2. 不整脈の診断と治療：最近のトピックス

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Key words：不整脈の3次元マッピング，心房細動，遺伝性不整脈，植え込み型除細動器

はじめに

不整脈は時に心臓性突然死を起こす点で恐れられている病気であり，最近の15年ばかりの間に，その診断と治療に関する種々の方法論が急速に進歩した分野である．この発展には，基礎電気生理学，分子生物学，遺伝学，医用工学，コンピュータ科学など複数領域の寄与が大きい．たとえば，医用工学の発展により，不整脈を可視化することができるようになった．これにより，1990年代から盛んに行われるようになったカテーテルを用いた心筋焼灼術（カテーテルアブレーション）の適応範囲がさらに広がった．さらにコンピュータサイエンスの急速な導入により当該分野でもシミュレーションが普及してきた．しかしながらより安全なアブレーション治療が可能となった現在でも，日常診療における不整脈の正しい診断は，循環器専門医にあっても時に困難なことがある．また，近年，トランスレーショナル医療の一環として，多くの遺伝性不整脈の病態が遺伝子レベル解明されてきた．あらたに，不整脈のなかにイオン・チャネル病という概念が組み込まれた．

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1. 2種類の不整脈：遺伝子診断

不整脈の分類には，種々のスケールがある．たとえば，症候性・無症候性，頻拍性・徐脈性あるいは，その起源（心房性・心室性）などの分け方である．さて，ここで2種類の不整脈という分類は，(1) 心筋梗塞や心筋症など器質的な心疾患に合併するものと，(2) 器質的な心臓障害を有さないものと言うものである．後者の中に，たとえばQT延長症候群やブルガダ症候群のように遺伝的な背景を有し，心臓の興奮と伝導・収縮に大きく関わるイオン・チャネルやその調節蛋白をコードする遺伝子の異常により発症するものがあることが判明してきた（次ページ：表）．

さらに，家族性心房細動，洞不全症候群，不整脈源性右室心筋症，比較的まれなAndersen症候群やカテコラミン誘発性多形性心室頻拍（CPVT），QT短縮症候群などでも原因遺伝子が続々と同定されている．このような進歩をふまえて，テーラーメイド治療を目指し原因遺伝子の診断が行われている．特に発端者の家族における遺伝子検査は予期せぬ突然死を防ぐためにも有用と考えられる．表に現在までに明らかとなっている遺伝性不整脈と原因遺伝子さらに障害されるイオン・チャネルをまとめる．

興味深いことに，日常診療では家族性より，

表．現在までに判明している遺伝性不整脈疾患の責任遺伝子

疾患名	染色体	分類	責任遺伝子	障害部位と電流
QT 延長症候群 (LQT)	11p15.5	LQT1	*KCNQ1*	遅延整流性外向きＫチャネル（遅い成分）I_{Ks}
	7q35-q36	LQT2	*KCNH2*	遅延整流性外向きＫチャネル（速い成分）I_{Kr}
	3p21-p23	LQT3	*SCN5A*	電位依存性 Na チャネル I_{Na}
	4q25-q27	LQT4	*ANK-B*	Ankyrin-B, $I_{Na/Ca}$
	21q22.1-p22	LQT5	*KCNE1*	遅延整流性外向きＫチャネル（遅い成分）I_{Ks}
	21q22.1-p22	LQT6	*KCNE2*	遅延整流性外向きＫチャネル（速い成分）I_{Kr}
	17q23	LQT7	*KCNJ2*	内向き整流性Ｋチャネル $I_{Kir2.1}$
	12p13.3	LQT8	*CACNA1C*	L 型 Ca チャネル I_{CaL}
Brugada 症候群	3p21-p23	BRU1	*SCN5A*	I_{Na}
Lenègre-Lev 病	3p21-p23	CCD1	*SCN5A*	I_{Na}
先天性洞不全症候群	3p21-p23	SSS1	*SCN5A*	I_{Na}
	15q24-q25	SSS2	*HCN4*	過分極誘発陽イオンチャネル
不整脈源性右室異形性症 (ARVD)	1q42-42	ARVD2	*RyR2*	リアノジン受容体
	6p24	ARVD8	*DSP*	Desmoplakin
	12p13	ARVD9	*PKP2*	Plakophillin
Naxos 病	17q21	NAXOS	*JUP*	Plakoblobin
家族性心房細動	11p15.5	PAF1	*KCNQ1*	I_{Ks}
	21q22.1-p22	PAF2	*KCNE2*	I_{Kr}
	3p21-p23	PAF3	*SCN5A*	I_{Na}
カテコラミン感受性心室頻拍 (CPVT)	1q42-42	CPVT1	*hRyR2*	リアノジン受容体
	1p13.3-p11	CPVT2	*CASQ2*	Ca 結合蛋白
QT 短縮症候群 (SQT)	7q35-q36	SQT1	*KCNH2*	I_{Kr}
	11p15.5	SQT2	*KCNQ1*	I_{Ks}
	17q23	SQT3	*KCNJ2*	I_{K1} (Kir2.1)

より高頻度に遭遇する２次性QT延長症候群の患者の中にも，表上段の家族性LQTSで明らかとなった関連遺伝子の異常を認めることがある．このような従来，後天性と考えられていた病態の中でも，低カリウムや徐脈・薬剤などにより病状が，torsade de pointesまで進んでしまうことがある．家族性であっても，その浸透率（発病率）は 30% 程度であり，このような遺伝子異常のキャリアーも，潜在性LQTSであり，薬剤をむやみに内服しない，下痢やダイエットなど低K血症を誘発するようなことを避けるなどの生活指導が大切である．このような意味からも発端者家族の遺伝子検査は重要である．ピルジカイニドなどNaチャネル阻害薬によりたまたま顕性化するブルガダ症候群においても，同じことが観察される．とくに，本症候群では，発作性心房細動の合併が多いため，つねに念頭に置いておく必要がある．

2. 薬物治療：心房細動を巡る論議

不整脈治療の主役は薬物であるが，たとえば，心房細動は甲状腺機能亢進症で高頻度に合併し甲状腺機能を正常化することにより治療できる場合がある．このように多彩な病気が基礎疾患となっていることがあり，不整脈の基礎疾患を見極め，もし，その治療が可能であれば，これが優先する．抗不整脈薬の使用も，より不整脈の病態に即した選択を行うようになってきている．従来のVaughn Williams分類は薬剤の活動電

位に対する作用から分類されていたが，新しいScilian Gambitの分類では不整脈の発症機序と維持機構をまず考えて一番のターゲットなる部位あるいはチャネル（受攻因子）を想定して薬剤を選択するべきとされる．

この関連で，心房細動の治療はリズムコントロールなのかレートコントロールなのか論議が盛んである．AFFIRM試験やRACE試験では，あえて洞調律に戻すのではなく，心拍調節（レートコントロール）群で有意差はないものの，生命予後が良い傾向が示された．この原因の一つとして，抗凝固療法が，後者ではより厳密に施行されており塞栓症の合併が少なかった点が挙げられた．現在，日本でのエビデンス集積のため，J-RHYTHM試験が進行中である．また，抗不整脈薬や抗凝固療法，β-ブロッカーなど心房細動の薬物療法の基本に加えて，心房リモデリングに関わるレニン・アンギオテンシン系を阻害するACE阻害薬やアンギオテンシン受容体ブロッカーがupstream治療として注目されている．心房細動を起こす器質の形成を阻止しようと言うものである．

3. 不整脈の視覚的診断

不整脈の正確な診断には，勿論，心電図記録が不可欠であるが，近年，実際に起こっている不整脈を目でみることができるようになった．電磁場を利用した3次元マッピング（Electro-anatomical mapping system：CARTO）を行い，心臓の構造と電気的な興奮順序を3次元的に表示できる．この方法は，後述のカテーテル・アブレーション治療を成功に導くのに役立っている．しかし，CARTOシステムのマッピングでは頻拍時の血行動態が安定していないと長時間のサンプリングのため困難である．保険認可の問題で本邦ではまだ使用できないが，この欠点を補うNon-contacting mappingと呼ばれる一心拍のサンプリングでデータを取得できるシステムも開発されている．

4. カテーテル・アブレーション：最近の展開

前述のように1990年前後より高周波をエネルギー源としたアブレーションが広まり，WPW症候群などに伴う上室性頻拍や心房粗動，特発性心室頻拍などの各種頻拍症が根治できるようになり，多くの患者に福音をもたらしている．さらに，1998年頃からは従来は困難とされていた心房細動などの難治性不整脈も根治可能となった．これらの進歩には，不整脈専門医の増加，アブレーション・デバイスの進歩，前述の3次元マッピングの導入などが大きく寄与している．

5. 心臓性突然死の予防治療

あらかじめ体内に植え込み，心臓性突然死の最大原因である心室頻拍・細動を電気ショックで停止し救命できるデバイスが，1996年から保険償還が認められ，一定の基準を満たした認定施設での治療が可能となっている．この植え込み型除細動器（ICD）治療を含め本邦における低心機能症例の心臓性突然死の実態を前向きに調査するPREVENT-SCDが全国レベルで開始されている．心不全症例では，その早い病期で心室性不整脈により突然死をきたすことが示されており，米国のVal-HeFT研究では，NYHAII～III度の心不全患者でEF35％以下を対象として，従来治療＋アミオダロンあるいはプラセボの2群とICD群の3群に分けて生命予後を検討している．5年間の観察期間では，ICD群でプラセボあるいはアミオダロン群に比べて有意に死亡率を低下させた．サブグループ解析でみると，しかしながら，非虚血性心不全では，ICDの死亡率低下は，ほか2群に比べて大きいものの有意差はなかった．結果の解釈はむずかしいが，ACC/AHAのガイドラインでは，この臨床研究をふまえて

NYHAII～III度でEF30％以下の心不全症例に関して一次予防としてのICDが勧められている．しかし，我が国では未だこのような症例に対する一次予防的ICD植え込みは認められていない．

6. 重症心不全のペーシング治療

超高齢化社会を迎えた本邦では今後いよいよ心不全患者が増加すると予想される．中には脚ブロックを合併し，心室内の興奮の伝わり方が不均一なために，収縮力が低下している場合がある．特殊なペースメーカを植え込み，右心室と同時に左室側からも電気刺激することにより，左心室全体の収縮に同時性を与えて治療する．通常，左側のペーシング電極は冠状静脈洞の分枝に挿入される．この治療法は心臓再同期療法（Cardiac Resynchronization Therapy；CRT）と呼ばれ，2004年に保険償還が認められ，やはり認定施設で植え込みが可能となった．さらに，ICDとCRT機能の両者を具備したデバイスも海外では使用されるようになっており，心不全・心臓突然死患者に対する新しいストラテジーを提供している．

2005年に報告されたCOMAPANION研究では，NYHAIII～IV度の心不全でQRS幅が120 msを超える患者1,500例余りを，CRT，CRT＋ICD，そして薬物療法のみの3群にわけて予後を検討している．薬物だけの群に比べてCRT群さらにそれ以上にCRT＋ICD群で，生命予後の改善が得られた．また，対象患者のQRS幅が広いほど，左脚ブロックが強いほどCRT＋ICDの効果が勝っていた．しかし，我が国では未だ，CRT＋ICDデバイスは認可されていない．

残念ながら，本邦では，不整脈を含む循環器診療のみならず，いわゆる最先端治療と呼ばれる領域は，非常な制約の基でしか施行できないのが現状である．志が高く優秀でやる気のある医師がこの分野で十分に活躍できる場を提供するためにも，なんらかの施策が必要と思われる．

参考文献

1) AFFIRM：Atrial Fibrillation Follow-up Investigation of Rhythm Management. N Engl J Med 347：1825–1833, 2002.
2) RACE：Rate Contorl vs. Electrical Cardioversion for Persisitent Atrial Fibrillation. N Engl J Med 347：1834–1840, 2002.
3) Haissaiguerre M, et al：Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 339：659, 1998.
4) Bardy GH, et al：Amiodarone or an implantable defibrillator for congestive heart failure（SCD-HeFT）. N Engl J Med 352：225, 2005.
5) Bristow MR, et al：Cardiac resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. N Engl J Med 350：2140, 2005.

