Cardiac Safety of Noncardiac Drugs

Cardiac Safety of Noncardiac Drugs

Practical Guidelines for Clinical Research and Drug Development

Edited by

Joel Morganroth, MD
Ihor Gussak, MD, PhD, FACC

eResearchTechnology Inc.,
Philadelphia, PA; Bridgewater, NJ; Peterborough, UK

Humana Press
Totowa, New Jersey

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512
www.humanapr.com

For additional copies, pricing for bulk purchases, and/or information about other Humana titles,
contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699;
Fax: 973-256-8341, E-mail: humana@humanapr.com; or visit our website at www.humanapress.com.

Production Editor: Mark J. Breaugh.

Cover design by Patricia F. Cleary.

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American National Standards Institute) Permanence of Paper for Printed Library Materials.

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

eISBN: 1-59259-884-6

Library of Congress Cataloging-in-Publication Data

Cardiac safety of noncardiac drugs : practical guidelines for clinical
research and drug development / edited by Joel Morganroth, Ihor Gussak.
p. cm.
Includes bibliographical references and index.
ISBN 1-58829-515-X (alk. paper)
1. Cardiovascular toxicology. 2. Heart--Effect of drugs on. 3.
Drugs--Side effects--Testing. 4. Electrocardiography. I. Morganroth, Joel.
II. Gussak, Ihor.
RC677.C36 2005
616.1'07--dc22

2004014055

Dedication

To our families, without whose support our professional activities would be diminished:

To my Wife Gail,
and Children Jason, Jennifer, and Jessica,
and to my Parents Ben and Grace

Joel Morganroth

To my Wife Hiie,
and Children Maria and Georg,
and to my Parents Bohdan and Maria

Ihor Gussak

PREFACE

Joel Morganroth, *MD*

Ihor Gussak, *MD, PhD*

It is generally easy to define the efficacy of a new therapeutic agent. However, what is even more difficult and more challenging yet more important is to define its safety when administered to millions of patients with multi-faceted diseases, co-morbidities, sensitivities and concomitant medications. The commonest cause of new drug discontinuations, cause for disapproval from marketing and removal from the market after approval is a drug's effect on cardiac repolarization which is essentially identified by increasing the duration of the QTc interval duration on the standard 12-lead electrocardiogram (ECG).

Cardiac Safety of Noncardiac Drugs: Practical Guidelines for Clinical Research and Drug Development is designed to present the current preclinical, clinical, and regulatory principles to assess the cardiac safety of new drugs based primarily on their effects on the ECG. Practical guidance to define cardiac safety at all stages of clinical research and drug development are featured and discussed by internationally recognized experts with academic, industrial, and regulatory experience. Each chapter contains the best available evidence, the author's personal opinions, areas of controversy, and future trends. Although some of the areas are highly specialized, this book has been designed for a broad audience ranging from medical and graduate students to clinical nurses, clinical trial coordinators, safety officers, data managers, statisticians, regulatory authorities, clinicians, and scientists.

The book is organized in a practical and easy to assimilate manner, with each chapter focusing on a particular aspect of cardiac safety. Part I contains an historical overview from a clinical and regulatory prospective. Part II is devoted to preclinical and pharmacogenomic aspects of cardiac safety in clinical research and drug development. Part III includes clinical methodologies and technical aspects of assessing cardiac safety of investigational drugs with the main focus on cardiac repolarization, especially as defined by the duration of the QTc interval. Part IV provides a comprehensive review of the application of electrocardiology in clinical research, including fundamentals of ECG interpretation in clinical trials, cardiac safety assessment in all phases of drug development, statistical analysis plans for ECG data obtained in formal clinical trials, and practical interpretation of the results. Finally, Part V presents a broad spectrum of domestic and international regulatory aspects in assessing the cardiac safety in clinical research and drug development.

The editors of *Cardiac Safety of Noncardiac Drugs* wish to recognize the significant contribution made by all of the contributing authors. The book is the result of a collaboration that has brought together the skills and perspectives of researchers, scientists, and clinicians. Finally, we hope that the book will become a primary reference for drug developers in all therapeutic areas as well as academicians consulting in this arena.

Joel Morganroth, *MD*
Ihor Gussak, *MD, PhD*

CONTENTS

Part IV. Application of Electrocardiology in Clinical Research

Part V. Regulatory Considerations

CONTRIBUTORS

AMY M. ANNAND-FURLONG, MS • *Senior Vice President, Regulatory Compliance, eResearchTechnology Inc., Philadelphia, PA, USA*

CHARLES ANTZELEVITCH, PhD, FACC, FAHA • *Executive Director and Director of Research, Gordon K. Moe Scholar, Experimental Cardiology Program Director, Masonic Medical Research Laboratory, Utica, NY, USA; Professor of Pharmacology, Upstate Medical University, Syracuse, NY*

FABIO BADILINI, PhD • *Chief Scientist, AMPS-LLC, New York, NY, USA*

MARTIN P. BEDIGIAN, MD • *Clinical Research and Development, Novartis Pharmaceuticals, East Hanover, NJ, USA*

ARTHUR M. BROWN, MD, PhD • *Professor, Physiology and Biophysics, Case Western Reserve University, MetroHealth Campus, Chairman and CEO, ChanTest, Inc., Cleveland, OH, USA*

BARRY D. BROWN, MS • *Product Integration Manager, Mortara Instrument, Inc., Milwaukee, WI, USA*

ROBERT BROWN • *Senior Vice President, eResearchTechnology Inc., Philadelphia, PA, USA*

JEAN-PHILIPPE COUDERC, PhD, MBA • *Assistant Professor of Medicine (Cardiology), Assistant Director of Heart Research Follow-up Program, University of Rochester Medical Center, Rochester, NY, USA*

GERALD A. FAICH, MD, MPH, FISPE • *President, Pharmaceutical Safety Assessments, Inc., Narberth, PA, USA*

SCOTT GRISANTI • *Senior Vice President, Business Development and Chief Marketing Officer, eResearch Technology Inc., Philadelphia, PA, USA*

IHOR GUSSAK, MD, PhD, FACC • *Vice President, Global Medical Affairs, eResearchTechnology Inc., Bridgewater, NJ; Clinical Associate Professor of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; Associate Editor, Journal of Electrocardiology, Bridgewater, NJ, USA*

ALAN S. HOLLISTER, MD, PhD • *Clinical Pharmacology Unit Director, GlaxoSmithKline, Philadelphia, PA, USA*

RICHARD JUDSON, PhD • *Chief Scientific Officer, Senior Vice President, Research & Development, Genaissance Pharmaceuticals, New Haven, CT, USA*

ROBERT S. KASS, PhD • *David Hosack Professor of Pharmacology, Chairman, Department of Pharmacology, Columbia University College of Physicians and Surgeons, New York, NY, USA*

ROBERT KLEIMAN, MD, FACC • *Senior Director of International Cardiology, eResearchTechnology Inc., Philadelphia, PA, USA*

RAYMOND JOHN LIPICKY, MD • *Director, LIPICKY LLC, Gaithersburg, MD; Former Director, Cardio-Renal Drug Products Division, Food and Drug Administration, USA*

JEFFREY S. LITWIN, MD, FACC • *Senior Vice President and Chief Medical Officer, eResearchTechnology Inc., Philadelphia, PA, USA*

PIERRE MAISON-BLANCHE, MD • *Hopital Lariboisiere, Paris, France*

TIMOTHY H MONTAGUE, MS • *Statistical and Data Sciences, GlaxoSmithKline, Collegeville, PA, USA*

JOEL MORGANROTH, MD • *Chief Scientist, eResearchTechnology Inc., Clinical Professor of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*

JUSTIN L. MORTARA, PhD • *Vice President of Sales and Marketing, Mortara Instrument Inc., Milwaukee, WI, USA*

ARTHUR J. MOSS, MD, FACC • *Professor of Medicine (Cardiology), Director of Heart Research Follow-Up Program, University of Rochester Medical Center, Rochester, NY, USA*

JEANNE M. NERBONNE, PhD • *Alumni Endowed Professor, Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, MO, USA*

NENAD SARAPA, MD • *Director, Clinical Pharmacology, La Jolla Laboratories, Pfizer Inc., San Diego, CA, USA*

RASHMI R. SHAH, MD, BSc, MBBS, FRCP, FFPM • *Senior Clinical Assessor, Medicines and Healthcare products Regulatory Agency, London, UK*

ANNETTE STEMHAGEN, DrPH, FISPE • *Vice President, Strategic Development Services, Covance Inc., Radnor, PA, USA*

WOJCIECH ZAREBA, MD, PhD • *Associate Professor of Medicine (Cardiology), Director of Clinical Research, Cardiology Unit, Associate Director of Heart Research Follow-up Program, University of Rochester Medical Center, Rochester, NY, USA*

I Introduction

1 Cardiac Safety of Noncardiac Drugs

Historical Recollections

Raymond John Lipicky, MD

CONTENTS

INTRODUCTION

This chapter is a set of recollections that would be difficult to document because they are based on my experience as a Food and Drug Administration (FDA) regulator and a pharmacologist with a special interest in cardiac repolarization. As is the common precedent, this is not related to considerations of antiarrhythmic drugs. Rather, this focus is upon drugs that have not been developed for the express purpose of modifying the behavior of cardiac ion channels, but as we all know many unexpectedly do.

