Cellular mechanisms underlying the effects of cilostazol, milrinone, isoproterenol and ajmaline on electrographic and arrhythmic manifestations of early repolarization syndrome, and comparative analysis of the cardiac electrophysiological effects of the optical isomers of mexiletine

PhD Thesis

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STUDIES RELATED TO THE OBJECT OF THE THESIS

(I.) **Patocskai B**, Barajas-Martinez H, Hu D, Gurabi Z, Koncz I, Antzelevitch C. Cellular and ionic mechanisms underlying the effects of cilostazol, milrinone, and isoproterenol to suppress arrhythmogenesis in an experimental model of early repolarization syndrome. *Heart Rhythm.* 2016 Jun; 13(6):1326-34.

(II.) **Patocskai B**, Koncz I, Gurabi Z, Antzelevitch C. Cellular mechanisms underlying the effect of cilostazol, milrinone and isoproterenol to supress arrhythmogenesis in an experimental model of early repolarization syndrome. In: Program and abstracts of the *23rd Annual Meeting of the Upstate New York Cardiac Electrophysiology Society and Upper Canada Cardiac Electrophysiology Society*; October 11, 2013, Toronto, Canada

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(VI.) **Patocskai B**, Gurabi Z, Györe B, Virág L, Mátyus P, Papp JGy, Varró A, Koncz I . Electrophysiological effects of the R- and S-enantiomers of mexiletine and their combination with sotalol in isolated canine papillary muscle. *(Manuscript in preparation.)*

(VII.) Gurabi Z, **Patocskai B**, Györe B, Virág L, Mátyus P, Papp JGy, Varró A, Koncz I. Different electrophysiological effects of the levo- and dextrorotatory isomers of mexiletine in isolated rabbit cardiac muscle. *Canadian Journal of Physiology and Pharmacology*. 2017 Feb. doi: 10.1139/cjpp-2016-0599. [Epub ahead of print]

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Abbreviations:

AP: transmembrane action potential
APA: amplitude of action potential
APD: action potential duration
ATP: adenosine-triphosphate
BrS: Brugada syndrome
cAMP: cyclic adenosine-monophosphate
CT: conduction time
ECG: transmural electrocardiogram
EG: bipolar electrogram recorded from the epicardial (or endocardial) surface
EDR: epicardial dispersion of repolarization
Endo: endocardium/ endocardial
Epi: epicardium/epicardial
ERP: early repolarization pattern
ERS: early repolarization syndrome
$\boldsymbol{I_{Ca}}$: L-type calcium current
$\boldsymbol{I_{K\text{-}ATP}}$: ATP-sensitive potassium current
$\boldsymbol{I_{Na}}$: cardiac voltage-gated fast sodium current
$\boldsymbol{I_{to}}$: transient outward current
IVF: idiopathic ventricular fibrillation
Jp: peak of the J wave (or the onset of end-QRS slur)
LV: left ventricle
MDP: maximal diastolic potential
P2R: phase 2 reentry
PDE-3: phosphodiesterase-3
PKA: protein kinase A (cAMP-dependent protein kinase)
$\boldsymbol{V_{max}}$: maximal upstroke velocity of phase 0
VT: polymorphic ventricular tachycardia
RV: right ventricle
RVOT: right ventricular outflow tract
SCD: sudden cardiac death
TDR: transmural dispersion of repolarization
VF: ventricular fibrillation

I. INTRODUCTION

I.1. Early repolarization syndrome and early repolarization pattern

I.1.1. Overview

Early repolarization syndrome is one of the main representatives of J wave syndromes, the unique group of electrical heart diseases characterized by the appearance of distinctive J waves in certain leads of the standard 12 lead ECG and increased vulnerability to malignant ventricular arrhythmias.

I.1.2. Diagnosis, classification and risk stratification

Early repolarization ECG-pattern (ERP) is characterized by J-point (J_p) elevation ≥0.1mV manifesting as a notch or slur on the final 50% of the downslope of the QRS complex (J wave) in at least two contiguous ECG-leads other than V1-V3.