遺伝性不整脈のゲノム解析

Genetic analysis of inherited arrhythmias

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◎遺伝性不整脈という概念は比較的新しい．そもそも判然とした遺伝性がわかっていた不整脈はRomano-Ward症候群とよばれる家族性QT延長症候群であった．1990年代に入って分子遺伝学や遺伝子工学の急速な進歩がQT延長症候群にも応用され，1995年になって，その病因が心筋の興奮・伝導・収縮をつかさどる蛋白分子であるイオンチャネルをコードする遺伝子の変異により招来される機能障害であることが判明した．イオンチャネルの病気，すなわち“チャネル病”であることが明らかとなった．その後，従来，遺伝性がはっきりしなかったり，あまり議論されてこなかった不整脈についても，つぎつぎと原因遺伝子が同定されている．なかにはチャネル蛋白ではなく，細胞骨格，デスモゾームやカルシウム結合蛋白の遺伝子異常までがみつかってきている．ここではこれらの不整脈に関して現在行われつつあるゲノム解析を紹介する．

Key word：イオンチャネル病，チャネル遺伝子，QT延長症候群，Brugada症候群，遺伝子検査

遺伝性不整脈には家族性QT延長症候群(以下，LQTS)，Brugada症候群，カテコールアミン誘発性多形性心室頻拍，Andersen症候群，QT短縮症候群など多種多様な病態が包含されるが，最近，その多くが心筋の興奮・伝導・収縮を司る蛋白分子であるイオンチャネルの病気，すなわち“チャネル病”であることが判明した(表1)．なかでも心電図上，著しいQT時間の延長と特異な多形性心室頻拍を起こす家族性LQTSは若年者にみられ，心臓突然死を起こすことから注目を集め，詳しく調べられた(表1-A)．また，Brugada症候群は体表面心電図で特異的な胸部右側誘導のST上昇と右脚ブロックを伴い，夜間に多く発症する心室細動で突然死をきたす．その一部ではNaチャネルの遺伝子(SCN5A)異常が同定されている(表1-B)．

本稿ではLQTSやBrugada症候群，その他の不整脈に関して，現在行われているゲノム解析を紹介する．

LQTS(先天性・後天性)(表1)

LQTSは，心電図上，著しいQT時間の延長と特異な多形性心室頻拍(torsade de pointes：TdP；図1)を起こす．家族性LQTSは10歳前後と若年者に発症し，TdPさらには心臓突然死を起こすため古くより注目を集めていた．常染色体劣性遺伝で，感音性難聴を伴うJervell Lange-Nielsen症候群と，常染色体優性遺伝で難聴を伴わないRomano-Ward症候群が先天性としてはよく知られている[1-3](表1)．周期性四肢麻痺や骨格異常を合併するAndersen症候群(LQT7)や，自閉症など多様な神経症状を合併するTimothy症候群(LQT8)はQT延長をきたすまれな疾患であるが，最近になって原因遺伝子が報告されている．一方，薬剤などに伴う後天性LQTS(表1)においても一部にはLQTS関連遺伝子の異常が潜んでいるが，チャネルの機能障害が軽度であるため，薬剤，徐脈，低カリウム血症など他のリスクファクターが重複してはじめて発病する可能性があることもわかってきた．

現在までに同定されたLQTSの原因遺伝子は8つであり，LQT1からLQT8に分類されている(表1)[4-10]．このなかで，LQT4のみがankyrinとよばれる膜構成蛋白の異常が原因であるが，その他は

表 1　イオンチャネル病の原因遺伝子とイオンチャネル機能

タイプ	遺伝子座	原因遺伝子	イオンチャネル
A. QT 延長症候群			
A-1. 先天性 QT 延長症候群			
Romano-Ward 症候群			
LQT1	11(11p15.5)	*KCNQ1*	I_{Ks}
LQT2	7(7q35-36)	*KCNH2*	I_{Kr}
LQT3	3(3p21-24)	*SCN5A*	I_{Na}
LQT4	4(4q25-27)	*Ankyrin-B*	Na-K ATP_{ase}, I_{Na-Ca}
LQT5	21(21q22.1-q22.2)	*KCNE1*	I_{Ks}
LQT6	21(21q22.1-q22.2)	*KCNE2*	I_{Kr}
LQT7	17(17q23)	*KCNJ2*	I_{K1}
LQT8	12(12p13.3)	*CACNA1C*	I_{Ca-L}
Jervell & Lange-Nielson 症候群			
JLN1	11(11p15.5)	*KCNQ1*(ホモ接合)	I_{Ks}
JLN2	21(21q22.1-q22.2)	*KCNE1*(ホモ接合)	I_{Ks}
A-2. 後天性 QT 延長症候群			
	11(11p15.5)	*KCNQ1*	I_{Ks}
	7(7q35-36)	*KCNH2*	I_{Kr}
	3(3p21-24)	*SCN5A*	I_{Na}
	21(21q21.1-q22.2)	*KCNE1*	I_{Ks}
	21(21q22.1-q22.2)	*KCNE2*	I_{Kr}
B. Brugada 症候群			
	3(3p21-24)	*SCN5A*	I_{Na}
C-1. 進行性心臓伝導障害(Lenègre 病)			
	3(3p21-24)	*SCN5A*	I_{Na}
C-2. 家族性洞機能不全症候群，洞停止，家族性房室ブロック	3(3p21-24)	*SCN5A*	I_{Na}
		HCN4	If
D. カテコールアミン誘発性多形性心室頻拍			
CPVT1	1(1q42-43)	*RyR2*	RyR2
CPVT2	1(1p11-13.3)	*CASQ2*	calsequestrin
E. 催不整脈性右室心筋症			
ARVC1	14(14q24.3)	*TGF-β3*	TGF-β3
ARVC2	1(1q42-43)	*RyR2*	リアノジン受容体
ARVC3	14(14q12-22)	不明	不明
ARVC4	2(2q32)	不明	不明
ARVC5	3(3p23)	不明	不明
ARVC6	10(10p12-14)	不明	不明
ARVC7	10(10q22)	不明	不明
ARVC8	6(6p24)	*DSP*	desmoplakin
ARVC9	12(12p11)	*PKP2*	plakophillin
Naxos 病	17(17q21)	*JUP*	plakoglobin
F. 家族性心房細動			
	11(11p15.5)	*KCNQ1*	I_{Ks}
	21(21q21.1-q22.2)	*KCNE2*	I_{kr}
	17(17q23)	*KCNJ2*	I_{K1}
	12(12p13)	*KCNA5*	I_{Kur}
G. QT 短縮症候群			
SQT1	7(7q35-36)	*KCNH2*	I_{kr}
SQT2	11(11p15.5)	*KCNQ1*	I_{Ks}
SQT3	17(17q23)	*KCNJ2*	I_{K1}

K チャネルあるいは Na チャネルなどのα，βサブユニットに関与する遺伝子変異である．前述の Andersen 症候群は LQT7 とされるが，最近の心電図解析[11]では QT 延長というよりむしろ QU 延長で，異常 U 波が特徴的であって，LQTS の 1 型とは考えられなくなってきている．

