Vulnerable substrate and multiple ion channel disorder in a diseased heart will be new targets for antiarrhythmic therapy

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ABSTRACT

Life-threatening arrhythmia remains a problem contributing to major death in cardiovascular diseases. Till date the antiarrhythmic drugs AAD, including the Class I and the pure Class III agents have not been recommended for controlling malignant ventricular arrhythmias in a diseased heart not because of low efficacy but because of an increase in mortality due to their toxic effects. A vulnerable substrate VS possessing some properties such as reduced NE and SOD activity, hypertrophied myoccardium and an increase in QT dispersion is reported to develop in non-infarcted zone of an infarcted heart. Hypertrophied ventricle and exaggerated cardiac arrhythmia can be produced on chronic medication with levothyroxin and this model shares some properties of VS. There is a significant difference in the pattern of disordered ion channels between the congenital long QT syndrome LQTS and the acquired heart disease. The affected ion channel in congenital LQTS is single. A novel mutation causing an early appearance of stop codon was discovered in HERG gene resultant with a single disarranged I_{K_s} channel leading to a prolonged QT interval. In contrast it is characterised with multi-channels and non-specific disorder in the hypertrophied myocardium in the acquired heart disease. The disordered ion channel is the consequence of the VS lesion influencing the lipid membrane in a diseased heart. The VS and multiple ion channel disorder are provided as new targets to treat cardiac arrhythmias in a diseased heart.

INTRODUCTION

An infarcted heart is vulnerable to develop malignant cardiac arrhythmias finally degenerating into sudden cardiac death by ventricular fibrillation VF possibly due to an existence of a vulnerable substrate VS in the diseased heart. The life-threatening arrhythmia markedly contributes to the mortality in cardiovascular diseases which remain a big problem in clinical treatment.

The pharmacological interventions to prevent acute myocardial infarction AMI and the chronic infarcted heart have been focused on using antiarrhythmic drugs AAD with definite ion channel blocking action. A CAST Cardiac Arrhythmias Suppressing Trial was conducted in 1455 patients for 300 d to test the effectiveness of sodium channel blocking agents the I_{K_s} group in suppressing malignant arrhythmias in the post-infarcted patients and the outcome was discouraging due to a high mortality rate of 4.5 % of 33 of n = 730 of flecainide/encainide over placebo 1.2 % of 9 of n = 725. The famous Sicilian gambit thereafter set up some theoretical considerations and the development of new AAD was shifted from the sodium channel blocking agents to a novel class the pure Class III blockade. The pure Class III agents prolong the APD significantly without affecting other ion channels and β-receptors. Dextro-sotalol which is devoid of the β-blocking action and is recognized as an ideal AAD was administered to test its ability to prevent post-infarcted patients from cardiac death. It was known as the SWORI Survival with oral
The unknown etiology of the VS becomes an obstacle in developing a novel AAD for eliminating ventricular arrhythmias in a diseased heart. Some of its properties have unravelled to some extent the mystery of an infarcted heart.

Depletion of norepinephrine in an infarcted heart Following an onset of acute myocardial infarction norepinephrine[NE] is rapidly released from the myocardium leading to a depletion status in an infarcted heart[1][7]. The enhanced release of NE is correlated with the presence of ischemic arrhythmias. Abrupt withdrawal of propranolol after chronic medication frequently induces cardiac arrhythmia and an increase in severity of cardiac arrhythmias is also linked with an increase in NE release[12][33]. A heart with overt congestive cardiac failure is at risk to develop cardiac arrhythmias and is also presented with a depletion of NE.

The spontaneous VF and programmed electric stimulation[PES]-induced ventricular tachycardia[VT] are not frequently induced in a normal heart. However these occur in a chronic infarcted heart due to the existence of a vulnerable substrate[VS][2]. A progressive and sustained depletion of NE in the non-infarcted region of a heart possibly exerts an important role in the formation of VS.

A rapid depletion of NE is noted in an infarcted zone[IZ] during the acute phase of myocardial infarction. The NE concentration in the non-infarcted zone[NIZ] remains intact after coronary occlusion because of no development of ischemia over there. A depleted NE in the NIZ can not be found until 24 h after AMI. It is progressive and sustained and eventually depletion of NE in the NIZ approaches to the level of the IZ[4]. It resembles the depletion of acetylcholine developed at the endplate after denervation of skeletal muscle causing an abnormal response to the transmitter. Under persisted depletion of NE in an infarcted heart and in a congestive cardiac failure the response to NE and the impulses of the sympathetic nerve could be altered. It is evidenced that the cardiac arrhythmias induced in an infarcted heart are greatly exaggerated in response to an isoproterenol challenge[1][3].

