Ginsenoside Re Binds to AR, ERα, and PR. Receptor competitor assay was performed to test whether ginsenoside Re could bind to the LBD of human AR, ERα, and PR. DHT, E_2, and P_4 showed dose-dependent displacement of fluorescently tagged receptor ligands; IC_{50} was 2.8 nM for DHT, 33.8 nM for E_2, and 50.0 nM for P_4 (Fig. 7). Ginsenoside Re also showed dose-dependent displacement of fluorescently tagged receptor ligands; the IC_{50} values were 56.2 μM for the AR, 59.0 μM for the ERα, and 80.6 μM for the PR (Fig. 7). The binding of ginsenoside Re to the AR, ERα, and PR was not saturable up to the concentration of 1 mM, suggesting that ginsenoside Re is a partial agonist of the AR, ERα, and PR.

Ginsenoside Re Does Not Activate Genotropic Action of AR and ERα. Genotropic action of E_2 is generally assessed by its effects on proliferation of the estrogen-responsive human breast cancer cell line MCF-7 (Lippman et al., 1976) and that of DHT via proliferation of the testosterone-responsive human prostate cancer cell line LNCaP (Hasen-

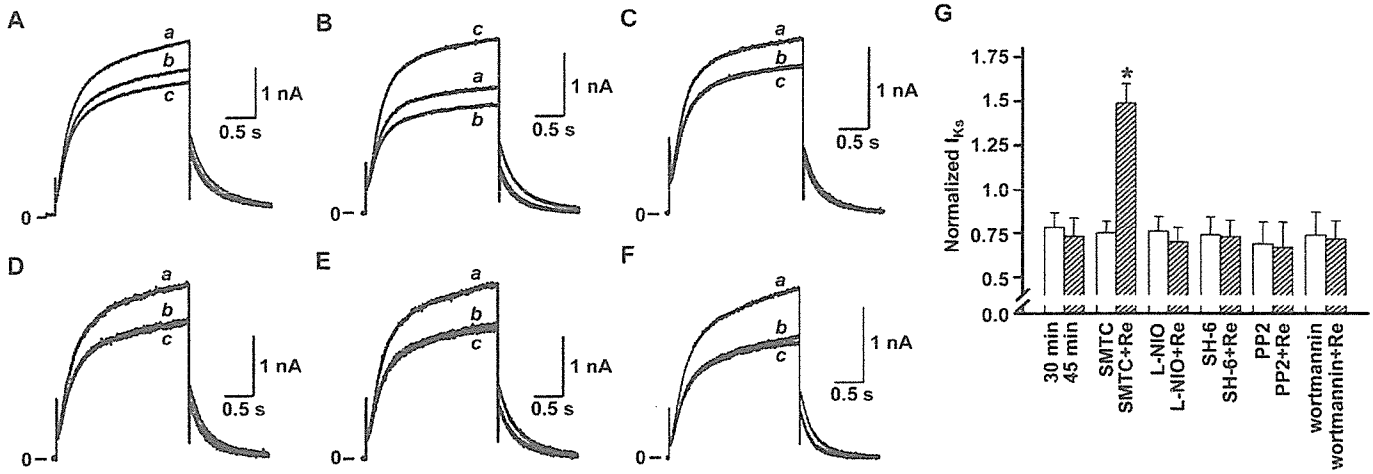

Fig. 4. Effects of preincubation with various blockers on ginsenoside Re (3 μM)-induced I_{Ks} enhancement. A, representative superimposed current traces in the time control experiment in the control state (trace a), at 30 min after start of experiment (b), and at 45 min after start of experiment (c). B–F, representative superimposed current traces in the control state (trace a), after incubation with various blockers (trace b), and after addition of ginsenoside Re (3 μM) in the continued presence of various blockers (trace c). B, SMTC (3 μM); C, L-NIO (1 μM); D, SH-6 (1 μM); E, PP2 (1 μM); and F, wortmannin (10 μM). G, averaged I_{Ks} after incubation with various blockers for 30 min, and 15 min after addition of ginsenoside Re in the continued presence of various blockers (45 min after start of experiment). I_{Ks} was normalized to the control value. *, $p < 0.05$ between before and after ginsenoside Re application.

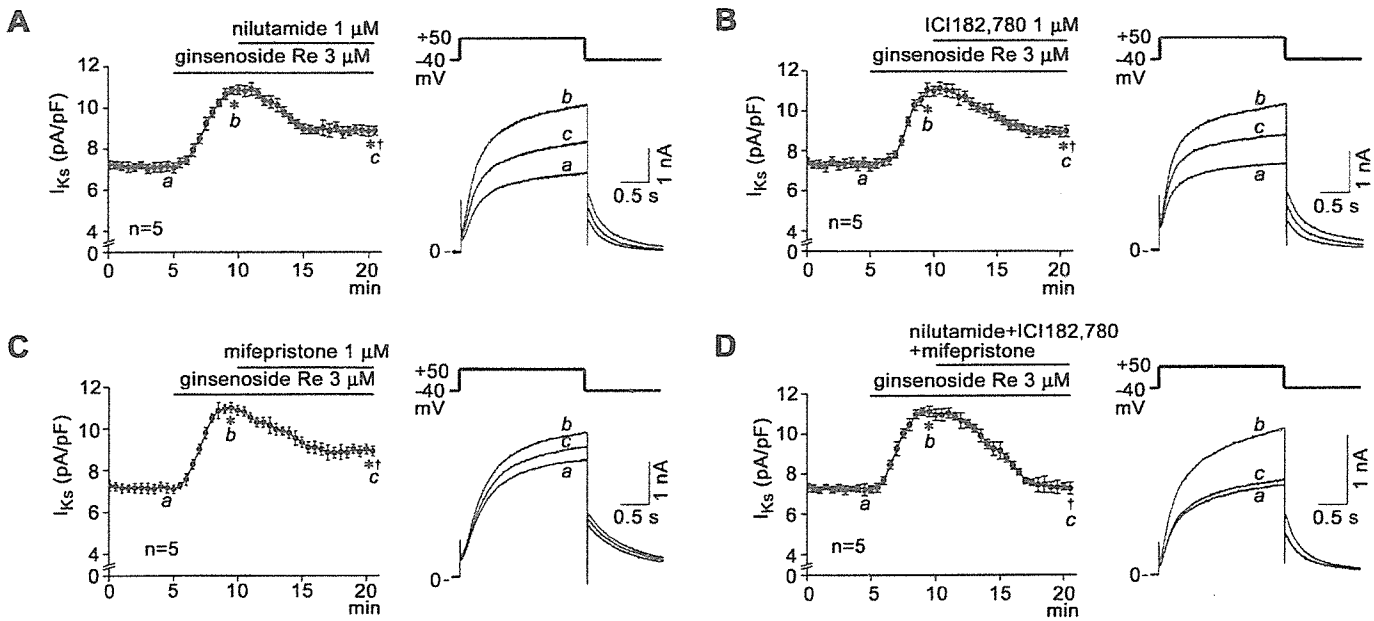

Fig. 5. Involvement of sex hormone receptors on I_{Ks} enhancement by ginsenosides. A–D, effects of nilutamide (A), an AR inhibitor, ICI182,780 (B), an ER inhibitor, mifepristone (C), a PR inhibitor, and a combination of nilutamide, ICI182,780, and mifepristone (D) on ginsenoside Re-induced I_{Ks} enhancement. Left, time course of experiments in 5 cells. *x*-Axis is time after start of experiments, and *y*-axis is averaged current density of I_{Ks}. I_{Ks} were continuously elicited by depolarizing pulses to +50 mV at 0.1 Hz. *, $p < 0.05$ versus control, †, $p < 0.05$ versus in the presence of ginsenoside Re. Right, representative superimposed current traces recorded at the timing indicated by italic lower-case alphabets.

son et al., 1985). We found that, unlike DHT or E_2, ginsenoside Re did not stimulate proliferation of LNCaP or MCF-7; rather, it partially inhibited DHP-induced LNCaP proliferation and E_2-induced MCF-7 proliferation (Fig. 8). Thus, ginsenoside Re is a partial antagonist, but not an agonist, of the genomic pathway of AR or ERα.

Ginsenoside Re Fails to Recruit CoActivator of AR, ERα, and PR. To further seek for the mechanism underlying lack of genomic action by ginsenoside Re, we examined whether ginsenoside Re triggered binding of a coactivator peptide containing a canonical LXXLL-motif (L = leucine, X = any amino acid) to the LBD of ERα, AR, and PR. We used a FRET indicator, *SCCoR*, in which agonist-induced recruitment of coactivator to the LBD of receptors was designed to induce FRET signals between enhanced CFP and enhanced YFP (Awais et al., 2004, 2006). We first confirmed that ginsenoside Re did not change FRET signals for AR-*SCCoR*, ERα-*SCCoR*, or PR-*SCCoR* (Fig. 9, A and D). Then, we found that ginsenoside Re significantly inhibited E_2-, DHT-, or P_4-induced FRET signals (Fig. 9, B–D), indicating that ginsenoside did not induce coactivator recruitment to AR, ERα, and PR, and rather inhibited agonist-induced coactivator recruitment.

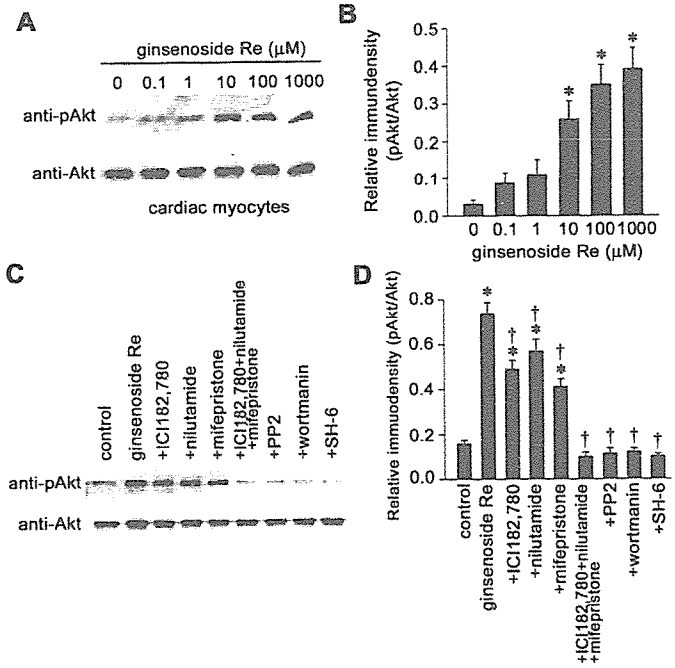

Fig. 6. Effects of ginsenoside Re on Akt phosphorylation. A, representative immunoblots showing dose-dependent effects of ginsenoside Re on Akt phosphorylation. B, densitometric analysis of dose-dependent phosphorylation of Akt by ginsenoside Re in three experiments. *, $p < 0.05$ versus without ginsenoside Re. C, representative immunoblotting showing effects of various blockers on Akt phosphorylation by ginsenoside Re. D, densitometric analysis of effects of various blockers on Akt phosphorylation by ginsenoside Re in three experiments. *, $p < 0.05$ versus in the control state; †, $p < 0.05$ versus in the presence of ginsenoside Re without any blockers.

Discussion

The present study provides convincing evidence to clarify a mechanism underlying the bioactivity of ginseng in cardiovascular system. We have previously reported that ginsenoside Re enhances I_{Ks} via a NO-dependent manner in isolated cardiac myocytes (Bai et al., 2003, 2004). In the present study, we found that ginsenoside Re releases NO via a nongenomic pathway of sex steroid receptors, resulting in I_{Ks} activation in cardiac myocytes. Ginsenoside Re does not activate the genomic pathway of sex steroid hormones, because it fails to recruit coactivators upon binding of ginsenoside to the LBD of sex hormone receptors. Thus, ginsenoside is a specific agonist for the nongenomic pathway of sex steroid receptors.

Our pharmacological experiments indicate that ginsenoside Re activates the nongenomic pathway of sex steroid receptors to activate eNOS and release NO. Because reliable methods to measure NO at nanomolar range have not been available until very recently (Sato et al., 2005), we did not directly assess NO release from cardiac myocytes induced by ginsenoside Re. However, our previous report that two different types of NO-trapper, carboxy-2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide and LNAC, abolished ginsenoside Re-induced I_{Ks} activation (Bai et al., 2004) supports the idea that I_{Ks} enhancement by ginsenoside Re is caused by NO. Ginsenoside Re-induced I_{Ks} activation was reversed by inhibitors of c-Src, PI3-kinase, Akt, and eNOS that are key signal molecules of the nongenomic pathway of sex steroid receptors (Figs. 2 and 3). In the preincubation with these inhibitors, ginsenoside Re did not activate I_{Ks} (Fig. 4). Akt phosphorylation by ginsenoside Re was suppressed by inhibitors of c-Src, PI3-kinase, and Akt (Fig. 6).

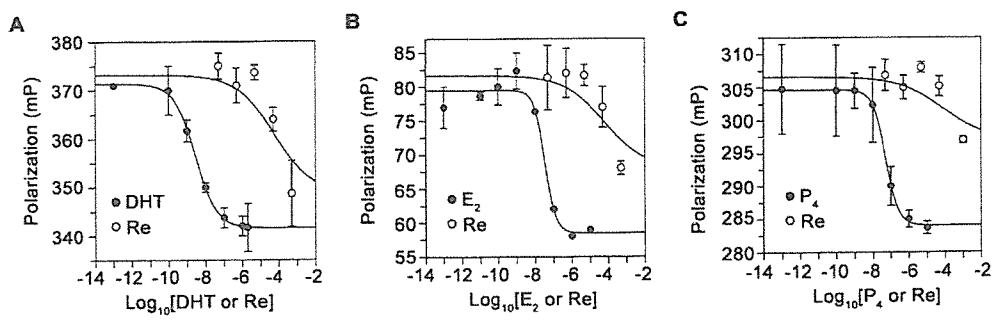

Fig. 7. Ginsenoside Re binds to the human AR, ERα, and PR. Fluorescently tagged receptor ligands bound to the LBD of the human AR were displaced by DHT and partially by ginsenoside Re (A); those to the LBD of the human ERα were displaced by E_2 and partially by ginsenoside Re (B); and those to the LBD of the human PR were displaced by P_4 and partially by ginsenoside Re (C). *x*-Axes are logarithm of concentration of DHT, E_2, P_4, and ginsenoside Re; *y*-axes are intensity of fluorescent polarization. Continuous lines are results of fitting of data to the Hill equation in the following formula using the least-squares method: $mP = mP_{0\%} - (mP_{0\%} - mP_{100\%})/[1 + (IC_{50}/[\text{ginsenoside Re}])^{n_H}]$, where mP is intensity of fluorescent polarization, $mP_{0\%}$ is mP without radioactive competitor, $mP_{100\%}$ is mP with the highest concentration of competitor (0.1 mM DHT, E_2, or P_4), and n_H is the Hill coefficient.