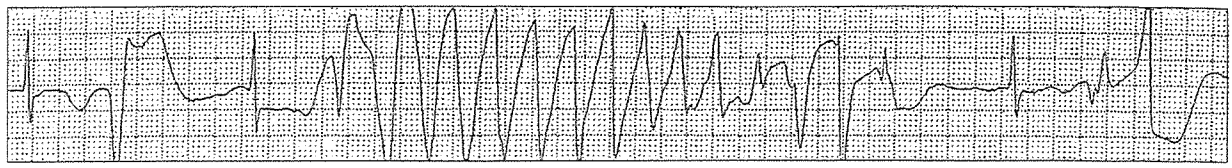

図 1　72 歳女性，薬剤誘発性 QT 延長症候群に認められた torsade de pointes

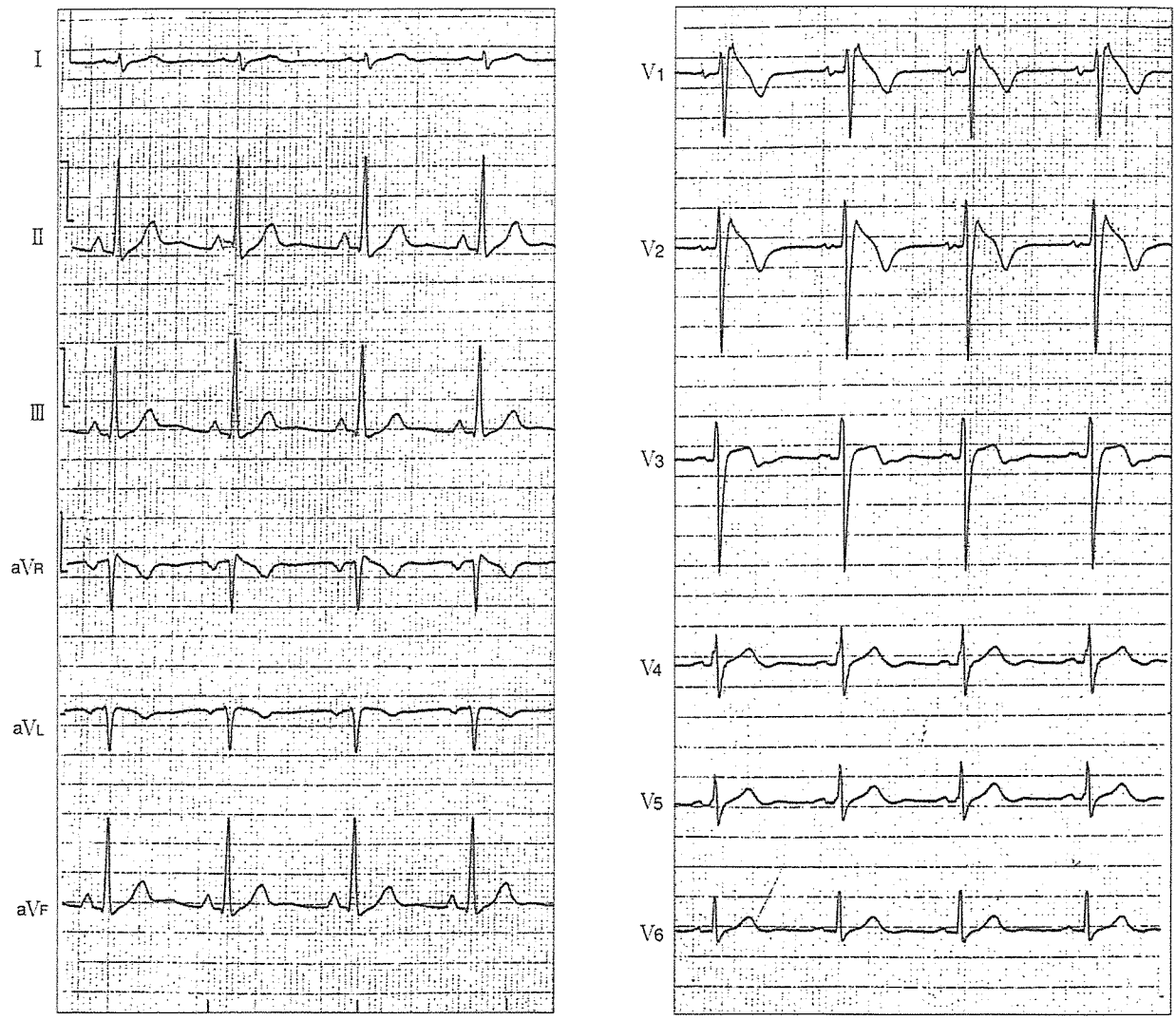

図 2　40 歳男性，突然死の家族歴を有する無症候性 Brugada 症候群

前述の Timothy 症候群は LQT8 に分類される．Keating らのグループが 2004 年に L 型 Ca チャネルの遺伝子異常を原因であると報告した[12]．心疾患で Ca チャネルの遺伝子異常が関与する最初の報告である．合指症，禿頭，小歯症，免疫不全，自閉症などの知能発育障害など，多臓器に障害が出現する．報告された Ca チャネルαサブユニットの変異(G406R)は，発現実験で L 型 Ca チャネルの不活性化障害を起こし，細胞内 Ca 濃度が上昇することが示された．このような病態から，多臓器障害や致死性不整脈を引き起こす，きわめて予後の悪い疾患である．

Brugada症候群(表 1-B)

器質的な心疾患を有さず，致死性不整脈を認める特発性心室細動症例のなかでも，Brugada 症候群は共通する特徴的な心電図波形(V1～V3 誘導の ST 上昇)(図 2)を示す症候群である．家族内突然死の多いこれらの症例に，1998 年 Chen らは，心筋 Na チャネルのαサブユニットをコードする SCN5A

に遺伝子異常を見出した[13]．これらの遺伝子変異によりNa電流が減少することが本症候群の病態であると理解されている．ただ，SCN5Aに遺伝子異常が見出される症例は本症候群の20%前後と低く，他の原因遺伝子が存在することが予想されてきたが，清水らはBrugada症候群がアジアに多い原因として，SCN5Aのプロモーター領域の遺伝子多型が関与していることを報告した[14]．この遺伝子多型によりNaチャネルの発現量が変化することが推測されている．SCN5A変異のないBrugada症候群の病因がすべて，この遺伝子多型のハプロタイプの違いで説明できるかについては，今後の検討が必要と思われる．著者らは，洞不全症候群や房室ブロックを合併した本症候群患者38名にSCN5A解析を行った結果，あらたな4変異(11%)を見出した．機能解析を行った結果，すべての変異はnon-functionであることを証明し，本症候群の中にも存在する臨床像の相違を報告した[15]．

進行性心臓伝導障害と家族性洞不全症候群(表1-C)

進行性心臓伝導障害はLenègre病ともよばれ，刺激伝導系の進行障害により脚ブロック，完全房室ブロックをきたす疾患で，SCN5Aの遺伝子異常が報告されている．先天性洞不全症候群ではSCN5AとHCN4遺伝子の変異が報告されているが，2006年にDiFrancescoらのグループは，洞性徐脈の家系に過分極誘発性陽イオンチャネルをコードするHCN4の遺伝子異常(S672R)を見出し報告した[16]．この家系内の変異キャリアは有意に徐脈であった(変異キャリアvs.健常人：52 vs. 73/min, $p<0.001$)．本変異はHCN4のコードするIfチャネル蛋白のcAMP結合部位に位置する変異であるが，発現実験ではcAMPに依存するチャネル活性には影響せず，チャネル活性の電位依存性を過分極側へ変異させることによる電流低下が原因であった．

カテコールアミン誘発性多形性心室頻拍(CPVT)(表1-D)

カテコールアミン誘発性多形性心室頻拍(catecholaminergic polymorphic ventricular tachycardia：CPVT)は，フランスのCoumelらによって最初に報告された，家族性に発症し運動に伴う多形性心室頻拍をきたす不整脈で，30歳までに約1/3の患者が死亡するといわれている．2001年になってPrioriらとLaitinenらは，独立して心筋細胞内のCaハンドリングに重要な働きをしているリアノジン受容体遺伝子(RyR2)の変異が疾患と関連することを報告した[17,18]．

ついで，Lahatらは常染色体劣性遺伝を呈するCPVTの家系で，リアノジン受容体が発現する筋小胞体のなかに存在するCa binding proteinであるcalsequestrin 2(CASQ2)の変異を発見し，報告した[19]．このタイプはCPVT2とよばれ，RyR2に異常のあるタイプはCPVT1とされる(表1)．このように，CPVTは心筋細胞内のCa濃度を調節する機構に齟齬が生じて発症する病態であることがわかってきた．

不整脈源性右室心筋症(ARVC)(表1-E)

不整脈源性右室心筋症(ARVC)は右室心筋の脂肪変性により心室性不整脈を認める疾患で，現在までに10個の原因遺伝子が報告されている．そのうちの1タイプ(ARVC2)は表1-DのCPVTで問題となるRyR2であるが，その報告は少ないようである．一方，Gerullらは120家系のARVC症例中32例(27%)にデスモゾーム複合体の1蛋白であるplakophilin-2に遺伝子異常を見出した[20]．最近，著者らも本遺伝子変異によるARVC症例を報告したが，その後，本例は突然死している[21]．このほかにもdesmoplakin，desmoglein-2(DSG2)などの遺伝子変異によるARVCが報告されており，細胞間接着に関与する蛋白が障害され，心筋症を発症するものと考えられる(表1)．

家族性心房細動[23](表1-F)

Brugadaら[22]がはじめて家族性心房細動の遺伝異常を報告したのは1997年であるが，現在までに明らかになっている原因遺伝子は4つで，KCNQ1[22]，KCNE2[23]，KCNJ2[24]の遺伝子変異は機能亢進(gain-of-function)による活動電位の短縮を引き起こし，ひいては心房筋の有効不応期を短縮させ心房細動の基盤を形成すると考えられている[22-24]．一方，KCNA5の変異(E375X)による機能異常は機能喪失

型(loss-of-function)で，心房筋の活動電位の延長と早期後脱分極を引き起こす[25]．イオンチャネルの変異ではないが，gap junctionの異常による家族性心房細動の報告[26]もあり，今後さらに病態が解明されていくものと考えられる．

QT短縮症候群(SQTS)(表1-G)

QT短縮症候群(short QT syndrome：SQTS)の記載は，LQTSに比べて新しい．2000年にGussakらがはじめて症例報告し，器質的心疾患がないにもかかわらず恒常的なQT短縮(QTc 300 ms以下)を呈し，心臓突然死の家族歴や不整脈症状と関連する．Brugadaらは，QT延長症候群の原因遺伝子でもあるKCNH2がQT延長症候群の原因遺伝子でもあることを2004年にはじめて報告した[27]．以後，KCNQ1, KCNJ2にも変異が発見された[28,29]．QT短縮はこれらのチャネルのgain-of-functionが原因で，再分極過程が速まり活動電位が短くなることが原因と考えられる．心房細動を合併することが多いのも特徴で，表1-Fの家族性心房細動とSQTSの原因遺伝子との間でその機能障害の特性は類似する点が多く，たがいにオーバーラップする症候群ともいえる．

おわりに

遺伝性不整脈疾患の原因遺伝子の発見は1990年代なかばにLQTSでの報告にはじまり，この10年でいわゆるcommon diseaseである心房細動にまで及んでいる．表1のリストは今後さらに長くなると思われる．これらの遺伝子異常の多くは，心臓の興奮活動に関与するイオンチャネルをコードしており，当然のことながら遺伝子異常の種類や機能異常の性質により心電図異常も異なってくる．心電図波形に関与するイオンチャネル遺伝子の“変調”が不整脈疾患やさまざまな心電図変化に関与していることがわかってきた．さらに，コンピュータサイエンスを駆使したシミュレーションを用いて，遺伝子異常と心電図や不整脈という表現型との連関が詳しく研究されるであろう．今後の成果が期待される．

文献

1) Jervell, A. and Lange-Nielsen, F.：*Am. Heart J.*, **54**：59-68, 1957.
2) Romano, C. et al.：*Pediatr. Clin. Pediatr.*, **45**：656-683, 1963.
3) Ward, O. C.：*J. Irish Med. Assoc.*, **54**：103-106, 1964.
4) Wang, Q. et al.：*Nat. Genet.*, **12**：17-23, 1996.
5) Splawski, I. et al.：*Cell*, **119**：19-31, 2004.
6) Mohler, P. J. et al.：*Nature*, **421**：634-639, 2003.
7) Barhanin, J. et al.：*Nature*, **384**：78-80, 1996.
8) Sanguinetti, M. C. et al.：*Nature*, **384**：80-83, 1996.
9) Neyroud, N. et al.：*Nat. Genet.*, **15**：186-189, 1997.
10) Schulze-Bahr, E. et al.：*Nat. Genet.*, **17**：267-268, 1997.
11) Zhang, L. et al.：*Circulation*, **111**：2720-2726, 2005.
12) Splawski, I. et al.：*Cell*, **119**：19-31, 2004.
13) Chen, Q. et al.：*Nature*, **392**：293-296, 1998.
14) Bezzina, C. R. et al.：*Circulation*, **113**：338-344, 2006.
15) Makiyama, T. et al.：*J. Am. Coll. Cardiol.*, **46**：2100-2106, 2005.
16) Milanesi, R. et al.：*N. Engl. J. Med.*, **354**：151-157, 2006.
17) Laitinen, P. J. et al.：*Circulation*, **103**：485-490, 2001.
18) Priori, S. G. et al.：*Circulation*, **103**：196-200, 2001.
19) Lahat, H. et al.：*Circulation*, **103**：2822-2827, 2001.
20) Gerull, B. et al.：*Nat. Genet.*, **36**：1162-1164, 2005.
21) Nagaoka, I. et al.：*Circ. J.*, **70**：933-935, 2006.
22) Chen, Y. H. et al.：*Science*, **299**：251-254, 2003.
23) Yang, Y. et al.：*Am. J. Hum. Genet.*, **75**：899-905, 2004.
24) Xia, M. et al.：*Biochem. Biophys. Res. Commun.*, **332**：1012-1019, 2005.
25) Olson, T. M. et al.：*Hum. Mol. Genet.*, **15**：2185-2191, 2006.
26) Gollob, M. H. et al.：*N. Engl. J. Med.*, **354**：2677-2688, 2006.
27) Brugada, P. et al.：*Circulation*, **109**：30-35, 2004.
28) Bellocq, C. et al.：*Circulation*, **109**：2394-2397, 2004.
29) Priori, S. G. et al.：*Circ. Res.*, **96**：800-807, 2005.