My personal recollections about the "QT" and torsades de pointes (TdP), start somewhere in the early 1980s regarding the drug lidoflazine, and in this instance my recollections are very vague. Lidoflazine, an anti-anginal calcium channel blocker was being considered (for symptomatic relief of angina) in the early 1980s. The initial principal issue was an unusually high incidence of atrial fibrillation as an adverse effect. There was, as I remember, neither QT discussion nor argument, but then (after initial deliberations were concluded) it became recognized, by an isolated publication and anecdote that TdP was associated with lidoflazine as well as prolongation of the QT_C interval. The role of this latter observation played in final decision making is not within my recollection (so, it must not have been important).

The next event I recall was a meeting held in Philadelphia *(1)* in 1992 to discuss cardiac repolarization with special emphasis on the clinical significance of QT interval measurements. The major unstated question was "how much QT prolongation was acceptable?"

From: *Cardiac Safety of Noncardiac Drugs:*
Practical Guidelines for Clinical Research and Drug Development
Edited by: J. Morganroth and I. Gussak © Humana Press Inc., Totowa, NJ

At that meeting, I declared that any QTc interval prolongation was "bad," even 5 ms. Bad was used in the sense that such a finding raised some element of uncertainty and by inference imparted a mortality risk for a new drug, and that risk could be critical in determining the approvability of a nonantiarrythmic drug, especially if the indication was simply symptom relief. Others said that there must be some degree of prolongation of the QTc interval that would not be important enough to impact on the drug's approvability, of course, trying to define an acceptable risk vs benefit ratio. No person, however, had data (including me) that could be brought to bear on either point of view. The debate and search lives on, although 5 ms as a cut-off has been declared invalid by recent decision making (6 ms must be acceptable, see moxifloxacin).

There are hundreds of references that could be cited and dozens of conferences that could be referenced regarding cardiac repolarization and regulatory inferences but, in my opinion, they all add little to my considerations here, although they do represent a large database of opinions as well as new important data, some of which are represented elsewhere in this book.

What I think has been established is that the QT_C interval duration itself is not a sufficient condition to cause TdP. It is necessary as one component of making the diagnosis, else the ventricular arrhythmia is called multiform or polymorphic ventricular tachycardia. Nonetheless, we also know that persons with hereditary defects of the IK_r cardiac ion channel can have (although some do not) a prolonged QTc interval (presumably from in-utero), but can go decades without developing TdP. Some additional factor (other than the absence of currents mediated by the IK_r channel) is necessary to set off this ventricular tachyarrhythmia. We also now know that those patients who actually have a short QT interval duration on the basis of a hereditary defect in cardiac ion channels may also be at risk for a ventricular arrhythmia *(2–4).* There seems to be no reason to only be concerned with QT_C prolongation, except that it has been historically associated with TdP.

Since the early 1990s, astemizole, cisapride, grepafloxacin, terfenadine, and terodiline (all of which prolonged the QTc interval duration) were withdrawn from the market because of drug-induced TdP, not because they affect the QT interval. Yet, other drugs such as bepridil, moxifloxacin, and newer antiarrhythmic drugs have been approved for marketing despite known effects on increasing QTc interval duration with some having known TdP associated with their use before approval. These decisions were primarily based on an analysis of the risk vs benefit of a drug. Such assessment is a difficult task and subject always to increasing knowledge and experience. The following nonexhaustive set of examples outline some of the issues, as I remember them, involved in such regulatory assessment. Bear in mind that decisions made were in the context of what was known or believed to be known at the time and only those aspects that have a relationship to QT issues are included. The examples are not organized chronologically, because regulatory actions were taken at various times in relationship to when factual information first became available.

DISCUSSION

Assessment of the Risk–Benefit Ratio

Bepridil Approved 1990 —Still on the Market

Bepridil, a calcium channel blocker, is an example of a regulatory judgment that, loosely interpreted, means if a drug has unusual benefits despite demonstrated increased

risk for mortality and, especially if there is no other member of the pharmacological class available for patients, that marketing approval can be achieved. Bepridil was at the time of approval known to produce dose-related increases in QT_C duration (about 8% or 30 to 70 ms change from baseline), as well as other ventricular arrhythmias. Moreover, in French post-marketing experience, Bepridil was known to produce TdP (over an 8 yr duration 124 verified cases were reported).

In more than one trial, bepridil demonstrated anti-anginal efficacy based on symptom-limited exercise tolerance trials. Approval, however, hinged entirely upon one trial in which randomized patients, who were intolerant to or continuing to have symptoms at maximum doses of diltiazem, were shown to respond better to bepridil when compared either to placebo or diltiazem. Thus, it was judged that bepridil was shown to have superior efficacy compared to other approved therapies and such alternatives were not available. Thus, bepridil was approved as a second line agent, approval, reserved for use when all others failed (in such a circumstance it was "better" than nothing because nothing else was available) despite its liability of known QTc prolongation and known TdP and known other ventricular arrhythmias.

Moxifloxacin Approved in 1999—Still on the Market

Moxifloxacin, a fluroquinolone antibiotic with a clear dose-related increase in QTc duration and a placebo-corrected mean change from baseline of 6 ms at 400 mg per day was approved. Its short-term use (up to 14 d) for a potentially life-threatening infection was the basis for marketing approval. A phase IV commitment that included a large simple trial was required. In more than 18,000 subjects, the trial revealed no TdP events (*see* Chapter 13). Additionally, post-marketing experience to date has noted about 15 TdP in close to 20 million patients treated, though most if not all had alternative explanations for the TdP event (e.g., concomitant use with sotalol, etc.).

Terfenadine Approved in 1985—Taken Off the Market in 1998, Cisapride Approved in 1993—Effectively Taken Off the Market in 2000

Surprisingly, the debates and endless reviews relating to terfenadine (a nonsedating antihistamine) and cisapride (a prokinetic gastrointestinal drug for reflux disease) were not related so much to whether or not TdP was related to their use (even though the incidence was rare, in the range of <1/10,000 to 100,000 exposures), but initially as to whether there was objective demonstration of prolongation of the QT_C interval duration. After further QT studies with terfenadine a mean change from baseline of about 6 ms on average was demonstrated. Although QT_C prolongation was known and production of TdP was uncontested, terfenadine remained on the market ("appropriately" labeled) until another nonsedating antihistamine that did not have the QT_C effects became available. This represents another example of the loosely interpreted principle that "something better than something else" (in this case nonsedating properties) was "worth" the risk.

At the time of cisapride approval, no ECG trial had been conducted. Retrospectively, the then existing data can be interpreted to show an effect on QTc duration in the same range as terfenadine existed. Cisapride was finally removed from active marketing when the FDA and the sponsor (each, initially unwilling to accept the rare reports as representing a risk) accepted that the increasing risk of voluntary post-marketing reports of TdP was not worth the benefit of some relief of dyspepsia.

Mibefradil Approved in 1997—Taken Off the Market in 1998

Mibefradil, the first selective T-type calcium channel antagonist was withdrawn from the market primarily because of drug–drug interactions (there were serious events associated with at least 24 other drugs commonly used in cardiovascular medicine). The interactions were produced from mainly CYP3A4 inhibition produced by mibefradil. The withdrawal from the market was neither based on its effects on cardiac repolarization nor the observed cases of TdP.