Early repolarization syndrome (ERS) is diagnosed when ERP coexists with documented aborted sudden cardiac death (SCD), ventricular fibrillation (VF) or polymorphic ventricular tachycardia (VT) with the exclusion of an organic heart disease. These traditional criteria have been recently refined and further specified by the Shanghai diagnostic score system, which pays regard also to the major risk factors for SCD associated with the syndrome, including J point elevation in the inferior leads, ERP with horizontal/descending ST segment, J_p ≥0.2mV especially with T wave inversion, dynamic J wave/ST changes, clinical history of suspected or documented VT/VF and family history of SCD or ERP.

The conventional *classification* of early repolarization is based on the localization of the J waves and the course of the subsequent ST segments. ST is considered *ascending* if it courses above J_t, when measured at 100ms duration (M interval). Individuals displaying ERP with ascending ST segment, especially only in the lateral leads, are generally at low risk for VT/VF. ST segment is to be evaluated as *horizontal/descending* when ST measured at the M interval tracks in line or below J_t. ERP with *horizontal/descending* ST segment is generally recognized as a more malignant pattern with an increased risk for VT/VF and cardiac mortality.

The pre- or coexistence of several other cardiac conditions with an early repolarization pattern have also been shown to predispose to sudden arrhythmic death or even increased all-cause mortality. These include Brugada- and short QT syndromes (or patterns), heart failure, hypothermia and ischemic heart diseases especially with newly emerging J wave.

I.1.3. Epidemiology

An ERP is a relatively common electrographic finding with a strong male predominance. Depending on anthropologic differences and the definition used for ERP, the prevalence ranges between 0.6% and 24% in the general population. J_p-elevation $\geq$ 0.2mV was described in 0.3-0.64% of the general population. Similarly to Brugada syndrome –the other member of J wave syndromes–, ERP is reportedly more prevalent in South-East Asia. ERP is more frequently observed among black and Australian aboriginal males but it is associated with lower arrhythmic risk in these anthropologic groups. VF/VT episodes occur mostly at increased vagal tone and during night or rest. Interestingly, the incidence of ventricular arrhythmias in the Japanese population has been reported to inversely correlate with air temperature, peaking in winter and early spring.

I.1.4. Cellular electrophysiology

The electrophysiological background for J waves has long been a matter of debate. The two main hypotheses are **repolarization** theory (1) and **depolarization** theory (2).

(1) In the sense of **repolarization** hypothesis, a net outward shift in the balance of currents active during the early phases of the cardiomyocyte's action potential (AP) leads to pronounced notch in the ventricular epicardium but not in the endocardium, resulting in voltage gradient across the ventricular wall, which depicts on the ECG as J wave. A further accentuation of the AP notch can lead to the highly proarrhythmic heterogeneous loss of the AP dome in the epicardium, giving rise to propagation of the second upstroke of the AP from sites where it is maintained to sites where it is lost. When this (concealed) phase 2 reentry (P2R) catches a vulnerable window opened up by increased dispersion of repolarization, it can serve as trigger for premature ventricular complexes (PVCs) and VT/VF.

(2) **Depolarization** hypothesis maintains that regional conduction delay is the cause underlying the electrographic manifestation of J wave. In this sense, ERP is accounted as a distinctly fragmented QRS complex, where J wave is actually an r' or R' wave.

Although repolarization and depolarization abnormalities are certainly not mutually exclusive and their joint effect can be synergistic or potentiating, as of now only repolarization defect models are capable of recapitulating and explaining all features of the

