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FORUM REVIEW ARTICLE

Ryanodine Receptor: A New Therapeutic Target to Control Diabetic Cardiomyopathy

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Abstract

Diabetes mellitus is a major risk factor for cardiovascular complications. Intracellular Ca^{2+} release plays an important role in the regulation of muscle contraction. Sarcoplasmic reticulum Ca^{2+} release is controlled by dedicated molecular machinery, composed of a complex of cardiac ryanodine receptors (RyR2s). Acquired and genetic defects in this complex result in a spectrum of abnormal Ca^{2+} release phenotypes in heart. Cardiovascular dysfunction is a leading cause for mortality of diabetic individuals due, in part, to a specific cardiomyopathy, and to altered vascular reactivity. Cardiovascular complications result from multiple parameters, including glucotoxicity, lipotoxicity, fibrosis, and mitochondrial uncoupling. In diabetic subjects, oxidative stress arises from an imbalance between production of reactive oxygen and nitrogen species and capability of the system to readily detoxify reactive intermediates. To date, the etiology underlying diabetes-induced reductions in myocyte and cardiac contractility remains incompletely understood. However, numerous studies, including work from our laboratory, suggest that these defects stem in part from perturbation in intracellular Ca^{2+} cycling. Since the RyR2s are one of the well-characterized redox-sensitive ion channels in heart, this article summarizes recent findings on redox regulation of cardiac Ca^{2+} transport systems and discusses contributions of redox regulation to pathological cardiac function in diabetes. *Antioxid. Redox Signal*. 15, 1847–1861.

Introduction

Cardiovascular dysfunction is a leading cause of mortality in diabetic individuals, in part due to a specific cardiomyopathy known as diabetic cardiomyopathy (107). Diabetic cardiomyopathy is a clinical condition diagnosed when ventricular dysfunction develops in patients with diabetes in the absence of coronary atherosclerosis and hypertension (44, 45) Several mechanisms involved in the development of cardiomyopathy have been postulated, including alterations in intracellular ion homeostasis and glucose metabolism, as well as enhanced oxidative stress (22). Therefore, the cardiovascular complications result from multiple parameters, including glucotoxicity, lipotoxicity, fibrosis, and mitochondrial uncoupling. The World Health Organization estimates that more than roughly 180 million people worldwide in 2005 suffer from diabetes mellitus, a figure that is likely to double within the next 20 years (www.eatlas.idf.org/). Among the 3.8 million deaths each year, about 2/3 are attributable to cardiovascular complications associated with the disease. The economic and human cost of this disease is also devastating with a significant increase.

Diabetic cardiomyopathy has been associated with both type 1 (insulino-deficient) and type 2 (insulino-resistant) diabetes and is characterized by both early-onset diastolic and late-onset systolic dysfunctions, including reduction in diastolic compliance, contractility, and rate of myocardial relaxation (99). Diabetic cardiomyopathy was initially classified as a dilated cardiomyopathy with prominent left ventricular enlargement and depressed systolic function. Over the last two decades, however, diastolic left ventricular dysfunction was identified as an early manifestation of diabetic cardiomyopathy (71, 160).

The pathogenesis of diabetic cardiomyopathy is undoubtedly multifactorial and complex. It includes alterations in cardiac energy metabolism showing a reduced glucose uptake and an increased free fatty acid oxidation related to mitochondrial uncoupling, as well as impaired Ca^{2+} homeostasis, both leading to deficient contractile activity. On the one hand in diabetes, the heart is becoming almost solely dependent on the metabolism of fatty acids. Such an increase in the myocardial uptake of fatty acids implies an increase in fatty acid oxidation and reduction in glucose oxidation resulting in a decrease in ATP production per mole of oxygen and an increase in mitochondrial uncoupling leading to an unfavorable energetic state together with an overproduction of reactive oxygen species (ROS)

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(6). On the other hand, in isolated ventricular myocytes, diabetes has long been reported to rapidly induce contractile dysfunctions associated with altered Ca^{2+} handling (26, 65, 103, 104). These effects are mostly attributed to reduced Ca^{2+} current (I_{Ca}) (98), reduced sarcoplasmic reticulum (SR)-Ca^{2+} load in relation with anomalous SR-pump activity (SERCA2a), and altered cardiac ryanodine receptors (RyR2) (16, 98, 159). Of note, the RyR2 alterations are minimized in female rats (158). Ca^{2+} handling is also impaired in the mitochondria of an animal model of obesity and type-2 diabetes, the *ob/ob* mice (39). Besides the slower action potential and reduced cardiac SR-Ca^{2+} release, in some reports the contractile deficit is attributed to left ventricular remodeling (161). Further redox status, known to impair contractile machinery (59, 129), may also account for the known depressed cardiac myofilament function observed in human diabetes. Indeed some authors consider that the observed left ventricular remodeling in diabetes may play a crucial role, whereas the slower action potential and reduced SERCA2a expression can explain the slower Ca^{2+} transient kinetics in diabetic rats but not the contractile deficiency (161).