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Altered Action Potential Dynamics in Electrically Remodeled Canine Atria

— Evidence for Altered Intracellular Ca^{2+} Handling —

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Background Electrical instability following sustained rapid excitation has been attributed to altered ion channels. Alterations of Ca^{2+} handling could also contribute to abnormal dynamics of action potential, favoring the initiation and perpetuation of arrhythmia.

Methods and Results Transmembrane action potentials and twitch force (TF) were recorded from normal (n=6) and remodeled (6-week atrial pacing at 400 beats/min, n=6) canine atria. When the cycle length (CL) was suddenly prolonged in normal atria, both TF and action potential duration (APD) increased on the first beat, and decreased subsequently. Opposite changes were observed with sudden CL shortening. These dynamics in both APD and TF were abolished by ryanodine, but augmented by cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum (SR) Ca^{2+} pump. In remodeled atria (RA), dynamic changes in APD were also concordant with dynamic changes in TF. The transient increases in APD and TF were enhanced, and the transient decreases were reduced compared to normal atria. The maximal slopes of APD and TF restitution curves were flatter and the magnitude of alternans was reduced in RA. The protein expression of SR Ca^{2+} ATPase and SR Ca^{2+}-release channel in RA was significantly reduced.

Conclusion Altered Ca^{2+} handling may underlie abnormal APD dynamics in RA. (*Circ J* 2006; **70:** 1488–1496)

Key Words: Action potentials; Atrial fibrillation; Calcium handling; Remodeling

An important advancement in our understanding of atrial fibrillation (AF) during the past decade was the demonstration that rapid atrial excitation alters atrial electrophysiology to promote AF[1–4] This process, termed "electrical remodeling", includes shortening and loss of rate-adaptation of the effective refractory period (ERP) as well as a reduction of conduction velocity[1] With respect to the cellular mechanisms underlying such electrical remodeling, most previous studies have focused on the altered action potential (AP) configuration during the steady state, resulting from altered function and expression of sarcolemmal ion channels. For example, a shortening in ERP, which reflects a shortening of the AP duration (APD), has been attributed primarily to a reduction of the L-type Ca^{2+} current ($I_{Ca,L}$) in association with a downregulation of pore-forming $I_{Ca,L}$ α-subunits[2,3]

In addition to changes in sarcolemmal ion channels, sustained rapid atrial excitation leads to profound changes in cellular Ca^{2+} handling[5] giving rise to significant contractile dysfunction. Such AF-related alterations in cellular Ca^{2+} handling also could contribute to abnormal electrophysiologic properties of the atria. The information available on this issue is still limited. Hara et al reported that the dynamics of APD, in response to an abrupt change of stimulation cycle length (CL) in canine atria with AF, were different from normal atria[4] They suggested an involvement of altered Ca^{2+} handling, because the difference in APD dynamics was abolished by ryanodine. The present study was designed to obtain further insight into the role of Ca^{2+} dynamics in modulating APD in atria subjected to chronic rapid atrial pacing.

We hypothesized that alterations to the Ca^{2+} handling in electrical remodeling could contribute to the abnormal APD dynamics favoring the initiation and perpetuation of fibrillation. Recent studies have shown that non-steady state AP characteristics have important implications for the development of arrhythmias[6–10] Several studies have suggested that APD restitution kinetics are an important determinant of stability in several types of re-entrant circuits[6–8] Burashnikov and Antzelevitch showed that late phase 3 triggered activity induced by early afterdepolarization (EAD) occurs after a long diastolic interval, causing immediate AF recurrence after AF termination in a cholinergically-mediated model of AF[9]

The purpose of this study was to test the following hypotheses: (1) that non-steady state APD dynamics, including APD restitution and APD transition after an abrupt change in CL, are related to intracellular Ca^{2+} handling in canine atria; and that (2) APD dynamics are altered in association with altered Ca^{2+} handling in the setting of electrical remodeling.

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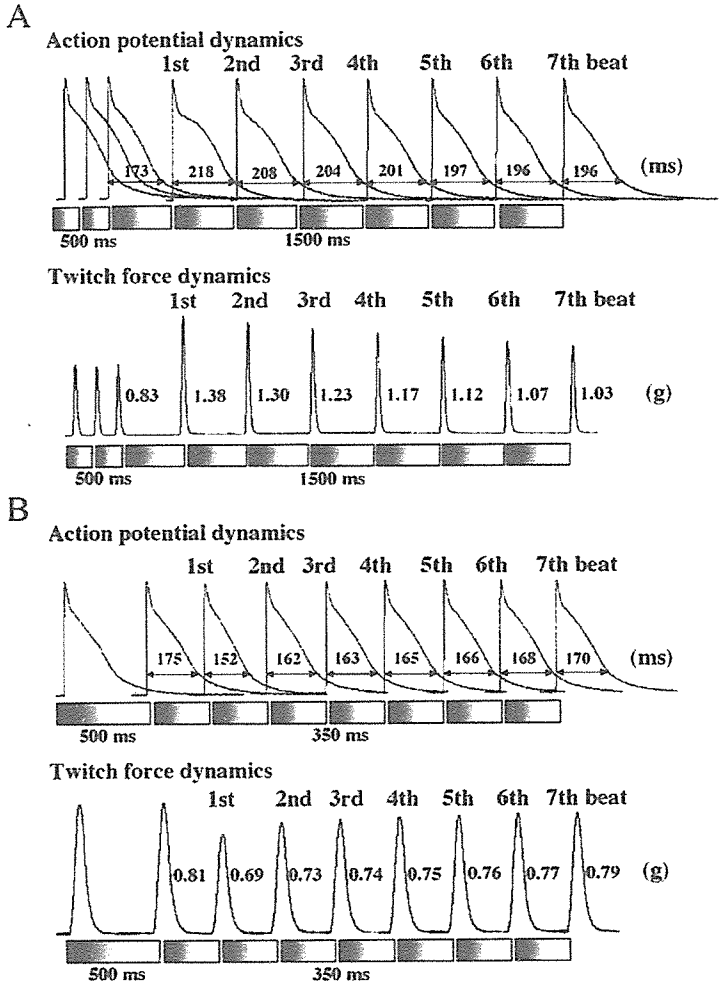

Fig 1. Representative action potentials and twitch contractions in normal canine atria subjected to a sudden increase in the cycle length (CL) from 500 to 1,500 ms (A) and to a sudden decrease in the CL from 500 to 350 ms (B).

For this purpose, we investigated the dynamics of APD and twitch force (TF) in response to abrupt CL changes in normal and remodeled canine atria (6–8 week rapid pacing), because TF dynamics are known to reflect dynamic changes in Ca^{2+} released from the sarcoplasmic reticulum (SR). We also examined the protein expression of SR Ca^{2+} ATPase (SERCA2), SR Ca^{2+}-release channel (RyR2) and sarcolemmal Na^+/Ca^{2+} exchanger (NCX1).

Methods

Animal Preparation

The investigation was approved by the Institutional Scientific Review Committee of the Keio University School of Medicine. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health. Adult beagle dogs (weighing 10–15 kg) of either sex were used for the experiments. A group of 6 dogs were anesthetized with sodium pentobarbital (20 mg/kg iv), and a left intercostal thoracotomy was performed. A bipolar pacing wire was positioned in the left atrial appendage through the opening in the pericardium. The electrode leads were tunneled subcutaneously to the back of the neck, where they were connected to a pacemaker (Medtronic, Minneapolis, MN, USA). After the incisions were closed, the animals were allowed to recover for 10 days. The dogs then received rapid atrial pacing (400 beats/min) with normal atrioventricular conduction for 6–8 weeks in a conscious and freely moving state. Clinical signs of heart failure were not observed. Another group of 6 age-matched beagle dogs without inserted instrumentation served as controls.

Recordings of APs and Twitch Contraction

On the day of the study, dogs were anesthetized with sodium pentobarbital (30 mg/kg iv) and the hearts were excised. Right atrial trabeculae were dissected and suspended in a Plexiglas tissue bath with the endocardial side up. The preparations were superfused with Tyrode's solution composed of (in mmol/L): NaCl 137, $NaHCO_3$ 12, KCl 4.0, $CaCl_2$ 2.7, $MgCl_2$ 0.5, NaH_2PO_4 1.8, and glucose 5.5. The solution was equilibrated with 95% O_2+5% CO_2 at 36.5°C to maintain the pH at 7.4.

Transmembrane APs were recorded with standard glass microelectrodes (3.0 mol/L KCl, 10–15 MΩ DC resistance) that were connected to a high-input impedance amplifier. Isometric contractile force was recorded by using a force-displacement transducer (Grass Instruments Co). The signals were displayed on an oscilloscope, digitized, and stored in a computer for offline analysis. APD was measured at 90% repolarization (APD_{90}).

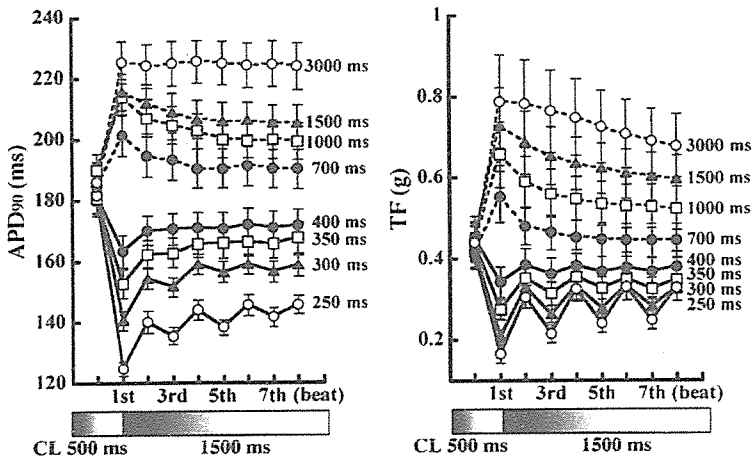

Fig 2. Effects of different cycle lengths (CLs) on the transitions of action potential duration was measured at 90% repolarization (APD_{90}) (Left panels) and twitch force (TF) (Right panels). The CL was changed abruptly from a basal level of 500 ms to several different levels (n=6). Note that the dynamic change in action potential duration occurred in parallel with dynamic changes in TF.