pattern and the syndrome. Our research group pioneered in this work by publishing several studies using J wave syndrome models in the recent past. The corner stone of early repolarization syndrome is the transmural difference of the AP morphology across the ventricular myocardium, with special focus on the notch at phase 1, which is shaped by the balance of depolarizing and repolarizing early currents. Among these currents, the transient outward potassium current (I_{to}) plays to most prominent role. The magnitude of the AP notch closely follows the I_{to} current density not just across the ventricular wall but also within the different regions of the ventricles. The higher I_{to} density in the right ventricle and the apical left ventricle explains why the global and inferior/inferolateral ERP implies more arrhythmic risk when compared with the lateral type. I_{to} current is under autonomic regulation via the cAMP/PKA pathway. The aggravating effect of increased vagal tone and the ameliorative impact of isoproterenol on ERP are attributed to their action to decrease or elevate the cardiomycyte's cAMP level, via activating muscarinic or adrenergic receptors, respectively.
Although I_{to} is crucial, it is not the only contributor of forming the action potential notch. Loss of function or block of the cardiac fast sodium current (I_{Na}) has been shown to augment AP notch and J waves by numerous studies. However, in the absence of a prominent AP notch and I_{to}, loss of I_{Na} in itself is incapable of producing any sign of J wave syndrome pattern. The other main depolarizing early current in ventricular cardiomyocytes is I_{CaL}. It plays the principal role in the creation of the 2nd upstroke and plateau-phase of an action potential with spike-and-dome morphology; therefore it is the ultimate "executor" of maintaining or losing AP dome and developing phase 2 reentry (P2R).

I.1.5. Genetic background

The outward shift in the balance of currents, as the cellular basis for ERS, has been attributed to mutations in genes causing a gain of function in ATP-dependent potassium current ($I_{K\text{-}ATP}$) (*KCNJ8* and *ABCC9*) or I_{to} or loss of function in I_{CaL} (*CACNA1C, CACNB2* and *CACNA2D1*) or I_{Na} (*SCN5A* and *SCN10A*). Gain of function mutations in the I_{to} genes has been also linked to IVF and sudden unexplained death. Familial clustering of ERS has been reported with an autosomal dominant inheritance pattern with incomplete penetrance. Population-based studies have also suggested a degree of inheritance of ERP in the general population but the familial inheritance of malignant ER pattern is questionable.

I.1.6. Current approaches to therapy and pharmacologic responses

Intracardiac (transluminal) implantable cardioverter defibrillator (ICD)

For individuals with a definitive diagnosis of ER**S** (survived sudden cardiac arrest or documented VF/VT episodes), an ICD implantation is strongly recommended to be the first-line therapy (*Class I* recommendation). For other patients displaying an ER**P**, ICD implantation requires careful consideration depending on the individual arrhythmic risk. Taking into account also the high complication rate of transluminal implantation and chronic presence of intracardiac and intravessel electrodes, an ICD is not recommended (*Class III*) for asymptomatic cases with an isolated ER**P**.

A *completely subcutaneous ICD* offers an alternative therapeutic option in the future for infants, young patients or patients with active lifestyle or severe complications. However, long-term clinical experience regarding its application lacks at the present.

Pharmacologic responses and therapy

ICD is not an optimal or available choice of therapy for infants and young children, or for low-risk patients, or for patients residing in regions of the world where an ICD is out of reach because of economic factors. The unique goal of a pharmacologic approach to therapy is to produce an inward shift in the balance of currents flowing during the early AP-phases of ventricular epicardium. No wonder, that most of the conventional antiarrhythmics are ineffective in supressing VF/VT episodes either as acute or long-term therapy.

In contrast, the adrenergic agonist **isoproterenol** is quite useful in combatting acute VT/VF or even electrical storms, whereas **quinidine –**in its I_{to} blocking concentration range**–** is effective in the long-term prevention of arrhythmic episodes and suppression of ERP. The cellular basis for their ameliorative effect has been described by our research team in a recent experimental study: Both agents are capable of reducing dispersion of repolarization and abolishing P2R activity, via diminishing AP notch and restoring AP dome by their virtue of boosting I_{Ca} (beta-adrenergic stimulation) and blocking I_{to} current, respectively. According to the latest HRS/EHRA/APHRS guideline, isoproterenol have a Class IIa recommendation for the acute suppression of electrical storms. Quinidine received also a Class IIa designation for long term prevention of arrhythmic episodes as adjuvant therapy to ICD.