Finally, inhibitors of AR, ERα, and PR inhibited I_{Ks} enhancement and Akt phosphorylation by ginsenoside Re (Figs. 5 and 6).

In this study, each inhibitor of AR, ERα, and PR only partially suppressed ginsenoside Re-induced I_{Ks} enhancement, whereas the combination of all three inhibitors completely abolished ginsenoside Re actions. Competitive binding assays revealed that ginsenoside Re bound to AR, ERα, and PR. FRET experiments showed that ginsenosides competitively inhibited DHT-, E_2-, and P_4-induced coactivator recruitment further imply that ginsenoside Re somehow interacts with the LBD of AR, ERα, and PR. Taken together, we speculate that sex hormone receptors might be primary targets of ginsenoside Re. However, we would not completely eliminate the possibility that ginsenoside activates some common signaling molecules downstream of AR, ERα, and PR rather than binding to each of three receptors. Ginsenoside Re required relatively higher concentration to competitively displace fluorescently tagged receptor ligands compared with the concentration to enhance I_{Ks}. Although it is possible that the concentration to interact with the LBD is different between receptors present in the cytosol (receptor binding assays) and those localized in the plasma membrane (electrophysiological experiments), ginsenoside could also act primarily on molecules other than sex hormone receptors. Because a phytosterol genistein is a well established nonspecific inhibitor of tyrosine kinases (Akiyama et al., 1987), c-Src, a tyrosine kinase that is a common downstream signal of the AR, ERα, and PR, may be a potential candidate for target of ginsenoside Re. Therefore, these points are not settled yet, and further experiments are certainly needed

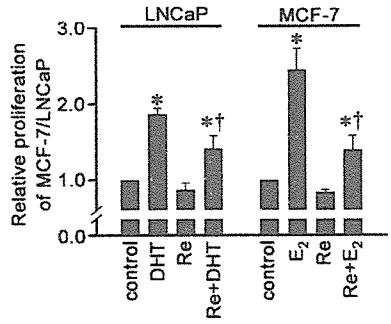

Fig. 8. Effects of ginsenosides on proliferation of MCF-7 and LNCaP. Relative proliferation of cells was calculated as (cell counts in the presence of drugs)/(cell counts in the control state). *, $p < 0.05$ versus control; †, $p < 0.05$ versus in the presence of DHT alone or E_2 alone.

Although our data indicate that ginsenoside Re does not activate the genomic pathway of sex hormone receptors, reported effects of ginsenoside on MCF-7 breast cancer cell growth are controversial. Ginsenoside Re induces expression of genes with estrogen-responsive element and proliferation of MCF-7 (Lee et al., 2003), whereas American ginseng inhibits MCF-7 breast cancer cell growth (Duda et al., 1999). Our data are consistent with the latter; ginsenoside Re does not enhance proliferation of MCF-7 cells or LNCaP cells. Experiments with FRET probes, *SCCoRs*, further provide supporting evidence that ginsenoside Re fails to activate the genomic pathway; ginsenoside Re does not induce coactivator recruitment upon binding to the LBD and inhibits coactivator recruitment induced by E_2, DHT, or P_4. A structural basis analysis seems to provide further supporting evidence. A structural basis of ERα/coactivator recognition is well documented from the analysis of the crystal structure of ERα-LBD bound to both an agonist diethylstilbestrol and a coactivator GRIP1 (Brzozowski et al., 1997; Shiau et al., 1998). The LBD pocket bound by an agonist is covered by helix 12 of

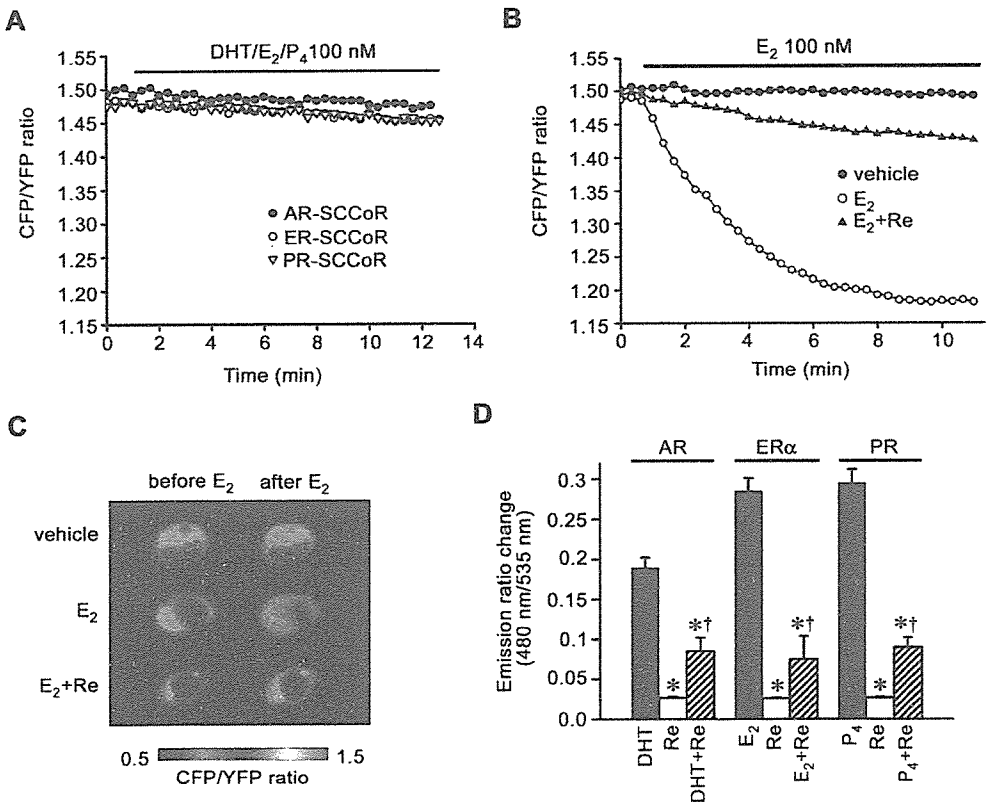

Fig. 9. Effects of ginsenoside on recruitment of coactivator examined by FRET experiments. A, representative time course of FRET responses of AR-*SCCoR*, ER-*SCCoR*, and PR-*SCCoR* upon addition of ginsenoside Re (10 μM). B, representative time course of FRET responses of ER-*SCCoR* upon E_2 (100 nM) in addition in the presence and absence of ginsenoside Re (10 μ). C, representative pseudocolor images of CFP/YFP emission ratios before (left) and after E_2 (100 nM) addition (right). D, averaged emission ratio change. *, $p < 0.05$ versus ligand alone.

LBD, creating a hydrophobic groove on the surface of LBD, where a coactivator can bind (Brzozowski et al., 1997; Shiau et al., 1998). The LBD pocket bound by a selective antagonist 4-hydroxytamoxifen disturbed motion of helix 12 and creation of a coactivator recognition groove, because of the presence of a bulky side chain in 4-hydroxytamoxifen. Because, like 4-hydroxytamoxifen, every ginsenoside has a bulky side chain (Fig. 1A), ginsenoside is unlikely to promote coactivator binding.

Hormone replacement therapy has been used for rapidly developing cardiovascular events, osteoporosis, disturbed cognition, and other symptoms in postmenopausal women; however, there are accompanying serious adverse events including high risk of estrogen-sensitive cancers (breast cancer, ovarian cancer, and certain types of lung cancer) (Barrett-Connor et al., 2005). Likewise, testosterone replacement therapy has recently been used for various symptoms in male menopause (andropause) with a risk of testosterone-sensitive prostate cancer (Hijazi and Cunningham, 2005). 4-Estren-3α,17β-diol (estren) is a synthetic compound that selectively induces nongenomic actions of estrogens and androgens without classic transcriptional activity (Kousteni et al., 2002). Conversely, 1,2,5-tris(4-hydroxyphenyl)-4-propylpyrazole (pyrazole) has potent transcriptional activity with minimal effects on nongenomic-induced events: estren, but not pyrazole, reversed bone loss in mice (Kousteni et al., 2002). From these findings, they propose that mechanism-specific ligands of steroid nuclear receptors represent a novel class of pharmacotherapeutics (Kousteni et al., 2002). Our data imply that ginsenoside is a naturally harvested, mechanism-specific agonist of sex steroid receptors. In the Eastern world, *P. ginseng* has been successfully prescribed for health problems associated with the post- and perimenopausal periods, which includes not only cardiac events, but also hot flashes, loss of bone matrix, and cognition disturbance (Punnonen and Lukola, 1984; Kropotov et al., 2002; Hartley et al., 2004; Low Dog, 2005). In the present study, we used ginsenoside Re at a concentration of 3 μ, because this is the concentration prescribed to patients in China (Bai et al., 2003). It does not necessarily reflect the plasma concentration in humans. Nevertheless, we expect that effects of ginsenoside described in the present article may provide a potential of ginsenoside as a medicinal seed for treatment of cardiac events, and potentially other symptoms, in postmenopausal women and postandropausal men.

Acknowledgments

We thank Dr. H. Kagechika (Tokyo Medical and Dental University), Dr. T. Hirano (Tokyo Medical and Dental University), Dr. Y. Ebizuka (The University of Tokyo), and Dr. M. Shibuya (The University of Tokyo) for helpful discussion and Dr. A. L. Bassett (University of Miami School of Medicine) and Dr. R. S. Kass (Columbia University, New York, NY) for reading the manuscript and correcting English. We also thank Dr. K. Akiyoshi (Tokyo Medical and Dental University) for helping conduct the competitive receptor-binding assay.

References

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, and Fukami Y (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* **262:**5592–5595.

Attele AS, Wu JA, and Yuan C-S (1999) Ginseng pharmacology: multiple constituents and multiple actions. *Biochem Pharmacol* **58:**1685–1693.

Awais M, Sato M, Sasaki K, and Umezawa Y (2004) A genetically encoded fluorescent indicator capable of discriminating estrogen agonists from antagonists in living cells. *Anal Chem* **76:**2181–2186.

Awais M, Sato M, Lee X, and Umezawa Y (2006) A fluorescent indicator to visualize activities of the androgen receptor ligands in single living cells. *Angew Chem Int* **45:**2707–2712.

Bai C-X (1993) Further research on the protective effects of ginsenoside Re on myocardial ischemia/reperfusion injury in rats. Master degree's thesis.

Bai C-X, Kurokawa J, Tamagawa M, Nakaya H, and Furukawa T (2005a) Nontranscriptional regulation of cardiac repolarization currents by testosterone. *Circulation* **112:**1701–1710.

Bai C-X, Namekata I, Kurokawa J, Tanaka H, Shigenobu K, and Furukawa T (2005b) Role of nitric oxide in Ca^{2+} sensitivity of the slowly activating delayed rectifier K^+ current in cardiac myocytes. *Circ Res* **96:**64–72.

Bai C-X, Sunami A, Namiki T, Sawanobori T, and Furukawa T (2003) Electrophysiological effects of ginseng and ginsenoside Re in guinea pig ventricular myocytes. *Eur J Pharmacol* **476:**35–44.

Bai C-X, Takahashi K, Masumiya H, Sawanobori T, and Furukawa T (2004) Nitric oxide-dependent modulation of the delayed rectifier K^+ current and the L-type Ca^{2+} current by ginsenoside Re, an ingredient of Panax ginseng, in guinea-pig cardiomyocytes. *Br J Pharmacol* **142:**567–575.

Baron S, Manin M, Beaudoin C, Leotoing L, Communal Y, Veyssiere G, and Morel L (2004) Androgen receptor mediates non-genomic activation of phosphatidylinositol 3-OH kinase in androgen-sensitive epithelial cells. *J Biol Chem* **279:**14579–14586.

Barrett-Connor E, Grady D, and Stefanick ML (2005) The rise and fall of menopausal hormone therapy. *Annu Rev Public Health* **26:**115–140.

Boyer M, Poujol N, Margeat E, and Royer CA (2000) Quantitative characterization of the interaction between purified human estrogen receptor alpha and DNA using fluorescence anisotropy. *Nucleic Acids Res* **28:**2494–2502.

Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, and Carlquist M (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature (Lond)* **389:**753–758.

Duda RB, Zhong Y, Navas V, Li MZ, Toy BR, and Alavarez JG (1999) American ginseng and breast cancer therapeutic agents synergistically inhibit MCF-7 breast cancer cell growth. *J Surg Oncol* **72:**230–239.

Gillis CN (1997) Panax ginseng pharmacology: a nitric oxide link? *Biochem Pharmacol* **54:**1–8.

Goldman P (2001) Herbal medicines today and the roots of modern pharmacology. *Ann Intern Med* **135:**594–600.

Goligorsky MS, Li H, Brodsky S, and Chen J (2002) Relationships between caveolae and eNOS: everything in proximity and the proximity of everything. *Am J Physiol* **283:**F1–F10.

Hartley DE, Elsabagh S, and File SE (2004) Gincosan (a combination of Ginkgo biloba and Panax ginseng): the effects on mood and cognition of 6 and 12 weeks' treatment in post-menopausal women. *Nutr Neurosci* **7:**325–333.