Norepinephrine modulating ion channels via the activation of α- and β-adrenoceptors Abnormal release of NE in the myocardium may trigger cardiac arrhythmias due to activation of α- and β-receptors as fol-
Activation of β-receptors. Some ion channels such as $I_K$, $I_{Cl}$, $I_Na$ the inward $I_{Na}$ current in the phase 4 of the action potential responsible for the local depolarization and $I_{Cl}$ are required for a process of phosphorization of the ion channels to increase the conductance of ion flux. The achievement of phosphorization is dependent upon the presence of cAMP and takes place at the nucleotide binding domain between the S6 of the transmembrane peptide chain and the -COOH end inside the cytosol[13]. The β-receptor activation promotes opening of the channels to increase these currents and eventually shortens the APD. The APD is determined mainly by the balance between the inward currents $I_{Ca}$ and $I_{Na}$ and the outward current $I_K + I_{to}$ simplified as $APD = I_{Na}$ and $I_{Ca} = I_K + I_{to}$. A shortened APD is a cause of inducing reentry mechanism and the activation of $I_{Na}$ and $I_{Ca}$ also possibly leads to the triggered activity for development of the early after depolarization EAD[14] which is recognized as a single cell model of the torsade de pointes. Both the shortening of APD and the formation of EAD are closely related to the appearance of cardiac arrhythmias. In general the trigger mechanism takes part in a given tachycardia when the reentry of impulse is the main mechanism involved.

The opening of the $I_K$ channel by the released norepinephrine via the activation of the β-receptor is likely to provoke arrhythmogenesis. An alleviation of arrhythmias can be achieved by a reduction in NE release and furthermore the APD prolonging effect of the pure Class III agent E4031 is lost in the presence of activated β-receptor by an isoproterenol challenge. Isoproterenol was able to eliminate the prolongation of APD by d-sotalol via β-receptor activation mechanism and d/L-sotalol retained the ability to lengthen the APD totally ignoring the isoproterenol challenge[15]. Epinephrine iv could cause dispersion of QT interval corrected QT interval in patients suspected with long QT syndrome LQTS[16] who are at high risk to ventricular arrhythmias under stress.

Activation of α-receptors. An increment in free Ca$^{2+}$ in the cytosol is possibly involved in the development of cardiac arrhythmias and is the consequence to both an opening of the receptor operated channel via the $α_{1A}$ receptor and an enhanced intracellular release of Ca$^{2+}$ by activated $α_{1B}$ receptor mediated by IP$_3$. Furthermore the temporary outward potassium current $I_{to}$[17] and the inward rectifier potassium current $I_{Kr}$[18] which affect the APD are opened under the influence of the activated α-receptors. The activation of both the α- and β-receptors was found to be linked with an exacerbated cardiac arrhythmia[19].

The imbalance of conductance in ion channels results from the abnormal distribution of cations in myocardium diminished K$^+$ and elevated Na$^+$ and Ca$^{2+}$ in the cytosol[20]. It forms the ionic basis for development of cardiac arrhythmias attributed at least partly to the release of NE.

Reduction in SOD activity involved in vulnerable substrate in the non-infarcted zone A reduction in the SOD activity in NIZ is not significantly observed in the acute phase until a couple of days after AMI. The process is progressive and long lasting reaching a very low level in the IZ in a chronic infarcted rat heart[21]. In contrast the MDA production in NIZ does not vary significantly. The ATP levels in the NIZ decrease only temporarily and return to the normal levels 24 h after AMI[18]. The energy supply remains normal and no excess peroxide product exists in the NIZ in a chronically infarcted heart. The infarcted heart is at risk to be attacked by oxidative stress due to a reduction in SOD activity. This may be one of the vulnerable properties of the NIZ. Drug interventions have been partially effective in increasing NE and SOD activity to improve the VS property[22]. The VS can be considered as a non-specific lesion in myocardium caused by infarction hypertrophy ischemia.