For many patients neither isoproterenol nor quinidine offers an optimal long-term solution. Their application can be even disadvantageous in many cases. Therefore, there is still a pressing need to find new, safe and effective pharmacologic therapeutic options. Recent

clinical case reports demonstrated the efficacy of novel candidates to control ERS-related VT/VF: The phospho-diesterase-3 (PDE3) inhibitor **Cilostazol**.

ERS is not the only clinical entity embraced under the umbrella of the term J wave syndromes: Brugada syndrome (BrS) is also characterized by electrographic J waves (or ST elevation), is associated with VT/VF and supposedly shares common cellular basis with ERS. Although overlap-phenotypes are also frequent, ERS mainly involves the LV, whilst BrS preferentially affects the right ventricular outflow tract (RVOT), so thus its ECG-sign is depicted in right precordial leads. Lately, clinical studies tested the effect of **ajmaline** on early repolarization pattern. Ajmaline is orginally a class I/A sodium channel blocker, but it exerts multiple effects also on other ion-channels including I_{to}. It is in wide-spread clinical use to unmask Brugada syndrome, because it usually provokes or accentuates the isolated J wave/ ST-elevation in V1-V3 ECG leads, the diagnostic ECG pattern of the syndrome. It has been also observed that the compound aggravates (i.e. prolongs and splits) epicardial bipolar electrogram abnormalities. Interestingly, the recent clinical studies of Bastiaenen et al. and Roten et al. reported the opposite effect on early repolarization pattern: administration of ajmaline infusion suppressed the manifestation of mild ERP.

I.1.7. Goals of the study

The first part of the present study was aimed at investigating the electrophysiological basis for the latest advances experienced in the field of ERS and ERP. Our principal aims were to (1) assess the cellular electrophysiological mechanisms underlying the antifibrillatory effects of cilostazol in ERS; (2) on this basis, test the applicability of a more potent PDE-3 inhibitor, milrinone; (3) compare their efficiency to the conventional choice of therapy, isoproterenol; (4) and to provide a direct test of the hypothesis that both PDE-3 inhibitors reduce I_{to} significantly contributing to their ameliorative effect in J wave syndromes. (5) As an additional goal, our study is sought to resolve the controversy regarding ajmaline's ambigous effects to augment or diminish the ECG pattern of J wave syndromes.

I.2. Optical isomers of mexiletine and their co-administration with sotalol

Mexiletine is a Class I/B sodium channel blocker with similar cardiac actions to lidocaine but with good oral availability and longer half-life. Its racemic form (containing both R- and S-mexiletine) is in clinical use for the suppression of ventricular extrasystoles and VT/VF. Racemic mexiletine use-dependently reduces the magnitude of fast sodium current,

decreases the maximal velocity (slope) of phase-0 depolarization (V-max) with a relatively fast onset and offset kinetics, markedly slows premature conduction, suppresses automacity in Purkinje fibers and shortens the durations of action potentials (APD). Lately, mexiletine has attracted attention afresh, due to its recently described potency to effectively terminate acquired long QT syndrome-related Torsades de Pointes (TdP) tachyarrhythmias and its lately emerged application opportunities in the therapy of certain neurologic and myotonic disorders.

Mexiletine is reasonable to apply in combination with the ClassII-ClassIII antiarrhythmic drug sotalol. The combination of the two compounds has been reported to have a higher antiarrhythmic efficacy and less proarrhythmic side effects compared to that when sotalol was applied alone. The benefits of this combination therapy have been attributed to the effect of mexiletine to counteract sotalol-induced APD-prolongation, early afterdepolarizations (EADs) and increased APD-range of premature action potentials.

As mentioned above, mexiletine is administered as racemic compound. Although, the differential effects of its stereoisomers on sodium current and excitability of skeletal muscle has been established more than twenty years ago, studies directed to exhaustive comparison of the electrophysiological effects of the R-(-) and S-(+) mexiletine isomers on cardiac ventricular muscle preparations were scarce until the recent past: In a lately published work of our research team we have investigated the electrophysiological effects of the levo- and dextrorotatory isomers of mexiletine in isolated **rabbit** cardiac muscle. We have observed a slower dissociation (offset) kinetics for R-(-) mexiletine from sodium channels than that for the S-(+) enantiomer.

