long-chain acylcarnitines and lysolipids, have been reported (48-53). Because the effects of excessive production of some of these metabolites on cardiac potassium and GJ channels were still unknown, we intended to determine the specific effects of H^+ and hydrogen peroxide (H_2O_2) on two major repolarizing currents: I_{Kr} and the ultrarapid potassium current (I_{Kur}). We also tested the effects of an early marker of ischemia, which possesses important deleterious properties on GJ intercellular coupling and intracellular calcium homeostasis: lysophosphatidylcholine (LPC).

External pH and oxygen free radicals are likely to contribute to arrhythmias during acute myocardial ischemia and reperfusion

Exposition of cardiac myocytes to low external pH (pH_o) decreases the resting potential and shortens the APD (54). We were the first to demonstrate that pH_o affects hERG, a potassium channel responsible for the cardiac I_{Kr} (55). Our main findings were that the hERG current amplitude was decreased while kinetics of activation and deactivation were accelerated at a pH of 6.0, and that activation was accelerated and voltage activation at which 50% of channels were activated was negatively shifted at a pH of 8.0. Because these effects were not prevented when the intracellular HEPES concentration was raised, they were not likely to be due to changes in intracellular pH. The precise mechanisms responsible for the observed changes are still unknown. They may involve surface charge screening by accumulation of positive charges (H^+),

which could explain the negative shift observed for hERG properties, titration of hydrogen acceptor groups (such as histidine residues) of hERG protein that may impose strains on the channel structure or repelling of K^+ ions by H^+ ions near the channel pore.

The reduction of oxygen generates extremely reactive mediators, the reactive oxygen species (ROS) that includes oxygen free radicals (56). The role of ROS in tissue damage has been demonstrated in ischemia, reperfusion, AF and heart failure progression (53,57-59). The presence of oxygen free radicals has been shown to increase the incidence of cardiac arrhythmias in animal models (60). The electrophysiological effects of ROS include a reduction in the action potential amplitude and the APD (61), but the underlying mechanisms are only partially understood.

Because the effects of ROS on I_{Kr} and I_{Kur} were unknown, we aimed to characterize the effects of H_2O_2 on the gating properties of hERG and $hK_v1.5$, the molecular correlate of a major atrial repolarizing current, I_{Kur} . H_2O_2 is an important producer of ROS that has a relatively long half-life and easily diffuses through hydrophobic membranes (62). We found that external application of H_2O_2 (30 μ M to 1000 μ M) accelerated activation and deactivation of hERG, and induced a negative shift in its activation current-voltage relationship curve (63). These effects were prevented by intracellular application of catalase, but superoxide dismutase only prevented acceleration of hERG activation. These results indicate that H_2O_2 diffuses inside the cell before acting on hERG and affects the deactivation process through a superoxide-independent mechanism. Moreover, changes in activation and deactivation kinetics were not concomitant, which further suggests that these effects were not produced by the same pathway or at the same site on hERG protein. Potential targets for H_2O_2 -mediated hERG oxidative strain can be the Per-Arnt-Sim structure of its *n*-terminus that is known to confer redox sensitivity for several proteins, the cysteine SH groups, membrane phospholipid peroxidation, and activation of intracellular cascade mediators such as protein kinase C and adenylyl cyclase. Gating properties of $hK_v1.5$ were also affected by H_2O_2 : activation kinetics were accelerated while the activation curve was negatively shifted (64). The shift in the current-voltage curve was voltage-dependent; the current increased when voltage was below +20 mV but decreased for higher depolarizing voltages. The presence of ROS scavengers (catalase or superoxide dismutase) in the intracellular medium blunted the effects of H_2O_2 at or below +20 mV, indicating that H_2O_2 diffuses into the intracellular medium before acting on $hK_v1.5$ and that its effect on the activation process may be mediated by the superoxide anion. As with hERG, oxidation of $hK_v1.5$ amino acid residues (such as cysteine) or elements of intracellular signal cascades known to regulate $hK_v1.5$ (such as adenylyl cyclase) represent mechanisms by which H_2O_2 may modulate this channel.

