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局部心肌缺血時細胞內鈣離子調控與心律不整發生機轉之研究

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Brief Report of Calcium handling and arrhythmogenesis during acute regional ischemia

The objective of this project is to explore the relationship between intracellular calcium (Ca_i) and membrane voltage (V_m) dynamics during acute regional ischemia. Hope this study could help us understand the mechanisms of ventricular fibrillation (VF) and may provide a novel target for the treatment of sudden cardiac death. Despite the pharmacological or non-pharmacological interventions to further clarify the relationship between V_m and Ca_i dynamics were not approved by NSC (the 2nd year project), we still completed our first year works and have the following results:

1. We developed a novel method to characterize the coupling between V_m and Ca_i in whole heart preparation.
2. Spontaneous calcium release (spCa) contributed to the generation of wavebreak formation during VF. (data not shown in this report due to the limitation of pages of brief report)
3. There are heterogeneous alterations in V_m and Ca_i dynamics during acute regional ischemia. Such alterations may further destabilize the V_m during acute ischemia.

All of these results were presented at the 25th Annual Scientific Sessions of Heart Rhythm Society in May, 2004; at San Francisco, California. (2 oral presentations and 1 poster presentation) We will complete the full manuscript of our works and send it for peer-review about 1-2 month later.

I. A Novel Method to Characterize Coupling Between Membrane Potential and Intracellular Calcium During Rapid Pacing in Whole Heart

Background

Intracellular calcium (Ca_i) fluctuations affect cardiac membrane potential (V_m) characteristics by modulating Ca-sensitive ionic currents, such as the L-type Ca channel, Na-Ca exchanger, and Ca-activated Cl and nonselective channels. Rapid pacing can induce dynamic instability as manifested by repolarization alternans of the V_m. However, the dynamic coupling between Cai and Vm at different heart rate and different location are still unclear. We investigated the role of Ca_i handling in pacing-induced dynamic instability in whole heart.

Methods

1. We performed dual optical mapping of V_m (RH237) and Ca_i (Rhod-2) in 6 isolated rabbit hearts during pacing with progressively faster rates.

   The optical mapping setup is similar to that of a previous V_m mapping system. The tissues were stained with a Ca indicator, Rhod-2 AM (Molecular Probes), and a voltage-sensitive dye, RH237 (Molecular Probes). Rhod-2 AM 0.5 mg will be dissolved in 0.5mL o f DMSO containing Pluronic F-127 (20% wt/vol). This solution will be diluted in 300 ML of Tyrode's solution with 5 ml fetal bovine serum and infused over 5-10 min. After the infusion, 15-30 min will be allowed for de-esterification of the dye. About 0.1 ml of 0.5 mg/ml RH237 solution in DMSO will be directly injected into the perfusion system. Electromechanical uncoupler, 5 μM cytochalasin D (Sigma Inc.), was used. Laser light of wavelength 532 nm (Verdi, Coherent Inc.) illuminate the tissues, and epifluoresence will be collected through a long-pass 715 nm cutoff filter (Nikon) for the voltage image and another 580±20 nm interference filter for calcium image with 2 high-speed CCD cameras (250 frames/s, Model CA D1-0128T, Dalsa Inc.). The digitized pixel intensity from the digital camera was transferred to a frame grabber board (Roadrunner, Bitflow Inc.) in a personal computer. Image acquisition was controlled by custom-designed software based on LabVIEW and the IMAQ Vision toolset (National Instrument). We mapped the LV anterior wall over 25x25 mm^2, acquiring 128 x 128 sites simultaneously for both V_m and Ca_i image.

   Because the two cameras take images of the heart from different angles, it is necessary to perform spatial registration in order to compare Vm and **[**Ca2+]**i** at the same location. We will use a projective transformation to find the corresponding pixels between the Vm and [Ca2+]i images. Let (x,y) be the coordinates of the Vm image, and (u,v) the coordinates of the Ca image, the projective transform is obtained by