The QT_C issues associated with mibefradil's original approval illustrate the difficulty in defining what to measure as well as how to interpret whatever one measures. The analysis is recounted here, cursorily, because to my knowledge this was the singular (and only) time that the FDA actually looked at raw ECG data and measured QT intervals from raw ECG recordings. The QT_C data we derived were only corrected by Bazett's formula, which was the standard in the mid to late 1990s.

In the original NDA routine analysis of routine ECGs obtained in the clinical trials (hypertension and angina were the target populations' disease indications) produced data that showed that mibefradil prolonged the QT_C interval and the prolongation was dose-related. Were that accepted as fact, mibefradil would not have been approved. Consequently, a great deal of attention was paid to analysis of the QT_C variable by both the sponsors and FDA, and perhaps because of that attention, the implications of the effects of mibefradil on the CYP3A4 system were largely overlooked.

All ECGs that were declared by the previous routine analysis to have had a prolongation of the QT_C was the material that was to be analyzed, by patient and week in study, by myself from original ECGs, calipers, an EXCEL spreadsheet, and two sponsor representatives. After 31 patients' ECGs were examined (probably in the order of 200 or so ECGs) I decided to stop looking at ECGs, because the same phenomenon was boringly and consistently observed in the first 31 patients. So boring and consistent were they, that I decided it was a waste of time to look at any more.

Basically, one had to be sensitive to minuscule U waves seen in the prerandomization records. Surprisingly, U waves could be detected in over 50% of the patients' ECGs. What was measured was both the QT and QU interval if there was a U wave detected (or suggested) in the prerandomized ECGs. Both intervals were then corrected for heart rate by the Bazett formula. In those patients in whom no U wave was detected at baseline, there was a prolongation in the QT_C because no QU had been able to be measured at baseline. In those individuals in whom a U wave was detectable at baseline, there was no change in QU_C although the QT_C was prolonged.

In each of the patients that had a U wave detectable at baseline, lining up the ECGs as a function of weeks in study (e.g., baseline, wk 1, wk 2, wk 4, wk 8, etc.) showed the U wave growing (as a function of time in study), and becoming indistinguishable from the T. Thus, we concluded and the Cardiac and Renal Drugs Advisory Committee concurred, that there was no effect of mibefradil on ventricular repolarization, although there was an alteration of morphology. We and our panel of advisors did not know how to consider this abnormal form of repolarization, and thus we decided that mibefradil should be approved for both hypertension and angina, and that nothing more than a description of the phenomenon should appear in the package insert.

Our conclusion was based on many factors in addition to the lack of effect on intervals. Included among those factors: mibefradil (after very careful exploration of a wide range of concentrations) did nothing but shorten the action potential duration in an in vitro

model; animal models of TdP were not able to demonstrate TdP induction by mibefradil; ECGs that showed similar phenomenology when verapamil was administered at high doses in man; some in-silico work produced by Dr. Denis Noble relating mibefradil's known effects on ion channels to surface ECGs; and finally by the relatively event free database associated with the mibefradil development program (though the one patient with a confounded case of TdP may be considered differently today).

Not long after approval (a few months) there were 14 cases of TdP reported to the voluntary adverse drug reaction system of the FDA. Only one of these reports was not confounded by many factors (such as concomitant cisapride use, concomitant bepridil use, congestive heart failure, etc.) and was not considered by the FDA at the time to be sufficient to substantively establish a causative relationship between use of mibefradil and TdP. Subsequently, a report by Glaser et al. *(5)* established a causal relationship by re-challenge. Thus, some agents may have a negative repolarization interval signal but induce repolarization changes with QTc-U wave complexes that may provide, as in the case of mibefradil, a substrate allowing TdP. Thus, careful consideration must be given to the process of ECG acquisition and exposition, and ECGs must be looked at in addition to any analysis of ECG intervals.

SUMMARY OF ANECDOTES

Although the above is an incomplete list of possible anecdotes, the list provided exemplifies that serious post-marketing actions have been based entirely upon clinical events of TdP, and that pre-marketing decision making is spotty at best and in error at worst. Of particular note is that the anecdotes, and my memory, do not include discussions related to the maximum (i.e., peak effect) on QTc in temporal relationship to dose, nor any form of *safety margin* (related to effects at maximum body burden of parent drug and/or metabolites). Such deficiencies in concept have been currently remedied, at least in the Division of Cardiac and Renal Drug Products.

Perhaps more importantly, despite particular recent emphasis on QT measurements (e.g., the Division of Cardiac and Renal Drug Products had around 50 consults per year in early 2000 related to effects of drugs on QT intervals from other FDA divisions), the imprecise value of the QT interval as a predictor of clinical events seems brutally clear. Most of those consultations resulted in determining that there was no definitive information in the data collected and that repeat measurements needed to be made

MEASUREMENT OF THE QT DURATION AND CORRECTION FOR HEART RATE

This book as well as other numerous publications delineates the rather staggering amount of information that has occurred as a consequence of the focus on QT measurements in the drug developing arena. Among the most important being the recognition that using a fixed exponent (in the form of $QT_c = (QT)/RR^{exp}$) is the poorest way to make the heart rate correction for QT to derive the corrected QT or QTc interval. An article by Browne and co-workers *(6)* analyzed the effects of atropine on the QT interval in patients with pacemakers. The effects of atropine when unpaced and the QT interval was estimated by Bazett correction to increase the QT by an average of 43 msec, whereas the actual effect of atropine in the same patients when they were paced was to decrease the QT interval by 24 ms (a 67 ms error).

It now appears reasonable to conclude that the relationship between QT interval and heart rate interval (RR) varies from individual to individual *(7),* and that the individual relationship is preserved over long times. Moreover, the exponent that can be obtained by fitting the RR QT data from each individual has wide variations from the single fixed exponent values that are commonly used to calculate the QT_C duration. Many examples exist in the author's experience where an apparent QT_C effect calculated by a fixed exponent Bazett or Fridericia method disappears when the individual correction formula method is used.

DIGITAL ECGS: THE CURRENT RECOMMENDATION

Almost all commercially available ECG machines digitize the analog signal recoded from the limb and precordial leads—the original data is recorded in digital format—and then converts the digitized ECG back to an analog waveform for purposes of writing it to paper (so that the ECG can be viewed in conventional format). Among the more rational recent events was the notion that digital ECGs (not paper) should be submitted in support of an NDA and/or other submissions to the FDA for any purpose. Numerous public meetings have been held where the details of this notion have been discussed. At present, the FDA is requesting raw ECG data in the FDA XML schema routinely for all ECG trials (*see* Chapter 16).

Thus, in November 2002 the FDA Concept Paper *(8)* was generated as an attempt to summarize what has been learned since the mid-1980s about the regulatory impact of drugs that affect the QTc interval. The use of digital rather than paper ECGs that are processed, stored, and available for review, as well as careful consideration of methods for ECG analysis and interpretation and the conduct of a trial dedicated to reveal the ECG changes in the target species (man) are detailed in this FDA publication.

EXPERIMENTAL DESIGN WHEN EVALUATING THE QT

Although terfenadine should have sensitized everyone to the P450 system, and indeed it is very unusual for any drug presented for approval today not to have carefully delineated those effects, as late as 1997 (mibefradil) an important signal was missed while pursuing a misleading analysis. Such oversights may again occur, but not when designing an appropriate QT evaluation according to current concepts. The concept of having maximum body burden of parent drug and metabolites at steady state (perhaps requiring the presence of a metabolic inhibitor) is now a clearly enunciated (and although sometimes not enforced) request of the FDA.

Among the more important developments, other than collecting and preserving raw data in original form, is defining a purpose for evaluation of the QT. The first principle is to make the measurement appropriately, thus avoiding the endless arguments over the prolongation or lack of prolongation of the QT. Although there may be no exact way to interpret the results, there should be no doubt as to the drug's effects on this variable as a function of dose. The next is the principle that appropriate experimental design must incorporate the use of a positive control. In other words, not finding an effect on QT for a new drug depends heavily upon the entire clinical trial and its analysis being such that it could find a prolongation, if it had been present. Not a novel concept, but now finally being applied.

Lastly, is the notion that the 12 lead ECGs should be recorded continuously (for new Holter technology methods, *see* Chapter 8) and analyzed at discrete time after dose. After the first analysis is complete, if one concludes that other time points should have been measured to better define the time course of effect, one has a recording that contains the

data; as opposed to having to repeat the trial because paper was not collected at those times.