Hasenson M, Hartley-Asp B, Kihlfors C, Lundin A, Gustafsson JA, and Pousette A (1985) Effect of hormones on growth and ATP content of a human prostatic carcinoma cell line, LNCaP-r. *Prostate* **7:**183–194.

Hijazi RA and Cunningham GR (2005) Andropause: is androgen replacement therapy indicated for the aging male? *Annu Rev Med* **56:**117–137.

Kim OS, Choi JH, Soung YH, Lee SH, Lee JH, Ha JM, Ha BJ, Heo MS, and Lee SH (2004) Establishment of in vitro test system for the evaluation of the estrogenic activities of natural products. *Arch Pharm Res (NY)* **27:**906–911.

Kaku T, Miyata T, Uruno T, Sako I, and Kinoshita A (1975) Chemico-pharmacological studies on saponins of *Panax ginseng* C.A. Meyer. II. Pharmacological part. *Arzneimittelforschung* **25:**539–547.

Kohn AD, Takeuchi F, and Roth RA (1996) Akt, a pleckstrin homology domain containing kinase, is activated primarily by phosphorylation. *J Biol Chem* **271:**21920–21926.

Kone BC (2000) Protein-protein interactions controlling nitric oxide synthases. *Acta Physiol Scand* **168:**27–31.

Kousteni S, Chen JR, Bellido T, Han L, Ali AA, O'Brien CA, Plotkin L, Fu O, Mancino AT, Wen Y, et al. (2002) Reversal of bone loss in mice by nongenotropic signaling of sex steroids. *Science (Wash DC)* **298:**843–846.

Kropotov AV, Kolodnyak OL, and Koldaev VM (2002) Effects of Siberian ginseng extract and ipriflavone on the development of glucocorticoid-induced osteoporosis. *Bull Exp Biol Med* **133:**252–254.

Lee Y, Jin Y, Lim W, Ji S, Choi S, Jang S, and Lee S (2003) A ginsenoside-Rh1, a component of ginseng saponin, activates estrogen receptor in human breast carcinoma MCF-7 cells. *J Steroid Biochem Mol Biol* **84:**463–468.

Lippman M, Bolan G, and Huff K (1976) The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res* **36:**4595–4601.

Low Dog T (2005) Menopause: a review of botanical dietary supplements. *Am J Med* **118:**98–108.

McCall TB, Feelisch M, Palmer RM, and Moncada S (1991) Identification of *N*-iminoethyl-L-ornithine as an irreversible inhibitor of nitric oxide synthase in phargocytic cells. *Br J Pharmacol* **102:**234–238.

Narayanan K and Griffith OW (1994) Synthesis of L-thiocitrulline, L-homothiocitrulline, and *S*-methyl-L-thiocitrulline: a new class of potent nitric oxide synthase inhibitors. *J Med Chem* **37:**885–887.

Porter AC and Vaillancourt RR (1998) Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. *Oncogene* **17:**1343–1352.

Punnonen R and Lukola A (1984) The effect of ginseng on serum total cholesterol, HDL-cholesterol and triglyceride levels in postmenopausal women. *Asia Oceania J Obstet Gynaecol* **10:**399–401.

Sanguinetti MC and Jurkiewicz NK (1992) Role of external Ca^{2+} and K^+ in gating of cardiac delayed rectifier K^+ currents. *Pflueg Arch Eur J Physiol* **420:**180–186.

Sato M, Hida N, and Umezawa Y (2005) Imaging the nanomolar range of nitric oxide with an amplifier-coupled fluorescent indicator in living cells. *Proc Natl Acad Sci USA* **102:**14515–14520.

Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, and Greene GL (1998) The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95:**927–937.

Weiss DJ and Gurpide E (1998) Non-genomic effects of estrogens and antiestrogens. *J Steroid Biochem* **31:**671–676.

Winslow LC and Kroll DJ (1998) Herbs as medicines. *Arch Intern Med* **158:**2192–2199.

Wymann MP and Pirola L (1998) Structure and function of phosphoinositide 3-kinases. *Biochim Biophys Acta* **1436:**127–150.

Zheng Y-J, Furukawa T, Ogura T, Tajimi K, and Inagaki N (2002) M phase-specific expression and phosphorylation-dependent ubiquitination of the ClC-2 channel. *J Biol Chem* **277:**32268–32273.

Address correspondence to: Dr. Tetsushi Furukawa, 2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101-0062, Japan. E-mail: t_furukawa.bip@mri.tmd.ac.jp

Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular and Cellular Cardiology 41 (2006) 753–761
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Review article

Potassium channel remodeling in cardiac hypertrophy

Tetsushi Furukawa *, Junko Kurokawa

Department of Bio-informational Pharmacology, Medical Research Institute, Tokyo Medical and Dental University, Japan

Received 19 July 2006; received in revised form 28 July 2006; accepted 31 July 2006
Available online 7 September 2006

Abstract

Cardiac hypertrophy is an adaptive process against increased work loads; however, hypertrophy also presents substrates for lethal ventricular arrhythmias, resulting in sudden arrhythmic deaths that account for about one third of deaths in cardiac hypertrophy. To maintain physiological cardiac function in the face of increased work loads, hypertrophied cardiomyocytes undergo K^+ channel remodeling that provides a prolongation in action potential duration and an increase in Ca^{2+} entry. Increased Ca^{2+} entry, in turn, activates signaling mechanisms including a calcineruin/NFAT pathway to permit remodeling of the K^+ channels. This results in a positive feedback loop between the K^+ channel remodeling and altered Ca^{2+} handling; this loop may represent a potential therapeutic target against sudden arrhythmic deaths in cardiac hypertrophy. The purposes of this review are to: (1) discuss types of K^+ channels and their mRNA that undergo remodeling in cardiac hypertrophy; (2) report on recent research on molecular mechanisms of K^+ channel remodeling; and (3) address physiological events underlying new therapeutic modalities to ameliorate arrhythmias and sudden death in cardiac hypertrophy.

Keywords: Ion channel; Arrhythmia; Calcineurin; NFAT3; Myocardial infarction

Contents

1. Introduction . . . 754
2. K^+ channel remodeling in cardiac hypertrophy . . . 754
   2.1. Large mammals . . . 754
   2.2. Small mammals (Table 1) . . . 754
   2.3. Implications of difference between large and small mammals . . . 755
3. Myocardial infarction (MI) . . . 755
4. Change in ion channel mRNA . . . 755
   4.1. Kv4.2, Kv4.3, and Kv1.4 . . . 755
   4.2. KChIP2 (Kv channel interacting protein 2) . . . 755
   4.3. Other K^+ channel mRNAs . . . 755
5. Molecular mechanism of electrical remodeling . . . 756
6. Regional heterogeneity of electrical remodeling . . . 756
   6.1. Transmural heterogeneity . . . 756
   6.2. Interregional heterogeneity . . . 756
7. Physiological and clinical significance . . . 757
   7.1. Is reduced I_{to-f} a cause of cardiac hypertrophy or epiphenomenon? . . . 757
   7.2. Clinical implications . . . 757
Acknowledgments . . . 757
References . . . 757

* Corresponding author.
E-mail address: t_furukawa.bip@mri.tmd.ac.jp (T. Furukawa).

0022-2828/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2006.07.021

1. Introduction

Left ventricular hypertrophy (LVH) is a common electrocardiographic finding, occurring in 3–4% of normal population [1]. In the Framingham Study, there were 197 sudden deaths over 26 years in a follow-up of 5209 subjects [2,3]. The multivariate sudden death prediction analysis revealed that ventricular premature beat which occurs concurrently with LVH is an independent risk factor [1]. Fatal ventricular arrhythmias caused by premature beats upon cardiac hypertrophy are most likely associated with an alternation in cellular electrophysiology and cardiac remodeling. Specifically, accumulated experimental data suggest that the alteration in activity of K^+ channels associated with cardiac hypertrophy is a major cause of electrophysiological remodeling and arrhythmogeneity [4–7].

Subsequent studies employing molecular analyses indicate that alterations in Ca^{2+}-dependent signaling pathways underlie the K^+ channel remodeling. The resultant positive feedback loop between K^+ channel remodeling and altered Ca^{2+} handling may provide a new therapeutic target against lethal arrhythmias and sudden death in cardiac hypertrophy.

2. K^+ channel remodeling in cardiac hypertrophy

Experimental cardiac hypertrophy has been induced in several species of animals with varieties of techniques. In these models, the most consistent electrical change which has been described in association with the chronic stage of cardiac hypertrophy is prolongation of action potential duration (APD) [8–10]. Although the APD prolongation upon hypertrophy is attributable to alterations in K^+ channels, the target type of K^+ channel appears to differ between large (feline and canine) and small mammals (rat, mouse, and ferret) as we will describe.

2.1. Large mammals

In feline right ventricular hypertrophy (RVH) induced by constriction of pulmonary artery, the amplitude of inward rectifier K^+ current (I_{K1}) is increased and that of the delayed rectifier K^+ current (I_K) is decreased [11,12]. In feline LVH induced by constriction of abdominal aorta, I_K amplitude is decreased as is the case for pulmonary artery, while I_{K1} amplitude is not altered [13,14]. Although in these studies transient outward current (I_{to}) was not analyzed [11–14], in the later study using feline RVH model, I_{to} was found to be enhanced [15]. In a canine model of biventricular hypertrophy induced by atrioventricular block (AVB), the current density of I_{Ks} is diminished in both left ventricular (LV) (~50%) and right ventricular (RV) cells (~55%) [16,17]. The current density of I_{Kr} is diminished in the RV cells, but not in LV cells [16,17] (Table 1).

2.2. Small mammals (Table 1)

In rats, mice, and ferrets, experimental LVH and/or RVH have been developed with various techniques shown in Table 1 [18–34]. In a majority of the models, I_{to} is reduced. Ito consists of two components: (1) I_{to} in which recovery from inactivation is rapid ($I_{to\text{-}f}$ or I_{to1}) and (2) that in which recovery from inactivation is slow ($I_{to\text{-}s}$ or I_{to2}) [35]. $I_{to\text{-}f}$, rather than $I_{to\text{-}s}$, is the target of remodeling in cardiac hypertrophy, with no changes in the voltage dependence of steady-state activation and

Table 1
Species and techniques used for induction of cardiac hypertrophy

Species	Methods	Current changes	Reference
Feline	Pulmonary artery constriction	$I_K\downarrow$, $I_{K1}\uparrow$	Kleiman et al. [11,12]
	Constriction of abdominal aorta	$I_K\downarrow$, $I_{K1}\rightarrow$	Furukawa et al. [13,14]
Canine	Atrioventricular block	$I_{Ks}\downarrow$, I_{Kr} in RV$\downarrow$	Volders et al. [16], Ramakers et al. [17]
Rats	Constriction of ascending aorta	$I_{to\text{-}f}\downarrow$, $I_K\rightarrow$, $I_{K1}\rightarrow$	Volk et al. [18]
	Constriction of transverse aorta	$I_{to}\downarrow$	Gomez et al. [19]
	Constriction of abdominal aorta	$I_{to}\downarrow$	Tomita et al. [20]
	Uninephrectomized deoxycorticosterone	$I_{to}\downarrow$	Coulombe et al. [21]
	acetate (DOCA) salt-drinking	$I_{to\text{-}f}\downarrow$, $I_{sus}\rightarrow$	Momtaz et al. [22]
	Spontaneous hypertensive rat (SHR)	$I_{to}\downarrow$, $I_{K1}\rightarrow$	Cerbai et al. [23]
	Daily injection of isoproterenol	$I_{to}\downarrow$	Meszaros et al. [24]
	Chronic high-altitude exposure	$I_{to\text{-}f}\downarrow$, $I_K\rightarrow$, $I_{K1}\rightarrow$	Chouabe et al. [25]
	Monocrotaline (MCT)-induced RVH	$I_{to}\downarrow$	Lee et al. [26]
	Growth hormone (GH)-secreting tumor	$I_{to}\downarrow$	Xu et al. [27]
Ferrets	Pulmonary artery constriction	$I_{to}\downarrow$	Ponteau et al. [28]
Mice	Pregnancy	$I_{to}\downarrow$	Eghbali et al. [29]
	Calsequestrin (CSQ) overexpression	$I_{to}\downarrow$, $I_{K1}\downarrow$	Knollmann et al. [30]
	$G\alpha_q$ overexpression	$I_{to}\downarrow$, $I_{K1}\downarrow$	Mitarai et al. [31]
	Nerve growth factor (NGF) overexpression	$I_{to}\downarrow$, $I_{Kur}\downarrow$	Heath et al. [32]
	L-type Ca^{2+} channel overexpression	$I_{to}\downarrow$	Bodi et al. [33]
	Tumor necrosis factor-α overexpression	$I_{to}\downarrow$, $I_{Kslow}\downarrow$	Petkova-Kirova et al. [34]
Rabbit	Atrioventricular block	$I_{Ks}\downarrow$, $I_{Kr}\downarrow$, $I_{to\text{-}f}\rightarrow$, $I_{K1}\uparrow$	Tsuji et al. [36]
	Perinephritis-induced hypertension	$I_{to}\downarrow$, $I_{K1}\downarrow$	McIntosh et al. [37]
	Aortic banding	$I_{to}\downarrow$, $I_{K1}\downarrow$, $I_K\rightarrow$	Gillis et al. [38]

inactivation, suggesting that the main effect of hypertrophy is a decrease in the copy number of I_{to-f} channel proteins. However, in the ferret RVH model, I_{to} exhibits delayed recovery from inactivation, implicating some changes in I_{to-f} constructing molecules, discussed in the section "Change in ion channel mRNA" [28].