   $$u = (Ax + By + C)/(Gx + Hy + I)$$
   $$v = (Dx + Ey + F)/(Gx + Hy + I)$$

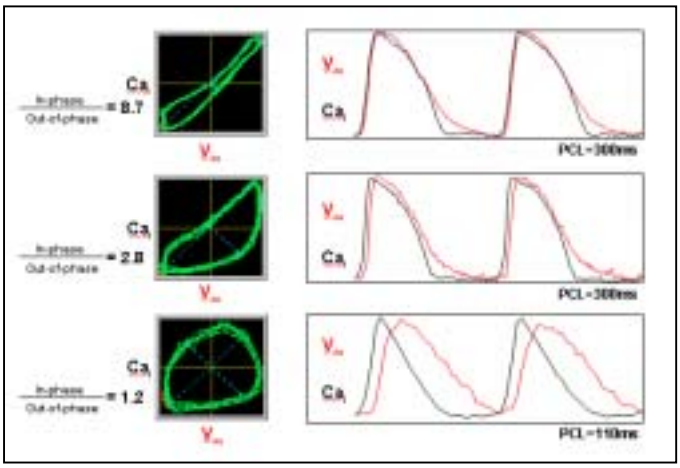
   Assume I = 1, multiply both equations, by denominator:

   $$u = [x\ y\ 1\ 0\ 0\ 0\ -ux\ -uy] * [A\ B\ C\ D\ E\ F\ G\ H]'$$
   $$v = [0\ 0\ 0\ x\ y\ 1\ -vx\ -vy] * [A\ B\ C\ D\ E\ F\ G\ H]'$$

   Therefore, with 4 or more correspondence point pairs, we can combine the u equations and the v equations for one linear system to solve for the registration matrix, [A B C D E F G H]. The users will manually select the initial correspondence points. After designating four point pairs, the correspondence matrix can be solved and will be used to automatically find

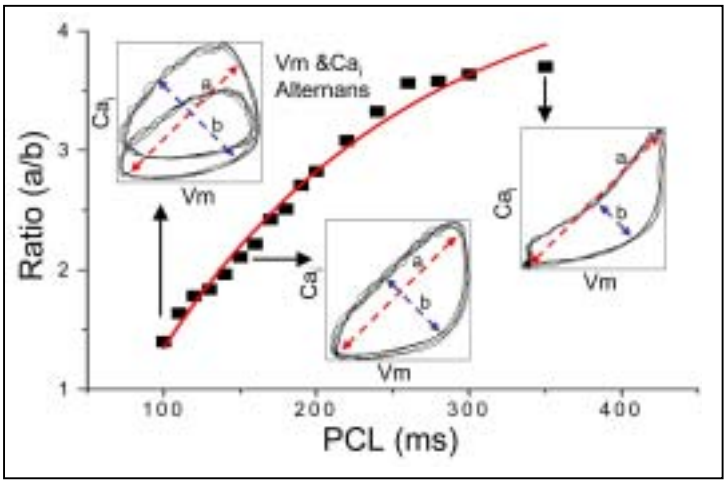
the corresponding points in the two maps. Accuracy of the registration can be examined by checking the correspondence of fixed judicial points. To overcome the lack of obvious geometrical features for registration, at least four artificial judicial points will be placed on the heart surface by inserting non-conducting cactus pins into the heart or using wet tissue markers. After the registration, pixels in the [Ca2+]i map are locked to the corresponding pixels in the Vm map. Comparison between the Vm and the [Ca2+]i signals can be made by selecting and dragging pixels in the Vm display window.

2. Phase plots of Ca_i vs V_m were constructed. The ratio of the in-phase (+45 deg) and the out-of-phase (-45 deg) axes of the phase trajectory was used to characterize V_m-Ca_i coupling. The following figure shows some examples of phase plot and its in-phase/out-of-phase ratios.



Results

1. The in-phase/out-of-phase ratio gradually decreased as the pacing CL was reduced, and exhibited a restitutional dynamics against CL (Figure).



2. We found a 40% dispersion of this ratio (5.2±2.1) on the anterior wall when pacing at 300ms. The following table shows the results of all 6 rabbits.

Ratio of In-phase/out-of-phase Axes of the Phase Plot on Anterior Wall During Pacing at CL =300 ms

	Mean	SD	Max	Min	Difference
Rabbit 1	5.38	3.67	19.25	2.61	16.64
Rabbit 2	4.80	2.02	11.05	2.99	8.06
Rabbit 3	7.60	3.22	14.07	3.79	10.28
Rabbit 4	4.81	1.16	7.53	3.46	4.07
Rabbit 5	4.50	1.76	10.85	2.34	8.51
Rabbit 6	4.04	0.85	5.70	2.41	3.28
Average	5.19	2.11	11.4	2.93	8.47

3. Out of phase Ca_i alternans were observed in 5 hearts under rapid pacing. The relationship between the amplitude of Ca_i transient and APD were quite variable. Larger Ca_i transients could be associated with either longer APD, shorter APD or no APD alternans. Thus, the Ca_i has its own independent dynamics that is magnified by rapid rate. Independent Ca_i dynamics resulted in increased spatial heterogeneity of Ca_i alternans, which may contribute to the generation of discordant APD alternans.