As microRNA (miRNAs) play a fundamental role in gene expression, alterations of a large number of miRNAs in a chronic disease process are possible (24). In a chronic neonatal rat model of diabetes demonstrating cardiomyopathy, in total, 14 miRNAs were upregulated and 28 miRNAs were downregulated. Exposure of cardiomyocytes to high levels of glucose produced hypertrophic changes and reduced expression of miRNA133a, known to play an important role in muscle cell differentiation and proliferation (41). Besides in insulin-resistant heart, miR-223 was consistently upregulated. miR-223 overexpression-induced glucose transporter protein 4 (Glut4) protein expression in cardiomyocytes was necessary and sufficient for increased glucose uptake (78).

Diabetes is also associated with alterations in the neurohumoral systems. It has been shown to elevate angiotensin II (Ang II) in human and in rat cardiac myocytes, and diabetic cardiomyopathy has been viewed as an Ang II-dependent process in which that peptide plays a critical role in myocyte death and hypertrophy (42). Diabetic myocardium also shows a significant increase in protein expression of the angiotensin II receptor type 1 (AT1) receptors that are functionally coupled, resulting in a stronger inotropic response upon stimulation with Ang II (105). Diabetes has also been associated with increased plasma aldosterone and improvement occurs in cardiac function following aldosterone blockade (43). Moreover, glucose and aldosterone potentiate each other deleterious effects (66). Not withdrawing the fact that diabetes produces particularly disparate outcomes for coronary heart disease, with risk of fatal cardiovascular heart disease 50% higher in diabetic women than in men (64), implies a significant role of estrogen. Indeed, Ang II increase is less in diabetic female compared to male rat, with estrogen appearing as a suppressor of the renin angiotensin system (RAS) (115).

In this review we focused on the role of diabetes-linked RyR2 disorder in the diabetic cardiomyopathy and the potential of RyR2 as a new therapeutic target in this heart dysfunction.

Ryanodine Receptors: Structure, Regulation, and Function

Cardiac contraction is directly controlled by Ca^{2+}. In the normal heart during the early plateau phase of the cardiac action potential, a small amount of Ca^{2+} enters through the L-type Ca^{2+} channel or dihydropyridine receptor (DHPR). This Ca^{2+} influx triggers a large-scale Ca^{2+} release from the SR through the Ca^{2+} release channel sensitive to ryanodine (RyR). Close spatial coupling between DHPR and RyR clusters and the relative insensitivity of RyR to be triggered by Ca^{2+} together ensures the stability of this positive-feedback system of Ca^{2+} amplification. This process, referred as cardiac excitation–contraction (E-C) coupling, is characterized by a transient increase in cytosolic-free Ca^{2+} ($[Ca^{2+}]_i$) from 100 n*M* to about 1 μ*M* (111). For termination of SR-Ca^{2+} release, RyR inactivation and SR-Ca^{2+} depletion play important roles by acting in a synergistic manner. The released Ca^{2+} binds to the troponin C that activates myofilaments and induces muscle contraction. Relaxation follows Ca^{2+} reuptake into the SERCA2a and subsequent *trans*-sarcolemmal Ca^{2+} removal through the Na^+/Ca^{2+} exchanger operating in its forward mode (Fig. 1).

The RyR is a huge tetrameric protein with each monomer constituted of ~5000 amino acids (M_w: 565 kDa). About 90% of the RyR polypeptide chain forms a bulky cytoplasmic domain that modulates the channel function. The remaining C-terminal region forms transmembrane and channel-pore regions. Three mammalian isoforms of RyR have been identified: RyR1 is found in skeletal muscle (84), RyR2 is predominantly expressed in cardiac muscle (94), whereas RyR3 is ubiquitously expressed at low levels. RyR exists as a scaffolding protein bound with many accessory proteins, producing a huge macromolecular complex. It associates with FK506-binding immunophilin protein (FKBP; mainly FKBP12.6, or calstabin2 in heart), protein kinase A (PKA), protein phosphatases 1 (PP1) and 2A (PP2A), calmodulin (CaM), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and the phosphodiesterase PDE4D3. Both junctin and triadin, which anchor calsequestrin, bind to the luminal site of the RyR2. Sorcin, a 22-kDa Ca^{2+}-binding protein, also binds to cardiac RyRs with high affinity, and its interaction with RyR2 is facilitated by annexin A7 in a Ca^{2+}-dependent manner (58). The neuronal isoform of nitric oxide (NO) synthase, nNOS, or NOS1 is also found to be constitutively expressed in cardiomyocytes, where it localizes to the SR and coimmunoprecipitates with RyR2 under physiological conditions (88). Spatial association of the DHPR and RyR2 macromolecular complex results in a key functional unit controlling cardiac E-C coupling (15, 83). FKBP12.6, PP2A, and PP1 are reported to be lost from the complex in heart failure (HF) in a paced dog model (87, 155) and in a rabbit model of aortic constriction (3) and in human HF (87).