Experimental Protocols

The preparations were equilibrated in the tissue bath until electrical and mechanical stability were achieved under constant stimulation through a pair of bipolar electrodes at a basic CL (BCL) of 500 ms. Pulses were 5 ms in duration and 1.5–2-fold the threshold intensity. The stimulation CL was changed suddenly from 500 to 200–3,000 ms; this procedure was repeated. Restitution of APD and TF was determined using single test pulses (S_2) delivered after every 40th basic pulse (S_1) at a BCL of 500 ms. The S_1S_2 coupling interval was progressively decreased from 3,000 to 200 ms.

Drugs used in the study included ryanodine, isoproterenol hydrochloride, and cyclopiazonic acid (CPA). Ryanodine was dissolved in dimethyl sulfoxide (DMSO) and diluted in distilled water to make a stock solution. The final concentration of DMSO in the bath did not exceed 1 μmol/L and had no effects on APs or contractions.

Western Blots

The protein expression of SERCA2, RyR2 and NCX1 was estimated by Western blot analysis using tissue homogenates of the right atrial trabeculae. The procedures were essentially the same as those described previously[11] Primary antibodies used for the Western blots were as follows: anti-SERCA2 (1:1,000, Affinity Bioreagents #MA3-919), anti-RyR2 (1:5,000, Sigma, #R-128), and anti-NCX1 (1:500, Affinity Bioreagents, #MA3-926). The immunoblots were developed with horseradish peroxidase-labeled goat anti-mouse IgG (BD Transduction Laboratories, #M15345) as a secondary antibody (1:2,000 for SERCA2 and NCX1; 1:10,000 for RyR2) for 1 h, followed by visualization using an ECL reagent. The density of the protein bands was quantified by using a CS Saver and Analyzer (ATTO & Rise Corporation) and the value was normalized to the amount of total protein.

Statistical Analysis

Statistical analysis was performed with the Student's t-test for paired or unpaired data, and ANOVA followed by Bonferroni's test was then used, as appropriate. All data are expressed as the mean±SE, unless otherwise specified. A value of $p<0.05$ was considered to be significant.

Results

APD and TF Dynamics in Normal Atria in Response to Sudden Changes in CL

Fig 1A shows representative recordings of APs and twitch contractions in normal canine atria subjected to a sudden increase in the CL from 500 to 1,500 ms. The APD_{90} increased abruptly from 173 ms (steady-state at CL 500 ms) to 218 ms with the first beat of the longer CL, which then shortened in steps during subsequent beats. This observation is consistent with the early phase of rate-adaptation described in a previous report (Hara et al[4]). The TF recorded simultaneously showed parallel changes. TF amplitude increased abruptly from 0.83 to 1.38 g with the first beat at the longer CL, which then decreased in a stepwise fashion during subsequent beats. In the present study, we analyzed APD and TF dynamics only in the early phase of rate-adaptation. When the pacing CL was abruptly shortened, the opposite effect was observed (Fig 1B). Both APD_{90} and TF were abruptly decreased with the first beat, which then increased during subsequent beats. Thus, APD and TF showed a transient increase when CL was abruptly prolonged, and a transient decrease when CL was abruptly shortened in normal atria.

Fig 2 shows the effects of different CLs on the transient changes in APD_{90} and TF. The CL was suddenly changed from the basal value of 500 ms to several different values. Transient changes in APD_{90} and TF of different magnitudes were observed for all of the stimulation protocols with peaks in the first beat of the new CL. The greater the difference between the basic and the new CL, the greater the magnitude of transient changes in both APD_{90} and TF was.

Note that both APD and TF showed alternans when the new CL was short, although APD alternans was less prominent than TF alternans. The shorter the new CL, the greater was the magnitude of alternans. The magnitudes of APD and TF alternans were the largest at the beginning of the new CL and gradually decreased to a plateau after the 10th beat.

Pharmacological Modification of Intracellular Ca^{2+}-Handling

Dynamic changes in APD in response to a sudden change in CL were thought to reflect beat-to-beat changes in intracellular Ca^{2+} handling because they were always accompanied by parallel changes in TF. To confirm this hypothesis,

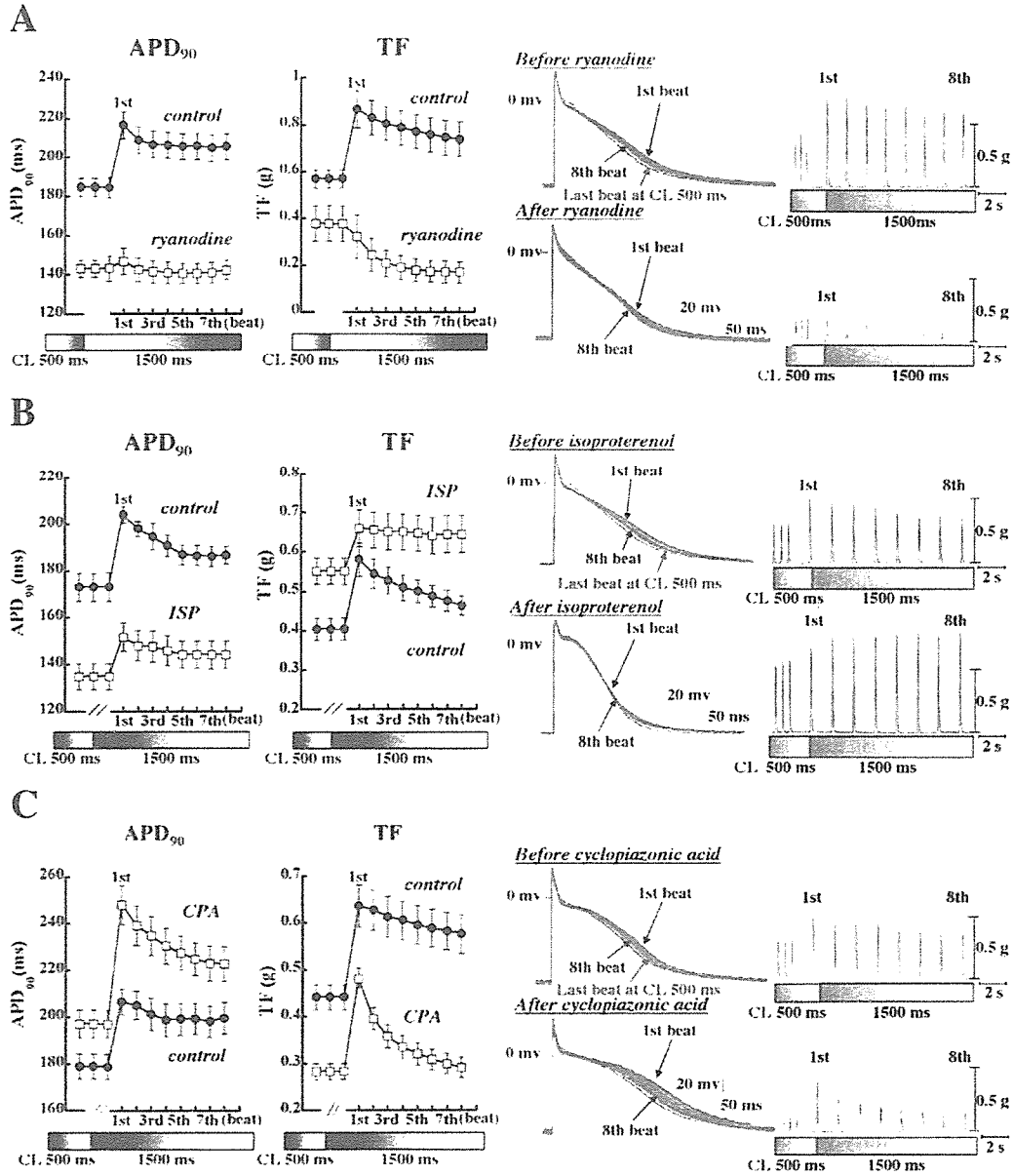

Fig 3. Effects of agents that modulate Ca^{2+} handling on action potential (AP) and twitch force (TF) dynamics in normal atria. (A) ryanodine (1 μmol/L, n=6). (B) isoproterenol (1 μmol/L, n=6). (C) cyclopiazonic acid (CPA) (10 μmol/L, n=6). (Left panels) Transition of the AP duration was measured at 90% repolarization (APD_{90}) and TF after abruptly changing the cycle length (CL) from 500 to 1,500 ms before and after the application of each agent. (Right panels) Representative experiments. Note that each agent had a similar effect on both AP duration and TF dynamics.

we examined the effects of ryanodine, isoproterenol and CPA on APD and TF dynamics after an abrupt prolongation of CL in normal atria.

Fig 3A shows the effects of ryanodine on the dynamic changes in APD_{90} and TF. Ryanodine binds specifically to the Ca^{2+}-release channel in the SR and at low concentrations (<30 μmol/L) locks the channel in an open state, causing Ca^{2+} leakage from the SR into the cytosol and subsequently out of the cell.[12] In atrial muscles treated with 1 μmol/L ryanodine, the transient increases in APD_{90} and TF in response to a sudden prolongation of the CL to 1,500 ms were completely abolished.

Fig 3B shows the effects of isoproterenol, a β-adrenergic receptor agonist. In the muscles treated with 1 μmol/L isoproterenol, the transient increases in the APD_{90} and TF were less prominent than in the control group. The percent increase in the first beat from the baseline in the absence and presence of isoproterenol was 16.2±1.2 vs 10.9±2.4%, respectively, for APD_{90} (n=6, $p<0.05$); and 45.1±2.5 vs 17.1±3.5%, respectively, for TF (n=6, $p<0.05$). Subsequent reductions in the APD and TF from the 1st to 8th beats during the longer CL were minimized. The percent reduction in the absence and presence of isoproterenol was 5.0±1.1 vs 2.7±0.2%, respectively, for APD_{90}; and 21.1±7.0 vs 2.9±1.2%, respectively, for TF (n=6, $p<0.05$).