2.3. Implications of difference between large and small mammals

In hearts of large animals, I_{to} is responsible for the initial rapid phase of action potential repolarization, discernible as a notch preceding the plateau phase; I_K, rather than I_{to}, plays a major role in terminal repolarization. I_{to} in rodents, however, is a major repolarizing current throughout the comparatively short cardiac action potential necessary to maintain extremely high heart rates. These findings reflect differential features of hypertrophy-induced electrical remodeling. Rabbits appear to be intermediate; in biventricular hypertrophy induced by AVB, the amplitudes of I_{Kr} and I_{Ks} are smaller than control [36,37], which is in line with the finding that APD changes induced by aortic stenosis in rabbits are corrected by an I_{Ks} specific blocker, dofetilide [38]. In rabbit LVH caused by perinephritis-induced hypertension, however, I_{to}, I_{K1}, and I_{sus} rather than I_{Kr} or I_{Ks} are diminished [39]. Although data on K^+ channel remodeling in human cardiac hypertrophy are lacking, that in failing hearts is reported on; I_{to} is down-regulated in LV and RV cells of failing hearts [40], and I_{Ks} is also down-regulated in RV cells of heart failure [41].

3. Myocardial infarction (MI)

After acute MI, infarcted zones (IZ) [42–44] and non-infarcted zones (NZ) [45–51] exhibit distinct electrical remodeling. The NZ after MI undergoes significant hypertrophy as a compensatory process in response to diminished mass of working muscle [52], which may provide an arrhythmogenic substrate at a site remote from the previous MI [53,54]. In the NZ of the rat MI model, I_{to-f} and I_K significantly decrease from 3 days to 16 weeks post-infarction without changes in I_{K1} or $I_{Ca,L}$ [45–50]. In experimental healed MI in dogs, hypertrophied cardiomyocytes in remote NZ have prolonged APD and reduced I_K [51]. Thus, the target K^+ channels in electrical remodeling in post-MI cardiac hypertrophy are similar to those of cardiac hypertrophy induced by other stimuli; APD is prolonged by I_{to-f} reduction in rat, and by I_K reduction in dog.

4. Change in ion channel mRNA

To determine which K^+ channel mRNAs are altered by cardiac hypertrophy, rodent hypertrophy models have been used due to their convenience for genetic engineering. In the rodent models, alterations in mRNA encoding the α-subunit of I_{to} (Kv4.2, Kv4.3, and Kv1.4) and its β-subunit (KChIP2) are consistent with the electrophysiologic findings indicated above.

4.1. Kv4.2, Kv4.3, and Kv1.4

Kv4.2 and Kv4.3 encode the pore-forming α-subunit of I_{to-f}, and Kv1.4 encodes the α-subunit of I_{to-s}. In mice, a heterotetramer of Kv4.2 and Kv4.3 α-subunits underlie I_{to} [55]. Kv4.2 and Kv4.3 mRNA are consistently down-regulated in each hypertrophy model, while effects on Kv1.4 mRNA are controversial. In LVH rats with abdominal aortic constriction [56] and renovasular hypertension [57] and in rats with RVH induced by monocrotaline treatment [26], Kv4.2 and Kv4.3 mRNA are the targets of remodeling, without a change in Kv1.4 mRNA. In murine cardiac hypertrophy induced by overexpression of L-type Ca^{2+} channel gene CACNA1C, Kv1.4 mRNA is markedly increased, although Kv4.2 and Kv4.3 mRNA are reduced [33]. Similar findings are reported in newborn and cultured rat ventricular myocytes. In newborn rat ventricular myocytes, Kv1.4 protein is expressed at a higher level than Kv4.2; as the age of culture progresses that does not associate with progression of cardiomyocyte hypertrophy, Kv1.4 is significantly diminished, while Kv4.2 increased becoming the predominant K^+ channel protein [58]. Such K^+ channel isoform switch is diminished by incubation with non-myocyte cell-conditioned growth medium or phenylephrine (PE) that induces cardiomyocyte hypertrophy, resulting in reversion of fetal phenotype of the K^+ channels [58].

4.2. KChIP2 (Kv channel interacting protein 2)

KChIP2 is an auxiliary K^+ channel β-subunit that binds to the N-terminus of Kv4 channels, including Kv4.2 and Kv4.3 [59,60]. KChIP2 appears to have a dual function [59,60]. KChIP2 is required for transport of Kv4 channels from endoplasmic reticulum to plasma membrane [59,60], which is in line with the finding that I_{to-f} expression completely disappears in KChIP2 knock-out mice ($KChIP2^{-/-}$) [61]. KChIP2 also regulates gating kinetics of the Kv4 channel; the presence of KChIP2 accelerates recovery from inactivation of I_{to-f} [59,60]. In mice with cardiac hypertrophy induced by transverse aortic constriction, KChIP2 mRNA is down-regulated [62]. In ferret RVH, I_{to-f} amplitude is reduced in association with delayed recovery from inactivation, which agrees with the loss of acceleration of recovery from inactivation due to the absence of KChIP2 [28].

4.3. Other K^+ channel mRNAs

Alterations in K^+ channel mRNAs other than I_{to-f} encoding mRNA (Kv4.2, Kv4.3, and KChIP2) also occur [56,63]. Among them, Kv1.5 alteration is most frequently observed [32,64,65]. In post-MI LVH, Kv1.5 mRNA is diminished. Kv1.5 remodeling appears to be related to thyroid hormone. Kv1.5 mRNA reduction in post-MI LVH is associated with reduction in serum triiodethyronine (T3) level [64]. Because the Kv1.5 gene has a thyroid hormone-responsive element in its promoter region, T3 induces expression of Kv1.5 in rat heart [66]. KChIP2 also interacts

with Kv1.5 and facilitates its cell surface expression [67]. Thus, down-regulation of KChIP2 mRNA may also contribute to the Kv1.5 remodeling. Although expression of Kv1.5 mRNA and protein is demonstrated both in atrium and ventricle [68,69], antisense oligodeoxynucleotides directed against Kv1.5 mRNA inhibit ultrarapid I_K (I_{Kur}) in adult human atrial myocytes, but do not affect I_{to} or I_K in human ventricular myocytes [70]. Thus, the role of Kv1.5 channel remodeling may be more significant in atrial fibrillation rather than in ventricular hypertrophy [71].

5. Molecular mechanism of electrical remodeling

Molecular mechanisms underlying down-regulation of I_{to} have been examined in rodents; roles of Ca^{2+}-dependent mechanisms involving calcineruin and NFAT3 are well documented. Calcineurin is a Ca^{2+}-dependent protein phosphatase, and its activity is increased in pathological conditions, including hypertrophy and MI [72]. Calcineurin inhibitor cyclosporin A ameliorates I_{to-f} reduction and decreases in Kv4.2/Kv4.3 mRNA expression in hypertrophied NZ cardiomyocytes of the rat MI model [73].

NFAT3 is normally phosphorylated in the cytoplasm [74]. When NFAT3 is dephosphorylated by calcineurin, it translocates into the nucleus where it can interact with GATA4 to activate transcription of hypertrophic response genes [75]. Kv1.5, Kv2.1, Kv4.2, Kv4.3, and KChIP2 genes all have putative NFAT binding sites in their promoter regions, and all are down-regulated in an NFAT3-dependent mechanism, but with different thresholds [76]. Kv4.2 is down-regulated by $\approx$1.6-fold increase in NFAT3 activity, and much higher increases (presumably $\geq$3-fold) in NFAT3 activity are required for down-regulation of Kv1.5, Kv2.1, Kv4.3, and KChIP2 [76]. NFAT activity is increased in experimental hypertrophy models [77], but its magnitude appears to vary among models [76], a potential explanation why some studies but not all report down-regulation of Kv1.5 and Kv2.1.

In cardiac hypertrophy, signaling pathways other than calcineruin and NFAT3 are also altered and may play a role in K^+ channel remodeling [78,79]. Myocyte enhancer factor-2 (MEF2) is another Ca^{2+} responsive transcription factor, implicated in development of cardiac hypertrophy [80,81]. In normal cardiac myocytes, MEF2 exhibits only basal activity, whereas hypertrophic neurohumoral stimuli, such as endothelin-1, stimulate MEF2 transcriptional activity by causing the nuclear export of class II histone deacetylases (HDACs), which associate with MEF2 and suppress its activity [78,82]. It has recently been reported that MEF2 directly interacts with NFAT, triggering ternary complex formation between MEF2, NFAT, and CBP/p300, and induces chamber dilatation and heart failure with only modest induction of cardiac hypertrophy [83]; however, it is not addressed whether MEF2 activation is involved in down-regulation of the K^+ channels in cardiac hypertrophy. Oxidative stress through an Rac-dependent NADPH oxidase activation and superoxide production is involved in destabilization of Kv4.3 mRNA [84,85]. Kv4.2 and Kv1.4 mRNA expression is also regulated by endogenous oxidoreductase systems [86,87]. In this context, it is interesting that oxidative stress suppresses DNA binding of several transcriptional factors including NFAT in certain cells [88]. Mitogen activated protein kinase (MAPK) is also involved in down-regulation of KChIP2 mRNA [62].

6. Regional heterogeneity of electrical remodeling

6.1. Transmural heterogeneity

APD and K^+ channel expression exhibits transmural heterogeneity in normal hearts, with I_{to-f} more prominent in epicardium than in endocardium [89–91]. In catecholamine-induced rat LVH [81] and rat LVH induced by aortic constriction [18], APD is predominantly prolonged in epicardial cells in accordance with greater reduction in I_{to-f} amplitude compared to endocardial cells. In hypertrophied cardiac myocytes that are remote from IZ in rat ventricles, reduction in I_{to-f} density and in Kv4.2 and Kv4.3 protein expression are also greater in epicardium than in endocardium [47].

Several molecular mechanisms have been proposed for transmural heterogeneity in I_{to-f} [92–96], one of which involves calcineurin and NFAT3 pathways [97]. Both diastolic and systolic $[Ca^{2+}]_i$, and calcineurin activity are higher in endocardium than in epicardium, resulting in the diminished I_{to-f} amplitude in endocardium in the normal heart, at least in mice [97]. Ascending aortic stenosis selectively increases the Ca^{2+} influx during action potential in epicardium [98]. Resultant elevated calcineurin activity may cause a prominent I_{to} reduction in epicardium compared to endocardium; this hypothesis seems to be supported by the finding that suppression of both I_{to} amplitude and Kv genes expression in post-MI hypertrophy is ameliorated by calcineurin inhibitor cyclosporin A in rat model [73] and in $NFAT^{-/-}$ mice [76].

6.2. Interregional heterogeneity

In addition to transmural heterogeneity, there is interregional heterogeneity of LV repolarization [19,99]. APD is shortest in LV apex, longest in septum, and intermediate in LV free wall, reflections of differential I_{to-f} amplitude [19]. LVH induced by abdominal aorta constriction in rats causes greater I_{to-f} reduction in LV apex and LV free wall, resulting in loss of interregional APD heterogeneity [19]. Interregional heterogeneity in APD, I_{to} and Kv4.2 expression between RV free wall and intraventricular septum also is eliminated by MI [100]. Underlying mechanisms, including roles of calcineruin and NFAT3, in loss of interregional heterogeneity are yet to be determined. Nevertheless, these findings indicate that both transmural and interregional heterogeneity in APD and I_{to-f} present in normal hearts work as an anti-arrhythmic; elimination of transmural and interregional heterogeneity would then cause electrical

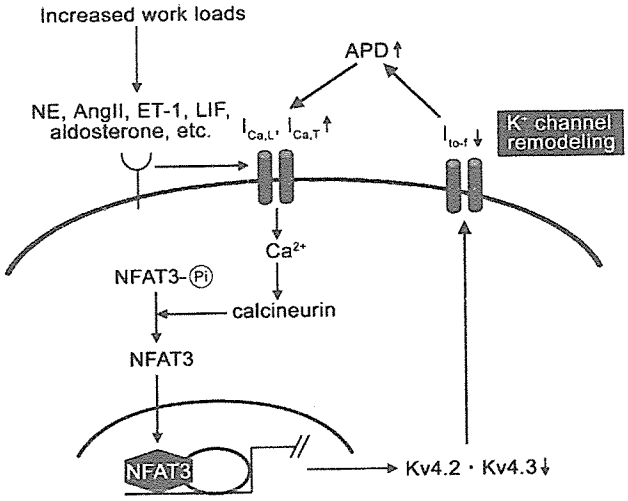

Fig. 1. Potential positive feedback between increased Ca^{2+} entry and I_{to} reduction. NE, norepinephrine; ET-1, nedothelin-1; LIF, leukemia inhibitory factor.

instability, making hypertrophied hearts more susceptible to lethal arrhythmias.

7. Physiological and clinical significance

7.1. Is reduced I_{to-f} a cause of cardiac hypertrophy or epiphenomenon?

Cardiac-specific functional knock-out of I_{to-f} with overexpression of a dominant-negative mutant W362F Kv4.2 in mice [101] and targeted deletion of Kv4.2 ($Kv4.2^{-/-}$) [102] does not induce hypertrophy, suggesting that pathological hypertrophy causes a reduction of I_{to} rather than the opposite.

In other studies, however, cardiac-specific overexpression of a dominant-negative Kv4.2 in mice caused dilated cardiomyopathy and heart failure, in addition to prolongation of APD [103,104].

Furthermore, overexpression of Kv4.2 in cultured neonatal rat cardiomyocytes prevents hypertrophy induced by PE [105]. Infection of adenovirus carrying the Kv4.3 gene (Ad.Kv4.3) reverses hypertrophy induced by Ang II in cultured neonatal rat ventricular myocytes [106]. In vivo infection of Ad.Kv4.3 abrogates the hypertrophy induced by aortic stenosis in rats [107]. All these findings implicate I_{to} reduction as a cause of hypertrophy, and restitution of I_{to} protects against hypertrophy, indicating the presence of a positive feedback loop between I_{to} reduction and progression of hypertrophy via a Ca^{2+}, calcineruin, and NFAT3-dependent pathway (Fig. 1) [108]. However, there is currently no clear explanation why some models show the association between I_{to} reduction and hypertrophy [103,104], and others do not [101,102].