SUMMARY

Perhaps the next decade or two will evolve a more sensible and defensible overall position. For now, evaluation of the QT (as primitive and as nonpredictive as it is) remains the singular means of dispensing "safety concerns." It is the sponsor's task to show that the drug is "safe," not FDA's task to show that the drug is "unsafe." Real risk of rare events can only be explicitly excluded by controlled clinical trials that involve tens to hundreds of thousands of randomized patients; an unachievable and impractical plan. At least from the perspective of QT effects, such "safety assurance" can be currently offered by suitably measuring the QT_C in a suitably designed trial that includes a positive control (*see* Chapter 11). As that becomes common to development programs, discussion can center around the measurement having suitable predictive value in contrast with current discussions that center around the question of is there an effect on the QT_C. Additionally, and aside from the quantitation of intervals, analysis and display of data must incorporate actually being able to see the intervals superimposed upon the raw data. That is a form of progress. Perhaps more will become available as the phenomenology of TdP is elucidated *(9)* and more systematic approaches to assessing risk are evolved.

Such considerations are especially important for treatments that are intended only for symptomatic relief, or where efficacy can be shown with only a small sample size. Alternatives are for a new chemical entity to convincingly show that it is the only therapy available, it is more effective than therapies currently available, its effect is favorable utilizing a morbid/mortal endpoint (and consequently there is no need to worry about rare serious adverse effects), or to conduct trials of at least 100,000 persons to establish that the new therapy is safe (the latter, although seemingly heroic, can only exclude events occurring at a frequency of less than 1 per 160,000 if no events are detected).

REFERENCES

1. Symposium on the QT Interval Prolongation: Is it harmful or beneficial? Edited by Joel Morganroth MD to be Published by Yorke Medical Publishers—August 26, 1993 as a monograph in the American Journal of Cardiology.
2. Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, Chaitman BR, Bjerregaard P. Idiopathic short QT interval: a new clinical syndrome? Cardiology 2000;94:99–102.
3. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation. 2004;109:30–35.
4. Schimpf R, Wolpert C, Bianchi F, Giustetto C, Gaita F, Bauersfeld U, Borggrefe M. Congenital short QT syndrome and implantable cardioverter defibrillator treatment: inherent risk for inappropriate shock delivery. J Cardiovasc Electrophysiol. 2003;14:1278–1279.
5. Glaser S, Steinbach M, Opitz C, Wruck U, Kleber FX. Torsades de pointes caused by Mibefradil. Eur J Heart Fail. 2001;3:627–630.
6. Browne KF, Zipes DP, Heger JJ, Prystowsky EN. Influence of the autonomic nervous system on the Q-T interval in man. Am J Cardiol. 1982;50:1099–1103.
7. Malik M, Farbom P, Batchvarov V, Hnatkova K, Camm AJ. Relation between QT and RR intervals is highly individual among healthy subjects: implication for hear rate correction of the QT interval. Heart 2002;87:220–228
8. Food and Drug Administration and Health Canada. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Preliminary Concept paper, November 15, 2002 http//www.fda.gov/cder/workshop.htm#upcoming
9. Fenichel, RR, Malik, M, Antzelevitch, C, Sanguinetti, M, Roden, DM, Priori, S, et alDrug-induced Torsades de Pointes and implications for drug development. J Cardiovasc Electrophsiol2004;15:1–21.

II Preclinical and Pharmacogenomic Cardiac Safety Evaluations

2 Molecular Physiology of Ion Channels That Control Cardiac Repolarization

Jeanne M. Nerbonne, PhD
and Robert S. Kass, PhD

CONTENTS

INTRODUCTION

The mammalian heart operates as an electromechanical pump, the proper functioning of which depends critically on the sequential activation of cells throughout the myocardium and the coordinated activation of the ventricles (Fig. 1). Electrical signaling in the heart is mediated through regenerative action potentials that reflect the synchronized activity of multiple ion channels that open, close, and inactivate in response to changes in membrane potential (Fig. 1). The rapid upstroke of the action potential (phase 0) in ventricular and atrial cells, for example, is attributed to inward currents through voltage-gated Na^+ (Nav) channels. Phase 0 is followed by a rapid phase of repolarization (phase 2), reflecting Nav channel inactivation and the activation of voltage-gated outward K^+ (Kv)

From: *Cardiac Safety of Noncardiac Drugs:*
Practical Guidelines for Clinical Research and Drug Development
Edited by: J. Morganroth and I. Gussak © Humana Press Inc., Totowa, NJ

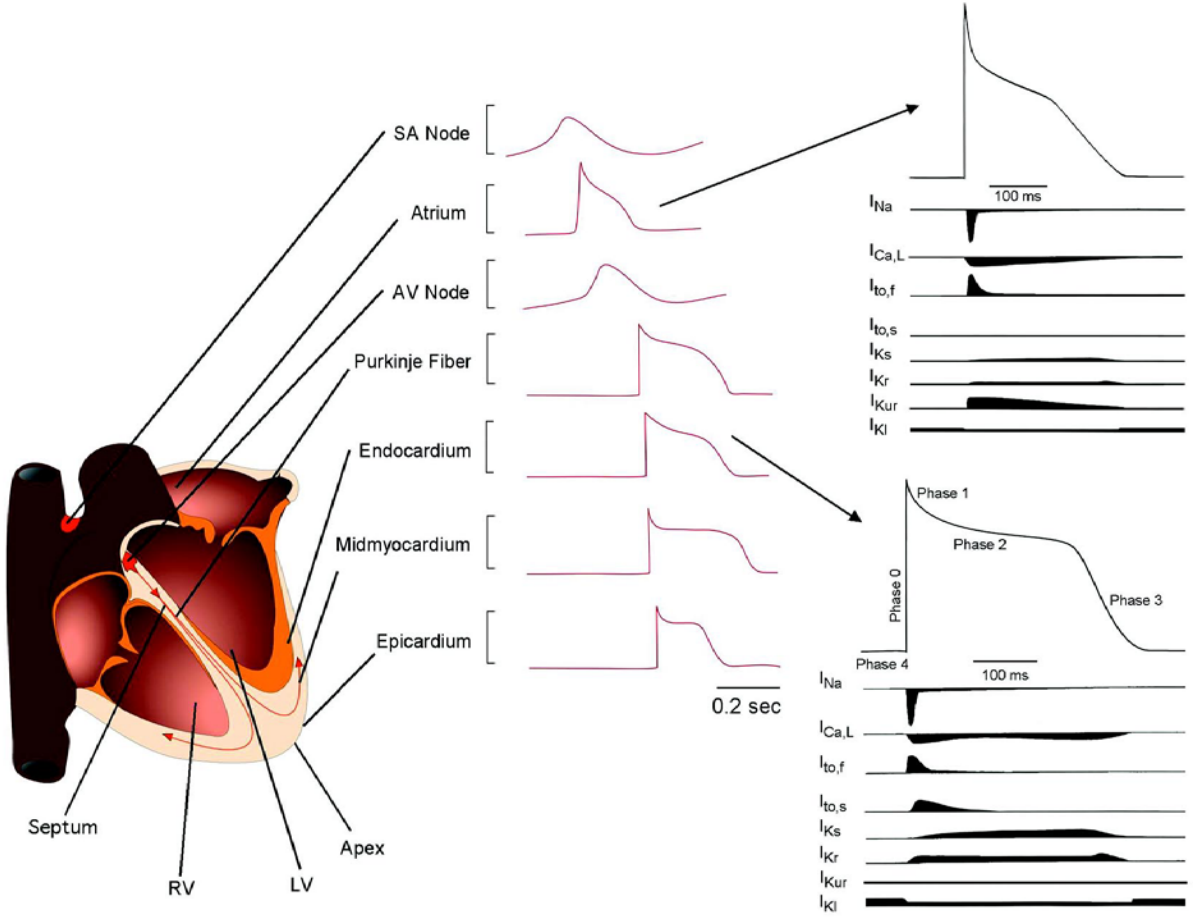

Fig. 1. Regulation of cardiac membrane excitability. Left Panel: schematic of the human heart and the waveforms of the action potentials recorded in different regions of the heart. Right panel: action potentials and underlying ionic currents in adult human atrial (top) and ventricular (bottom) myocytes. The contributions of some K^+ currents, such as I_{Kr} and I_{Kur}, are distinct in atrial and in ventricular cells.

currents (Fig. 1). In ventricular cells, this transient repolarization or *notch* influences the height and duration of the action potential plateau (phase 2), which depends on the balance of inward (Ca^{2+} and Na^{+}) currents and outward (K^{+}) currents. The main contributor of inward current during the plateau phase is Ca^{2+} influx through high threshold, L-type voltage-gated Ca^{2+} (Cav) channels. The (L-type Ca^{2+}) channels undergo Ca^{2+} and voltage-dependent inactivation and, as these channels inactivate, the outward K^{+} currents predominate resulting in a second, rapid phase (phase 3) of repolarization back to the resting potential (Fig. 1). The height and duration of the plateau, as well as the time-and voltage-dependent properties of the underlying Na^{+}, Ca^{2+}, and K^{+} channels determine action potential durations in individual cardiac cells. Changes in the properties or the densities of any of these channels, owing to underlying cardiac disease or as a result of the actions of cardiac and noncardiac drugs, therefore, is expected to have dramatic effects on action potential waveforms, refractory periods, and cardiac rhythms.