The corresponding (second) part of the present study was directed to compare the effects of mexiletine's **R-(-)** and **S-(+) optical isomers** on the basic electrophysiological parameters of **canine** papillary muscle, with special focus on their effect either to shorten normal action potential duration (applying alone) or to re-abbreviate APD-prolongation of an acquired cause (coadministering with sotalol), thus making an attempt to predict their consequential potency to reverse LQTS-related TdP tachyarrhythmias in a comparative manner.

II. METHODS

II.1. J wave syndrome models

II.1.1. Coronary-perfused canine ventricular wedge preparation

Transmembrane action potentials (AP) were simultaneously recorded from epicardial (Epi) and endocardial (Endo) regions of coronary-perfused canine left-ventricular (ERS-model) or right ventricular (Brugada syndrome model) wedge preparations, together with a transmural pseudo-ECG. In the setting of experiments with ajmaline, bipolar epicardial surface-electrograms (EGs) were additionally recorded.

II.1.2. Pharmacologic models

ERS model: The I_{to}-agonist NS5806 (7-15 μM) and I_{Ca}-blocker verapamil (2-3 uM) were used to induce an ERP and VT/VF. Following stable induction of arrhythmogenesis, the PDE-3 inhibitors cilostazol and milrinone or isoproterenol were added to the coronary perfusate and then washed-out. In a second series of experiments, ajmaline alone was added to the coronary perfusate (without the previous administration of the provocative agents).

A Brugada syndrome model was designed to mimic a gain of function of the ATP-sensitive potassium current ($I_{K\text{-}ATP}$) using the $I_{K\text{-}ATP}$ agonist pinacidil (1-5 μM), and a loss of function of fast sodium channel current (I_{Na}) using the Class IA I_{Na} blocker ajmaline (2-10 μM). A second Brugada syndrome model was created in order to compare the inducibility of preparations displaying small or pronounced AP notch by applying ajmaline (10 μM) in the presence and absence of the I_{to} agonist NS5806 (7 μM).

II.1.3. Voltage-clamp measurement of I_{to}

Cardiomyocytes were isolated from the epicardium of the canine left ventricle as described in details by Zygmunt et al. I_{to} was measured at 36.5 ºC using the conventional whole-cell patch clamp techniques as specified by the seminal work of Hamill et al. I_{to} was analyzed using series of 370 ms voltage steps ranged from -40 mV to +40 mV, each preceded by a 40 ms prepulse to -30 mV to discharge the sodium current. Holding potential was maintained at -80 mV.

II.1.4. Measurements and calculations

J wave and AP notch area as well as transmural and epicardial dipersion of repolarization (TDR and EDR) were measured at each experimental step to quantify the grade of progression of J wave syndrome phenotype and arrhythmic substrate.

II.2. Stereoisomers of mexiletine and their co-administration with sotalol

II.2.1 Isolated canine papillary muscle preparation

Transmembrane APs were recorded with the use of conventional glass microelectrode technique from superfused canine right papillary muscles preparation. After obtaining control measurements, the preparation was superfused either with 20 μM sotalol, or 20 μM R-(-) mexiletine or S-(+) mexiletine. In a second series of experiments, control recordings were followed by the combined application of sotalol and R- or S-mexiletine. The preparations were stimulated at 1000 ms basic cycle length. The following parameters were measured at each experimental step: resting membrane potential, AP amplitude, AP duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization and the maximum rate of rise of the AP upstroke at phase 0 (Vmax).

II.3. Statistical analysis

Results are presented as mean ± S.E.M. throughout the study. Statistical analysis was performed using paired Student's *t*-test and one-way ANOVA for repeated measurements followed by pairwise comparisons corrected using the Holm-Sidak method, as appropriate. Statistical significance was considered at $p< 0.05$.

