Effects of Sarcolemmal Ca^{2+} Entry, Ryanodine Function, and Kinase Inhibitors on a Rabbit Model of Heart Failure

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SUMMARY

QT prolongation may increase the risk of torsades de pointes (TdP). Early afterdepolarizations (EADs) and transmural dispersion of repolarization have been known to serve as physiological substrates and predictors for TdP. Abnormal Ca^{2+} cycling is the proximate cause of EADs, and Ca^{2+} cycling is abnormal in heart failure (HF). However, the mechanisms for drug-induced TdP in HF are poorly understood. The purpose of this study was to search for torsadogenic-modifying effects of verapamil, ryanodine, KB-R7943, W-7, KN-93, and H-8 on ventricular premature depolarizations (VPD) and TdP in rabbits with HF. Rabbits with HF were pretreated with propranolol followed by test articles before continuous infusion of dofetilide to induce TdP.

In the control hearts, VPD and TdP were induced in all rabbits and the onsets of VPD and TdP were 3.6 ± 1.3 minutes and 10.3 ± 1.4 minutes, respectively. Dofetilide lengthened RR, QT and QTc. Verapamil, ryanodine and H-8 significantly delayed onset of VPD ($P < 0.05$) and suppressed TdP ($P < 0.01$). KB-R7943, W-7, and KN-93 accelerated onset of TdP. Blockades of L-type Ca^{2+} channel, ryanodine channel, and protein kinase A prevent dofetilide-induced TdP, suggesting roles for intracellular Ca^{2+} overload and Ca^{2+} signaling pathways in drug-induced TdP. (Int Heart J 2010; 51: 285-290)

Key words: Calcium, QT, Heart failure, Protein kinase A, Rabbit, Torsades de Pointes

Prolongation of the action potential duration (APD), the result of a reduction in net repolarizing current or an increase in depolarizing current, translates to lengthening of the QTc interval on the electrocardiogram, the culprit marker for torsades de pointes (TdP). It has been shown previously that early afterdepolarizations (EADs) and transmural dispersion of repolarization (TDR) are the candidates for causes of TdP.^{1,2} While TDR serves as a substrate for the initial EADs and subsequent re-entrant tachycardia,² EADs can trigger a new action potential (AP) and augment electrical heterogeneity in neighboring regions of myocardium. Thus, EADs can provide not only the trigger but also the substrate for the initiation and perpetuation of TdP.³

It was suggested that EADs are associated with reactivation of long lasting Ca^{2+} channels (I_{CaL}) in the setting of APD prolongation.⁴ A Ca^{2+} entry blocker has been shown to attenuate clofilium-induced TdP in anesthetized rabbits.⁵ Recently, the $\text{Na}^+/\text{Ca}^{2+}$ exchange (NCX) was suggested to produce triggered arrhythmias in heart failure (HF) by prolonging the plateau phase of the AP, thereby providing time for the reactivation of I_{CaL} which then causes the EADs.⁶ The effects of NCX blocker on ventricular arrhythmias are still controversial; however, KB-R7943, a specific blocker of the reverse mode of NCX, has been shown to suppress arrhythmia in guinea pigs.⁷ In the setting of HF, abnormalities in Ca^{2+} handling, reduction in cardiac contractility, and diminution in β -adrenergic receptor responsiveness due to an increase in circulating catecho-