Conclusion

Our results suggest that there is a spatiotemporal heterogeneity between the coupling of V_m and Ca_i in intact heart. Phase plot to characterize the dynamic Vm-Cai coupling may provide a novel tool to quantify such heterogeneity. The V_m-Ca_i decoupling process under rapid pacing may underlie pacing-induced wavebreak and ventricular fibrillation.

II. Heterogeneous Alterations in Membrane Potential and Intracellular Calcium Dynamics During Acute Regional Ischemia in Rabbit Ventricles

Background

The majority of sudden deaths are attributed to VF, often triggered by acute ischemic events. Ca_i dynamics affected V_m through Ca2+ -sensitive membrane currents: $I_{Ca(L)}$, I_{NaCa}, $I_{Cl(Ca)}$, $I_{ns(Ca)}$. Abnormal Ca_i handling during acute ischemia had been demonstrated in previous studies, which could predispose myocardium to arrhythmias. However, the effects of acute ischemia on Ca_i and V_m dynamics and their interaction remain poorly understood. Whether or not these changes are restricted in ischemic zone (IZ) was also unclear.

In this study we specifically tested the following hypotheses related to the Ca_i and V_m dynamics during acute regional ischemia.

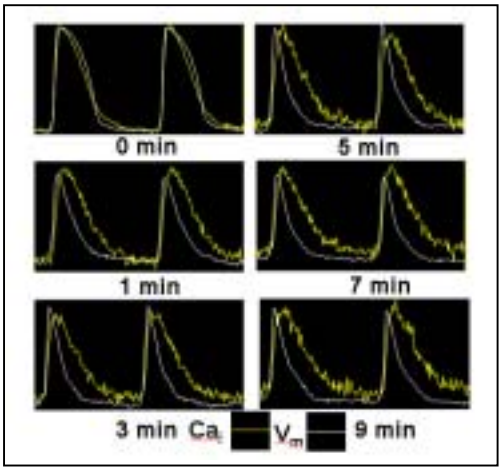
1. The alterations with time in Ca_i transients and action potential (AP) is different during the acute ischemia. The magnitude of difference increases with time.
2. The Ca_i transient reveals its own restitution characteristics during ischemia, which is independent of AP duration (APD) restitution.
3. Spatial heterogeneity of Ca_i alternans was increased in both IZ and non-IZ during regional ischemia.
4. The Ca_i dynamics became disassociated from V_m dynamics in IZ, resulting in APD oscillation without a steep APD restitution.

Methods

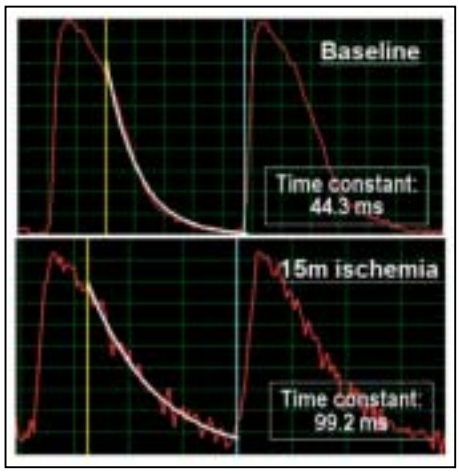
1. Six New Zealand white rabbits, weighing 3-5 kg, were injected with 1000 units of heparin and anesthetized with ketamine (20 mg/Kg) and xylazine (5 mg/Kg) through an intravenous injection. After a midline sternotomy, the heart was harvested, and the ascending aorta will be cannulated and perfused with 37°C Tyrode's solution Coronary perfusion pressure will be regulated between 80 and 95 mmHg. A quadripolar catheter will be placed into the endocardium of RV apex for pacing.
2. Dual optical mapping was performed in isolated rabbit heart model. (See above)
3. Study protocol: Before inducing ischemia, APD restitution was determined by a modified dynamic pacing protocol with progressively shorter pacing cycle length. Branches of left coronary artery were ligated to create regional ischemia. The dynamic pacing protocol was repeated at 15 min after coronary ligation.
4. Data analysis: Restitution curves at 25 selected sites on the anterior wall were constructed simultaneously before and 15 min after coronary ligation. Each restitution curve is constructed by plotting APD80 or total duration of Cai transient against the preceding diastolic interval (DI). In-phase and out-of-phase alternans of Cai transients and APD were also registered during dynamic pacing.