7.2. Clinical implications

Interruption of the Ca^{2+}-dependent positive feedback loop may be a potential new therapeutic target against arrhythmias and sudden death in cardiac hypertrophy [109–112]. Hypertrophy-stimulating neurohumoral factors, such as noradrenaline [113–115], Ang II [116,117], endothelin-1 [118,119], leukemia inhibitory factor [120], and aldosterone [121,122] activate the L-type Ca^{2+} channels and/or the T-type Ca^{2+} channels, resulting in increases in $[Ca^{2+}]i$ and calcineurin activity and triggering hypertrophic stimuli. Elimination of these triggers by inhibition of angiotensin-converting enzyme (ACE) [123–126], the type-1 Ang II (AT1) receptor [127–131], a mineralcorticoid receptor [132,133], and an endothelin-A receptor [134] causes regression of cardiac hypertrophy and successfully prevents arrhythmogeneity of cardiac hypertrophy. L-arginine, the biological precursor of nitric oxide (NO) [135], also attenuates DOCA-induced LVH in rats, which is in line with recent data that NO released from eNOS protects against Ca^{2+} overload [136,137].

Calcineurin inhibitor prevents cardiac hypertrophy in mice [138]. However, these are results from rodent hypertrophy models and are yet to be verified in larger mammals. For example, in canine complete AV block-induced hypertrophy, AT1 receptor antagonist irbesartan does not reverse the hypertrophy or arrhythmogenesis [139]. Thus, the therapeutic efficacy of such pharmacological approaches requires further careful examination in large mammals including the human.

Acknowledgments

We like to thank Dr. Arthur L. Bassett (University of Miami) for reading and proofing our manuscript. This work was supported in part by the Grant-in-Aid for scientific Research on Priority Areas (17081007), and Grant from the Ministry of Education, Science, Culture, Sports and Technology of Japan (18390231, 17790167), health sciences research grants (H18-Research on Human Genome-002) from the Ministry of Health, Labour and Welfare, Japan, and research grant from the Vehicle Racing Commemorative.

References

[1] Kannel WB. Incidence and epidemiology of heart failure. Heart Failure Rev 2000;5:167–73.
[2] Kannel WB, McGee DL, Schatzkin A. An epidemiological perspective of sudden death. 26-year follow-up in the Framingham Study. Drugs 1984;28(Suppl 1):1–16.
[3] Haider AW, Larson MG, Benjamin EJ, Levy D. Increased left ventricular mass and hypertrophy are associated with increased risk for sudden death. J Am Coll Cardiol 1998;32:1454–9.
[4] Boyden PA, Jeck CD. Ion channel function in disease. Cardiovasc Res 1995;29:312–8.
[5] Wickenden AD, Japrielian R, Kassiri Z, Tsoporis JN, Tsushima R, Fishman GI, et al. The role of action potential prolongation and altered intracellular calcium handling in the pathogenesis of heart failure. Cardiovasc Res 1998;37:312–23.
[6] Nabauer M, Kaab S. Potassium channel down-regulation in heart failure. Cardiovasc Res 1998;37:324–34.
[7] Swynghedauw B. Molecular mechanisms of myocardial remodeling. Physiol Rev 1999;79:215–62.
[8] Uhley HN. Study of the transmembrane action potential, electrogram, electrocardiogram, and ventrocardiogram of rats with left ventricular hypertrophy. Am J Cardiol 1961;7:211–7.

[9] Bassett AL, Gelband H. Chronic partial occlusion of the pulmonary artery in cats. Change in ventricular action potential configuration during early hypertrophy. Circ Res 1973;32:15–26.
[10] Bassett AL, Gelband H. Electrical and mechanical properties of cardiac muscle during chronic right ventricular pressure overload. Recent Adv Stud Cardiac Struct Metab 1974;4:3–20.
[11] Kleiman RB, Houser SR. Outward currents in normal and hypertrophied feline ventricular myocytes. Am J Physiol 1989;256:H1450–61.
[12] Kleiman RB, Houser SR. Outward currents in hypertrophied feline ventricular myocytes. Prog Clin Biol Res 1990;334:65–83.
[13] Furukawa T, Bassett AL, Furukawa N, Kimura S, Myerburg RJ. The ionic mechanism of reperfusion-induced early afterdepolarizations in feline left ventricular hypertrophy. J Clin Invest 1993;91:1521–31.
[14] Furukawa T, Myerburg RJ, Furukawa N, Kimura S, Basssett AL. Metabolic inhibition of $I_{Ca,L}$ and I_K differs in feline left ventricular hypertrophy. Am J Physiol 1994;266:H1121–31.
[15] Ten Eick RE, Zhang K, Harvey RD, Bassett AL. Enhanced functional expression of transient outward current in hypertrophied feline myocytes. Cardiovasc Drugs Ther 1993;Suppl. 3:611–9.
[16] Volders PG, Sipido KR, Vos MA, Kulcsar A, Verduyn SC, Wellens HJ. Cellular basis of biventricular hypertrophy and arrhythmogenesis in dogs with chronic complete atrioventricular block and acquired torsade de pointes. Circulation 1998;98:1136–47.
[17] Ramakers C, Vos MA, Doevendans PA, Schoenmakers M, Wu YS, Scicchiotano S, et al. Coordinated down-regulation of KCNQ1 and KCNE1 expression contributes to reduction of I_{Ks} in canine hypertrophied hearts. Cardiovasc Res 2003;57:486–96.
[18] Volk T, Nguyen TH, Schultz JH, Faulhaber J, Ehmke H. Regional alterations of repolarizing K^+ currents among the left ventricular free wall of rats with ascending aortic stenosis. J Physiol 2001;530:443–55.
[19] Gomez AM, Benitah JP, Henzel D, Vinet A, Lorente P, Delgado C. Modulation of electrical heterogeneity by compensated hypertrophy in rat left ventricle. Am J Physiol 1997;272:H1078–86.
[20] Tomita F, Bassett AL, Myerburg RJ, Kimura S. Diminished transient outward currents in rat hypertrophied ventricular myocytes. Circ Res 1994;75:296–303.
[21] Coulombe A, Momtaz A, Richer P, Swynghedauw B, Coraboeuf E. Reduction of calcium-independent transient outward potassium current density in DOCA salt hypertrophied rat ventricular myocytes. Pflughers Arch 1994;427:47–55.
[22] Momtaz A, Coulombe A, Richer P, Mercadier JJ, Coraboeuf E. Action potential and plateau ionic currents in moderately and severely DOCA-salt hypertrophied rat hearts. Mol Cell Cardiol 1996;28:2511–22.
[23] Cerbai E, Barbieri M, Li Q, Mugelli A. Ionic basis of action potential prolongation of hypertrophied cardiac myocytes isolated from hypertensive rats of different ages. Cardiovasc Res 1994;28:1180–7.
[24] Meszaros J, Ryder KO, Hart G. Transient outward current in catecholamine-induced cardiac hypertrophy in the rat. Am J Physiol 1996;271:H2360–7.
[25] Chouabe C, Espinosa L, Megas P, Chakir A, Rougier O, Freminet A, et al. Reduction of $I_{Ca,L}$ and I_{to} density in hypertrophied right ventricular cells by simulated high altitude in adult rats. J Mol Cell Cardiol 1997; 29:193–206.
[26] Lee JK, Kodama I, Honjo H, Anno T, Kamiya K, Toyama J. Stage-dependent changes in membrane currents in rats with monocrotaline-induced right ventricular hypertrophy. Am J Physiol 1997;272:H2833–42.
[27] Xu XP, Best PM. Decreased transient outward K^+ current in ventricular myocytes from acromegalic rats. Am J Physiol 1991;260:H935–42.
[28] Ponteau D, Gomez JP, Fares N. Depressed transient outward current in single hypertrophied cardiomyocytes isolated from the right ventricle of ferret heart. Cardiovasc Res 1995;30:440–8.
[29] Eghbali M, Deva R, Alioua A, Minosyan TY, Ruan H, Wang Y, et al. Molecular and functional signature of heart hypertrophy during pregnancy. Circ Res 2005;96:1208–16.
[30] Knollmann BC, Knollmann-Ritschel BE, Weissman NJ, Jones LR, Morad M. Remodeling of ionic currents in hypertrophied and failing hearts of transgenic mice overexpressing calsequestrin. J Physiol 2000;525:483–98.
[31] Mitarai S, Reed TD, Yatani A. Changes in ionic currents and beta-adrenergic receptor signaling in hypertrophied myocytes overexpressing Gαq. Am J Physiol 2000;279:H139–48.
[32] Heath BM, Xia J, Dong E, An RH, Brooks A, Liang C, et al. Overexpression of nerve growth factor in the heart alters ion channel activity and beta-adrenergic signaling in an adult transgenic mouse. J Physiol 1998;512:779–91.
[33] Bodi I, Muth JN, Hahn HS, Petrashevskaya NN, Rubio M, Koch SE, et al. Electrical remodeling in hearts from a calcium-dependent mouse model of hypertrophy and failure: complex nature of K^+ current changes and action potential duration. J Am Coll Cardiol 2003;41:1611–22.
[34] Petkova-Kirova PS, Gursoy E, Mehdi H, McTiernan CF, London B, Salama G. Electrical remodeling of cardiac myocytes from mice with heart failure due to overexpression of tumor necrosis factor-α. Am J Physiol 2006;290:H2098–107.
[35] Brahmajothi MV, Campbell DL, Rasmusson RL, Morales MJ, Trimmer JS, Nerbonne JM, et al. Distinct transient outward potassium current (I_{to}) phenotypes and distribution of fast-inactivating potassium channel α subunits in ferret left ventricular myocytes. J Gen Physiol 1999;113:581–600.
[36] Tsuji Y, Opthof T, Yasui K, Inden Y, Takemura H, Niwa N, et al. Ionic mechanisms of acquired QT prolongation and torsades de pointes in rabbit with chronic complete atrioventricular block. Circulation 2002;106:2012–8.
[37] McIntosh MA, Cobbe SM, Kane KA, Rankin AC. Action potential prolongation and potassium currents in left-ventricular myocytes isolated from hypertrophied rabbit hearts. J Mol Cell Cardiol 1998; 30:43–53.
[38] Gillis AM, Mathison HJ, Kulisz E, Lester WM. Dispersion of ventricular repolarization in left ventricular hypertrophy: influence of afterload and dofetilide. J Cardiovasc Electrophysiol 1998;9:988–97.
[39] Gillis AM, Goenzon RA, Mathison HJ, Kulisz E, Lester WM, Duff HJ. The effects of barium, dofetilide and 4-aminopyridine (4-AP) on ventricular repolarization in normal and hypertrophied rabbit heart. J Pharmacol Exp Ther 1998;285:262–70.
[40] Kaab S, Dixon J, Duc J, Ashen D, Nabauer M, Beuckelmann DJ, et al. Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. Circulation 1998;98:1383–93.
[41] Li GR, Lau CP, Leung TK, Nattel S. Ionic current abnormalities associated with prolonged action potentials in cardiomyocytes from diseased human right ventricles. Heart Rhythm 2004;4:460–8.
[42] Dun W, Baba S, Yagi T, Boyden PA. Dynamic remodeling of K^+ and Ca^{2+} currents in cells that survived in the epicardial border zone of canine healed infracted heart. Am J Physiol 2004;287:H1046–54.
[43] Jiang M, Cabo C, Yao J, Boyden PA, Tseng G. Delayed rectifier K currents have reduced amplitudes and altered kinetics in myocytes from infracted canine ventricles. Cardiovasc Res 2000;48:34–43.
[44] Dun W, Boyden PA. Diverse phenotypes of outward currents in cells that have survived in the 5-day-infarcted heart. Am J Physiol 2005;289: H667–73.
[45] Qin D, Zhang ZH, Caref EB, Boutjdir M, Jain P, El-Sherif N. Cellular and ionic basis of arrhythmias in postinfarction remodeled ventricular myocardium. Circ Res 1996;79:461–73.
[46] Gidh-Jain M, Huang B, Jain P, El-Sherif N. Differential expression of voltage-gated K^+ channel genes in left ventricular remodeled myocardium after experimental myocardial infarction. Circ Res 1996;79:669–75.
[47] Rozanski GJ, Xu Z, Zhang K, Patel KP. Altered K^+ current of ventricular myocytes in rats with chronic myocardial infarction. Am J Physiol 1998;274:H259–65.
[48] Kaprielian R, Wickenden AD, Kassiri Z, Parker TG, Liu PP, Backx PH. Relationship between K^+ channel and $[Ca^{2+}]i$ in rat ventricular myocytes following myocardial infarction. J Physiol 1999;517:229–45.
[49] Yao JA, Jiang M, Fan JS, Zhou YY, Tseng GN. Heterogeneous changes in K currents in rat ventricles three days after myocardial infarction. Cardiovasc Res 1999;44:132–45.