The RyR2 release channel is regulated, in addition to cytosolic and luminal Ca^{2+}, by endogenous effectors such as Mg^{2+}, ATP, reactive oxygen, and nitrogen molecules as well as by FK-506 binding proteins mainly FKBP12.6. The accessory protein FKBP12.6 binds to RyR2 with a stoichiometric ratio of 1 FKBP12.6 to 1 RyR2 monomer, or 4 FKBP12.6 to the RyR2 tetramer. FKBP12.6 is suggested to stabilize a closed state of the RyR2 channel and to reduce RyR2 sensitivity to Ca^{2+}. Studies from both Marks's and Yano's groups (86, 87, 155) demonstrated that altered stoichiometry between RyR2 and FKBP12.6 leads to SR-Ca^{2+} leak and cardiac contractile dysfunction. The dissociation of FKBP12.6 from RyR2 also functionally uncouples multiple RyR2 and disturbs both the simultaneous opening of RyR2 during systole and their

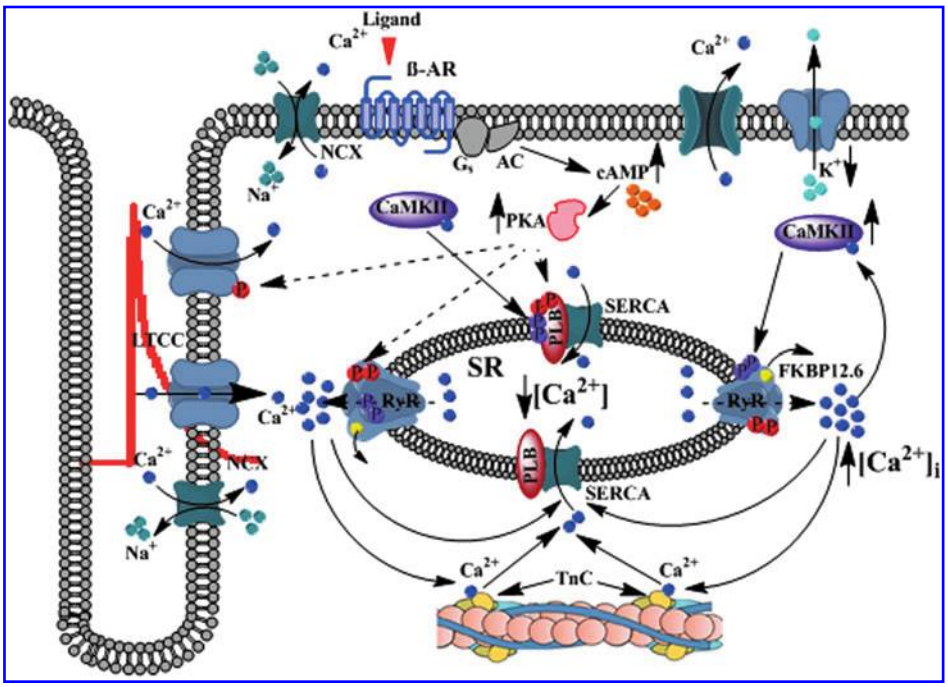

FIG. 1. Schematic representation of the regulation of Ca^{2+} cycling in cardiomyocytes. In cardiomyocytes, Ca^{2+} influx through L-type Ca^{2+} channels and released from intracellular stores plays an important role in the regulation of muscle contraction and electrical signals that determine the heart rhythm, and excitation–contraction coupling is an important intermediate step between initiation of the action potential and induction of contraction. This process is predominantly controlled by Ca^{2+} released from the sarcoplasmic reticulum (SR) *via* the ryanodine receptors (RyR2). The spatio-temporal parameters and velocity of the Ca^{2+} transient are regulated by phosphorylation of nodal Ca^{2+} cycling regulators in cardiomyocytes. Two kinases, protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), play a major role along with phosphatases (PP1 and PP2A) to control local phospho-reactions. β-adrenergic receptor signaling activates PKA and CaMKII signaling. *Red line* is a schematic action potential occurring along the T-tubular system. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

simultaneous closing during diastole. Sorcin inhibits both the spontaneous activity of RyRs by directly reducing the mean open time and the frequency of open events in quiescent cells (observed as Ca^{2+} sparks) and the I_{Ca}-triggered activity of RyRs that gives rise to $[Ca^{2+}]_i$ transient (37).

Phosphorylation-dependent regulation of RYR2

RyR2 activity is also regulated by evolutionarily highly conserved signaling pathways that control the E-C coupling in the heart. Phosphorylations of RyR2 at Ser2030 by cAMP-dependent protein kinase, PKA, and at Ser2809 (corresponding to Ser2808 in human) by PKA and CaMKII have been described. According to Marks's group, RyR2 phosphorylation at Ser2809 plays a key role in regulating the channel in response to stress following activation of the sympathetic nervous system, the fly-or-fight response (87). Dysregulation of the RyR2 by hyperphosphorylation is characterized by improper control of SR-Ca^{2+} release and diastolic Ca^{2+} leak. Hyperactivation of PKA, by causing an increased phosphorylation at Ser2809 of the RyR2, induces dissociation of the FKBP12.6 subunit from RyR2.