lamine were reported in both humans and animals.⁸⁻¹⁰ The underlying mechanisms of HF are still unclear; however, protein kinases (eg, cAMP dependent protein kinase, PKA; Ca^{2+} /calmodulin dependent protein kinase II, CaMKII) are known to regulate cardiac function and it is possible that altered activity of protein kinases is responsible for the disturbed Ca^{2+} homeostasis in HF.¹⁰ It was found that PKA activity and its protein level in cardiac muscle were significantly increased in genetically cardiomyopathic hamsters.¹¹ Altered Ca^{2+} homeostasis in HF is also implicated in arrhythmogenesis.¹² Recent studies have shown that PKA and CaMKII signaling pathways are involved in arrhythmia induction, especially in the presence of certain conditions such as cardiovascular remodeling.^{8,13} Many studies have demonstrated that a PKA inhibitor (H-8), calmodulin antagonist (W-7), and CaMKII inhibitor (KN-93) can suppress EADs and attenuate arrhythmias.¹⁴⁻¹⁶ This evidence implies that PKA inhibition, CaMKII inhibition, and calmodulin antagonism could prevent TdP and therefore might be useful as novel antiarrhythmic drugs. However, these modalities have not been evaluated in a systematic manner in a uniform preparation. In addition, these compounds were not tested in the setting of HF, with increased adrenergic drive. The purpose of this study was to search for torsadogenic-modifying effects of verapamil, ryanodine, KB-R7943, W-7, KN-93, and H-8 on ventricular premature depolarizations (VPD) and TdP in the conscious failing rabbit heart known to be prone to develop TdP. Our specific hypotheses are: 1) reduced sarcolemmal Ca^{2+} en-

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try produced by verapamil and KB-R7943 prevents the development of TdP; 2) blocking of ryanodine receptor (RyR) of SR by ryanodine attenuates the incidence of TdP; and 3) the calmodulin antagonist, PKA blocker, and the CaMKII inhibitor prevent the development of TdP but do not alter drug-induced lengthening of QTc. This study might provide understanding of the mechanisms of enhanced arrhythmogenicity in hearts of patients with HF, and to develop novel therapeutic or prophylaxis strategies against the development of TdP.

METHODS

This study was approved by the Institutional Animal Care and Use Committees of the Ohio State University. A total of 43 male rabbits were used. All weighed between 2.4 and 2.8 kg. All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The details of producing heart failure in rabbits have been previously described.¹⁷⁾ Echocardiographic examination of left ventricular function was performed before and 4 weeks after coronary artery ligation. A reduction in shortening fraction of approximately 15% from the baseline preoperative value was required for entry into the study. A significant but relatively small (~15%) reduction in shortening fraction was sought so that rabbits would not die from heart failure. All echocardiographic images were acquired and analyzed by a single experienced operator. The mean $\pm$ SEM was calculated for left ventricular shortening fraction (SF), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), left ventricular end-diastolic wall thickness (LVEDWT), and left ventricular end-systolic wall thickness (LVESWT). Values obtained before and 4 weeks after surgery were compared using a paired *t*-test.

Experimental protocol: Thirty-nine surviving rabbits with failing hearts were randomized into 7 groups: 1) vehicle (*n* = 8), 2) verapamil (*n* = 5), 3) KB-R7943 (*n* = 4), 4) ryanodine (*n* = 5), 5) W-7 (*n* = 8), 6) KN-93 (*n* = 4), and 7) H-8 (*n* = 5). Rabbits were placed in a padded sling which provided necessary but minimal restraint and permitted recording of a bipolar transthoracic electrocardiogram. The right and left thoracic limb electrodes were attached to the right and left hemithoraces, the electrocardiograph was switched to limb lead I, and a bipolar transthoracic ECG was obtained on a Biopac MP100 Data Acquisition Unit (Biopac Systems, Inc., Santa Barbara, CA). Catheters were inserted into the right marginal ear veins and into the left central ear artery for infusion of drug and for recording of arterial blood pressure, respectively. All animals were allowed to stabilize in the sling for 10 minutes before the start of any protocol. After 10 minutes stabilization, all rabbits were pretreated with propranolol (0.5 mg/kg i.v.) in order to avoid reflex tachycardia, and this has been shown to not interfere with arrhythmia induction.¹⁸⁾ Ten minutes later, a continuous infusion of either vehicle (0.5 mL/kg), verapamil (0.3 mg/kg), KB-R7943 (3 mg/kg), ryanodine (10 μ g/kg), W-7 (50 μ mol/kg), KN-93 (0.1 μ mol/kg), or H-8 (10 μ mol/kg) was started and continued over a period of 10 minutes. Immediately after the end of infusion of vehicle or test articles, dofetilide was infused for at most 15 minutes at a rate of 0.04 mg/kg/min or until the first occurrence of TdP. Electrocardiograms were