[50] Huang B, Qin D, El-Sherif N. Early down-regulation of K^+ channel genes and currents in the postinfarction heart. J Cardiovasc Electrophysiol 2000;11:1252–61.
[51] Yuan F, Pinto JM, Li Q, Wasserlauf BJ, Yang X, Bassett AL, et al. Characteristics of I_K and its response to quinidine in experimental healed myocardial infarction. J Cardiovasc Electrophyiol 1999;10: 844–54.
[52] Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial infarction in rats. infarct size, myocytes hypertrophy, and capillary growth. Circ Res 1986;58:26–37.
[53] Patterson E, Holland K, Eller BT, Lucchesi BR. Ventricular fibrillation resulting from ischemia at a site remote from previous myocardial infarction. A conscious canine model of sudden coronary death. Am J Cardiol 1982;50:1414–23.
[54] Furukawa T, Moroe K, Mayrovitz HN, Sampsell R, Furukawa N, Myerburg RJ. Arrhythmogenic effects of graded coronary blood flow reductions superimposed on prior myocardial infarction in dogs. Circulation 1991;84:368–77.
[55] Guo W, Li H, Aimond F, Johns DC, Rhodes KJ, Trimmers JS, et al. Role of heteromultimers in the generation of myocardial transient outward K^+ currents. Circ Res 2002;90:586–93.
[56] Capuano V, Ruchon Y, Antoine S, Sant MC, Renaud JF. Ventricular hypertrophy induced by mineralcorticoid treatment or aortic stenosis differentially regulates the expression of cardiac K^+ channels in the rat. Mol Cell Biochem 2002;237:1–10.
[57] Takimoto K, Li D, Hershman KM, Li P, Jackson EK, Levitan ES. Decreased expression of Kv4.2 and novel Kv4.3 K^+ channel subunit mRNA in ventricles of renovascular hypertensive rats. Circ Res 1997;81:533–9.
[58] Guo W, Kamiya K, Honjo M, Kodama I, Toyama J. Regulation of Kv4.2 and Kv1.4 K^+ channel expression by myocardial hypertrophic factors in cultured newborn rat ventricular cells. J Mol Cell Cardiol 1998;30:1449–55.
[59] An WF, Bowlby MR, Betty M, Cao J, Ling HP, Mendoza G, et al. Modulation of A-type potassium channels by a family of calcium sensors. Nature 2000;403:553–6.
[60] Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. Physiol Rev 2005;85:1205–53.
[61] Kuo HC, Cheng CF, Clark RB, Lin JJ, Lin JL, Hoshijima M, et al. A defect in the Kv channel-interacting protein 2 (KChIP2) gene leads to a complete loss of I_{to} and confers susceptibility to ventricular tachycardia. Cell 2001;107:801–13.
[62] Jia Y, Takimoto K. Mitogen-activated protein kinases control cardiac KChIP2 gene expression. Circ Res 2006;98:386–93.
[63] Walsh KB, Sweet JK, Parks GE, Long KJ. Modulation of outward potassium currents in aligned cultures of neonatal rat ventricular myocytes during phorbol ester-induced hypertrophy. J Mol Cell Cardiol 2001;33:1233–47.
[64] Ojamaa K, Kenessey A, Shenoy R, Klein I. Thyroid hormone metabolism and cardiac gene expression after acute myocardial infarction in the rat. Am J Endocrinol Metab 2000;279:E1319–24.
[65] Matsubara H, Suzuki J, Inada M. Shaker-related potassium channel, Kv1.4, mRNA regulation in cultured rat heart myocytes and differential expression of Kv1.4 and Kv1.5 genes in myocardial development and hypertrophy. J Clin Invest 1993;92:1659–66.
[66] Abe A, Yamamoto T, Isome M, Ma M, Yaoita E, Kawasaki K, et al. Thyroid hormone regulates expression of shaker-related potassium channel mRNA in rat heart. Biochem Biophys Res Commun 1998;245:226–30.
[67] Li H, Guo W, Mellor RL, Nerbonne JM. KChIP2 modulates the cell surface expression of Kv1.5-encoded K^+ channels. J Mol Cell Cardiol 2005;39:121–32.
[68] Dixon JE, McKinnon D. Quantitative analysis of potassium channel mRNA expression in atrial and ventricular muscle of rats. Circ Res 1994;75:252–60.
[69] Barry DM, Trimmer JS, Merlie JP, Nerbonne JM. Differential expression of voltage-gated K^+ channel subunits in adult rat heart. relation to functional K^+ channels? Circ Res 1995;77:361–9.
[70] Feng J, Wible B, Li GR, Wang Z, Nattel S. Antisense oligodeoxynucleotides directed against Kv1.5 mRNA specifically inhibit ultrarapid delayed rectifier K^+ current in cultured adult human atrial myocytes. Circ Res 1997;80:572–9.
[71] Van Wagoner DR, Pond AL, McCarthy PM, Trimmer JS, Nerbonne JM. Outward K^+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. Circ Res 1997;80: 772–81.
[72] Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 1998;93:215–28.
[73] Deng L, Huang B, Qin D, Ganguly K, El-Sherif N. Calcineurin inhibition ameliorates structural, contractile, and electrophysiologic consequences of postinfarction remodeling. J Cardiovasc Electrophysiol 2001;12:1055–61.
[74] Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineruin, and NFAT. Genes Dev 2003;17:2205–32.
[75] Morin S, Charron F, Robitaille L, Nemer M. GATA-dependent recruitment of MEF2 proteins to target promoters. EMBO J 2000;19:2046–55.
[76] Rossow CF, Minami E, Chase EG, Murry CE, Santana LF. NFATc3-induced reduction in voltage-gated K^+ currents after myocardial infarction. Circ Res 2004;94:1340–50.
[77] Xia Y, McMillin JB, Lewis A, Moore M, Zhu WG, Williams RS, et al. Electrical stimulation of neonatal cardiac myocytes activates the NFAT3 and GATA4 pathways and upregulates the adenylosuccinate synthetase 1 gene. J Biol Chem 2000;275:1855–63.
[78] Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, et al. Local InsP3-dependent perinuclear Ca^{2+} signaling in cardiac myocytes excitation–transcription coupling. J Clin Invest 2006;116: 675–81.
[79] Xu J, Gong NL, Bodi I, Aronow BJ, Backx PH, Molkentin JD. Myocyte enhancer factors 2A and 2C induced dilated cardiomyopathy in transgenic mice. J Biol Chem 2006;281:9152–62.
[80] Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. Cell 2002;110:479–88.
[81] Vega RB, Harrison BC, Meadows E, Roberts CR, Papst PJ, Olson EN, et al. Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylases 5. Mol Cell Biol 2004;24:8374–85.
[82] McKinsey TA, Zhang CL, Lu J, Olson EN. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature 2000;408:106–11.
[83] van Oort RJ, van Rooji E, Bourajjaj M, Schimmel J, Jansen MA, Nagel vanderR, et al. MEF2 activates a genetic program promoting chamber dilatation and contractile dysfunction in calcineruin-induced heart failure. Circulation 2006;114:298–308.
[84] Zhang TT, Takimoto K, Stewart AF, Zhu C, Levitan ES. Independent regulation of cardiac Kv4.3 potassium channel expression by angiotensin II and phenylephrine. Circ Res 2001;88:476–82.
[85] Zhou C, Ziegler C, Birder LA, Stewart AF, Levitan ES. Angiotensin II and stretch activate NADPH oxidase to destabilize cardiac Kv4.3 channel mRNA. Circ Res 2006;98:1040–7.
[86] Rozanski GJ, Xu Z. Glutathione and K^+ channel remodeling in postinfarction rat heart. Circ Res 2005;567:177–90.
[87] Li X, Li S, Xu Z, Lou MF, Anding P, Liu D, et al. Redox control of K^+ channel remodeling in rat ventricle. J Mol Cell Cardiol 2006;40:339–49.
[88] Flescher E, Tripoli H, Salnikow K, Burns FJ. Oxidative stress suppresses transcription factor activities in stimulated lymphocytes. Clin Exp Immunol 1998;112:242–7.
[89] Litovsky SH, Antzelevitch C. Transient outward current prominent in canine ventricular epicardium but not endocardium. Circ Res 1998; 62:116–26.
[90] Furukawa T, Myerburg RJ, Furukawa N, Bassett AL, Kimura S. Differences in transient outward currents in feline endocardial and epicardial myocytes. Circ Res 1990;67:1287–91.

[91] Fedida D, Giles WR. Regional variations in action potentials and transient outward current in myocytes isolated from rabbit left ventricle. J Physiol 1991;442:191–209.
[92] Bryant SM, Shipsey SJ, Hart G. Normal regional distribution of membrane current density in rat left ventricle is altered in catecholamine-induced hypertrophy. Cardiovasc Res 1999;42:391–401.
[93] Rosati B, Pan Z, Lypen S, Wang HS, Cohen I, Dixon JE, et al. Regulation of KChIP2 potassium channel beta subunit gene expression underlies the gradient of transient outward current in canine and human ventricle. J Physiol 2001;533:119–25.
[94] Rosati B, Grau F, Rodriguez S, Li H, Nerbonne JM, McKinnon D. Concordant expression of KChIP2 mRNA, protein and transient outward current throughout the canine ventricle. J Physiol 2003;548:815–22.
[95] Zicha S, Xiao L, Stafford S, Cha TJ, Han W, Varro A, et al. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. J Physiol 2004;561:735–48.
[96] Costantini DL, Arruda EP, Agarwal O, Kim KH, Zhu Y, Zhu W, et al. The homeodomain transcription factor Irx5 establishes the mouse cardiac ventricular repolarization gradient. Cell 2005;123:347–58.
[97] Rossow CF, Dilly KW, Santana LF. Differential calcineurin/NFAT3 activity contributes to the I_{to} transmural gradient in the mouse heart. Circ Res 2006;98:1306–13.
[98] Volk T, Noble PJ, Wagner M, Noble D, Ehmke H. Ascending aortic stenosis selectively increases action potential-induced Ca^{2+} influx in epicardial myocytes of the rat left ventricle. Exp Physiol 2005;90:111–21.
[99] Cheng J, Kamiya K, Liu W, Tsuji Y, Toyama J, Kodama I. Heterogeneous distribution of the two components of delayed rectifier K^+ current: a potential mechanism of the proarrhythmic effects of methanesulfonanilide class III agents. Cardiovasc Res 1999;43:135–47.
[100] Kaprielian R, Sah R, Nguyen T, Wickenden AD, Backx PH. Myocardial infarction in rat eliminates regional heterogeneity of AP profiles, I_{to}, I_K currents, and $[Ca^{2+}]_i$ transients. Am J Physiol 2002;283: H1157–68.
[101] Barry DM, Xu H, Schuessler RB, Nerbonne JM. Functional knockout of the transient outward current, long-QT syndrome, and cardiac remodeling in mice expressing a dominant-negative Kv4 α subunit. Circ Res 1998;83:560–7.
[102] Guo W, Jung WE, Marionneau C, Aimond F, Xu H, Yamada KA, et al. Targeted deletion of Kv4.2 eliminates $I_{to,f}$ and results in electrical and molecular remodeling, with no evidence of ventricular hypertrophy or myocardial dysfunction. Circ Res 2005;97:1342–50.
[103] Wickenden AD, Lee P, Sah R, Huang Q, Fishman GI, Backx PH. Targeted expression of a dominant-negative Kv4.2 K^+ channel subunit in the mouse heart. Circ Res 1999;85:1067–76.
[104] Kassiri Z, Zobel C, Nguyen TT, Molkentin JD, Backx PH. Reduction of I_{to} causes hypertrophy in neonatal rat ventricular myocytes. Circ Res 2002;90:578–85.
[105] Zobel C, Kassiri Z, Nguyen TT, Meng Y, Backx PH. Prevention of hypertrophy by overexpression of Kv4.2 in cultured neonatal cardiomyocytes. Circulation 2002;106:2385–91.
[106] Lebeche D, Kaprielian R, Hajjar R. Modulation of action potential duration on myocytes hypertrophic pathway. J Mol Cell Cardiol 2006;40:725–35.
[107] Lebeche D, Kaprielian R, del Monte F, Tomaselli G, Gwathmey JK, Schwartz A, et al. In vivo cardiac gene transfer of Kv4.3 abrogates the hypertrophic response in rats after aortic stenosis. Circulation 2004;110:3435–43.
[108] Sanguinetti MC. Reduced transient outward K^+ current and cardiac hypertrophy. Causal relationship or epiphenomenon? Circ Res 2002; 90:497–9.
[109] Lynch Jr JJ, Sanguinetti MC, Kimura S, Bassett AL. Therapeutic potential of modulating potassium currents in the diseases myocardium. FASEB J 1992;6:2952–60.
[110] Shieh CC, Coghlan N, Sullivan JP, Gopalakrishman M. Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol Rev 2000;52:557–94.
[111] Xu X, Salata JJ, Wang J, Wu Y, Yan GX, Liu T, et al. Increasing I_{Ks} corrects abnormal repolarization in rabbit models of acquired LQT2 and ventricular hypertrophy. Am J Physiol 2002;283:H664–70.
[112] Wasson S, Reddy HK, Dohrmann ML. Current perspectives of electrical remodeling and its therapeutic implications. J Cardiovasc Pharmacol Ther 2004;9:129–44.
[113] Lubic SO, Giacomini KM, Giacomini JC. The effects of modulation of calcium influx through the voltage-sensitive calcium channel on cardiomyocytes hypertrophy. J Moll Cell Cardiol 1995;27:917–25.
[114] Walsh KB, Kass RS. Regulation of a heart potassium channel by protein kinase A and C. Science 1998;242:67–9.
[115] Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, et al. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. Science 2002;295:496–9.
[116] Aiello EA, Cingolani HE. Angiotensin II stimulates cardiac L-type Ca^{2+} current by a Ca^{2+}- and protein kinase C-dependent mechanism. Am J Physiol 2001;280:H1528–36.
[117] Ichiyanagi O, Ishii K, Endoh M. Angiotensin II increases L-type Ca^{2+} current in gramicidin D-perforated adult rabbit ventricular myocytes: comparison with conventional patch-clamp method. Pflughers Arch 2002;444:107–16.
[118] Furukawa T, Ito H, Nitta J, Tsujino M, Adachi S, Hiroe M, et al. Endothelin-1 enhances calcium entry through T-type calcium channels in cultured neonatal rat ventricular myocytes. Circ Res 1992;71:1242–53.
[119] Kelso E, Spiers P, McDermott B, Scholfield N, Silke B. Dual effects of endothelin-1 on the L-type Ca^{2+} current in ventricular cardiomyocytes. Eur J Pharmacol 1996;308:351–5.
[120] Takahashi E, Fukuda K, Miyoshi S, Murata M, Kato T, Ita M, et al. Leukemia inhibitory factor activates cardiac L-type Ca^{2+} channels via phosphorylation of serine 1829 in the rabbit Cav1.2 subunit. Circ Res 2004;94:1242–8.
[121] Benitah JP, Vassort G. Aldosterone upregulates Ca^{2+} current in adult rat cardiomyocytes. Circ Res 1999;85:1139–45.
[122] Lalevee N, Bebsamen MC, Barrere-Lemaire S, Perrier E, Nargeot J, Benitah JP, et al. Aldosterone increases T-type calcium channel expression and in vitro beating frequency in neonatal rat cardiomyocytes. Cardiovasc Res 2005;67:216–24.
[123] Kreher P, Ristori MT, Corman B, Verdetti J. Effects of chronic angiotensin I-converting enzyme inhibition on the relations between ventricular action potential changes and myocardial hypertrophy in aging rats. J Cardiovasc Pharmacol 1995;25:75–80.
[124] Kahonen M, Makynen H, Wu A, Arvola P, Pekki A, Posti I. Angiotensin-converting enzyme inhibition attenuates arterial constrictor responses in experimental hypertension. J Pharmacol Exp Ther 1996;277:1701–9.
[125] Yokoshiki H, Kohya T, Tomita F, Tohse N, Nakaya H, Kanno M, et al. Restoration of action potential duration and transient outward current by regression of left ventricular hypertrophy. J Moll Cell Cardiol 1997; 29:1331–9.
[126] Rials SJ, Xu X, Wu Y, Marinchak RA, Kowey PR. Regression of LV hypertrophy with captopril normalizes membrane currents in rabbits. Am J Physiol 1998;275:H1216–24.
[127] Kohya T, Yokoshiki H, Tohse N, Kanno M, Nakaya H, Saito H, et al. Regression of left ventricular hypertrophy prevents ischemia-induced lethal arrhythmias. Beneficial effect of angiotensin II blockade. Circ Res 1995;76:892–9.
[128] Cerbai E, Crucitti A, Sartiani L, De Paoli P, Pino R, Rodorigeuz ML. Long-term treatment of spontaneous hypertensive rats with losartan and electrophysiological remodeling of cardiac myocytes. Cardiovasc Res 2000;45:388–96.
[129] Rials SJ, Xu X, Wu Y, Liu T, Marinchak RA, Kowey PR. Restoration of normal ventricular electrophysiology in renovascular hypertensive rabbits after treatment with losartan. J Cardiovasc Pharmacol 2001; 37:317–23.
[130] Cerbai E, De Paoli P, Sartiani L, Lonardo G, Mugelli A. Treatment with irbesartan counteracts the functional remodeling of ventricular myocytes from hypertensive rats. J Cardiovasc Pharmacol 2003;41:804–12.
[131] Martens JR, Reaves PY, Lu D, Katovich MJ, Bereck KH, Bishop SR, et al. Pretension of renovasular and cardiac pathophysiological