Electrophysiological studies have detailed the properties of the major voltage-gated inward (Na^{+} and Ca^{2+}) and outward (K^{+}) currents (Table 1) that determine the heights and the durations of cardiac action potentials. In contrast to the Na^{+} and Ca^{2+} currents, there are multiple types of myocardial K^{+} currents, particularly Kv currents. At least two types of transient outward currents, $I_{to,f}$ and $I_{to,s}$, and several components of delayed rectification, including I_{Kr} ($I_{K(rapid)}$) and I_{Ks} ($I_{K(slow)}$), for example, have been distinguished (Table 1). There are marked regional differences in the expression patterns of these currents, differences that contribute to regional variations in action potential waveforms *(1–3).* The time- and voltage-dependent properties of the Kv currents in myocytes isolated from different species and/or from different regions of the heart are similar, however, suggesting that the molecular correlates of the underlying channels are also the same. The pore forming (α) and accessory (β, δ, and γ) subunits encoding myocardial Na^{+}, Ca^{2+}, and K^{+} channels have been identified, and considerable progress has been made in defining the relationships between these subunits and functional cardiac Na^{+}, Ca^{2+}, and K^{+} channels.

The densities and the properties of voltage-gated cardiac Na^{+}, Ca^{2+}, and K^{+} currents change during development, reshaping action potential waveforms *(4)* and modifying the sensitivity to cardiac, as well as noncardiac, drugs. Alterations in the densities and properties of voltage-gated Na^{+}, Ca^{2+}, and K^{+} currents also occur in a number of myocardial disease states *(5–12).* These changes can lead directly or indirectly to arrhythmia generation, as well as influence the sensitivity of individuals to the effects of cardiac and noncardiac drugs that influence the properties and/or the functional expression of these channels. There is, therefore, considerable interest in defining the properties of myocardial ion channels, as well as in delineating molecular mechanisms controlling the regulation, the modulation, and the functional expression of these channels.

INWARD VOLTAGE-GATED NA^{+} CURRENTS IN THE MYOCARDIUM

Voltage-gated Nav channels open rapidly on membrane depolarization and underlie the rising phases of the action potentials in ventricular and atrial myocytes (Fig. 1). The threshold for Nav channel activation is quite negative (–55 mV), and activation is steeply voltage-dependent *(13).* In addition, Nav channels inactivate rapidly and, during the plateau phase of ventricular action potentials, most of the Nav channels are in an inactivated and nonconducting state *(14–16).* There is, however, a finite probability (approx 1%) of channel reopening at voltages corresponding to the action potential plateau *(14–17).* Although the resulting plateau (or "window") Nav current is small in magnitude *(18),*

Table 1
Cardiac Currents Contributing to Action Potential Repolarization

Channel Type	*Current Name*	*Activation*	*Inactivation*	*Recovery*	*Species*	*Tissue*[1]
NaV	I_{Na}	very fast	fast	fast	cat, dog, ferret, human, mouse, rat	A, P, V, SAN[3], AVN[3]
CaV	$I_{Ca(L)}$	fast	moderat	fast	cat, dog, ferret, human, mouse, rat	A, P, V, SAN, AVN
	$I_{Ca(T)}$	fast	fast	slow	cat, dog, guinea pig, rat	A, P, SAN, AVN
Kv(I_{to})	$I_{to,f}$	fast	fast	fast	cat, dog, ferret, human, mouse, rat	A, P, V
	$I_{to,s}$	fast	moderate	slow	ferret, human, mouse, rat, rabbit	V (A, AVN, SA)[4]
Kv(I_K)	I_{Kr}	moderate	fast	slow	cat, dog, guinea pig, human, mouse, rabbit, rat	A, P, V, SAN, AVN
	I_{Ks}	very slow	no	—	dog, guinea pig, human, rat	A, P, V, SAN
	I_{Kur}	very fast	very slow	slow	dog, human	A
	$I_{K,slow1}$	very fast	slow	slow	mouse	A, V
	I_{Kp}	fast	no	—	guinea pig	V
	$I_{K,slow2}$	fast	very slow	slow	mouse	A, V
	I_K	slow	slow	slow	rat	V
	I_{ss}	slow	no	—	dog, human, mouse, rabbit, rat	A, V, AVN
Kir	I_{KI}	—	—	—	cat, dog, ferret, human, mouse, rabbit, rat	A, P, V

[1] A = atrial; P = Purkinje; V = ventricular; SAN = sinoatrial node; AVN = atrioventricular node.
[2] Inactivation is Ca^{2+}-, as well as voltage dependent.
[3] Seen in some, but not all, AV and SAN cells.
[4] Seen in atrial and nodal cells only in rabbit.

particularly when compared with the Nav current during phase 0, it does contribute to maintaining the depolarized state, and plays a role in action potential repolarization, particularly in the ventricles.

Although the Nav channel "window" current has been recognized as a determinant of cardiac action potential waveforms for a great many years now *(19,20),* the identification of inherited mutations in the genes encoding myocardial Nav channels and the delineation of the molecular consequences of these mutations *(14–16)* have clearly demonstrated that plateau Nav currents play a very important role in action potential repolarization. Interestingly, there are regional differences in the expression of the persistent Nav current component *(21)*, differences that may contribute to regional heterogeneities in action potential amplitudes and durations *(1–3),* as well as impact arrhythmia susceptibility.

INWARD VOLTAGE-GATED MYOCARDIAL CA^{2+} CURRENTS

Two broad classes of voltage-gated Ca^{2+} (Cav) currents/channels, low-voltage-activated (LVA) and high-voltage-activated (HVA), Cav channels, have been distinguished based primarily on differences in the (voltage) threshold of channel activation *(22).* Similar to Nav channels, the LVA Cav channels activate at relatively hyperpolarized membrane potentials, and these channels activate and inactivate rapidly. HVA Cav channels, in contrast, open on depolarization to membrane potentials more positive than – 20 mV, and these channels inactivate in tens to hundreds of milliseconds. There is considerable variability in the detailed kinetic and pharmacological properties of HVA Ca^{2+} channels expressed in different cell types, and multiple HVA channel types, referred to as L, N, P, Q, or R, have been described *(22,23)*. LVA channels are also often referred to as T (transient) type Ca^{2+} channels *(23).*

In mammalian cardiac myocytes, L-type HVA Cav currents predominate *(24).* In response to membrane depolarization, L-type cardiac Cav channels open with a delay relative to the Nav channels, and these channels contribute little to phase 0 (Fig. 1). The Ca^{2+} influx through the L-type Cav channels, however, triggers the release of Ca^{2+} from intracellular Ca^{2+} stores and excitation-contraction coupling *(24).* At positive potentials, L-type Cav channels undergo rapid voltage- and Ca^{2+}-dependent inactivation, contributing to the termination of action potential plateau and repolarization. It is clear, therefore, that cardiac and noncardiac drugs that modulate the influx of Ca^{2+} through these channels could have profound effects on action potential waveforms and the generation of normal cardiac rhythms.