analyzed for heart rate, QT, and rhythm. The QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of the T wave. The values for QT interval were corrected for heart rate using the equation: $QT_c = QT/(RR)^{0.72}$, which has been shown previously to be appropriate for these conscious rabbits in our laboratory.¹⁹⁾ RR and QT durations were measured manually by using on screen cursors during baseline, 10 minutes after propranolol injection, at the end of vehicle or treatment with test articles, and at 5 minutes of dofetilide infusion or before arrhythmias presented. Measurements were made for 30 consecutive cardiac cycles, and the average was used. The ECG intervals were measured in beats that originated from the sinoatrial node. Rhythm was classified as normal sinus rhythm, VPD or TdP. TdP was defined as a polymorphic ventricular tachycardia where clear twisting of the QRS complexes around the isoelectric axis was seen. Arterial blood pressure was continuously recorded for measurement of mean arterial blood pressure (MBP). At the end of the experiment, the rabbits were euthanized with 50 mg/kg of pentobarbital given intravenously.

Drugs: Dofetilide (Pfizer, Groton, CT) was prepared as a solution in pyrogen-free 0.9% NaCl acidified with 0.1 M hydrochloric acid, at a stock concentration of 0.1 mg/mL. Propranolol hydrochloride (Sigma, St. Louis, MO) was dissolved in 0.9% NaCl. Verapamil hydrochloride (Sigma), W-7 and H-8 (Biomol International, Plymouth Meeting, PA, USA) were dissolved in 5% dextrose solution. KB-R7943 mesylate (Tocris Bioscience, Ellisville, MO, USA), ryanodine, and KN-93 (Biomol International) were dissolved in 0.1% DMSO. All drugs were freshly prepared each day. The doses of dofetilide and pretreated compounds were chosen based on the findings of previous publications.^{5,7,14-17,20)}

Statistics: Values are expressed as the mean $\pm$ SEM and *n* indicates the number of animals. Student's *t* test and one-way analysis of variance (ANOVA) were applied when appropriate. Post hoc comparisons were performed with Tukey's test. Fisher's exact test was used to compare the incidence of VPD and TdP. A *P* < 0.05 was considered to be significant.

RESULTS

Effects of coronary ligation on echocardiogram: Coronary ligation in the rabbit provided a reproducible model of left ventricular systolic dysfunction. Effects of myocardial infarction on wall motion and geometry were detected clearly 4 weeks after coronary ligation. Hypokinesis was observed at the posterior and lateral walls of the left ventricle (LV). LV shortening fraction decreased after coronary artery ligation from 32.64% to 25.21%, a 22.7% decrease (*P* < 0.001). Over the 4 weeks after myocardial infarction, marked dilation in left ventricular systolic and diastolic chamber dimensions were observed. As a result, LVEDD and LVESD were significantly increased (46.2%; *P* < 0.001 and 63.7%; *P* < 0.001, respectively) whereas LVEDWT and LVESWT were not significantly different.

Induction of TdP: In the vehicle group, TdP developed in all rabbits after continuous infusion with 0.04 mg/kg/min of dofetilide. Rabbits developed bradycardia and prolongation of QT interval, VPD, and TdP after dofetilide administration (Figure 1). Figure 2 shows the types of dofetilide-induced ventricular arrhythmias, and the Table shows the time between dosing of

Figure 1. Transthoracic electrocardiogram and evolutionary changes from rabbits receiving vehicle (0.1% DMSO at 0.5 mL/kg), verapamil (0.3 mg/kg), KB-R7943 (3 mg/kg), ryanodine (10 μ g/kg), W-7 (50 μ mol/kg), KN-93 (0.1 μ mol/kg), and H-8 (10 μ mol/kg) at baseline, after propranolol, after test articles, and at the first torsades de pointes (TdP) or after 15 minutes of continuous infusion of dofenilide (0.04 mg/kg/min). Notice that failing hearts exposed to KB-R7943, W-7, and KN-93 developed TdP. On the other hand, failing hearts exposed to verapamil, ryanodine, and H-8 did not develop TdP. Each segment of tracing is of 2.5 seconds.