We then concluded that the modulation of major cardiac potassium channels by two important mediators of ischemia (ie, pH and ROS) leads to a faster activation and/or an increase in current amplitude of hERG and $hK_v1.5$, allowing a greater amount of K^+ ions to flow through these channels in the early phase 3 of the action potential. This produces a reduction in APD that leads to an acceleration of the deactivation process of hERG, reducing potassium current at the end of the action potential and early during the diastole, which may preclude the protective effect of the slow deactivation against

premature beats (the persistence of a relatively low membrane resistance at the beginning of the phase 0 may neutralize the effects of anomalous depolarizing currents). Both phenomena are likely to contribute to the onset of ischemia-induced cardiac arrhythmias. Moreover, considering the oxidative stress produced by AF, the H_2O_2 -induced changes in $\text{hK}_{v1.5}$ reported in our study may be involved in the self-perpetuating tendency of the disease. Our results support a relationship between the well-accepted free radical theory of aging and the age-related prevalence of AF. They also support the use of antioxidant therapy in the development of myocardial diseases such as cardiac ischemia.

LPC – a major cause of electrical uncoupling and cardiac arrhythmias early in ischemia

With increasing knowledge of how the constituent myocytes relate to the function of the myocardium as a whole, it has become apparent that intermyocyte coupling is the principal determinant of intercellular conductance and myocardial conduction velocity (65). Thus, research attention has focused on the cardiac GJ, the subcellular structure responsible for low resistance electrical coupling between cardiac muscle cells (66).

The first studies on the effects of ischemia on intercellular coupling were performed in 1976, when Bredikis et al (67) measured the passive electrical properties in the rabbit heart papillary muscle under hypoxic conditions. They showed that internal longitudinal resistance (the sum of the cytoplasmic and junctional resistances over a length of a cardiac fiber) increases 1.4 times after 10 min of hypoxia. It was Kléber et al (68) who showed a rapid increase in internal resistance after 15 min to 20 min of ischemia, which may have contributed to the occurrence of arrhythmias observed at this duration of ischemia. Dekker et al (69) have more recently shown that the onset of cellular uncoupling that occurs during ischemia is always preceded by an increase in intracellular calcium. We also demonstrated that connexin (Cx) 43 content and spatial distribution are significantly changed during brief episodes of ischemia (70).

LPC, a product of phospholipid degradation, accumulates during the first few minutes of cardiac ischemia (71). In humans, its concentration significantly increases in the coronary sinus after 4 min of rapid atrial pacing and in tissue samples obtained during open heart surgery after 5 min of ischemia (72,73). LPC can reproduce the effects of ischemia on the cardiac action potential, and a direct relationship has been demonstrated between the local blood LPC concentration and the precocity and severity of arrhythmias during ischemia (74). However, the effects of this compound on GJ were still unknown. Using the double whole-cell patch-clamp technique, we were the first to demonstrate that LPC, at concentrations reached during cardiac ischemia (ie, 5 μM to 50 μM), impairs electrical coupling between cardiac myocytes (75). A complete cell uncoupling was observed during LPC superfusion after a concentration-dependent delay, suggesting that this lipophilic compound accumulates in cell membranes. The presence of 5 mM external Mg^{2+} postponed the decrease in junctional conductance, suggesting that effects of LPC could be mediated by an increase in intracellular Ca^{2+} (Mg^{2+} is known to limit the effects of intracellular Ca^{2+} overload [76]). Action potential amplitude and APD were decreased during LPC superfusion, but we showed that cell excitability was preserved

when cell coupling was reduced sufficiently to block action potential transfer. Taking into account the time lag of LPC action, our results are consistent with the delay of ischemia-induced increase in internal resistance in papillary muscle reported by Kléber et al (68). Thus, in addition to changes in pH_o and long-chain acylcarnitines, which also have been shown to reduce junctional conductance (77,78), LPC can be a major cause for electrical uncoupling and cardiac arrhythmias early in ischemia.