Fig 3C shows the effects of CPA, a specific inhibitor of

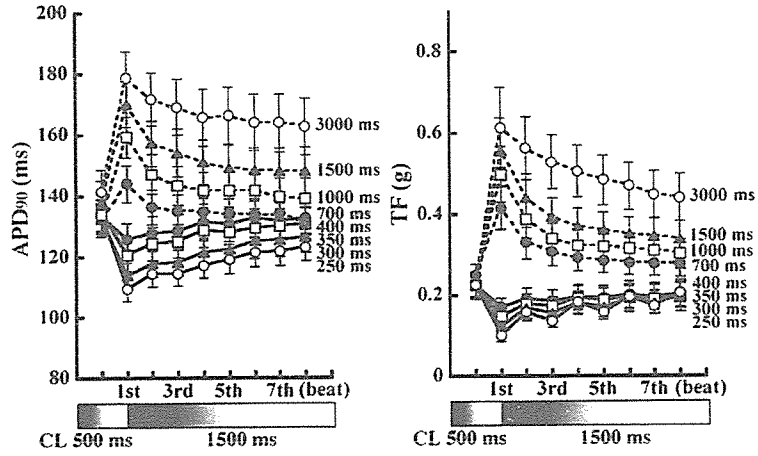

Fig4. Transitions of action potential duration was measured at 90% repolarization (APD_{90}) (Left panel) and twitch force (TF) (Right panel) in remodeled atria (n=6). The cycle length (CL) was changed abruptly from a basal level of 500 ms to several different levels.

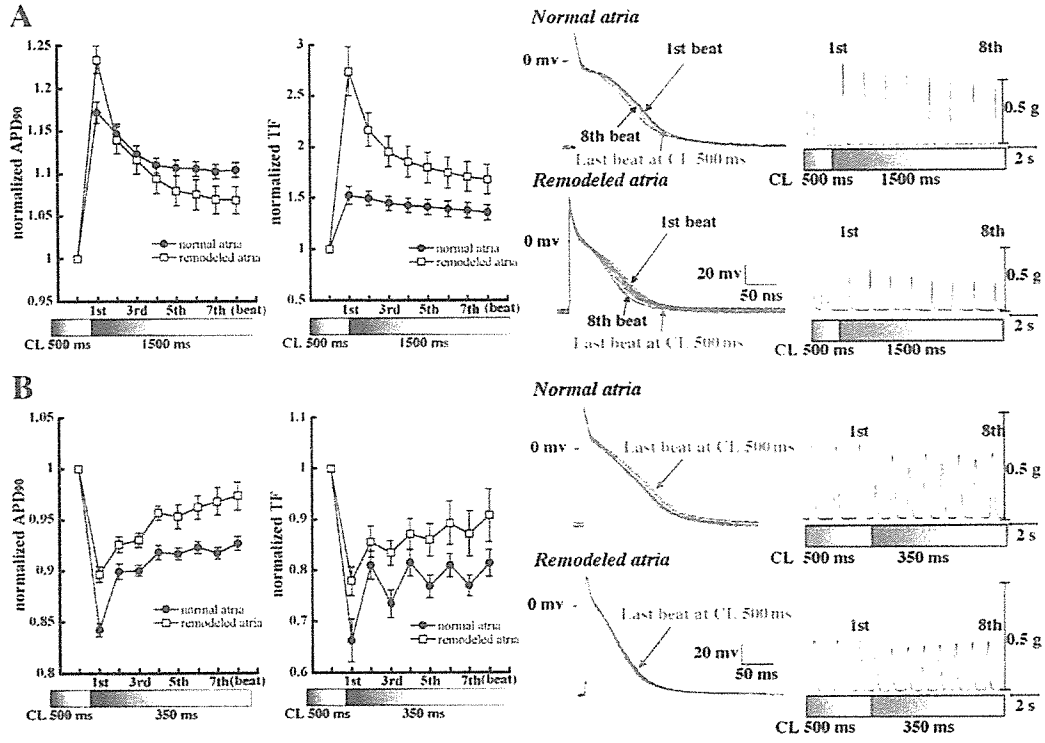

Fig5. Comparison of the transitions of the action potential duration was measured at 90% repolarization (APD_{90}) and twitch force (TF) after abruptly changing the cycle length (CL) from 500 to 1,500 ms (A) and after changing the CL from 500 to 350 ms (B) between normal and remodeled atria (n=6, respectively). Ordinates are normalized to the same values as those during bipolar electrodes at a basic CL of 500 ms. Representative experiments are also shown.

SR Ca^{2+}-ATPase. In the muscles treated with 10 μmol/L CPA, the transient increases in the APD_{90} and TF were more prominent than in the control group. The percent increase in the first beat from the baseline value in the absence and presence of CPA was 15.8±1.8 vs 25.8±0.94%, respectively, for APD_{90} (n=6, $p<0.05$); and 41.2±5.2 vs 75.7±10.3%, respectively, for TF (n=6, $p<0.05$). Subsequent reductions in the APD and TF from the 1st to 8th beats also were enhanced. The percent reduction in the absence and presence of CPA was 3.5±1.0 vs 10.2±0.7%, respectively, for APD_{90} and 8.6±5.4 vs 40.7±4.6%, respectively, for TF (n=6, $p<0.05$).

Therefore, drugs that modulate intracellular Ca^{2+} handling had similar effects on APD and TF dynamics, further supporting the concept that APD dynamics are associated with Ca^{2+} handling.

AP and TF Dynamics in Electrically Remodeled Atria

Fig4 shows APD and TF dynamics in remodeled atria after an abrupt change of CL from 500 ms to various CLs. Although the shapes of transition curves appeared similar to normal atria, the magnitudes of the transient changes in APD and TF were different (Figs2,4).

In Fig5A, transitions for the APD_{90} and TF after abruptly prolonging the CL from 500 to 1,500 ms are compared between normal and remodeled atria. In this figure, ordi-

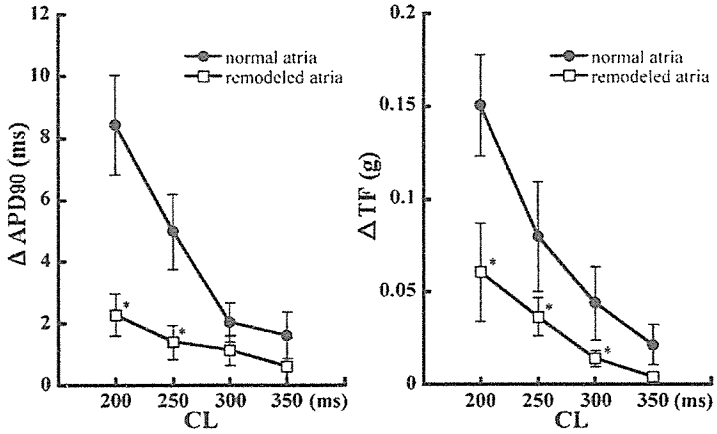

Fig 6. Comparison of the magnitudes of action potential duration was measured at 90% repolarization (APD_{90}) (A) and twitch force (TF) alternans (B) between normal and remodeled atria (n=6, respectively) at each cycle length (CL). The difference between long and short action potential duration (TF) after the 10th beat was calculated as the magnitude of alternans. *p<0.05 comparing normal and remodeled atria at each CL. The magnitude of both action potential duration and TF changes was significantly attenuated in remodeled atria. *p<0.05.

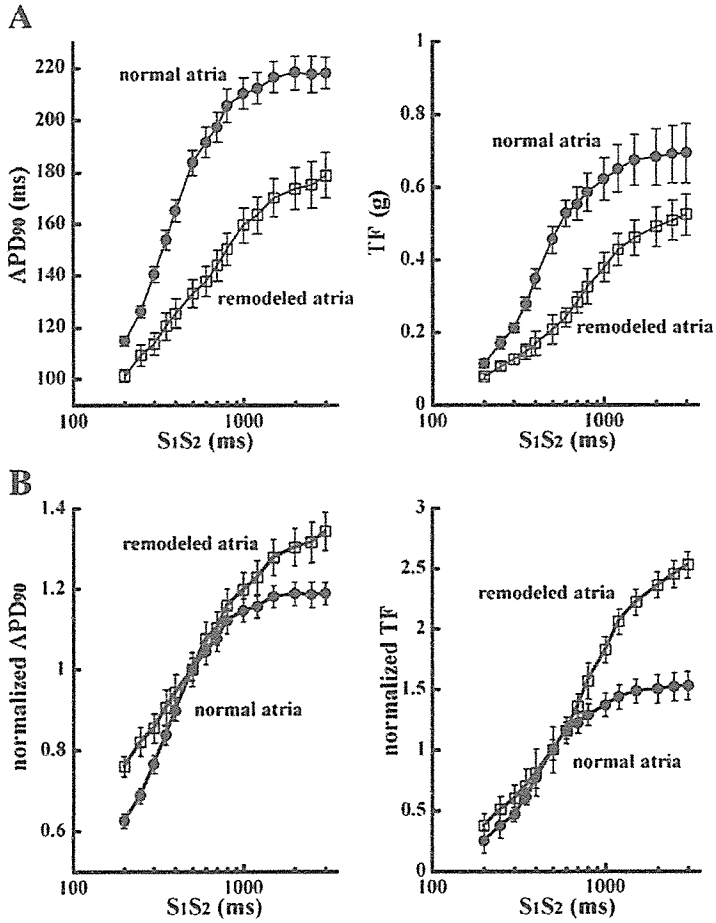

Fig 7. (A) Restitution curves for action potential duration was measured at 90% repolarization (APD_{90}) and twitch force (TF) at bipolar electrodes at a basic cycle length (BCL) of 500 ms in normal and remodeled canine atria (n=6, respectively). (B) Ordinates are normalized to the same values as those during a BCL of 500 ms. The slopes of action potential duration and TF restitution curves in remodeled atria were flatter when the S_1S_2 interval was shorter than the BCL, and steeper when the single test pulses (S_2) delivered after every 40th basic pulse (S_1) (S_1S_2) interval was longer than the BCL.

nates are normalized to the same values as those during a CL of 500 ms. Representative experiments are also shown. Although the steady-state APD_{90} and TF (BCL 500 ms) in remodeled atria were lower compared to normal atria, the transient increases in the APD_{90} and TF were significantly augmented. The percent increase in the first beat from the baseline values in normal and remodeled atria was 16.1±1.2 vs 25.4±4.6%, respectively, for APD_{90} (n=6, p<0.05); and 56.1±9.1 vs 174±23.8%, respectively, for TF (n=6, p<0.05). The percent reduction from the 1st to 8th beats during the longer CL in normal and remodeled atria was 5.0±0.7 vs 12.4±1.8%, respectively, for APD_{90}; and 11.8±4.9 vs 34.7±8.0%, respectively, for TF (n=6, p<0.05). These changes in the AP and TF dynamics in remodeled atria were similar to the effects of CPA in normal atria.