III. RESULTS

III.1 J wave syndromes

III.1.1. Pharmacologic induction of early repolarization pattern

Addition of the transient I_{to} agonist NS5806 (7-15 μM) and the calcium channel blocker verapamil (2-3 μM) to the coronary perfusate led to accentuation of the action potential notch in epicardium but not endocardium. This heterogeneous accentuation resulted in augmentation of the electrocardiographic J wave secondary to amplification of the transmural voltage gradients. Increased concentrations of provocative agents caused a further increase of J wave area and notch-index leading to all-or-none repolarization at the end of phase 1 of the Epi AP. Loss of the Epi AP dome at some sites but not others resulted in a prominent increase in epicardial dispersion of repolarization (EDR) and transmural dispersion of repolarization (TDR). The voltage gradient between the abbreviated Epi AP and the relatively normal Endo AP produced a prominent ST segment elevation. A pronounced APD

gradient developed between sites at which the dome was maintained and where the dome was lost, thus creating a vulnerable window within epicardium as well as between epicardium and endocardium across the left ventricular wall. Propagation of the AP dome from regions at which it was maintained to regions at which it was lost, caused local re-excitation via a P2R mechanism, leading to the development of closely coupled extrasystoles and polymorphic VT/VF.

III.1.2. Ameliorative effects of cilostazol, milrinone and isoproterenol

Addition of cilostazol (10 μM), milrinone (2.5 μM) or isoproterenol (0.1-1 μM) to the coronary perfusate restored the AP dome at all epicardial sites, reduced epicardial and transmural dispersion of repolarization, decreased J point and ST segment elevation and terminated all arrhythmic activity. All three agents, by virtue of their action to produce an inward shift of balance of currents, reversed the effect of the provocative agents, restoring all electrophysiologic parameters towards normal. Cilostazol (10 μM), milrinone (2.5 μM) and isoproterenol (0.1-1 μM) restored the AP dome at all epicardial sites, thus reducing notch index, J wave area, as well as epicardial and transmural dispersion of repolarization.

Cilostazol (10 μM) abolished VT/VF in 7 of 8 preparations, whereas milrinone (2.5 μM) abolished VT/VF in 6 out of 7 cases, and isoproterenol terminated VT/VF in 7 of 8 experiments. In each cases, washout of the drug resulted in re-appearance of arrhythmic activity.

III.1.3. Effect of cilostazol and milrinone to reduce I_{to}

The next step in our study was the evaluation of the effect of cilostazol and milrinone on I_{to} current of canine left ventricular epicardial myocytes using whole cell patch clamp techniques. Both PDE3 inhibitors caused a markedly reduced macroscopic I_{to} current density, contributing to their ameliorative effect in J wave syndrome models. It should be noted, that milrinone exerted a similar reduction on peak I_{to} as cilostazol, but already in a much lower concentration. At +40 mV, cilostazol (10 μM) reduced peak I_{to} current density by 44.4 % ($n=6$, $p<0.02$), whereas milrinone (2.5 μM) reduced I_{to} by 40.4 % ($n=8$, $p<0.02$).

III.1.4. Bidirectional effects of ajmaline on J wave syndrome pattern

The size of baseline AP notch and J wave showed a relative high variability in our preparations (e.g. apical LV vs. basal LV and RV vs. LV), providing us a great opportunity to diversify the behavior of J waves in response to ajmaline perfusion depending on the intrinsic

level of the tissue's AP notch. In preparations displaying small basal AP notch, ajmaline slightly decreased the area of J wave and AP notch, presumably due to the multiple effects on other various currents including I_{to} as well as widening of the QRS engulfing the J wave. These observations are consistent with clinical studies reporting improvement in the ECG manifestation of early repolarization pattern following ajmaline-infusion. For quantifying this association we compared the AP notch and J wave area of "inducible" and "non-inducible" cases of Brugada model experiments. Preparations that failed to develop the Brugada syndrome ECG pattern (BrP) and arrhythmic activity ("non-inducible"), either spontaneously or in response to PES, displayed significantly lower J wave and AP notch area at baseline and after the addition of the provocative agents, than those in which the provocative agents were successful in inducing the ECG and arrhythmic manifestations of BrS ("inducible"). At baseline, inducible vs. non-inducible values were 4.5±1.1 vs. 1.1±0.2 ($p=0.0238$) for J wave area, and were 9.4±1.5 vs. 2.4±0.3 ($p=0.002$) for AP notch area.