In additional experiments designed to identify the mechanisms mediating LPC-induced uncoupling, we found that ethanol protects against this detrimental effect (79). We then performed complementary experiments using the fura-2 fluorescence technique to assess LPC and ethanol effects on the intracellular calcium dynamics in cardiac myocytes (80). We demonstrated that ethanol was able to delay and reverse LPC-induced calcium overload, an indicator of cell injury. LPC-induced increase in membrane permeability was also postponed by ethanol (ie, the fura-2 retention time, which reflects increased membrane permeability). We showed that LPC-induced Ca^{2+} overload is primarily mediated by action on the sarcolemma. Using different specific blockers of ionic channels and transporter proteins (such as La^{3+} and Li^+), we concluded that the contribution of the L-type calcium current and Na-Ca exchanger to LPC-induced Ca^{2+} overload are unlikely. Opposing effects of LPC and ethanol may be due to an interaction at the level of insertion in membrane leaflets (LPC, as opposed to ethanol, does not flip quickly into the inner monolayer), changes in the LPC physical state (ie, formation of micelles) or changes in Ca^{2+} delivery to the cytoplasm. The protective effects of ethanol were obtained at clinically relevant concentrations (ie, 22 mM or 100 mg/dL) and may offer an explanation for its well-established cardioprotective effects in humans reported in numerous epidemiological studies (for moderate consumption) and experimental (acute and chronic) animal models (81,82).

In another series of experiments (80), we found that ethanol may protect cells by decreasing the critical micellar concentration (the concentration at which individual lipid molecules assemble to form micelles), thus increasing the proportion of LPC in micelles. When we studied the effect of different alcohols, we found that the increase in critical micellar concentration of LPC by *n*-alcohols was proportional to chain length (83). However, as the alcohol chain length increased, the protection against LPC-induced Ca^{2+} overload decreased and disappeared completely with *n*-pentanol, implying that micelle formation was not involved in the extension of the lag time.

GJs modulation by rapid pacing

It has been suggested that the electrical coupling of cardiac cells is perturbed during fibrillation or rapid pacing of cardiac fibers (67). Maurer and Weingart (84) measured GJ coupling on a pair of isolated cardiac cells using the double patch-clamp method. However, the experimental protocol was limited to 20 depolarizations at 12.5 Hz during 1.6 s and at conditions relatively far from physiological ones: only one cell from the pair was stimulated, while the other was maintained in a resting state. Calcium chloride and EGTA in the patch pipettes maintained intracellular P_{Ca} at 7.6 (avoiding the normal increase in intracellular calcium during contraction), and the junctional resistance spontaneously increased fourfold in 17 min. A possible

Figure 1) Contraction cycle of a pair of ventricular myocytes isolated from a guinea pig heart. The axis going through the two patch-clamp electrode tips remained stable during the contraction. The arrows show the contraction and relaxation phases, respectively

regulation of GJ coupling during cardiac contraction, especially at high frequencies such as those reached during tachycardia and fibrillation, is still a matter of debate. We used the double patch-clamp technique on isolated cardiac cell pairs (see references 85 and 86 for technical details). After establishment of the double whole-cell configuration, we stimulated both cells in a transmural electric field. The current intensity was raised until cells contracted in response to each stimulus. Before performing the double patch-clamp technique, a stable transverse axis near the middle of the cells (in the side-to-side configuration) was carefully identified, apart from which cells shortened. Mechanical stability of the double whole-cell during stimulation was obtained by applying both electrode tips on this stable transverse axis (Figure 1). Different nominal intracellular P_{Ca} levels were tested, and an intracellular P_{Ca} of 6.5 enabled the cells to contract in response to the stimulations. We used strong (four to 10 times the threshold) and long (5 ms) stimulations to destabilize the voltage clamp for a short period of time and to allow cell contraction, and measured the junctional resistance during the stimulation period. The results presented in Figure 2 show that the junctional conductance was progressively reduced during the 4 min stimulation period at 2.5 Hz. Our results suggest that high frequencies of cardiac contraction tend to reduce GJ coupling and, thus, may contribute to abnormal electrical conduction. Eventually, this uncoupling may also provoke an electrical insulation of ectopic foci with a rapid triggering rate.

GENETIC BACKGROUND INFLUENCES SUSCEPTIBILITY TO ARRHYTHMIAS

Implication of the genotype is now well recognized as an important factor responsible for several inherited arrhythmogenic diseases (87). However, the clinical consequences of genetic defects are variable, suggesting that they are not the only determinants of disease manifestation. Other factors such as common polymorphisms, aging, sex, metabolic imbalance such as diabetes, and stresses such as a major surgery, could modulate the time when a mutation-related disease is revealed.