Several investigators soon proposed that CaMKII-dependent, rather than PKA-dependent, phosphorylation was critical for RyR2-FKBP12.6 dissociation and the consequent diastolic Ca^{2+} leak. CaMKII is known to phosphorylate several Ca^{2+} handling proteins, including RyR2, PLB, and L-type Ca^{2+}-channels with multiple functional consequences (80). Notably, besides the originally reported Ser2808/2809 (shared amino acids with a putative PKA site), CaMKII can phosphorylate several other sites in RyR2, including Ser2815, resulting in more active RyR2 channels (54, 139). Thus, these authors proposed that CaMKII, activated by β-adrenergic stimulation, is responsible for the SR-Ca^{2+} leak. The increased spark frequency and low diastolic Ca^{2+} content observed in CaMKII-transgenic mice further supported this view (29). However, the reported effects of CaMKII are diverse. Thus, recently, it was shown that overexpression of CaMKII suppresses Ca^{2+} sparks and Ca^{2+} waves in cultured rat cardiomyocytes, thereby affording a negative feedback that stabilizes local and global Ca^{2+}-induced Ca^{2+} release in the heart (153). It is also shown that CaM, independent of the kinase activation, can activate at low physiological concentrations (50–100 n*M*), and at higher ones inactivate RyR2 channels in bilayers (116). In addition in cardiac cells, the authors demonstrated that CaM stimulates SR-Ca^{2+} release and this effect is mediated by RyR2. CaMKII expression and activity are increased in HF (3, 162). Concurrently, SR-Ca^{2+} leak is enhanced. The spontaneous Ca^{2+} release was prevented by CaMKII inhibition, but not by PKA inhibition (3).

The regulation of RyR2 activity by protein phosphatases was emphasized by Valdivia's group (132) and Marks' group (83). In HF, long-term hyperphosphorylation of RyR2 can be maintained through a reduction in the protein abundance of PP1 and PP2A, contributing to an increased S2808 phosphorylation state. Further, a splice variant of the PDE4 family, PDE4D3, containing an N-terminal targeting motif for mAKAP, forms a PKA-mAKAP-PDE4D3 signaling molecule with PDE4D3 contributing to the RyR2 complex. In human HF, PDE4D3 levels in the RyR2 complex were reduced by 43% (75). Prolonged RyR2 phosphorylation *via* inhibition of PP1 appears cardioprotective (149). However, Terentyev *et al.* (127) reported that increased intracellular PP1 activity stimulates RyR-mediated SR-Ca^{2+} release leading to depleted SR-Ca^{2+} stores in cardiac myocytes. In a recent study, Huke and Bers (63) measured phosphorylation with at least two

different antibodies per site and demonstrated that S2808-phosphorylated levels appear highly antibody dependent. RyR2 was substantially phosphorylated in quiescent rat cardiomyocytes at S2808 and less so at S2814, unphosphorylated at S2030. On the other hand during stimulation with isoproterenol, S2808 was phosphorylated by PKA and S2814 by CaMKII. PP1 appears to be the main phosphatase dephosphorylating S2808/S2814, but PP2A may also dephosphorylate S2814. In a recent study, Terentyev *et al.* (126) showed a promotion of cardiac arrhythmogenesis with overexpressing miR-1, which is targeting PP2A-regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2.

Several groups have challenged the role of FKBP12.6 as a channel stabilizer in various experimental conditions. In the same pacing-induced HF dog model as initially used by Marks's group, Jiang *et al.* observed no change in RyR2/FKBP12.6 association or single channel behavior (67). Phosphorylation at Ser2808 did not dissociate FKBP12.6 from RyR2 (144), whereas the constitutive phosphorylation of Ser2808 by mutations (S2808D) failed to disrupt the FKBP12.6–RyR2 interaction (119). The significant role of PKA phosphorylation of another site (Ser-2030) on hypersensitized channel to activation by luminal Ca^{2+} in disease-linked RyR2 channel disorder has also been stressed out (146). The effects could be dependent upon the level of RyR2 phosphorylation, 100% but not 75%, is required to increase the probability of RyR2 channel to be in the open state (20). In HF, despite reduced SR-Ca^{2+} load and RyR2 expression, increased Ca^{2+} sparks frequency might be resulted from the fact that PKA phosphorylation increases the sensitivity of RyR2 to luminal Ca^{2+} (145). Also, the onset of SR-Ca^{2+} leak would be fully dependent on phospholamban phosphorylation and the subsequent increase in SR-Ca^{2+} load rather than on RyR2 phosphorylation (76). Finally, using FKBP12.6 null mice, it is concluded that dissociation of FKBP12.6 from the RyR2 complex does not play a significant role in β-adrenergic-stimulated Ca^{2+} release in heart cells, whereas this mechanism does underlie the action of cADPR (163).