Figure 2. Effects of pretreatment with vehicle (0.1% DMSO; 0.5 mL/kg), verapamil (0.3 mg/kg), KB-R7943 (3 mg/kg), ryanodine (10 μ g/kg), W-7 (50 μ mol/kg), KN-93 (0.1 μ mol/kg) or H-8 (10 μ mol/kg) on the incidence of dofenilide-induced torsades de pointes (TdP) and ventricular premature depolarizations (VPD) in conscious rabbits with HF. All rabbits were pretreated with propranolol (0.5 mg/kg) to avoid reflex tachycardia before beginning infusion of each test article. Differences in the incidence of TdP and VPD between the vehicle group and the test article groups were statistically evaluated using Fisher's exact test. Notice that TdP inducibility is reduced with a calcium channel blocker (verapamil), sarcoplasmic reticulum calcium release channel blocker (ryanodine), and protein kinase A inhibitor (H-8). ** $P < 0.01$ compared to vehicle treated group.

Table. Effects of Pretreatment Compounds (Vehicle, Verapamil, KB-R7943, Ryanodine, W-7, KN-93, and H-8) on the Onset of Arrhythmias (Ventricular Premature Depolarizations and Torsades de Pointes) in Conscious Rabbit With Failing Heart

	Onset of arrhythmias (minutes)	
	Ventricular premature depolarizations	Torsades de pointes
Vehicle	3.6 $\pm$ 1.3	10.3 $\pm$ 1.4
Verapamil	11.7 $\pm$ 3.0*	NA
KB-R7943	2.1 $\pm$ 0.4	3.0 $\pm$ 0.3**
Ryanodine	7.5 $\pm$ 1.3*	10.2*
W-7	4.1 $\pm$ 0.7	5.0 $\pm$ 1.8*
KN-93	1.7 $\pm$ 0.1	2.6 $\pm$ 0.6**
H-8	7.9 $\pm$ 1.6*	13.9*

The data are presented as the mean $\pm$ SEM. NA indicates no torsades de pointes has been induced after pretreatment with verapamil; a, SEM cannot be calculated since $n = 1$. * $P < 0.05$ versus vehicle, ** $P < 0.01$ versus vehicle.

Figure 3. Plots of baseline adjusted RR interval for baseline, after propranolol, after pretreatment [ie, vehicle (0.1% DMSO; 0.5 mL/kg), verapamil (0.3 mg/kg), KB-R7943 (3 mg/kg), ryanodine (10 μ g/kg), W-7 (50 μ mol/kg), KN-93 (0.1 μ mol/kg) or H-8 (10 μ mol/kg)], and after dofenilide (0.04 mg/kg/min). All rabbits were pretreated with propranolol (0.5 mg/kg) to avoid reflex tachycardia before starting the infusion of each test article. The data are presented as the mean $\pm$ SEM. * $P < 0.05$ compared to baseline value of each test article.

dofenilide and onset of arrhythmia. VPD occurred in all rabbits given dofenilide (except for 1 rabbit treated with verapamil) whether or not they received vehicle, verapamil, KB-R7943, ryanodine, W-7, KN-93, or H-8. TdP occurred in 8 out of 8 rabbits receiving vehicle and dofenilide. TdP did not occur in rabbits pretreated with verapamil, and occurred only in 1 out of 5 rabbits receiving dofenilide and pretreated with either ryanodine or H-8. Rabbits pretreated with W-7 or KN-93 but not KB-R7943 developed TdP less frequently. The time to the first VPD was significantly increased for verapamil, ryanodine, and H-8 treated animals compared with vehicle treated animals. Rabbits treated with KB-R7943, W-7, or KN-93 had a significantly shortened time to the development of first TdP when compared to vehicle treated animals.