Figure 2) Currents recorded using the double whole-cell patch-clamp technique on a pair of isolated ventricular cells. The junctional current was elicited by the application of a 30 mV transjunctional voltage, and was recorded during the control period (control), following a 4 min period of stimulation (stim) at 2.5 Hz (two stim artifacts are visible) and 3 min after the end of the stim period (poststim)

Identification of allelic variants responsible for AF

AF is the most common sustained cardiac rhythm disturbance. Several mutations (seven chromosomal loci mapped and four genes) have been associated with a few AF cases (88-90), but all the studies available so far have addressed a limited number of patients, which precludes identification of genes by genetic linkage analysis. We initially performed a sequence analysis of Cx40, an isoform specifically expressed in human atria, in two French-Canadian families presenting the familial form of AF, but we found no correlation with the disease (91). However, other case-control association studies (92) in patients with structural heart disease and AF have suggested a predisposition to AF for patients with a Cx40 gene polymorphism. We also searched for mutations in $hK_v1.5$, a major repolarizing current of the human atria. In a population of 185 French-Canadians undergoing coronary bypass, we found three heterozygous single nucleotide polymorphisms (SNPs): R87Q, A251T and P307S (93,94). Functional analysis of the variants obtained after direct mutagenesis and expression in the CHO cell line showed that two variants (R87Q and P307S) affected the expression level of the channel in a dominant-negative manner. Both variants reduced the slow inactivation that develops during depolarization, while only R87Q accelerated the channel opening. These results were not reproduced in the HEK293 cell line, suggesting that the beta subunit present in the CHO cells (and in human atrial cells), but absent in HEK293 cells, is essential to observe the changes induced by the SNPs. Considering the role of $hK_v1.5$ in human atrial repolarization and that two of the SNPs we identified could potentially lead to abbreviation of the atrial action potential, we also assessed the possibility that these SNPs could predispose to the development of postoperative AF (94). Of the 185 patients screened after their coronary bypass, 96 had postoperative AF. The frequencies of SNPs were higher in patients with postoperative AF, but a larger patient population was required to reach statistical significance. We think that a genetic background associated with surgical-related factors, such as the oxidative stress, predisposes to the development of postoperative AF. Our findings should be considered to be a pilot study for the assessment of the relationship between $hK_v1.5$ SNPs and AF and to provide a better insight into the role of the genetic basis of AF. Another group from Vanderbilt University (USA) has identified a number of other $hK_v1.5$ variants in an ethnically

different population (Caucasians, African-Americans and Asians), including polymorphisms conferring drug resistance to commonly used antiarrhythmic drugs (95). They also proposed a structural mechanism for this observed drug resistance, thus providing a genetically related explanation for failure of drug treatment of common atrial arrhythmias (96).

CONCLUSIONS

Over the past decade, the Quebec Heart Institute has provided a significant amount of original data in the field of basic cardiac electrophysiology. Indeed, the identification and characterization of the QT-prolonging properties of a substantial number of widely used drugs has contributed to their withdrawal and, more importantly, has redefined the whole drug approval process by the regulatory authorities, such as Health Canada and the American Food and Drug Administration. It has also contributed to a better understanding of the cellular pathophysiological mechanisms involved in the genesis of

ischemia-related arrhythmias by characterizing the role of proarrhythmic factors such as pH, ROS and membrane phospholipid degradation products. We have also extended our studies to genetic predisposition to arrhythmias and pharmacogenetics. We have identified a number of allelic variants likely to be involved in the pathogenesis of AF and in the way it may respond to drug treatment. Because a number of important questions still remain, notably in the field of environmental predisposition to cardiac arrhythmias, we are currently focused on different risk factors for heart rhythm disturbances, such as diabetes, obesity and the nearly epidemic metabolic syndrome afflicting Canadians of all ages.

ACKNOWLEDGEMENTS: Dr Drolet is the recipient of a New Investigator Scholarship Award from the Heart and Stroke Foundation of Canada. Dr Simard is the recipient of a Chercheur-Boursier Scholarship Award from the Fonds de la recherche en santé du Québec.

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