In contrast, the transient decreases in the APD_{90} and TF in response to a sudden shortening in the CL were significantly reduced in remodeled atria. When the CL was shortened from 500 to 350 ms, the percent decrease in the first beat from the baseline value in normal and remodeled atria was 15.7±0.6 vs 10.3±0.8%, respectively, for APD_{90} (n=6, p<0.05); and 34.2±3.5 vs 21.9±2.9, respectively, for TF

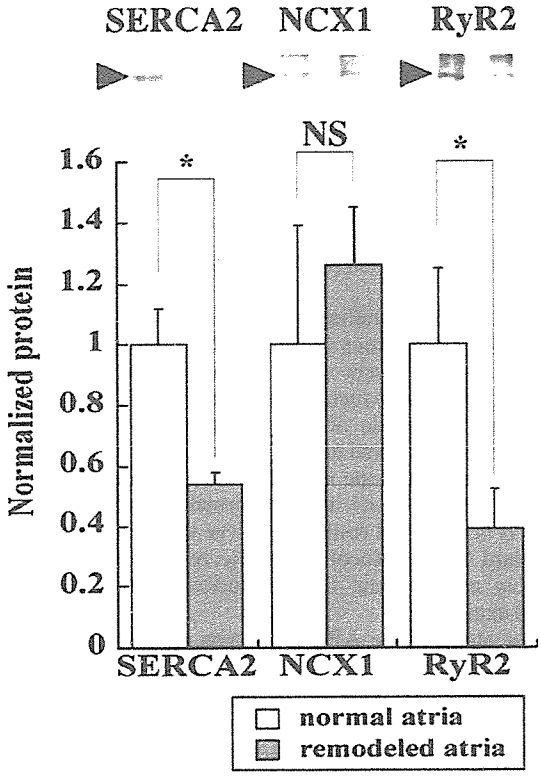

Fig 8. Histogram and representative Western blots reflecting the expression of SR Ca^{2+} ATPase (SERCA2; 110 kDa), SR Ca^{2+}-release channel (RyR2; 565 kDa) and sarcolemmal Na^+/Ca^{2+} exchanger (NCX1; 120 kDa) in normal (n=4) and remodeled atria (n=4). Remodeled atria had lower expressions of SERCA2 and RyR2 compared with normal atria (*$p<0.05$). No significant difference in NCX1 expression was observed. *$p<0.05$.

(n=6, $p<0.05$).

The magnitudes of APD and TF alternans were remarkably reduced in the remodeled atria (Figs 2,4). In Fig 6, the magnitudes of APD and TF alternans were compared between normal and remodeled atria. The differences between the long and short APD and TF after the 10th beat were calculated as the magnitudes of alternans.

Restitution Kinetics for APD and TF

The restitution kinetics for APD and TF would be altered, because APD and TF with the first beat after an abrupt shortening or prolongation of CL constitute restitution curves. Fig 7A shows the restitution curves for APD_{90} and TF in normal and remodeled canine atria. The restitution curve for APD was altered in parallel with that of TF in remodeled atria. In Fig 7B, ordinates are normalized to the same values as those during a BCL of 500 ms. The slopes of APD and TF restitution curves in remodeled atria were flatter when the S_1S_2 interval was shorter than the BCL, and steeper when the S_1S_2 interval was longer than the BCL. Such changes in the slopes in remodeled atria can be explained by the finding that the transient decrease in the APD and TF after a sudden CL shortening was reduced, and that the transient increase after a sudden CL prolongation was enhanced.

As a whole, the maximal slope of the APD restitution curve in the remodeled atria was significantly reduced compared with that in normal atria. The mean maximal slope of APD restitution curve calculated in each preparation in normal and remodeled atria was 0.29±0.02 vs 0.19±0.03, respectively (n=6, $p<0.05$).

Protein Expression Influencing the Intracellular Calcium Handling in Remodeled Atria

The expression of the SERCA2, NCX1, and RyR2 in remodeled atria were compared with those in normal atria (Fig 8). Both SERCA2 and RyR2 were significantly decreased in remodeled atria (n=4). There was no significant difference in the amount of NCX between the 2 groups.

Discussion

Most investigations of atrial electrophysiological remodeling have focused on steady state conditions at a constant pacing CL, and the underlying cellular mechanisms have been solely attributed to alterations in sarcolemmal ion channels. In the present study, we showed that dynamic AP characteristics are also altered in electrical remodeling because of altered intracellular Ca^{2+} handling. This is the first study to show a relationship between abnormal Ca^{2+} handling and altered dynamics of AP repolarization in electrical remodeling.

AP Dynamics After an Abrupt Change in CL

When the CL was abruptly prolonged in normal atria, the APD of the first beat at the new CL abruptly increased and then decreased in a step-wise fashion during subsequent beats. In contrast, when the CL was abruptly shortened, the APD of the first beat abruptly decreased and then increased during subsequent beats. Abrupt changes in APD with the first beat may be caused partly by the incomplete recovery of sarcolemmal ion channels, such as I_{Ks}, I_{to} and I_{CaL}. However, it is difficult to explain the mechanism responsible for the stepwise changes in the APD based on the recovery of ion channel function. In the present study, we found that the time-course of APD changes paralleled the time-course of TF changes, and that drugs that modulate intracellular Ca^{2+} handling (ryanodine, isoproterenol, CPA) had similar effects on APD and TF dynamics. These results suggest that APD dynamics are associated with Ca^{2+} handling. The underlying mechanisms responsible for the TF dynamics after an abrupt CL change are well understood[12] When the CL is abruptly increased, the initial TF is increased because a greater fraction of SR Ca^{2+} is released because of the longer pacing interval. During the longer diastolic interval, more Ca^{2+} is taken back up into the SR and becomes available for release[13] This larger SR Ca^{2+} release stimulates more Ca^{2+} extrusion via the Na^+/Ca^{2+} exchanger (NCX). Consequently, there is a progressive decline in the amount of SR Ca^{2+}, which is reflected by the step-wise decline in TF[12] Based on the above theory, we hypothesize that APD dynamics are caused by changes in NCX current that reflect changes in Ca^{2+} released from the SR. As NCX generates a net inward current during Ca^{2+} extrusion, APD dynamics can be explained by the NCX current. The APD of the first beat increases because of a large NCX current, which is caused by the release of a greater fraction of SR Ca^{2+}. The APD gradually decreases because the NCX current gradually decreases as a result of a progressive decline in the amount of SR Ca^{2+} released.

The hypothesis that APD dynamics reflect changes in the Ca^{2+} released from the SR explains many phenomena

observed in the present study. In Fig 2, the APD and TF increased transiently with the prolongation of CL, because more Ca^{2+} was taken back up into the SR during the longer diastolic interval and then released. Ryanodine abolished the transient increase in the APD and TF. Because ryanodine causes calcium leakage from the SR during the diastolic interval, the large Ca^{2+} release from the SR and the subsequent large NCX current after the abrupt CL prolongation can no longer occur. Isoproterenol, which stimulates the SR Ca^{2+} pump and increases the sarcolemmal Ca^{2+} current, attenuated the abrupt increase and step-wise decline in both APD and TF. As isoproterenol stimulates Ca^{2+} re-uptake into the SR, most of the Ca^{2+} released from the SR may have been taken back up, and made available for release during the diastolic interval at the baseline CL. Thus, suddenly prolonging the CL had less effect on Ca^{2+} uptake during diastole and the changes in APD and TF were blunted. Furthermore, because most of the Ca^{2+} released from the SR is recirculated into the SR, minimum Ca^{2+} may be extruded via NCX in the presence of isoproterenol[12] Thus, the amount of SR Ca^{2+} does not decrease progressively, which explains the loss of a step-wise decline in the APD and TF. CPA had effects opposite to those of isoproterenol, which can be explained by the partial inhibition of the SR Ca^{2+} pump. A complete blockade of the SR Ca^{2+} pump might deplete SR Ca^{2+} and transient increases of APD and TF might not occur. Thus, it is plausible that APD dynamics are caused by changes in NCX current that reflects changes in Ca^{2+} released from the SR.

Recently, Burashnikov and Antzelevitch showed that late phase 3 EAD-induced triggered activity occurred after a long diastolic interval following the termination of rapid atrial excitation in a cholinergically-mediated model of AF[9] Although we did not observe EADs in the present study, our study provides a theoretical basis for their results; that is, a phase 3 EAD would be mediated by the inward NCX current through accentuated SR release after a long preceding diastolic interval.

Altered AP Dynamics in Remodeled Atria

APD dynamics after an abrupt CL change were altered in remodeled atria. The transient increase in the APD after a sudden CL prolongation was enhanced, and the transient decrease after a sudden CL shortening was reduced, compared with normal atria. These changes in APD dynamics were parallel to the changes in the TF dynamics, suggesting that altered AP dynamics in remodeled atria are caused by altered intracellular Ca^{2+} handling.

Although remodeling of Ca^{2+} handling appears to be responsible for the altered AP dynamics, the precise mechanisms are not clear from the present study. Because we only measured TF, we could not determine which process of Ca^{2+} handling was altered. The reduced SR Ca^{2+}-pump activity might, in part, explain the change of APD dynamics, because the expression of SERCA2 was reduced and CPA administered in normal atria mimicked the APD and TF dynamics of remodeled atria. Increased activity of NCX could also explain the enhanced transient increase in the APD in remodeled atria, although the expression of NCX did not increase significantly in the present study. Upregulation of NCX has been shown in atrial myocardium of patients with AF[14] Further studies will need to be conducted in order to understand the precise mechanisms of remodeling of Ca^{2+} handling, including direct measurements of Ca^{2+} transients and analysis of the fractions of Ca^{2+} transported by the SR, NCX, and slow inward channel.

The enhanced transient increase of APD and TF after CL prolongation in remodeled atria may reflect enhanced Ca^{2+} extrusion from the cell. This enhanced Ca^{2+} extrusion as well as reduced I_{CaL} is a protective mechanism against Ca^{2+} overload. Thus, remodeling of Ca^{2+} handling can be regarded as an adaptive response to the Ca^{2+} overload caused by AF.

Restitution Kinetics and Alternans of APD in Remodeled Atria

Altered Ca^{2+} handling in electrical remodeling changed the restitution kinetics for APD. The maximal slope of the APD restitution curve was flatter in remodeled atria. This would lead to a reduced magnitude of APD alternans. In fact, the magnitude of APD alternans was significantly reduced in remodeled atria.

The maximal slope of the APD restitution curve in the present study was <1 in both normal and remodeled atria. This is because the restitution curve was determined with a standard S_1S_2 protocol. Dynamic restitution would more closely approximate the restitution relationship during rapid excitation[15]

The slope of the APD restitution curve and the magnitude of APD alternans have been shown to be important determinants of stability in several types of re-entrant circuits[6–8] In the ring model of atrial re-entry, Frame and Simpson showed that a steep slope favored large CL oscillations and termination of re-entry, whereas a flat slope decreased oscillations and stabilized re-entry[8] In contrast, a steep slope precipitated the breakup of single spiral waves into multiple smaller spirals[6,7] A flatter slope in the remodeled atria may stabilize single anatomical re-entry, but may decrease the number of functional multiple wavelets[4,16]

Clinical Implications

It is known that the recurrence of AF occurs early (several days) after pharmacological or electrical cardioversion, which is attributable to electrical remodeling. So far, the shortening of steady-state APD has been solely attributed to this phenomenon, but altered non-steady-state AP dynamics shown in the present study (altered APD restitution and APD transition) may also contribute to the early recurrence of AF.