Is hypertrophy the morphological basis for the VS Morphological changes in the NIZ were evident and presented by hypertrophy in the wall of the left ventricle and septum 4 days after infarction in rat heart[23]. Hypertrophied heart may be considered as a sort of ischemia as an increase in the distance for oxygen diffusion between capillary to the center of a myocyte results in an uneven repolarization. An increase in the length of impulse conduction may play a role to cause diversity in repolarization of a hypertrophied heart. The APD in hypertrophied ventricle developed by either partial ligation of the abdominal aorta or chronic medication of levothyroxin[24] and the MAPD mono-phasic APD[24] after AMI were reportedly prolonged indicating that repolarization had been altered in diseased hearts. It is thus reflected that the ion channels responsible for the repolarization process are altered in hypertrophied hearts. An increase in the dispersion of QT in SHR where a potential of ventricular hypertrophy exists is associated with a risk of arrhythmogenesis[25]. The expression of mRNA
of K⁺ current family in a failing heart was altered mainly in a reduced manner. Ventricular hypertrophy is thus an independent risk to develop cardiac arrhythmia in which the altered transmembrane signaling accumulated collagen and fibrosis in myocardium contribute to inhomogeneous electrophysiology. The vulnerable property of arrhythmogenesis of an infarcted heart is partly mediated by hypertrophy of the ventricle.

DISORDER OF ION CHANNELS AND CARDIAC ARRHYTHMIAS

Recently the awareness of molecular mechanisms involved in congenital LQTS greatly promoted the understanding of mechanisms of affected ion channels underlying the malignant ventricular arrhythmias.

Awareness of individual mutation causing ion channel dysfunction in LQTS A great deal of information has been developed in molecular biology to understand the insights of the mode underlying the congenital LQTS1–3,15,18. There are some mutations in individual ion channel related genes discovered as an etiological factor to present a long QT interval in ECG in individual member of the affected family. Sudden cardiac death by malignant cardiac arrhythmias is induced by two kinds of mutations which develop abnormal potassium and sodium ion channels in the myocardium. The Iks the slow component of IK current is altered by mutations in either the KvLQT1 gene causing a disordered α-sub-unit the LQT1 or hMINK gene causing dysfunction of the β-sub-unit.18 The abnormal IKr the rapid component of IK caused by mutations of HERG gene in LQT2 is currently the target for extensive investigation. The cardiac sodium channel could be rendered abnormal as the consequence of mutation of SCN5A in LQT3 and R1623Q.

We have also discovered an early appearance of a stopping codon found in a member from a LQT family. It causes a loss of function via a very short channel and presents an inherited LQTS in the ECG. Other mutations one in a normal QT persons HERG gene caused by an insertion of three additional amino acids and the other by cysteine replacing serine in HMINK gene were also found. However these resulted in no change in QT interval. Probably the two mutations are not crucial enough to alter the ion channel function.

Disordered multiple ion channels in hyper-
trophied ventricle The function of ion channels is changed by the development of hypertrophy in a diseased heart. A reduction in If and IK1 is one of the main manifestation of a failing enlarged heart. In a hypertrophied model produced by chronic levotiroxin cardiac arrhythmias caused by ischemia/reperfusion were markedly exaggerated with high incidence of ventricular fibrillation. Thus this model of ventricular hypertrophy was adopted to investigate the changes in ion channels to unravel the possible correlation between disordered ion channels and severe cardiac arrhythmias. The APD was lengthened accompanied with ventricular hypertrophy and no significant changes in Vmax of the action potential were observed. By using the whole cell holding technique the Ica of this type of hypertrophied myocardium was enhanced. This can explain the prolonged APD. An alteration in conductance however is not limited to a single ion channel thus along with the changes in Ica the IK and INa were also altered. The INa channels were also affected non-specifically showing a diminished state. There was a decrease in INa an increase including a transient decrease in IK and an increase in Ica as summarized in Tab 1. The pattern of the diseased ion channels in a hypertrophied ventricle is totally different from the inherited LQTS. It is also likely to be different in some aspects of disordered ion channels in different heart diseases but the basic fact is likely to be the same that a disorder of ion channels is presented in a diseased heart is multiple in nature. A reduction in INa is interesting indicating that an abnormal process is not only in-

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volved in repolarization but also in depolarization of an affected myocardium. An increment in $I_{Ca}$ in a hypertrophied heart is agreement with the increased $Ca^{2+}\rightarrow Mg^{2+}$ ATPase activity in the sarcolemmal and mitochondrial membrane in the hypertrophied rat heart produced by chronic levotiroxyn.