DIVERSITY OF VOLTAGE-GATED MYOCARDIAL K^+ CURRENTS

Voltage-gated K^+ (Kv) channel currents influence the amplitudes and durations of cardiac action potentials and, in most cells, two classes of Kv currents have been distinguished: 1. transient outward K^+ currents, I_{to}, and 2. delayed, outwardly rectifying K^+ currents, I_K (Table 1). I_{to} channels activate and inactivate rapidly and underlie the early phase (phase 1) of repolarization, whereas I_K channels determine the latter phase (phase 3) of repolarization (Fig. 1). These are broad classifications, however, and there are multiple Kv currents (Table 1) expressed in cardiac cells. Differences in the expression patterns and the properties of these currents contribute to the observed variations in action potential waveforms recorded in different cardiac cell types (Fig. 1) and in different species *(1–3).*

The early phase (phase 1) of repolarization is attributed to the activation of Ca^{++}-independent, 4-aminopyridine-sensitive transient outward K^+ currents, variably referred to as I_{to}, I_{to1}, or I_t *(25,26).* Electrophysiological and pharmacological studies, however, have now clearly demonstrated that there are actually two distinct cardiac transient outward K^+ currents, $I_{to,\,fast}$ ($I_{to,f}$) and $I_{to,slow}$ ($I_{to,s}$) *(27–30).* Rapidly activating and inactivating transient outward K^+ currents that are also characterized by rapid recovery from steady-state inactivation are referred to as $I_{to,\,fast}$ ($I_{to,f}$) *(28).* The rapidly activating transient outward K^+ currents that recover slowly from inactivation are referred to as $I_{to,slow}$ ($I_{to,s}$) *(28).* $I_{to,f}$ is a prominent repolarizing current in ventricular and atrial cells in most species *(27–37),* and is readily distinguished from other Kv currents, including $I_{to,s}$, using the spider K^+ channel toxins, *Heteropoda* toxin-2 or -3 *(38).* The fact that the properties of $I_{to,f}$ in different species and cell types are similar led to the suggestion that the molecular correlates of the underlying ($I_{to,f}$) channels are the same *(25),* and considerable experimental evidence in support of this hypothesis has now been provided. Nevertheless, there are differences in the detailed biophysical properties of $I_{to,f}$ channels *(39),* suggesting that there likely are subtle, albeit important, differences in the molecular compositions of these channels in different cells/species.

In rabbit myocardium, the prominent transient outward K^+ current (I_t) inactivates slowly and recovers from steady-state inactivation very slowly *(40–42),* and would be classified as $I_{to,s}$. In some species, $I_{to,f}$ and $I_{to,s}$ are co-expressed and differentially distributed *(28–30).* In all cells isolated from adult mouse right (RV) and left (LV) ventricles, for example, $I_{to,f}$ is expressed, whereas $I_{to,s}$ is undetectable *(28–30).* In the mouse interventricular septum, in contrast, $I_{to,f}$ and $I_{to,s}$ are co-expressed in approx 80% of the cells, and in ≈ 20% of the cells, only $I_{to,s}$ is evident.

Delayed rectifier Kv currents, I_K, have also been characterized extensively in cardiac myocytes and, in most cells, multiple components of I_K (Table 1) are co-expressed. Two prominent components of I_K, I_{Kr} ($I_{K,rapid}$) and I_{Ks} ($I_{K,slow}$), for example, were first distinguished in guinea pig myocytes based on differences in time- and voltage-dependent properties *(43–47).* I_{Kr} activates rapidly, inactivates very rapidly, displays marked inward rectification and is selectively blocked by several class III antiarrhythmics *(44,47).* In contrast, no inward rectification is evident for I_{Ks}, and this current is not blocked by the compounds that affect I_{Kr} *(44,47).* In human *(48,49),* canine *(50),* and rabbit *(51)* ventricular cells, both I_{Kr} and I_{Ks} are expressed and contribute to repolarization. In adult rodent hearts, however, neither I_{Kr} nor I_{Ks} is a prominent repolarizing Kv current, and there are additional components of I_K (Table 1). In rat ventricular myocytes, for example, there are novel delayed rectifier Kv currents, referred to as I_K and I_{ss} (Table 1) *(33,52).* In mouse ventricular myocytes, three distinct Kv currents, $I_{K,slow1}$, $I_{K,slow2}$, and I_{ss}, are co-expressed *(28,53–59).* It is clear, therefore, that in efforts focused on evaluating the possibility that there will be unwanted cardiac effects of drugs with clinical potential, it will be important to select the experimental species used in the assays carefully.

In rat *(60),* canine *(61),* and human *(62,63)* atrial myocytes, a novel, rapidly activating and slowly inactivating outward K^+ current, referred to as I_{Kur} ($I_{Kultra\ rapid}$), is expressed (Table 1). It has been suggested that the expression of I_{Kur}, together with $I_{to,f}$ in atrial myocytes, contributes to the more rapid repolarization evident in atrial, compared with ventricular, myocytes (Fig. 1). However, in guinea pig *(64)* and mouse *(53,57–59)* ventricular myoctyes there are voltage-gated outward K^+ currents with biophysical properties similar to atrial I_{Kur}. The rapidly activating μ*M* 4-AP-sensitive component of mouse ventricular $I_{K,slow}$, $I_{K,slow1}$ *(57,59)* should probably be renamed I_{Kur} (Table 1). Impor-

tantly, I_{Kur} is not expressed in human ventricular myocytes or in Purkinje fibers, suggesting that I_{Kur} channels might represent a therapeutic target for the treatment of atrial arrhythmias without complicating effects on ventricular function or performance. The potential of this pharmacological strategy, however, will have to be determined by the atrial specificity/selectivity of the reagents to be developed.

REGIONAL AND DEVELOPMENTAL DIFFERENCES IN ACTION POTENTIAL WAVEFORMS AND IONIC CURRENTS

There are marked regional differences in action potential waveforms in the myocardium (Fig. 1), and these contribute to the normal propagation of activity through the heart and the generation of normal cardiac rhythms. An important determinant of the observed regional differences in action potential waveforms is heterogeneity in Kv current expression *(1–3)*. There are, for example, large variations in ventricular $I_{to,f}$ densities *(27–29,31,32,35,36,65–67)*. In (canine) LV, $I_{to,f}$ density is five- to sixfold higher in epicardial and midmyocardial, than in endocardial, cells *(65)*. The density of $I_{to,s}$ is quite variable *(27–30),* being detected only in endocardial *(27)* and septum *(28,29)* cells. There are also regional differences in I_{Ks} and I_{Kr} densities. In (canine) LV, for example I_{Ks} density is higher in epicardial and endocardial cells than in M cells *(49)*. In cells isolated from the (guinea pig) LV free wall, I_{Kr} density is higher in subepicardial, than in midmyocardial or subendocardial, myocytes *(68)*. At the base of the LV, in contrast, I_{Kr} and I_{Ks} densities are significantly lower in endocardial than in midmyocardial or epicardial cells *(69)*. These differences contribute to the variations in action potential waveforms recorded in different regions (right vs left; apex vs base) and layers (epicardial, midmyocardial, and endocardial) of the ventricles. In addition, these electrophysiological differences clearly suggest that there will be regional differences in the physiological effects of drugs that affect the properties and/or the functional expression of cardiac Kv channels, differences that could increase the propensity to develop life-threatening arrhythmias.

During postnatal development, myocardial action potentials shorten markedly *(4)*. In ventricular myocardium, for example, phase 1 repolarization becomes more pronounced with age, and functional $I_{to,f}$ density is increased *(52,70–77)*. In addition, action potentials in neonatal cells are insensitive to 4-AP, and voltage-clamp recordings reveal that $I_{to,f}$ is undetectable, whereas, in cells from 60 d postnatal animals, $I_{to,f}$ is present and phase 1 repolarization is clearly evident *(71)*. $I_{to,f}$ density is also low in neonatal mouse *(75)* and rat *(52,70,72,74,76)* ventricular myocytes, and increases several fold during early postnatal development. In rat, the properties of the currents in 1–2 d ventricular myocytes *(76)* are also distinct from those of $I_{to,f}$ in postnatal d 5 to adult cells *(52)* in that inactivation and recovery from inactivation are slower. Indeed, the properties of the transient outward currents in postnatal d 1–2 rat ventricular cells *(76)* more closely resemble $I_{to,s}$ than $I_{to,f}$. In rabbit ventricular myocytes, transient outward K^+ current density increases and the kinetic properties of the currents also change during postnatal development *(73)*. In this case, however, the rate of recovery of the currents is ten times faster in neonatal (mean recovery time ~ 100 ms) than in adult (mean recovery time ~ 1300 ms) cells *(73)*. The slow recovery of the transient outward currents underlies the marked broadening of action potentials at high stimulation frequencies in adult (but not in neonatal) rabbit ventricular myocytes *(73)*. These observations suggest that $I_{to,f}$ is prominent in neonatal rabbit cells and that $I_{to,s}$ dominates repolarization in adult cells. In addition, these observations again

reveal species differences in the ionic currents shaping action potential waveforms, again demonstrating the importance of the selection of species in efforts focused on determining drug effects in the myocardium.