Results

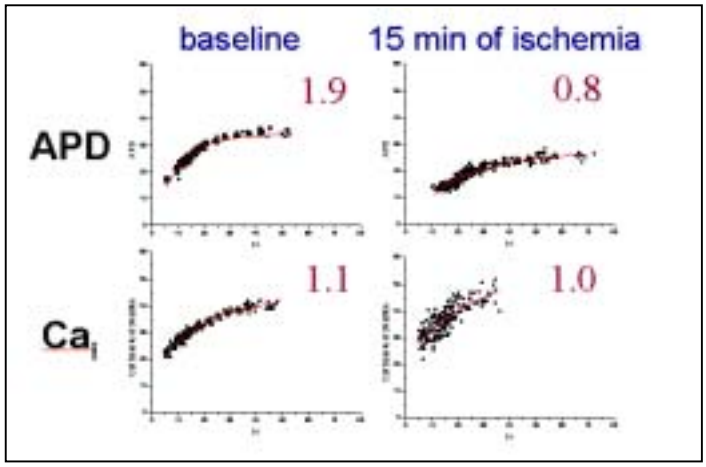
1. After ischemia, the APD progressively shortened and the Cai transient duration progressively prolonged (Figure). The magnitude of difference between APD and Cai transient duration was increased with time.



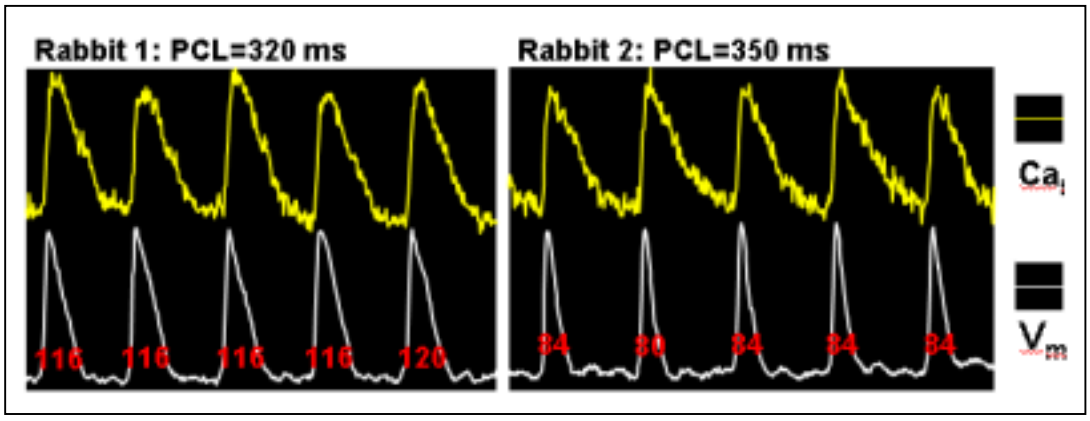
2. The following figure showed an example of Ca_i transient before and after coronary ligation. The rising phase of Ca_i transient was slightly lengthened and the falling phase of Ca_i transient was marked prolonged. After the exponential curves fitting the falling phase of Cai transients in all rabbits, the half-time of falling phase of Cai transients after ischemia were significantly longer than that of baseline condition. (15 min of ischemia: 87±10 ms, baseline: 53±3 ms; $p<0.01$)



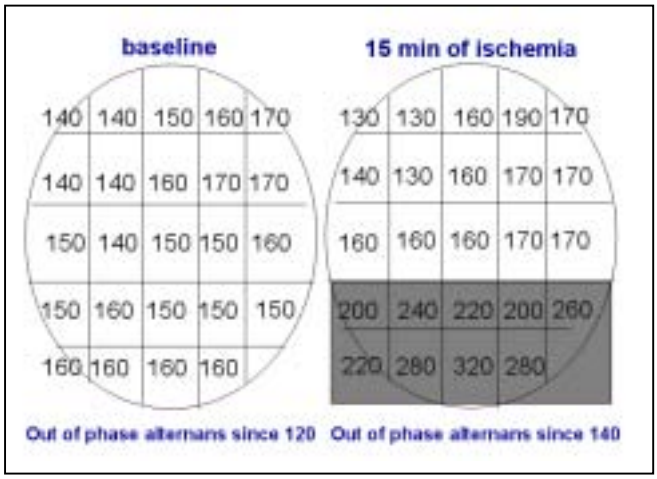
3. In baseline condition, the restitution curve of APD is steeper than that of Ca_i transient duration at the same site. (APD:1.4±0.4, Ca_i: 1.0±0.3; $p<0.001$) After ischemia, the slope of APD restitution curve in IZ was flattened but the slope of Ca transient restitution curve was less changed. (Figure)