Further, recent works report a reduced amount of cytosolic FKBP12.6 such that the relative amounts of FKBP12/12.6 to RyR without significant alterations in S2808-phosphorylation RyR2 status, as in the hearts of transgenic mice expressing human mineralocorticoid receptor or wild-type (WT) mice treated with aldosterone compared with control WT, whereas no difference was found in the ratio of phosphorylated RyR to total RyR among the three groups (50). This appears to be also true when analyzing the comparative effects of streptozotocin (STZ)-induced diabetes in control and $G\alpha_{11}$ and $G\alpha_q$-knockout animals (62). Finally, using quantitative immunoblots, Bers's group determined that intact cardiomyocytes the endogenous FKBP12.6 content is much lower than RyR and FKBP12 ones. They concluded that only 10%–20% of RyR2s have FKBP12.6 associated, but virtually all myocyte FKBP12.6 is RyR2-bound (because of very high affinity). PKA-dependent RyR2 phosphorylation has no significant effect on binding of either FKBP12 or FKBP12.6 to RyR2 in myocytes, whereas only FKBP12.6 but not FKBP12 inhibits basal RyR2 activity (53). To note, the RyR-channel instability was initially related to the tremendous decrease of FKBPs in HF (93), before a reduced FKBP/RyR stoichiometry was emphasized by the same group and others.

Regulation of interdomain interaction within RyR2

The concept that interactions between the N-terminal domain and the central domain of RyR1 are involved in Ca^{2+} channel regulation has emerged from recent domain peptide probe studies (150, 151). According to this concept, in the resting or nonactivated state, the N-terminal and central domains make close contact at several subdomains (domain zipping). The conformational constraints imparted by the zipped configuration of these two domains stabilize and maintain the closed state of Ca^{2+} channel. In RyR2 of cardiac disease patients, some mutations are located in the regions corresponding to the skeletal N-terminal and central domains harboring most of the malignant hyperthermia mutations that cause an increased Ca^{2+} leak. RyR1 and RyR2 shares about 60% similarities; this suggests that RyR2 shares a common domain-mediated channel regulation mechanism with RyR1 and that the increased Ca^{2+} leak of diseased RyR2 channels may be explained by the altered mode of interdomain interactions.

Stimulation *via* E-C coupling or pharmacological agents weakens these critical interdomain contacts, resulting in loss of conformational constraints (domain unzipping) and thus lowering of the energy barrier for Ca^{2+} channel opening. Weakening of these interdomain interactions may also occur *via* mutation or with the use of synthetic domain peptides. Experimental data presented strong evidence that synthetic domain peptides such as DPc10 corresponding to key subdomains of RyR2 are capable of mimicking diseased conditions of the RyR2 channel by interfering with the interdomain interaction (92). Interestingly, the binding region of FKBP12.6 to RyR2, the residues sequence 2361–2496, (82) is included in the sequence of DPc10 (2460–2495). Thus, there could be a close mechanistic relationship between the PKA-mediated FKBP12.6 dissociation and abnormal domain–domain interactions such as that seen in the DPc10-mediated channel hypersensitization. In pacing-induced dog failing heart, the domain unzipping has already occurred, together with FKBP12.6 dissociation and Ca^{2+} leak (92).

Redox-dependent modification of RyR2

ROS are involved in the regulation of cardiovascular function under physiological and pathological conditions (142, 164). In general, oxidizing conditions increase RyR2 activity and so stimulate SR Ca^{2+} release (2, 32, 129, 164). These effects are reversed by reducing reagents such as dithiothreitol (33, 34, 129). Redox reactions by biological oxidants and antioxidants have been shown to alter the kinetics of Ca^{2+}-induced Ca^{2+} release in the heart tissue (9, 49, 124, 129). Besides several potential phosphorylation sites, the tetrameric RyR2 channel contains ~84 free thiols and is S-nitrosylated *in vivo*. S-Nitrosylation of up to 12 sites (3 per subunit) led to progressive channel activation that was reversed by denitrosylation (148). RyR2s are activated also by reactive nitrogen species (118). For example, nNOS is expressed in SR and can supply NO to RyR2 in the immediate vicinity for S-nitrosylation, which increases RyR open probability in both skeletal and cardiac muscle (120) and leads to increased Ca^{2+} release (148). Thus, sulfydryl-oxidizing agents, hydrogen peroxide (H_2O_2) and diamide, diminished RyR2-FKBP12.6 binding. A cysteine-null mutant FKBP12.6 retained redox-sensitive interaction with RyR2, suggesting that the effect of the redox reagents is exclusively *via* sites on the RyR2 (166).

NOS1-knockout mice have a reduced RyR2 S-nitrosylation with no change in the stoichiometry and binding of FKBP12.6 and RyR2 phosphorylation (31). Diastolic Ca^{2+} levels are elevated in NOS1-knockout mice and this is accompanied by a proarrhythmic phenotype. The inducible isoform, iNOS, or NOS2 is expressed under pathophysiological conditions such as ischemia/reperfusion, aging, and HF. It is known to target RyR2. NOS2-NO depresses β-adrenergic stimulated RyR function through a cGMP-independent pathway (eg, NO- and/or peroxynitrite-dependent redox modification) although the detailed mechanism was not more clarified (165). Mice deficient in the endothelial NO synthase isoform, eNOS, or NOS3 did not exhibit alterations in any of these parameters (51).