**THERAPEUTIC INTERVENTIONS FOR THE ION CHANNELS DISORDER**

In congenital LQTS where mutations in the related gene specially cause an altered individual ion channel propranolol effectively shortens the QTc interval in the congenital LQT3 Mutations in the HERG gene. Lidocaine and mexiletine are the drugs of choice to reduce QTc in congenital LQT3 suitable enough to prevent sudden cardiac death.

In a diseased heart abnormal functioning of channels is secondary to a primary lesion in myocyte such as a damaged lipid membrane where ion channels assemble. Thus ion channel disorder caused by VS is non-specific and multiple vs the single channel affected in inherited LQTS. An AAD affecting multiple ion channels seems promising to restore the diseased state of the multichannel. As a matter of fact amiodarone which exerts a blockade on several ion channels is beneficial for the ventricular arrhythmias in an infarcted heart. The superiority of sotalol in controlling ventricular life-threatening arrhythmias as compared with $d$-sotalol which possesses a pure $I_{Kr}$ blocking action is based on its $\beta$-blocking activity. The influence of $\beta$-blockers on the life-threatening arrhythmias is based on several factors. The non-specific blockade of multiple channels can be beneficial against the over-activity of sympathetic nerve system which always precedes tachyarrhythmias. The multiple ion channel disorder is the result of pathological changes in the lipid membrane and myocardium so a relief in the ischemic lesion by pharmacological interventions up-stream to the ion channel disorder can offer an alternative route to diminish the ion channel dysfunction. Without direct interaction with ion channel pharmacological interventions are likely to help in suppressing arrhythmias with less toxic effects seen on the ion channels.

A novel antiarrhythmic agent CPU-86017 possessing a blocking action on multiple ion channels is effective in suppressing arrhythmia in various animal models. It exerts a blockade on the $I_{Na}$, $I_{Ca}$ and $I_{K}$ all three. It exerts a bi-phasic impact on the APD prolonging and shortening at low and high concentrations respectively. This phenomena is similar to that exhibited by azimilide which causes a blockade on the $I_{Kr}$ and $I_{Ca}$ creating a bi-phasic change in APD. A prolongation of APD caused by blocking of $I_{Kr}$ is opposed by blocking of $I_{Ca}$ which shortens APD at higher concentrations. This property improves its electrophysiological property of use-dependence to suppress arrhythmias. This biphasic influence on the APD is suitable for preventing both the appearance of very long APD which is predisposed to torsades de pointes and a short APD where the reentry of impulse may emerge.

It is potentially toxic for an antiarrhythmic agent to interact with an individual ion channel. Therefore an AAD affecting multiple ion channels in a diseased heart or a drug acting up-stream of the diseased ion channels may be favorable for cardiac arrhythmias and may hopefully reduce the incidence of proarrhythmias.

**REFERENCES**


36. MA YP. Part I. Ion channel mechanisms of arrhythmias caused by hypertrophied cardiac myocytes changes of sodium and potassium ion channels in hypertrophied cardiac myocytes induced by L-thyroxin-intra. Nanjing, China Pharm Univ 1999.


39. Chen DD, Dai DZ, Lu J, Zhang XK. Pranopanol and bepridil attenuation levothyroin-induced rat cardiac hypertrophy and mitochondrial Ca2+. Mg2+ -ATPase activity eleva-
病变心脏中的易损基质及多离子通道损害为抗心律失常药的新靶点

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关键词 抗心律失常药; 心肌梗死; 肥大; 去甲肾上腺素; 左甲状腺素; 离子通道;...

心血管疾病主要死因是致命性心律失常，对它的治疗仍是一个问题。当前β类及!

c 类药均不推荐用于控制室性心律失常，由于药效差及增加死亡率，对病变心脏有毒性。

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),如去甲肾上腺素耗竭,活性降低,心肌肥大,(&)延长及GD离散度增加等。

慢性给左甲状腺素可形成心肌肥大及加重缺血再灌注性心律失常，此模型有.'的特点。

离子通道的病变类型在先天性<GD.与后天性心脏病中有明显的不同。

先天性<GD.是单一离子通道的改变。我们发现在>=N;中一个新突变L提前终止密码，使GD延长。而后天性心脏病的心肌肥大是多离子通道病变而非特殊性。

后天性心脏病中离子通道病变是继发于.

病变对脂质膜的影响及多离子通道的病变可作为药物治疗病变心脏心律失常的新靶点。

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