As discussed above, the slope of the APD restitution curve can influence the stability of re-entrant arrhythmias. Although the precise mechanisms of AF have not been understood, a variety of mechanisms such as focal drivers, mother rotors with fibrillatory conduction, and multiple re-entrant circuits are thought to play a role in the initiation and maintenance of AF. The flatter slope in electrical remodeling may stabilize AF in patients where a stable rotor plays an important role in the maintenance of AF[4,16]

AF recurrence immediately after the termination of the arrhythmia (IRAF) is frequently observed. Burashnikov and Antzelevitch suggested that late phase 3 EAD-induced triggered activity might be a possible mechanism of IRAF[9] This EAD occurs after a long diastolic interval following the termination of rapid atrial excitation. As was discussed above, it might be mediated by the inward NCX current through accentuated SR Ca^{2+} release. In the present study, the transient increase in APD after a sudden CL prolongation was enhanced in remodeled atria. This suggests that triggered activity caused by EADs and IRAF might occur more easily in remodeled atria.

Conclusions

In the present study, we showed that: (1) not only steady-state AP but also non-steady-state AP dynamics is altered in electrical remodeling; and that (2) this altered AP dynamics is caused by altered Ca^{2+} handling rather than altered sarcolemmal ion channels. Because non-steady-state AP characteristics have important implications for the development of arrhythmias, the present study provides new insight into arrhythmogenesis in atrial electrical remodeling. Moreover, the present study predicts that intracellular Ca^{2+} handling might be a new therapeutic target for the treatment or prevention of AF.

References

1. Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA. Atrial fibrillation begets atrial fibrillation: A study in awake chronically instrumented goats. *Circulation* 1995; **92:** 1954–1968.
2. Yue L, Feng J, Gaspo R, Li G, Nattel S. Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ Res* 1997; **81:** 512–520.
3. Yue L, Melnyk P, Gaspo R, Wang Z, Nattel S. Molecular mechanisms underlying ionic remodeling in a dog-model of atrial fibrillation. *Circ Res* 1999; **84:** 776–784.
4. Hara M, Shvilkin A, Rosen MR, Danilo P Jr, Boyden PA. Steady-state and nonsteady-state action potentials in fibrillating canine atrium: Abnormal rate adaptation and its possible mechanisms. *Cardiovasc Res* 1999; **42:** 455–469.
5. Sun H, Chartier D, Leblanc N, Nattel S. Intracellular calcium changes and tachycardia-induced contractile dysfunction in canine atrial myocytes. *Cardiovasc Res* 2001; **49:** 751–761.
6. Karma A. Electrical alternans and spiral wave break up in cardiac tissue. *Chaos* 1994; **4:** 461–472.
7. Qu Z, Weiss JN, Garfinkel A. Cardiac electrical restitution properties and stability of reentrant spiral waves: A simulation study. *Am J Physiol* 1999; **276:** H269–H283.
8. Frame LH, Simson MB. Oscillations of conduction, action potential duration and refractoriness: A mechanism for spontaneous termination of reentrant tachycardias. *Circulation* 1998; **78:** 1387–1390.
9. Burashnikov A, Antzelevitch C. Reinduction of atrial fibrillation immediately after termination of the arrhythmia is mediated by late phase 3 early afterdepolarization-induced triggered activity. *Circulation* 2003; **107:** 2355–2360.
10. Hiromoto K, Shimizu H, Furukawa Y, Kanemori T, Mine T, Masuyama T, et al. Discordant repolarization alternans-induced atrial fibrillation is suppressed by verapamil. *Circ J* 2005; **69:** 1368–1373.
11. Liu W, Yasui K, Opthof T, Ishiki R, Lee JK, Kamiya K, et al. Development changes of Ca(2+) handling in mouse ventricular cells from early embryo to adulthood. *Life Sci* 2002; **71:** 1279–1292.
12. Bers DM. Control of cardiac contraction by SR and sarcolemmal Ca fluxes. *In*: Lederen WJ, editor. Excitation-Contraction Coupling and Cardiac Contractile Force, 2nd ed. Amsterdam, Netherlands: Kluwer Academic Publishers; 2001: 245–272.
13. Wohlfart B, Noble MIM. The cardiac excitation-contraction cycle. *Pharmacol Ther* 1982; **16:** 1–43.
14. Schotten U, Greiser M, Benke D, Buerkel K, Ehrenteidt B, Stellbrink C, et al. Atrial fibrillation-induced atrial contractile dysfunction: A tachycardiomyopathy of a different sort. *Cardiovasc Res* 2002; **53:** 192–201.
15. Koller ML, Riccio ML, Gilmour RF. Dynamic restitution of action potential duration during electrical alternans and ventricular fibrillation. *Am J Physiol* 1998; **275:** H1635–H1642.
16. Kim BS, Kim YH, Hwang GS, Pak HN, Lee SC, Shim WJ, et al. Action potential duration restitution kinetics in human atrial fibrillation. *J Am Coll Cardiol* 2002; **39:** 1329–1336.

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Letter to the Editor

Human cardiac ryanodine receptor mutations in ion channel disorders in Japan

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Abstract

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is characterized by adrenergic induced bidirectional or polymorphic ventricular tachycardias. Some of CPVT families were reported to be associated with cardiac ryanodine receptor gene (*RyR2*) mutations. However, association between RyR2 and other arrhythmogenic disorders is not clarified. In this study, we analyzed 83 Japanese patients including patients with long-QT syndrome, Brugada syndrome, idiopathic ventricular fibrillation, arrhythmogenic right ventricular caridiomyopathy and CPVT. Genetic screening of *RyR2* revealed 3 distinct mutations among 4 families with CPVT (75% of incidence). However, no mutation was found in other groups. This is the first report to demonstrate prevalence of RyR2 mutations in various arrhythmogenic disorders in Japan. RyR2 mutations were detected frequently in CPVT but not in other diseases.
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Keywords: Calcium channel; Mutation; Delayed after depolarization; Ventricular tachycardia; Sudden death

1. Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an autosomal dominant inherited disorder characterized by adrenergic induced polymorphic ventricular tachycardias (PVT) and associated with sudden cardiac death in childhood and adolescence. The cardiac ryanodine receptor gene (*RyR2*) has been shown to be linked to CPVT [1]. To date, more than 30 *RyR2* missense mutations have been reported in CPVT and arrhythmogenic right ventricular caridiomyopathy (ARVC) [1–3]. However, the prevalence of *RyR2* mutation in other arrhythmogenic disorders such as long-QT syndrome, Brugada syndrome is not known. The purpose of this study is to clarify the impact of *RyR2* mutations on various ion channel disorders in Japan.

2. Materials and methods

We performed genetic analysis of RyR2 against 83 Japanese patients. Those were 24 patients with long-QT syndrome (LQT), 48 patients with idiopathic ventricular fibrillation (IVF) including Brugada syndrome (BrS), 4 patients with CPVT and 7 patients with ARVC. Those patients were diagnosed according to typical clinical characteristics of the disease. The KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 were also screened but no mutation in such genes in these patients. Blood sample was obtained from each patient after a given informed consent of genetic analysis. We used PCR-SSCP method

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followed by DNA sequencing or direct sequencing. We also screened each identified mutation against more than 100 unrelated healthy controls to exclude the possibility of a polymorphism.

3. Result

We identified 3 distinct *RyR2* mutations among 4 genetically unrelated families with CPVT and 2 of those mutations were novel. (Fig. 1) However, no mutation was found in LQT, IVF and BrS patients.

3.1. Family 1

A 20-years-old male was referred to the hospital because of recurrent syncope occurring during physical or emotional stress. Syncope first developed at 10-years of age and recurrently over the last 10 years. PVT followed by ventricular fibrillation (VF) was documented during EP study. The patient had no family history of syncope or sudden death. Clinical evaluation including cardiac catheterization revealed no structural abnormality. Resting ECG was normal. Stress test performed on a treadmill induced premature ventricular contractions (PVC) in a pattern of bigeminy. Bi-directional ventricular tachycardia (BVT) was induced by isoproterenol infusion. The arrhythmia was suppressed with β-blocker using Propranolol. PCR-SSCP analysis of the proband revealed an abnormal band in exon 47 of *RyR2* and DNA sequencing confirmed a G to A transition at the position of 7202 leading to amino acid change of histidine for arginine 2401; R2401H [3].

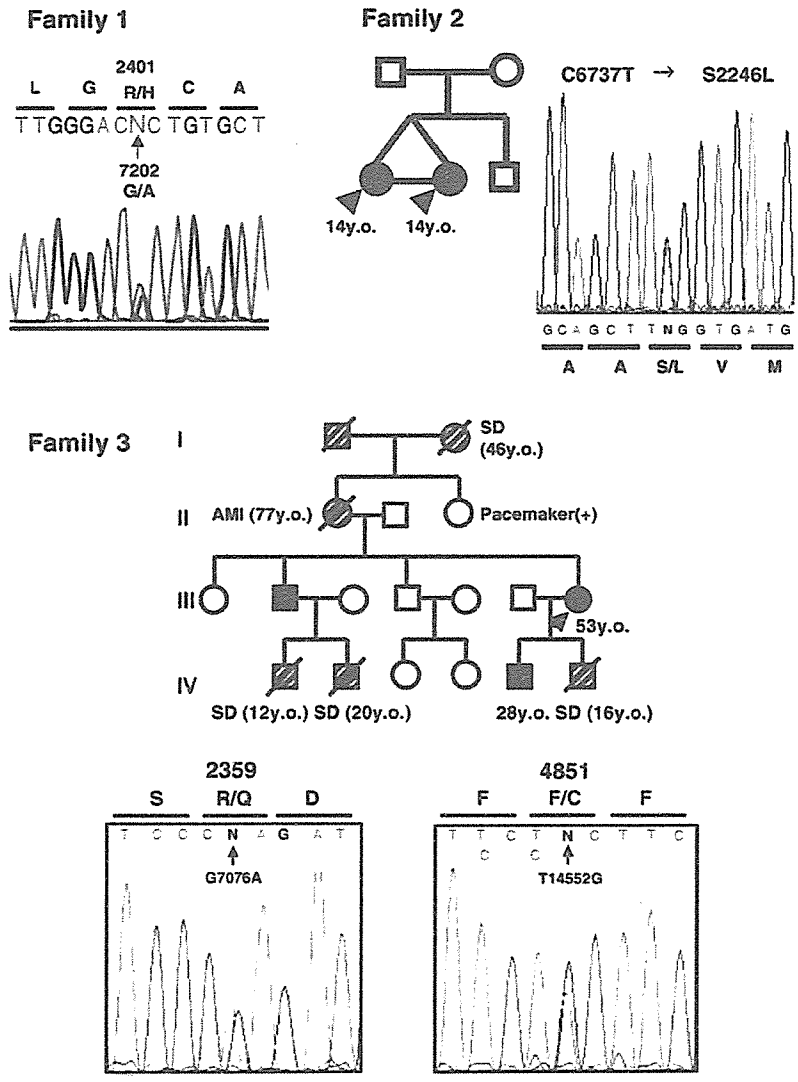

Fig. 1.