Effects of verapamil, KB-R7943, ryanodine, W-7, KN-93, and H-8 on electrocardiogram variables: Figure 3 and Figure 4

Figure 4. Plots of baseline adjusted QT (a) and QTc interval (b) for baseline, after propranolol, after pretreatment [ie, vehicle (0.1% DMSO; 0.5 mL/kg), verapamil (0.3 mg/kg), KB-R7943 (3 mg/kg), ryanodine (10 μ g/kg), W-7 (50 μ mol/kg), KN-93 (0.1 μ mol/kg) or H-8 (10 μ mol/kg)], and after dofetilide (0.04 mg/kg/min). All rabbits were pretreated with propranolol (0.5 mg/kg) to avoid reflex tachycardia before starting the infusion of each test article. QT was corrected for heart rate by the following equation: $QTc = QT/RR^{0.72}$. The data are presented as the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to baseline value of each test article. †† $P < 0.01$ compared with vehicle group at the same time point.

show plots of baseline adjusted RR interval, QT interval, and QTc interval for baseline, after propranolol, after vehicle, or after each test article, and after dofetilide. KB-R7943 significantly lengthened the RR interval when compared to baseline ($P < 0.001$). Dofetilide significantly lengthened the RR interval after pretreatment with vehicle ($P < 0.05$) and verapamil ($P < 0.05$) when compared to the baseline of each group. Dofetilide produced not only dramatic lengthening of QT in all groups, with the exception of KB-R7943, but also prolonged QTc in all groups except KB-R7943 and verapamil. The QTc lengthened more in the groups receiving KN-93 ($P < 0.01$) and H-8 ($P < 0.01$) than in the group receiving vehicle. The QTc intervals were significantly shortened in the verapamil treated group ($P < 0.05$) receiving dofetilide compared to the vehicle treated group.

Effects of verapamil, KB-R7943, ryanodine, W-7, KN-93, and H-8 on mean arterial blood pressure: Figure 5 shows plots of baseline adjusted mean systemic arterial blood pressures (MBP) for each group of rabbits. MBP was not significantly changed in any group, except rabbits with failing hearts that were pretreated with verapamil and H-8. Rabbits pretreated with verapamil and H-8 had precipitously and significantly decreased MBP when compared to baseline values ($P < 0.001$ and $P < 0.01$, respectively). In those rabbits, MBP were also significantly decreased when compared to the vehicle treated group at the same time ($P < 0.05$ and $P < 0.01$, respectively). In response to dofetilide, only the verapamil treated group had a significant decrease in MBP.

DISCUSSION

Rabbits with HF have been shown to develop TdP, and we believe it is a more realistic (ie, less contrived) model than either hearts with complete heart block or subjected to α_1 -agonists. Very few people with heart block develop TdP and very few people with TdP receive α_1 -agonists, whereas HF occurs in more than 5 million Americans and is a known torsadogenic substrate.¹⁷⁾ Echocardiography has showed that coronary ligation in rabbits produced cardiac dysfunction and

Figure 5. Plots of baseline adjusted mean arterial blood pressure (MBP) for baseline, after propranolol, after pretreatment [ie, vehicle (0.1% DMSO; 0.5 mL/kg), verapamil (0.3 mg/kg), KB-R7943 (3 mg/kg), ryanodine (10 μ g/kg), W-7 (50 μ mol/kg), KN-93 (0.1 μ mol/kg) or H-8 (10 μ mol/kg)], and after dofetilide (0.04 mg/kg/min). The data are presented as the mean $\pm$ SEM. ** $P < 0.01$, and *** $P < 0.001$ compared to baseline value of each test article. † $P < 0.05$ and †† $P < 0.01$ compared with vehicle group at the same time point.

marked left ventricular dilation consistent with the results of previous studies with this model.²¹⁾ More than 90% of rabbits operated upon survived the duration of the study (39 out of 43). No rabbit with HF ever developed TdP unprovoked by torsadogenic articles.

Prevention of dofetilide-induced TdP by verapamil, ryanodine, and H-8: Alterations in PKA, CaMKII, and calmodulin have been implicated in the induction of EADs.¹⁶⁾ It is well known that EADs, and reentry in regions of electrical inhomogeneity are responsible for the generation of TdP.^{5,22)} Several interventions that prolong the APD may cause EADs. Early EADs are probably caused by Ca^{2+} entry through the I_{CaL} whereas late EADs depend on intracellular Ca^{2+} ($[Ca^{2+}]_i$) overload produced from the SR.²⁰⁾ The present study shows that occurrence of dofetilide-induced TdP arrhythmias was prevented by administration of verapamil, ryanodine, and H-8. These compounds decrease $[Ca^{2+}]_i$ influx, block ryanodine channels and block PKA activity, and their inhibition of TdP point to the involvement of Ca^{2+} mishandling (Ca^{2+} entry, Ca^{2+} overload) and/or increased PKA activation as the initiating mechanism of TdP. It is well-known that ryanodine has a biphasic effect. The concentration of ryanodine used in this study is based on a previous publication that showed it blocked ryanodine receptors in rabbits.³⁾ That the suppression of TdP in this study did not correlate with lengthening of QTc interval induced by dofetilide supports the contention that QTc is not a satisfactory culprit marker for TdP.