Delayed rectifier K^+ current expression also changes during postnatal development. For example, both I_{Kr} and I_{Ks} are readily detected in neonatal mouse ventricular myocytes *(77),* whereas these currents are not detected in adult cells *(28,29).* Because I_{Kr} and I_{Ks} are prominent repolarizing K^+ currents in adult human cardiac cells, developmental changes in the expression and/or the properties of these currents will lead to marked changes in action potential waveforms and altered sensitivity to drugs that affect the properties and the functioning of these channels.

INWARDLY RECTIFYING K^+ CHANNELS CONTRIBUTE TO ACTION POTENTIAL REPOLARIZATION

In addition to Kv currents, the inwardly rectifying K^+ (Kir) current (I_{K1}) plays a role in myocardial action potential repolarization (Table 1), and there are marked regional differences in I_{K1} expression in atria, ventricles and conducting tissues *(78,79).* In atrial and ventricular myocytes and in cardiac Purkinje cells, I_{K1} plays a role in establishing the resting membrane potential, the plateau potential and contributes to phase 3 repolarization (Fig. 1). The strong inward rectification evident in these channels is attributed to block by intracellular Mg^{2+} *(80)* and by polyamines *(81,82).* The fact that channel conductance is high at negative membrane potentials underlies the contribution of I_{K1} to resting membrane potentials *(79).* The voltage dependent properties of I_{K1} channels, however, are such that the conductance is very low at potentials positive to approx –40 mV *(78).* Nevertheless, because the driving force on K^+ is high at depolarized potentials, I_{K1} channels do contribute outward K^+ current during the plateau phase of the action potential, as well as during phase 3 repolarization (Fig. 1), particularly in ventricular cells. Cardiac and noncardiac drugs that affect the properties or the functioning of I_{K1} channels, therefore, could have rather profound effects on myocardial action potential waveforms, propagation, and rhythmicity and these effects are expected to be region specific, owing to the differential expression of these channels.

MOLECULAR CORRELATES OF VOLTAGE-GATED CARDIAC NA^+ (NAV) CHANNELS

Functional cardiac Nav channels reflect the coassembly of Nav pore-forming (α) subunits and accessory (β) subunits. The Nav channel α subunits (Fig. 2A) belong to the "S4" superfamily of voltage-gated ion channel genes. Although a number of Nav α subunits have been identified, Nav1.5 (*SCN5A*) is the one predominantly expressed in the myocardium, and Nav1.5 is the locus of mutations linked to one form of inherited long QT syndrome, LQT3 (Fig. 2A), as well as Brugada syndrome and conduction defects *(14–17).* Each Nav α subunit has four homologous domains (I to IV), and each domain contains six α-helical transmembrane repeats (S1–S6) (Fig. 2A). The cytoplasmic linker between domains III and IV is a pivotal component of Nav channel inactivation, and a critical isoleucine, phenylalanine, and methionine (IFM) motif in this linker has been identified as the inactivation gate *(84–86).*

During the plateau phase of ventricular action potentials, approx 99% of the Nav channels are in an inactivated, nonconducting state in which the inactivation gate is

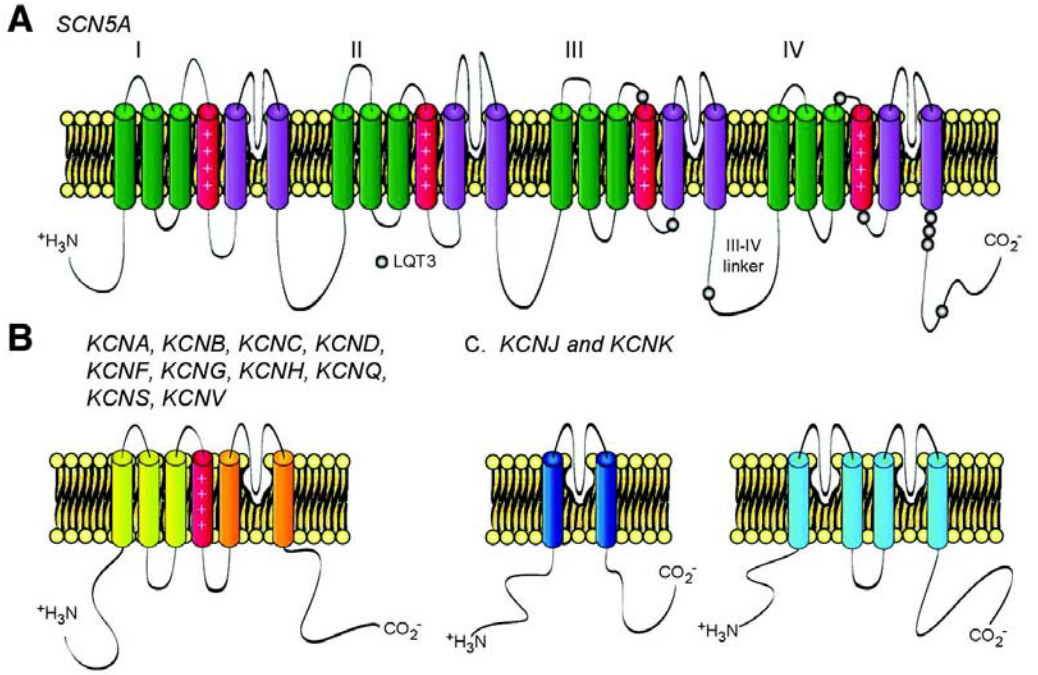

Fig. 2. Pore-forming (α) subunits of cardiac ion channels. Membrane topologies of the α subunits encoding Nav (A), Kv (B), and Kir channels (C) are illustrated. A four transmembrane, two-pore domain K^+ (K2P) channel α subunit is also illustrated in C.

thought to occlude the inner mouth of the pore through specific interactions with sites on S6 *(87)* or the S4-S5 loop *(88)* in domain IV. Inherited LQT3 mutations (i.e., ΔKPQ) in the domain III–IV linker in Nav1.5 disrupt inactivation *(89).* This (ΔKPQ) and other LQT3 mutations result in sustained (bursting) Nav current activity *(89),* resulting in action potential prolongation in theoretical models *(90)* and in mice genetically engineered with LQT3 mutant Nav channels *(91).* Analysis of other *SCN5A* mutations, linked both to LQT3 and the Brugada syndrome, however, has revealed that this is not the only mechanism by which altered Nav channel function can prolong cardiac action potentials. A critical role for the carboxy (C)-terminal tail of Nav1.5 channel in the control of channel inactivation, for example, has now been defined *(92–94).* Point mutations in the C-terminus shift the voltage-dependence of inactivation, promote sustained Na^+ channel activity, change the kinetics of both the onset of and recovery from inactivation, and alter drug-channel interactions *(95–98).* Single channel studies reveal that the C-terminus has pronounced effects on repetitive channel openings *(99).* Modeling studies suggest that this (C-terminal) domain can adopt a predominantly α-helical structure and that only the proximal region of the C-terminus, which contains this helical domain, appears to measurably affect channel inactivation. Interactions likely occur, therefore, between the structured region of the C-terminus and other components of the channel protein, and these interactions appear to function to stabilize the channel in an inactivated state at depolarized membrane potentials. Drugs that affect these interactions, therefore, will alter Nav channel inactivation, influence action potential waveforms, and affect rhythmicity.