To confirm these findings, we compared the effect of high dose ajmaline (10μM) in the absence and presence of the I_{to} agonist NS5806, in the same preparations. The results supported our conclusion that the effect of ajmaline is dependent on the magnitude of AP notch prior to introduction of ajmaline. This also provides an explanation for the RVOT-predominance of Brugada syndrome since this region of the heart displays the most prominent AP notch. The development of abnormal electrogram activity was resulted from inhomogeneous accentuation of the AP notch, loss of the AP dome and P2R activity. This association was further supported by the observation that at the maximal effect of high-dose ajmaline, loss of the epicardial AP dome throughout the entire epicardium and subepicardium led to loss of the fractionated EG activity, despite the further prolongation of QRS and further slowing of transmural conduction.

III.2 Effects of R- and S-mexiletine alone and in combination with sotalol

Significant differences between the effects of mexiletine's enantiomers on the studied electrophysiological parameters could not be observed. Both optical isomers of mexiletine had similar potency to moderate sotalol-induced APD-prolongation.

IV. DISCUSSION

IV.1 J wave syndromes

IV.1.1. Mechanisms underlying the action of cilostazol, mirinone and isoproterenol

Our data indicates that both PDE3-inhibitors, cilostazol and milrinone, exert significant I_{to}-blocking action, pointing to this as an important mechanism for their ameliorative effect in ERS, in addition to their previously well-described virtue of augmenting I_{Ca}. The much greater potency of milrinone is consistent with the results of previous studies reporting that the same concentration of milrinone produces a greater increase in cytosolic cyclic adenosine monophosphate than does cilostazol, possibly because milrinone blocks both PDE-3 and PDE-4. I_{to} has previously been reported to be regulated by the cAMP/ phosphokinase-A pathway, suggesting that inhibition of I_{to} may also apply to isoproterenol (and other sympathomimetics) in addition to its boosting effect on I_{Ca} via direct stimulation of the beta adrenergic receptors. Future experiments should be directed at a test of this hypothesis. It is noteworthy that cilostazol, milrinone and isoproterenol all produce positive inotropic and chronotropic effects. The elevation in heart rate would also be expected to indirectly decrease I_{to} because the current is relatively slow to recover form inactivation. However, it also should be noted that all of these agents have the potential to enhance not only automaticity, but triggered activity as well, and thus may promote extrasystolic activity that may have unfavorable outcomes in certain cases.

Lately published studies from our group have provided evidence in support of a preferential accentuation of the AP notch in LV epicardium as the cellular basis for electrographic and arrhythmic manifestations of ERS. Isolated accentuation of the epicardial but not endocardial AP notch leads to the development of transmural gradients across the LV wall and thereby the appearance of prominent J point elevation, distinct J waves, or slurring of the descending limb of the QRS complex.

In the present study, we pharmacologically modeled the genetic defects and attendant ionic changes with the use of verapamil to block I_{Ca} and NS5806 to augment I_{to}. NS5806-induced augmentation of I_{to} sensitized our preparations to the effects of verapamil consistent with the association of a higher density of this current in the inferior wall with a higher arrhythmic risk. Addition of verapamil further accentuates the AP notch, leading to the development of a more prominent J point and ST segment elevation. Increased concentration of these agents can then elicit all-or-none repolarization, leading to loss of the AP dome at some epicardial sites but not others, resulting in an epicardial dispersion of repolarization (EDR). Propagation of the AP dome from sites at which it was maintained to sites at which it was lost created a local re-excitation via a P2R mechanism within the left ventricular epicardium. Loss of the dome in the epicardium also creates a transmural dispersion of

repolarization (TDR) giving rise to a vulnerable window across the ventricular wall which, when captured by a closely coupled extrasystole generated in the epicardium, induces VT/VF.