Verapamil: The preventive effect of verapamil in our study supports the thought that Ca^{2+} influx via I_{CaL} channel plays a pivotal role in induction of TdP. The protective effect of verapamil against TdP induction in failing heart rabbit may be due to the direct effect of verapamil on I_{CaL} channels or its effects on reduction of ventricular transmural dispersion of repolarization as reported by Milberg, *et al.*²³⁾ Pretreatment with vera-

pamil was also associated with reductions in MBP, the QTc interval, and the torsadogenicity of dofetilide. This indicates that verapamil may influence the conditioning process for TdP (ie, lengthening of QTc interval, elevation of MBP). However, Milberg and colleagues²³⁾ suggested that the effectiveness of an intervention to abbreviate the QT interval is not necessarily congruent with its efficacy to reduce the incidence of arrhythmogenesis. These data suggest that, in the setting of HF, Ca^{2+} influx via I_{CaL} is the major cause of TdP. This can be via many processes, for example the window current which causes late Ca^{2+} influx leading to EAD. In addition, increased Ca^{2+} influx will lead to SR Ca^{2+} overload, which may lead to spontaneous release leading to a Ca^{2+} -activated nonselective transient inward current (I_{ti}).^{24,25)}

KB-R7943: Recent studies suggest that, in heart failure, stores of SR Ca^{2+} decrease, release of Ca^{2+} from SR to cytosol decreases, cytosolic Ca^{2+} decreases, and inotropy decreases. However, the NCX activity increases. This increased NCX activity tends to compensate for the decrease in SR Ca^{2+} and to provide inotropic support.^{26,27)} It has been shown by Weber, *et al*²⁸⁾ that in failing human myocytes, there is substantial Ca^{2+} entry through NCX as the AP becomes prolonged. The increased activity of the NCX has been implicated in triggered arrhythmias in a number of conditions, especially when the AP is prolonged and at membrane potentials negative to -35 mV, a potential at which activation of the I_{CaL} window current is unlikely.^{4,29)} Taken together, these concepts support the hypothesis that blocking the reverse mode of NCX might be an effective strategy to suppress TdP in the failing heart rabbit. In the present study, KB-R7943 did not demonstrate an inhibitory effect on dofetilide-induced TdP. On the other hand, KB-R7943 expedited the onset of VPD and TdP in our model. The mechanism(s) underlying this phenomenon is(are) still unclear. The dose of KB-R7943 used in this study was based on positive findings in a guinea pig study,⁷⁾ and it is possible that these doses were not appropriate for the rabbit. Furthermore, KB-R7943 significantly prolonged the RR interval in this failing heart rabbit which could precipitate the effects of dofetilide to induced arrhythmias. KB-R7943 does not inhibit the forward mode of NCX, which may contribute to I_{ti} with SR Ca^{2+} overload, and cannot be discounted in our model.