It has been reported that glutathione (GSH) acts as a cofactor for the stimulation of calcium release mediated by H_2O_2, generated by the NADPH oxidase in cardiac myocytes (125). The formation of mixed disulfides between GSH and cysteine SH residues, leading to RyR1 *S*-glutathionylation, can be induced *in vitro* by incubation with GSH plus H_2O_2 (7). Moreover, *in vitro* activation of NADPH oxidase increases both RyR2 *S*-glutathionylation and the Ca^{2+}-release activity of SR-enriched cardiac vesicles, suggesting that these changes are produced by NADPH oxidase-dependent ROS generation (108). Cardiac RyR2, which are endogenously *S*-glutathionylated, increase their *S*-glutathionylation levels after tachycardia-induced preconditioning such that enhanced RyR2 *S*-glutathionylation represents an important mechanism to sustain faster rates of Ca^{2+} release *in vivo* in response to increased cardiac activity (108). It remains to be studied whether the stimulatory effect of *S*-glutathionylation on Ca^{2+} release is due to activation of the RyR2 protein itself or to modifications of inhibitory interactions with RyR2-associated proteins, as shown for RyR1 (7).

Further, redox reactions indirectly regulate excitation–transcription coupling. Part of the released Ca^{2+} might be diverted to elicit excitation–transcription coupling according to a simple pathway given in Figure 2. Thus, membrane depolarization was shown to activate the GATA4 transcription factor only when the cells are pretreated with H_2O_2 (91).

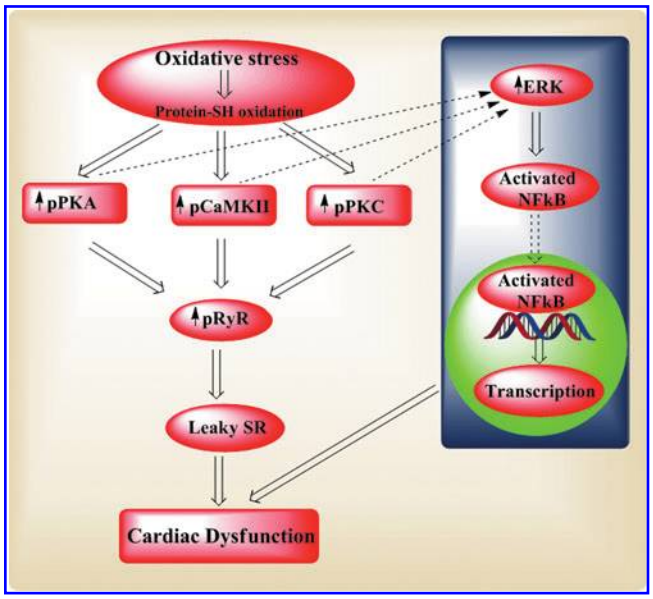

FIG. 2. Possible mechanisms that link diabetes with cardiovascular remodeling. Hyperglycemia determines an overproduction of superoxide by the mitochondrial electron transport chain and favors increased expression of NADPH. Consequently, hyperglycemia results in overproduction of superoxide and nitric oxide. Both lead to increased oxidative stress in the cells, associated with protein-SH oxidation. Increased oxidative stress in cardiomyocytes can lead to increases in the phosphorylation levels of both PKA and CaMKII as well as activation and then translocalization of protein kinase C (PKC) into sarcolemma. All three kinases cause hyperphosphorylation of RyR2. This results in leaky SR and finally depressed cardiac contractility. Activation of these three kinases *via* phosphorylation and protein-SH oxidation influences signaling molecules such as extracellular-regulated kinase (ERK) and NF-κB, and then transcription in the cell. These redox-sensitive processes contribute to cardiovascular damage and remodeling in diabetes. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Benzothiazepine derivatives as pharmacological modulators of RyR2

Modulation of cardiomyocyte Ca^{2+} handling by RyR2 is long known to occur by caffeine and tetracaine, which increase RyR2 open probability (55, 117). More recently, flecainide was reported to prevent catecholamine polymorphic ventricular tachycardia as a result of decreasing RyR2 conductance and RyR2 open time (61, 138). However, these observations made on SR vesicles containing the RyR2 complex do not clarify the precise mechanisms leading to alterations in RyR2 behavior.

JTV519, today known as K201, is a 1,4-benzothiazepine derivative that has been found to be more effective than other Ca^{2+} channel antagonists at reducing Ca^{2+}-induced myocardial damage. It was initially shown to improve contractility and prevent the development of HF in paced dog heart probably by preventing dissociation of FKBP12.6 from RyR2 (154). JTV519 treatment increased calstabin binding to PKA-hyperphosphorylated RyR2 and cardiac function in dogs with pacing-induced HF (149) and as well in calstabin2$^{-/-}$- and PDE4$^{-/-}$-deficient mice (140, 154). Using surface plasmon resonance (Biacore) to measure equilibrium binding kinetics, it is shown that PKA-dependent phosphorylation or K201 application in the closed RyR conformation reduced the affinity for FKBP12/12.6. Ryanodine binding showed the open conformational state to be unchanged by phosphorylation but reduced by K201, the latter effect being independent of FKBP12/12.6 binding (17).