changes in hypertension by angiotensin II type 1 receptor antisense gene therapy. Proc Natl Acad Sci U S A 1998;95:2664–9.

[132] Perrier E, Kerfant BG, Lalevee N, Bideaux P, Rossier MF, Richard S, et al. Mineralcorticoid receptor antagonism prevents the electrical remodeling that precedes cellular hypertrophy after myocardial infarction. Circulation 2004;110:776–83.

[133] Joffe HV, Adler GK. Effects of aldosterone and mineralocorticoid receptor blockade on vascular inflammation. Heart Fail Res 2005;10:31–7.

[134] Allan A, Fenning A, Levick S, Hoey A, Brown L. Reversal of cardiac dysfunction by selective ET-A receptor antagonism. Br J Pharmacol 2005;146:846–53.

[135] Fenning A, Harrison G, Rose'meyer R, Hoey A, Brown L. L-Arginine attenuates cardiovascular impairment in DOCA-salt hypertensive rats. Am J Physiol 2005;289:H1408–16.

[136] Nitta J, Furukawa T, Marumo F, Sawanobori T, Hiraoka M. Subcellular mechanism for Ca^{2+}-dependent enhancement of delayed rectifier K^+ current in isolated membrane patches of guinea pig ventricular myocytes. Circ Res 1994;74:96–104.

[137] Bai C-X, Namekata I, Kurokawa J, Tanaka H, Shigenobu K, Furukawa T. Role of nitric oxide in Ca^{2+}-sensitivity of the slowly activating delayed rectifier K^+ current in cardiac myocytes. Circ Res 2005;96:64–72.

[138] Sussman MA, Lim HW, Gude N, Taigen T, Olson EN, Robbins J, et al. Prevention of cardiac hypertrophy in mice by calcineurin inhibition. Science 1998;281:1690–3.

[139] Schoenmarkers M, Ramakers C, van Opstal JM, Leunissen JD, Londono C, Vos MA. Asynchronous development of electrical remodeling and cardiac hypertrophy in the complete AB block dog. Cardiovasc Res 2003;59:351–9.

Special Report

突然死の性差

—前 編—
研究の現状

Sex Difference of Sudden Death : The Current Status of Researches

東京医科歯科大学難治疾患研究所
生体情報薬理分野
古川 哲史
Tetsushi FURUKAWA

Key Words
突然死（sudden death），不整脈（arrhythmia），QT間隔（QT interval），性ホルモン（sex hormone）

はじめに

最近，循環器疾患の性差が注目されている。心筋梗塞・狭心症などの虚血性心疾患が男性に多いことはよく知られているが，興味深いことに突然死の主因となる不整脈にも特徴的な性差が存在する。特に，体表面心電図におけるQT間隔の延長を伴う不整脈“QT延長症候群”は女性に多く[1]，近年の研究の進展に伴い性差医療の実現化の兆しがみられる。実際に欧米では2005年から薬剤安全性評価への臨床応用がなされている。

1．先天性QT延長症候群の性差

QT延長症候群は体表面心電図でQT間隔が延長し，トルサード・ド・ポアンと呼ばれる特徴的な形態の不整脈をもたらす症候群である。主として常染色体優性遺伝形式の家族性を示す先天性QT延長症候群と，家族性を示さず薬剤の副作用などを原因とする後天性QT延長症候群に分類されるが，どちらも女性における発症頻度が男性に比べて高い。

1）男性ホルモン（テストステロン）の関与

先天性QT延長症候群の不整脈イベントを年齢別にみると，思春期を迎える15歳頃までは性差が認められないが，15歳を過ぎると男性の不整脈イベントが減少し，相対的に女性で多くなる（図1a）[2]。また，健常者におけるQT間隔を年齢別でみると，15歳頃までは男女差がないが，15歳を過ぎると男性でQT間隔が短縮し相対的に女性で長くなる[3]。さらに，テストステロンによるドーピ

東京医科歯科大学難治疾患研究所生体情報薬理分野 〒101-0062 東京都千代田区神田駿河台2-3-10

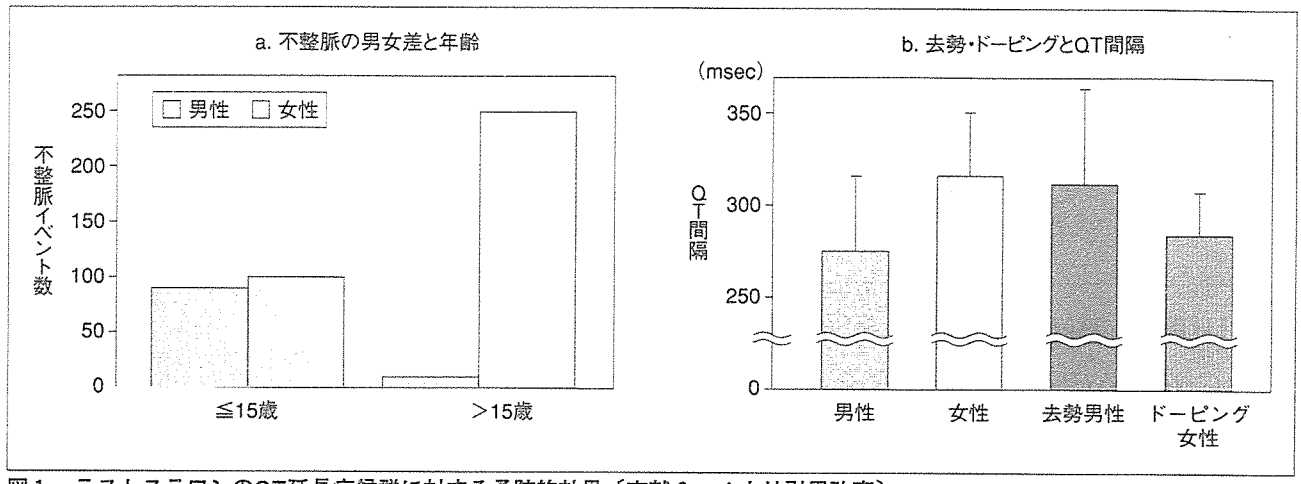

図1　テストステロンのQT延長症候群に対する予防的効果〔文献2，4より引用改変〕

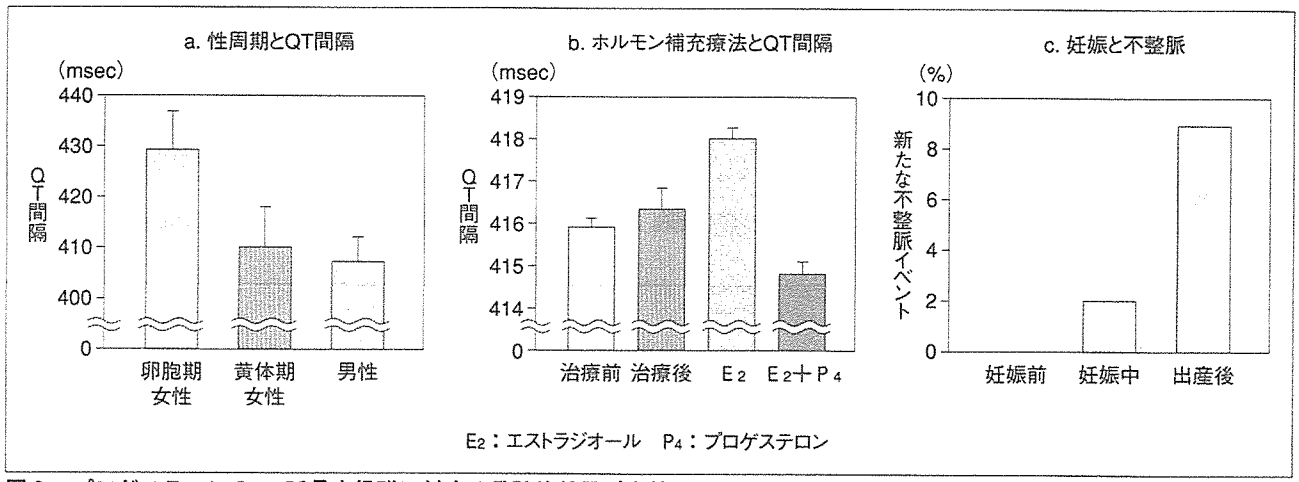

図2　プロゲステロンのQT延長症候群に対する予防的効果〔文献5，6，7より引用改変〕

ングを行った女性アスリートでは，QT間隔が短縮し，男性と変わらなくなる（図1b.）[4]。これらの臨床データは男性ホルモン（テストステロン）がQT延長症候群に対して保護的に働くことを示唆している。

2）黄体ホルモン（プロゲステロン）の関与

性周期によるQT間隔の違いをみると，QT間隔は黄体期で短い（図2a.）[5]。更年期障害に対するホルモン補充療法において，エストロゲン単独療法の場合はQT間隔の延長が認められるが，エストロゲン・プロゲステロン併用療法ではかえってQT間隔が短縮する（図2b.）[6]。さらに先天性QT延長症候群患者における不整脈イベントの出現率をみると，妊娠中に比べてプロゲステロンの激減する産後に不整脈イベントが起こる頻度が有意に高い（図2c.）[7]。これらのエビデンスから，黄体ホルモン（プロゲステロン）もテストステロン同様，QT延長症候群に対して保護的に働くことが示唆される。

2．薬剤誘発性QT延長症候群の性差

表に挙げたように，極めて多くの心臓薬・非心臓薬がQT間隔の延長を伴うトルサード・ド・ポアンにより失神・突然死を来たす。単一の機序として，市販薬の発売中止の最も頻度の高い原因となっており，また薬剤開発途上での中断の原因としても多いことから，製薬業界で

表　薬剤誘発性QT延長症候群をもたらす薬物

ハイリスクドラッグ	ロウリスクドラッグ	
抗不整脈薬	制吐剤	抗ヒスタミン薬
ベプリジル	ドンペリドール	アセタミゾール
ブレチリウム	ドロペリドール	テルフェナジン
ジソミラミド	抗痙攣剤	やせ薬
ドフェチリド	リチウム	エフェドリン
フレカイニド	フェニトイン	フェンタミン
イブチリド	向精神薬	フェンフラミン
プロカインアミド	ピモジド	抗喘息薬
キニジン	ジプラシドン	エピネフリン
ソタロール	フェノチアジン	イソプロテレノール
	三環系抗うつ薬	サルブタモール
	イミプラミン	抗がん剤
	アミトリプチリン	タモキシフェン
	抗生物質	三酸化ヒ素
	クラリスロマイシン	
	エリスロマイシン	その他
	抗真菌薬	免疫抑制薬
	ケトコナゾール	シクロスポリン
	抗マラリア薬	腸管運動促進薬
	ハロファントリン	シサプリド
	クロロキン	高脂血症治療薬
	キニン	プロブコール

＊頻度の高い薬剤とポピュラーな薬剤のみ掲載

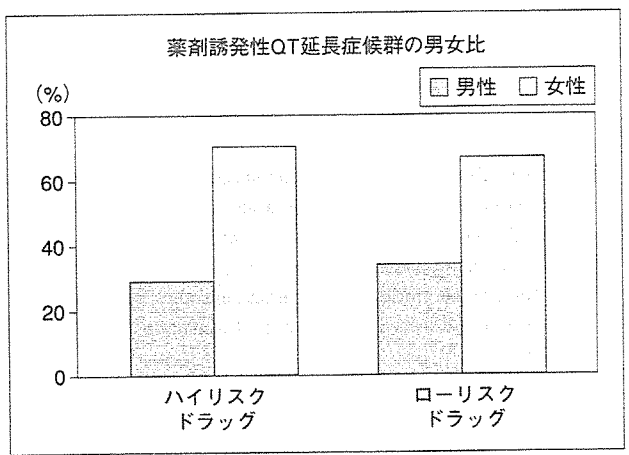

図3　薬剤誘発性QT延長症候群の男女差〔文献8より引用改変〕

は“QT延長問題”として緊急の解決課題に挙げられている。いずれの薬剤も*herg*遺伝子によりコードされるI_{Kr}チャネルと呼ばれるカリウムチャネルをターゲットとする。表に挙げた薬剤のうち抗不整脈薬はハイリスクドラッグに分類され，1%以上の頻度でトルサード・ド・ポアンを来たす。それ以外の薬剤はローリスクドラッグに分類され，0.1%以下の頻度でトルサード・ド・ポアンを来たすが，いずれの薬剤も女性でトルサード・ド・ポアンのリスクが高く，約70%（男性比で2倍）が女性に起こる（図3）[8]。