IV.1.2. Mechanisms underlying the effect of ajmaline to unmask or blunt J wave

The term J wave syndromes include ERS and Brugada syndrome, because their electrocardiographic and arrhythmic manifestations are associated with accentuation of J waves. By testing the effects of ajmaline on ERS and Bugada syndrome models, our results point out again the crucial role of epicardial action potential notch in the pathophysiology of J wave syndromes. Depending on the size of the notch, ajmaline is capable of both diminishing and accentuating the pattern by its virtue to block both depolarizing currents like I_{Na} and I_{Ca} and repolarizing currents like I_{to} and $I_{K\text{-}ATP}$. In preparations exhibiting a relatively small action potential notch, ajmaline mildly diminished ERP and failed to produce any sign of BrS. These observations explain why ajmaline produces ST-elevation exclusively in the right precordial ECG leads of BrS patients, but fails to provoke a Brugada pattern in other ECG-leads or in healthy subject, or in individuals with early repolarization pattern.

When the action potential notch was great enough, in addition to ajmaline's accentuating impact on Brugada syndrome ECG pattern, the drug prolonged the delay of late potentials in the epicardial bipolar electrogram recordings, as these post-QRS potentials were the depictions of the delayed 2^{nd} upstrokes of the epicardial APs and phase-2-reentries. The splitting and fragmentation of the epicardial bipolar EG and appearance of late potentials following the addition of ajmaline is very similar to those recorded by Sacher et al. in the epicardium of the RVOT of a BrS patient. Although their report interpreted this phenomenon as a proof of depolarization abnormality, in our experiments, ajmaline exerted these effects via accentuation of the AP notch and induction of P2R, and not via a conduction slowing. In support of this associtation, abnormal EG activity disappeared when the second upstroke of the AP and P2R has been lost homogeneously throughout the entire epicardium and subepicardium, despite further slowing of conduction. Our conclusion that late potentials were the consequence of repolarization defects was further verified by their behavior in response to cilostazol, milrinone and isoprotenol: All three agents concordantly suppressed the fragmentatation, delay and amplitude of post-QRS potentials, secondary to their virtue of reducing of AP notch and terminating P2Rs.

Although repolarization models can recapitulate and explain every aspect of the syndrome, we have to emphasize that our studies are not aimed at proving the exclusivity of repolarization hypothesis. There is no doubt that depolarization abnormalities can contribute

to development of J waves and related arrhythmogenesis, and several factors can modulate the transmural heterogeneity and the degree of both repolarization and depolarization abnormalities, including electrotonic coupling, "source-sink" relationship, transmural differences in tissue resistivity and transmural and regional distribution of other ion channels and gap junctions.

IV.1.4. Limitations of the study

As with any study involving experimental pharmacologic and animal models, extrapolation of our data to the clinic must be approached with great caution.

IV.2. Enantiomers of mexiletine and their co-administration with sotalol

In our present work, our primary aim was to compare the ability of the two enantiomers to abbreviate APD, especially when APD is abnormally prolonged secondary to an „acquired" condition like I_{Kr}-block by sotalol. A possibly more pronounced AP shortening effect of one of the enantiomers would have had a remarkable clinical impact. A recent study by Badri et al. reported that mexiletine is a promising treatment approach to terminate refractory TdP tachyarrhthmia in several acquired forms of LQTS. A mexiletine enantiomer with higher abbreviating-potency would supposedly offer a more effective therapeutic option for these individuals.

In isolated canine papillary muscle preparation, significant differences in the effects of mexiletine's levo- and dextrorotatory isomers could not be observed, suggesting that the separate application of neither enantiomer offers a therapeutic advantage compared to the use of the racemic form. However, it has to be emphasized that we cannot exclude the possibility that in human heart or in cardiomyocytes of other ventricular regions, layers or functions, the two optical isomers would display meaningfully divergent electrophysiological effects. It is also noteworthy that we did not implement direct tests on TdP- or other arrhythmia models; therefore or conclusions are extrapolative. Further researches in this field are most welcome.

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