K201 was also proposed to reverse domain unzipping and reduce Ca^{2+} leak (92). The K201-binding site was recently identified at domain 2114–2149 of RyR2. K201 reverses the mode of interdomain interaction from defective unzipped to normal zipped configuration and stops Ca^{2+} leak without requiring rebinding of FKBP12.6 to RyR2 in dog HF (152). The authors further suggest that K201 primarily modifies domain–domain interaction and by establishing a stable conformation it then favors FKBP12.6 binding to RyR2.

In Duchenne Muscular Dystrophy, dystrophin deficiency leads to abnormal intracellular Ca^{2+} homeostasis in both cardiac and skeletal muscles. The NO/cGMP signaling pathway exhibits early signs of alteration before any overt evidence of cardiomyopathy. RyR2s from these hearts were S-nitrosylated and depleted of FKBP12.6, resulting in leaky

RyR2 channels and a diastolic SR-Ca^{2+} leak. Inhibiting the depletion of calstabin2 from the RyR2 complex with the Ca^{2+} channel stabilizer S107 (rycal), a novel RyR2-specific benzothiazepine derivative compound, inhibited the SR-Ca^{2+} leak and prevented arrhythmias *in vivo* (40). Similarly, S107, which binds to RyR1 and recovers the binding of calstabin-1 to the nitrosylated channel, inhibits SR-Ca^{2+} leak, improves muscle function, and increases exercise performance in these dystrophic-deficient mice (13).

Moreover, JTV519 exhibits multiple other cellular effects, including inhibition of SR-Ca^{2+} uptake (5, 47), whereas during ischemia, the JTV519-dependent attenuation of the large decrease in ATP content was prevented by NOS inhibitors (72).

RyR2-Dysfunction in Diabetic Cardiomyopathy

Diabetes-induced cardiac dysfunction is characterized by a decrease in myocardial function independent of vascular disease. Alterations in Ca^{2+} signaling within the cardiac muscle cells have been a hallmark of diabetic cardiomyopathy. The defects identified in the mechanical activity of the hearts from type 1 diabetic animals are attributed to a decrease in systolic $[Ca^{2+}]_i$ and a lengthening of the systolic $[Ca^{2+}]_i$ transient that result primarily from dysfunction of the SR (26, 73), besides a reduction in L-type Ca^{2+} current (98). Data regarding Ca^{2+} handling in type 2 diabetes are limited and ambiguous. For example, a rat model of insulin-resistant obese type 2 diabetes (cp/cp) shows enhanced SR-Ca^{2+} uptake, but SERCA2a levels and SR-Ca^{2+} load are unaltered (90). By contrast, other well-known models of type 2 diabetes such as Otsuka Long Evans Tokushima Fatty rats (1) and *db/db* mice (12) present defects in both function and expression of SERCA2a. The amplitude of caffeine-releasable Ca^{2+} is also lowered in diabetic myocytes as a result of a reduced SERCA2a (74). A phenotypic rescue with SERCA2 by gene remodeling in type 2 diabetic cardiomyopathy was recently reported (70).

Altered Ca^{2+} homeostasis may also result from a dysfunction of RyR2, and leads to an altered Ca^{2+}-induced Ca^{2+} release. In an early study it was reported that non-cross-linking advanced glycation end products on RyR2 increase with chronic diabetes, and that formation of these post-translational complexes, nonenzymatic glycation products, was minimized with insulin treatment (16). Further studies in cardiomyocytes isolated from STZ-induced diabetic rats demonstrated that the altered spatio-temporal properties of the Ca^{2+} spark, representative of a single, or a small cluster of RyR2 channels opening, were parallel to the changes of Ca^{2+} transients, and these findings were associated to the hyperphosphorylated level of RyR2 and a depleted level of total FKBP12.6 (112, 159). Pereira *et al.* (98) studying Ca^{2+}-induced Ca^{2+}-release and E-C coupling in *db/db* obese type 2 diabetic mice found that Ca^{2+} sparks were less frequent than in +/+ myocytes, partly because of a depression of SR-Ca^{2+} load but also because of a reduced expression of RyR2 revealed by [^{3}H]ryanodine binding assay. Interestingly, residual functional RyR2 from diabetic rat hearts exhibited increased sensitivity to Ca^{2+} activation leading to a twofold increase in spontaneous Ca^{2+}-spark frequency (112). Altogether, RyR2 becomes leaky during diabetes and this defect may be responsible to the reduced SR-Ca^{2+} load. Further, diastolic Ca^{2+} release could also serve as a substrate for delayed afterdepolarizations, contributing to the increased incidence of arrhythmias and sudden cardiac death in type 1 diabetes.