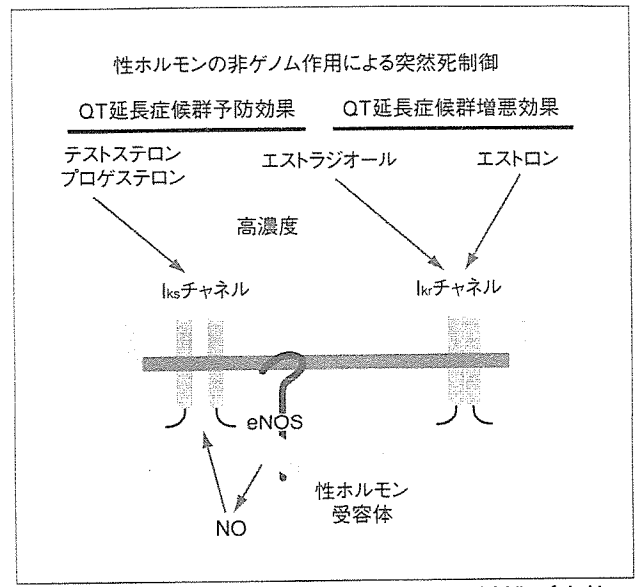

図4　性ホルモン非ゲノム作用による不整脈制御〔文献12より引用改変〕

3．ホルモンによる心電図調節

性ホルモンの一般的な作用メカニズムは，細胞質内に存在する性ホルモン受容体に結合し，性ホルモン/性ホルモン受容体複合体が核内移行し，性ホルモン応答領域を有した遺伝子の転写活性化をもたらす“ゲノム作用”である。最近，性ホルモンが非生殖組織，特に神経・循環器系ではゲノム作用で説明できない秒～分単位の迅速な作用を示すことが注目されている。詳細なシグナル伝達経路に関しては議論の余地が残されているが，細胞膜局在の性ホルモン受容体を介して内皮型一酸化窒素合成酵素（eNOS）を活性化する経路が主要な経路の1つと考えられている。

1）ゲノム作用による心電図調節

ゲノム作用による心筋イオンチャネルの制御の研究では，去勢することにより心筋カリウムチャネル遺伝子（*Kv1.5*・*Kir2.1*）の発現が減少するという報告から[9,10]，テストステロンレベルがカリウムチャネルの維持に必要

と考えられる。ところが，去勢あるいは卵巣摘出ラットにテストステロンを長期投与した実験では，心筋カリウムチャネル遺伝子（*Kv1.5・KCNE1*）の発現低下がみられ[11]，相反する成績となっている。このように，性ホルモンのゲノム作用に関してはいまだ不明な点が多く残されている。

2）非ゲノム作用による心電図調節

非ゲノム作用による心筋イオンチャネル調節機構は，かなり明らかになってきた（図4）[12]。テストステロン・プロゲステロンは急性効果としてQT間隔を短縮する。これはテストステロン・プロゲステロンがQT延長症候群に対して保護的に働くという臨床データとよく一致する。ターゲットとなる心筋イオンチャネルは，I_{Ks}チャネルと呼ばれるカリウムチャネルとカルシウムチャネル（$I_{Ca,L}$チャネル）であり，いずれもeNOS活性化による一酸化窒素産生の経路を介して調節される。

エストラジオールは急性効果として，QT間隔に対して2相性の作用を示す。低濃度ではQT間隔を延長し，高濃度では短縮する。高濃度エストラジオールによるQT短縮はテストステロン・プロゲステロン同様に一酸化窒素を介する作用である。低濃度によるQT間隔の延長は薬剤誘発性QT延長症候群のターゲットとなるI_{Kr}チャネルがターゲットである。これはeNOS活性化・一酸化窒素作用を介さず，直接I_{Kr}チャネルを抑制することにより，QT間隔を延長する。このエストラジオール作用は女性において薬剤誘発性QT延長症候群が多い根拠となる。

おわりに

QT延長を伴う不整脈は，先天性の症例の頻度は高いとは言えないが，薬剤誘発性QT延長症候群は頻度の高い疾患であり，上記の性差のメカニズムを臨床に応用する性差医療の実現化が期待される。薬剤安全性試験に臨床応用されている欧米のモデルは，性周期・生殖年齢等を考慮しない静的モデルであり，これらの情報も考慮した動的で精密な不整脈性差医療モデルが日本から発信されることが期待される。

本論文中の性ホルモン非ゲノム作用に関連した研究は，厚生労働科学研究費補助金（H18-ゲノム-一般-002）の助成を得て行われた。

参考文献

1）Hashiba K：Sex differences in phenotypic manifestation and gene transmission in the Romano-Ward syndrome. Ann. NY Acad. Sci 644：142-156, 1992.
2）Locati EH, Zareba W, Moss AJ, et al：Age-and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome：findings from the International LQTS Registry. Circulation 97：2237-2244, 1998.
3）Rautaharju PM, Zhou SH, Wong S, et al：Sex differences in the evolution of the electrocardiographic QT interval with age. Can. J. Cardiol 8：690-695, 1992.
4）Bidoggia H, Maciel JP, Capalozza N, et al：Sex differences on the electrocardiographic pattern of cardiac repolarization：possible role of testosterone. Am. Heart J 140：678-683, 2000.
5）Nakagawa M, Ooie T, Takahashi N, et al：Influence of menstrual cycle on QT interval dynamics. PACE Jun 29：607-613, 2006.
6）Kadish AH, Greenland P, Limacher MC, et al：Estrogen and progestin use and the QT interval in postmenopausal women. Ann Noninvasive Electrocardiol 9：366-374, 2004.
7）Rashba EJ, Zareba W, Moss AJ, et al：Influence of pregnancy on the risk for cardiac events in patients with hereditary long QT syndrome. LQTS Investigators. Circulation 97：451-456, 1998.
8）Makkar RR, Fromm BS, Steinman RT, et al：Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. JAMA 270：2590-2597, 1993.
9）Brouillette J, Trepanier-Boulay V, Fiset C：Effect of androgen deficiency on mouse ventricular repolarization. J. Physiol 546：403-413, 2003.
10）Carnes CA, Dech SJ：Effects of dihydrotestosterone on cardiac inward rectifier K(+) current. Int. J. Andol 25：210-214, 2002.
11）Drici MD, Burklow TR, Haridasse V, et al：Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart. Circulation 94：1471-1474, 1996.
12）Bai CX, Kurokawa J, Tamagawa M , et al：Nontranscriptional regulation of cardiac repolarization currents by testosterone. Circulation 112：1701-1710, 2005.

Special Report

突然死の性差

—後編—
治　療

Sex Difference of Sudden Death : Treatment

東京医科歯科大学難治疾患研究所
生体情報薬理分野
古川　哲史
Tetsushi　FURUKAWA

Key Words
突然死（sudden death），不整脈（arrhythmia），QT間隔（QT interval），性ホルモン（sex hormone）

はじめに

突然死の最も多い原因は不整脈であり，不整脈の中でQT延長症候群は女性に[1, 2]，ブルガダ症候群と呼ばれる特発性心室細動[3]と心房細動[4]は男性に多い。QT延長症候群においても，女性の不整脈発作は性周期の卵胞期[5, 6]や出産後[7]に増えること，一方でカテコラミン誘発性不整脈が妊娠中に多いことが知られている[8]。これらの性差に関する基礎データをもとにした性差医療が，QT延長症候群の薬剤治療に関して臨床応用されている。

1．薬剤誘発性不整脈の性差医療：FDA（米国食品医薬品局）の施策

前編（前号）でも述べたが，QT延長症候群は体表面心電図でQT間隔が延長し，トルサード・ド・ポアン（TdP）と呼ばれる特徴的な形態の不整脈をもたらす[9]。家族性を示す先天性QT延長症候群と，薬剤の副作用などを原因とする後天性QT延長症候群に分類される。薬剤誘発性QT延長症候群は失神・突然死をもたらすことから，単独の原因としては薬剤の発売中止となる最も多い原因であり，製薬業界では“QT延長問題”として喫緊の解決課題となっている。QT延長をもたらす薬剤は多種多様であり，抗不整脈薬（不整脈を抑えるための薬剤であるが，不整脈誘発を副作用とする）を中心とする高リスク群（＞1％の頻度）と，抗ヒスタミン薬などの非心臓薬を中心とする低リスク群（＜0.1％の頻度）に分類される[9]。テストステロンは非ゲノム作用を介してQT延長症候群に対して保護的作用を示す。これらの基

東京医科歯科大学難治疾患研究所生体情報薬理分野　〒101-0062 東京都千代田区神田駿河台2-3-10

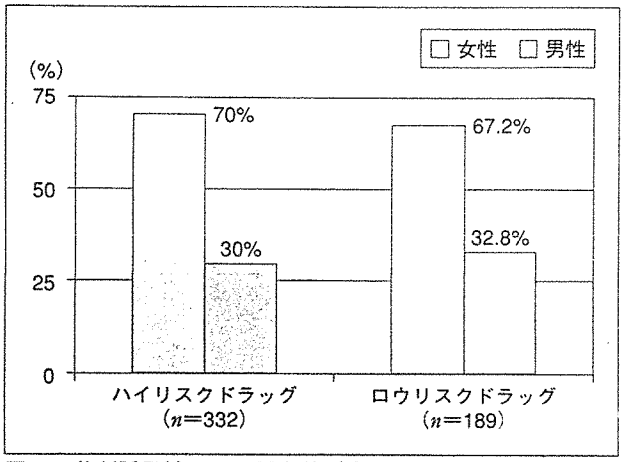

図1　薬剤誘発性TdPの男女差〔文献2，4より引用改変〕

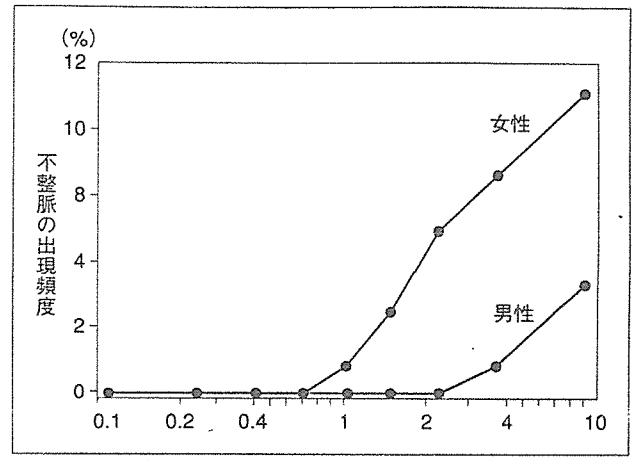

図2　QT延長薬の投与量と不整脈発現頻度〔文献5，6，8より引用改変〕

礎データをもとにQT延長問題に対する対策として，多くの製薬会社がFDAに対してテストステロンパッチの認可を求めたが，テストステロンの有するリスクを考慮して断念された経緯がある。これに代わり，FDAでは心電図 *in silico* モデルを用いた薬剤安全性評価システムの臨床応用を認可している。

薬剤誘発性QT延長症候群のハイリスクドラッグ・ロウリスクドラッグとも，男性に比べて女性で2倍以上の頻度で不整脈発作をもたらす（図1）[10]。FDAのモデルでも性差を考慮したオックスフォード大学Noble博士により構築された *in silico* モデルを用いている。男性に比べて女性で心臓カリウムチャネル活性が10～15%弱いことをモデルに取り入れることにより，女性でQT間隔が5msec程度長くなっている。これに加えてQT延長薬を少量から投与していくと，女性ではEC_{50}濃度量を投与すると不整脈の発現がみられるが，男性ではEC_{50}の3倍程度投与しないと不整脈が発現しない（図2）。これにより，QT延長をもたらす表のような薬剤の投与量は，男性ではEC_{50}の2倍量，女性ではEC_{50}の1/2倍程度が安全投与量と考えてよいかもしれない。

2．性周期を考慮した独自の心臓 *in silico* モデル

前編でも取り上げたが，QT延長を伴う不整脈はテストステロン・エストロゲン・プロゲステロンなど複数の性ホルモンの影響を強く受けている[11]。そこで，FDAで使われている男女差だけを考慮したモデルではなくて，性周期・性年齢・妊娠を考慮したモデル，さらに発展させると，性ホルモン血中濃度を反映したモデルが望まれる。そこで，プロトタイプとして性周期に伴う平均的血中ホルモン濃度を反映したモデルの構築を試みた。

表　薬剤誘発性QT延長症候群をもたらす薬剤（Vol.3，No11より再掲）

ハイリスクドラッグ	ロウリスクドラッグ	
抗不整脈薬	制吐剤	抗ヒスタミン薬
ベプリジル	ドンペリドール	アセタミゾール
ブレチリウム	ドロペリドール	テルフェナジン
ジソピラミド	抗けいれん剤	やせ薬
ドフェチリド	リチウム	エフェドリン
フレカイニド	フェニトイン	フェンタミン
イブチリド	向精神薬	フェンフラミン
プロカインアミド	ピモジド	抗喘息薬
キニジン	ジプラシドン	エピネフリン
ソタロール	フェノチアジン	イソプロテレノール
	三環系抗うつ薬	サルブタモール
	イミプラミン	抗がん剤
	アミトリプチリン	タモキシフェン
	抗生物質	三酸化ヒ素
	クラリスロマイシン	
	エリスロマイシン	その他
	抗真菌薬	免疫抑制薬
	ケトコナゾール	シクロスポリン
	抗マラリア薬	腸管運動促進薬
	ハロフェントリン	シサプリド
	クロロキン	高脂血症治療薬
	キニン	プロブコール

＊頻度の高い薬剤とポピュラーな薬剤のみ掲載