Ryanodine: A substantial amount of evidence suggests that the abnormal regulation of $[\text{Ca}^{2+}]_{\text{i}}$ by the SR is an underlying mechanism for late EADs, since ryanodine abolished these EADs.³⁾ In the present study, Ca^{2+} overload was manipulated by blocking the Ca^{2+} release channel of the SR with ryanodine, resulting in significant delayed onset of VPD and attenuation of dofetilide-induced TdP, suggesting a role for SR Ca^{2+} release and Ca^{2+} overload in induction of TdP in failing heart rabbit. However, we did not measure Ca^{2+} sparks and Ca^{2+} transient, an indication of Ca^{2+} overload, in dofetilide-induced TdP. Similarly, in a dog model of pacing-induced TdP, Verdun and coworkers²⁰⁾ showed that pacing-induced TdP could be prevented when the dogs were given ryanodine. Thus, Ca^{2+} leakage from the SR is an important pathway for inducing TdP. **H-8 and KN-93:** Recently, Anderson¹³⁾ suggested that PKA phosphorylation of I_{CaL} , PLB, and RyR increases $[\text{Ca}^{2+}]_{\text{i}}$ and subsequently activates CaMKII, causing development of afterdepolarizations. In our study, H-8, a PKA inhibitor, delayed the onset of VPD and inhibited dofetilide-induced TdP, suggesting that, at least in our conscious model of rabbits with

heart failure, PKA activation has a proarrhythmic effect and PKA inhibition could be used as a target for antiarrhythmic drug development. Our results are also in agreement with the results of Mazur, *et al*¹⁶⁾ who demonstrated a preventive effect of a PKA inhibitor on a rabbit model of TdP.

Interestingly, that infusion of a PKA inhibitor delays the onset of VPD and suppresses TdP without shortening QTc interval induced by dofetilide, is consistent with other studies¹⁶⁾ and supports the concept that prolongation of action potential duration alone is not sufficient to initiate arrhythmias and lengthening of QTc is not a good surrogate marker of TdP.

In contrast to PKA, CaMKII activity increases in response to elevated intracellular Ca^{2+} , and subsequently augments the incidence of afterdepolarizations. In the present study, KN-93 was used to block CaMKII. KN-93 slightly prevented the dofetilide-induced TdP; however, this was not significant. In addition, KN-93 expedited the onset of TdP. Although one study, in transgenic CaMKII δ_{c} mice having HF, demonstrated that CaMKII inhibition prevented isoproterenol-induced arrhythmias, no mention was made of prophylaxis against TdP.³⁰⁾ Therefore, to the best of our knowledge, no one has previously studied the ability of CaMKII inhibition to prevent TdP in HF. The possible explanations could be 1) a potential obstacle of systemic kinase inhibition for therapy of cardiac arrhythmias due to the ubiquitous nature of CaMKII; 2) The cell membrane permeability data together with effective intracellular concentration of KN-93 are unknown; and 3) inadequate data are present on the specificity of KN-93 on CaMKII as well as side effects when administered systemically. It is known that CaMKII can increase I_{CaL} ; however, the major effect of CaMKII is to sensitize RyR opening.³¹⁾ Thus, with CaMKII inhibition, SR Ca^{2+} load dramatically increases. This increased SR Ca^{2+} load, in the presence of PKA activation, may lead to the increased onset of TdP. Thus, in the setting of heart failure, although CaMKII seems to play a role in decreasing TdP, the PKA phosphorylation of the L-type Ca^{2+} channel seems to be the major pathway.

W-7: Increased activity of calmodulin after prolongation of repolarization has been implicated as a cause of afterdepolarizations in many studies, and the calmodulin antagonist W-7 has been demonstrated to prevent TdP initiated by coadministration of methoxamine and clofilium in anesthetized rabbit.^{15,16)} Unexpectedly, our results show that W-7 expedites the onset of TdP and can not protect dofetilide-induced TdP in conscious rabbits with heart failure. It might be possible that the altered kinetics of calmodulin in heart failure are different from those in normal hearts, which in turn may have consequences for the induction of VPD and TdP. Also, it is known that calmodulin can inhibit RyR opening.³¹⁾ In the presence of W-7, RyR opening should increase, which could lead to increases in the prevalence and early onset of TdP.

Conclusions: The present experiments in failing hearts indicate that calcium entry via I_{CaL} channel, calcium overload by SR Ca^{2+} release, and blocking of PKA may play a major role in the appearance of TdP suggested by the preventive effect of verapamil, ryanodine and H-8. Furthermore, these findings may have implications for the clinical therapy of TdP and for the development of effective antiarrhythmic agents. The differences in these results compared to those of Gbadebo, *et al*¹⁵⁾ and Mazur, *et al*¹⁶⁾ may be explained by differences in failing versus nonfailing hearts.

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