Modeling studies *(100,101)* have also provided insights into the mechanistic basis of the pathophysiology of other LQT3 mutations. The I1768V mutation, for example, does not cause channel bursting, but rather speeds recovery (from inactivation) at hyperpolarized potentials. Computational analysis predicts that this mutation will have a significant effect during action potential repolarization, a prediction that was verified experimentally *(102)*. Similarly, subtle changes in Nav channel gating are caused by a commonly occurring *SCN5A* variant (S1102Y) which is associated with an elevated arrhythmia risk in African Americans *(103)*. This variant causes subtle changes in channel activation and inactivation that are not likely to alter myocyte functioning in mutation carriers, unless these carriers are treated with drugs that block cardiac K^+ channels (103). In this case, computational analysis, in combination with the experimental data, suggests a novel mechanism underlying susceptibility to drug-induced QT prolongation *(103)*.

Functional Nav channels (Fig. 3) are thought to reflect the coassembly of Nav α subunits with accessory Nav β subunits *(104)*, and three different Nav β subunit genes, *SCN1b (105,106), SCN2b (107,108)*, and *SCN3b (109)* have been identified. Co-expression of either *SCN1b* or *SCN3b* with *SCN5A* affects Nav channel kinetics and current densities *(110),* and *SCN2b (111)* co-expression affects the Ca^{2+} permeability of functional Nav channels *(112)*. The fact that Navβ subunits interact with ankyrin B *(113),* a cytoskeletal adaptor protein *(114),* suggests that an important function of these subunits may be to regulate Nav channel function through the cytoskeleton. Consistent with this hypothesis, electrophysiological recordings from myocytes isolated from ankyrin $B^{+/-}$ hearts reveal increased Nav channel bursting *(115)*. Interestingly, molecular genetic studies have revealed that a loss-of-function mutation in ankyrin B (E1425G) underlies LQT4 *(116)*.

MOLECULAR CORRELATES OF VOLTAGE-GATED CARDIAC CA^{2+} (CAV) CHANNELS

Similar to Nav channels, Cav channel pore-forming (α) subunits belong to the "S4" superfamily of voltage-gated ion channel genes, and these subunits combine with auxiliary β and $\alpha_2\delta$ subunits to form functional Cav channels (Fig. 3). Four distinct subfamilies of Cav α_1 subunits, Cav1, Cav2, Cav3, and Cav4 *(117),* have been identified, each with many subfamily members. Expression studies reveal that these genes encode Cav channels with distinct time- and voltage-dependent properties and pharmacological sensitivities. Functional expression of any of the Cav1 α subunits, Cav1.1, Cav1.2, Cav1.3, or Cav1.4, for example, reveals L-type HVA Ca^{2+} channel currents, which activate at approx –20 mV and are selectively blocked by dihydropyridine Ca^{2+} channel antagonists. One member of this Cav1 subfamily, Cav1.2, is composed of 44 invariant and six alternative exons *(118)*. Cav1.2 encodes the α_{1C} ($\alpha_1$1.2) protein, and three different isoforms of the α_{1C} protein, $\alpha_1$1.2a, $\alpha_1$1.2b, and $\alpha_1$1.2c *(119,120),* have been identified. Although nearly identical (>95 %) in amino acid sequences, these isoforms are differentially expressed, and the cardiac specific isoform is $\alpha_1$1.2a *(119)*.

There are two distinct types of Cav accessory subunits, Cavβ and Cav$\alpha_2\delta$ subunits. The β subunits are cytosolic proteins that are believed to form part of each functional L-type Cav channel protein complex (Fig. 3). Four different Cavβ subunits, Cavβ_1 *(121,122)*, Cavβ_2 *(123,124)*, Cavβ_3 *(123–125),* and Cavβ_4 *(125,126)* have been identified. Each Cavβ subunit has three variable regions (the carboxyl terminus, the amino terminus, and small region in the center of the linear protein sequence) flanking two highly conserved domains. The conserved domains mediate the interaction(s) with Cavα_1 subunits, and the variable domains determine the functional effects of Cavβ subunit

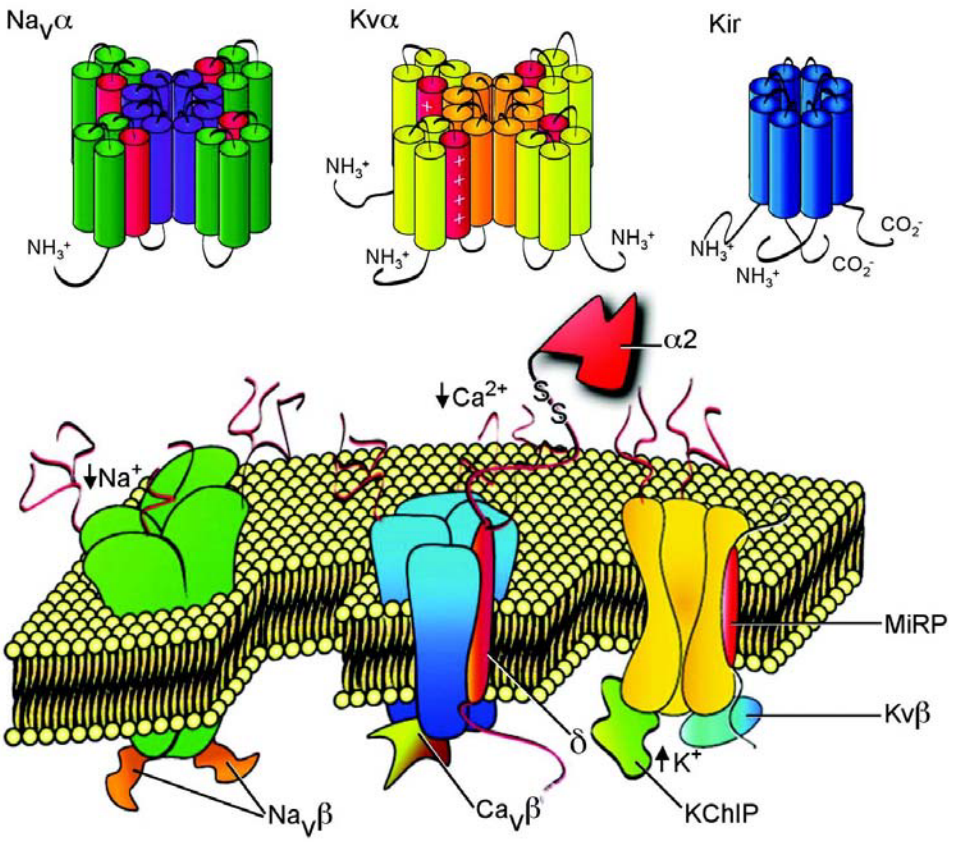

Fig. 3. Molecular compositions of functional cardiac Nav, Cav, and Kv channels. Upper panel: the four domains of Nav (and Cav) α subunit form monomeric Nav (and Cav) channels, whereas four Kv or Kir α subunits combine to form tetrameric Kv and Kir channels. Lower panel: schematic illustrating functional cardiac Nav, Cav, and Kv channels, composed of the pore-forming α subunits and a variety of accessory subunits.

co-expression *(127)*. In co-expression studies, all four Cavβ subunits associate with Cavα_1 subunits and modify the amplitudes, as well as the time- and voltage-dependent properties, of the currents *(128–133)*.

In addition to Cavβ subunits, a disulfide-linked, transmembrane accessory subunit, Cav$\alpha_2\delta$, is also found in the complex of functional Cav channels (Fig. 3). The first Cav$\alpha_2\delta$ subunit was cloned from skeletal muscle *(134)*, and there are several members of the Cav$\alpha_2\delta$–1 subfamily, as well as two homologous, Ca$_v\alpha_2\delta$–2 and Ca$_v\alpha_2\delta$–3, subfamilies *(135)*. The Cav$\alpha_2\delta$ subunits are heavily glycosylated proteins that are cleaved posttranslationally to yield disulfide-linked α_2 and δ proteins. The Cavα_2 domain is extracellular and the Cavδ domain has a large hydrophobic region, which inserts into the membrane (Fig. 3) and anchors the Cav$\alpha_2\delta$ complex *(136–138)*. The functional roles of Cav$\alpha_2\delta$ are somewhat variable and seem to depend on the identities of the co-expressed Cavα_1 and Cavβ subunits and the expression environment. In general, co-expression of Cav$\alpha_2\delta$–1 alters channel gating and increases current amplitudes, compared with the currents produced on expression of Cavα_1 and Cavβ subunits alone *(135,136,138–140)*. The increase in current density reflects improved targeting of Ca$_v\alpha_1$ subunits to the membrane, an effect attributed to the α_2 subunit domain *(141)*.