4. Alternans threshold of Ca_i transients became lower in both IZ and non-IZ.
   - The Ca_i alternans occurred at long pacing cycle length (PCL) in IZ without detectable APD alternans. (Figure)

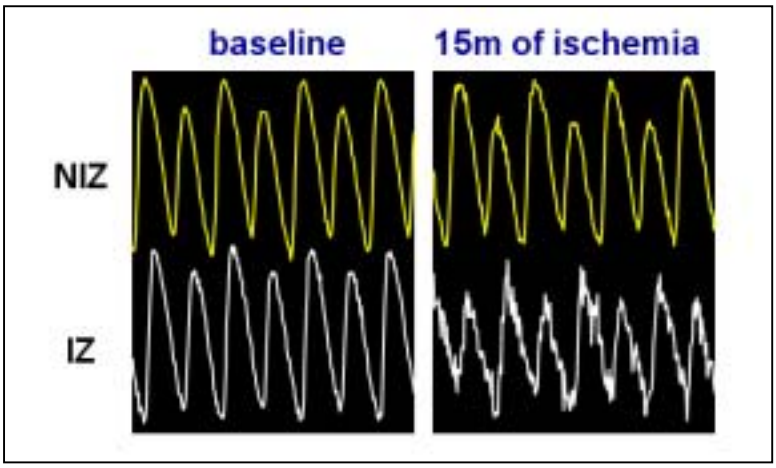


   - The longest PCL at which Ca_i alternans occurred was longer in IZ (352±30 vs. 168±19 ms; p<.001) after 15 min of ischemia compared to baseline condition. The longest PCL at which Ca_i alternans occurred was also longer in non-IZ (218±24 vs. 168±8 ms; p<.01) after 15 min of ischemia compared to baseline condition. (Figure)

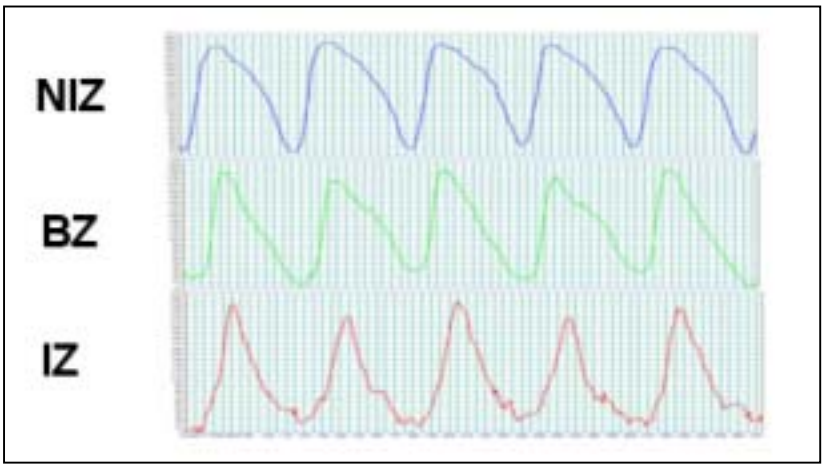


   - The longest PCL for the detectable out-of-phase Ca_i transient alternans

between the IZ and NIZ (Figure) also increased after ischemia (173±44 vs. 125±6 ms).



5. APD alternans occurred in the IZ but not in NIZ at the same PCL (Figure)
   - APD restitution was flattened in IZ
   - DI was longer in IZ
   - Other mechanism rather than APD restitution underlie the APD alternans in IZ
   - Increased spatial heterogeneity of Cai alternans and early onset of Cai alternans with longer DI during regional ischemia may contribute to the developing APD alternans in IZ.



Conclusion

The magnitude of difference between APD and Ca_i transient duration was increased with time after ischemia. The Ca_i transient reveals its own restitution characteristics, independent of APD restitution, during ischemia; which further enhanced the dynamic heterogeneity between the Ca_i and V_m dynamics. We demonstrated that the spatial heterogeneity of Ca_i transient increased in both IZ and NIZ during acute regional ischemia. Furthermore, the Ca_i dynamics

became disassociated from Vm dynamics in IZ, which could result in APD oscillation without a steep APD restitution and destabilizing the electrical wavefronts.