Gender Difference and RyR2 in Diabetic Rats

It has long been recognized that incidence and prevalence of certain diseases vary with sex. Differences exist between women and men in the impact of risk factors, symptoms, and therapeutic responses (11). Cardiovascular diseases are the leading cause of death in adult women in developed countries (11, 68). In the Framingham Heart Study, diabetes increased the prevalence of congestive HF more in women than in men and with a greater difference in younger than in 65-year-old patients (69). Reduced antioxidant activity and increased oxidative stress occur early after the diagnosis of type 1 diabetes, specifically in women accounting for an increased susceptibility of diabetic women to cardiovascular complications (85). The greater effect of diabetes in women compared to men as a risk factor for congestive HF is in agreement with a greater contribution of diabetes to arteriosclerosis in women (103). Estrogen is long known to have cardioprotective effect (89), in part because it increases the release of NO from vascular endothelial cells by regulating the mitochondrial respiratory chain and thus the redox status (23). Recently, White *et al.* (141) proposed that estrogen is neither good nor bad, but simply stimulates NOS activity. It is the biochemical environment around NOS that will determine whether estrogen produces a beneficial (NO) or deleterious (superoxide) product, and can account for vasodilatation in young or vasoconstriction in aged, whereas L-arginine decreases with aging.

Animal studies also reveal sex differences in cardiac performance and responses to pathological conditions (57). However, differences in age, heart size, physiologic status, and other factors confound comparisons leading to variable and conflicting conclusions (110). Reports on sex difference in both electrical and mechanical cardiac activities in control conditions are rather subtle but suggest a tendency for greater Ca^{2+} fluxes in females. Female rats, compared to male, demonstrated a weak enhanced contractile activity that could be, in most part, accounted for by a slower time course of decay of the $[Ca^{2+}]_i$ transient, whereas peak $[Ca^{2+}]_i$ transient was similar (158). Spatio-temporal parameters of Ca^{2+} sparks in cardiomyocytes isolated from control females were significantly larger and slower than in control males (158). This was associated with a larger content in both RyR2 and FKBP12.6 proteins. The present data are in line with the study of Chu *et al.* (27) that showed higher RyR2 protein and mRNA levels as well as higher Na^+/Ca^{2+} exchanger and Ca^{2+} channel protein levels in females than matched males. Diabetes reduced RyR2 phosphorylation and FKBP12.6 content in females in agreement with a lesser diabetes-induced depression of Ca^{2+} sparks amplitude such that the sex differences were more marked in diabetes (158). Our data can demonstrate that these differences can be attenuated significantly after estrogen treatment of the cells isolated from male rat heart (Fig. 3). Shimoni and colleagues had proposed that thiol oxidation was less in female diabetic animals accounting for lesser variations in K^+ currents (114, 115). This could be due to cytoprotective effects of estrogen related to its antioxidant properties (21, 121).

These observations should be compared with the report that disruption of the FKBP12.6 gene results in cardiac hypertrophy in male mice, but not in females (147). Female FKBP12.6-null mice treated with tamoxifen, an estrogen receptor antagonist, developed cardiac hypertrophy similar to that of male mice. The authors concluded that FKBP12.6 modulates cardiac E-C coupling and that estrogen plays a protective role in the hypertrophic response of the heart to Ca^{2+} dysregulation (147).

Therapeutic Approaches to RyR2-Dysfunction in Diabetic Cardiomyopathy

General overview

Diabetic cardiomyopathy, characterized by cardiac hypertrophy and contractile dysfunction, eventually leads to HF. Oxidative stress, being an imbalance between endogenous ROS and antioxidant systems in favor of the former, is involved in the etiology of diabetes-induced downregulation of heart function. Moreover, there is a close relationship between impaired insulin signaling and alteration in heart function *via* depressed endogenous antioxidant defense mechanism. Contractile dysfunction and abnormal Ca^{2+} handling in the diabetic heart may also result from the activation of humoral systems. Large clinical trials suggest excessive neurohumoral stimulation as a central mechanism in the pathogenesis of diabetic heart disease (35, 77).

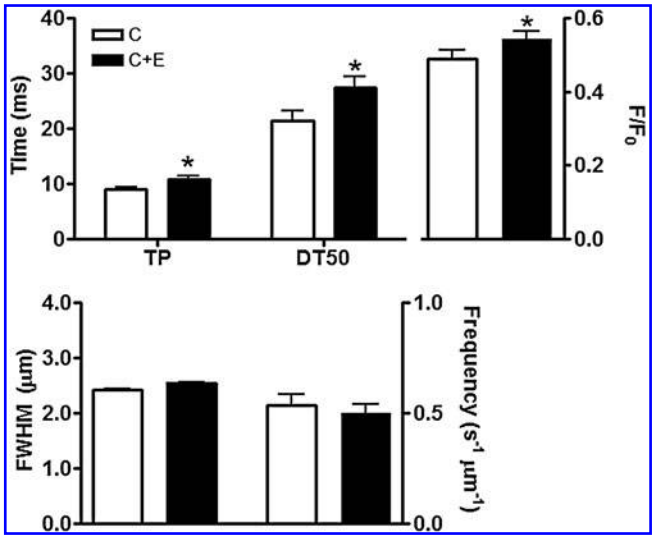

FIG. 3. Effect of 17β-oestradiol on the spatio-temporal parameters of local Ca^{2+} release in male cardiomyocytes. Previously, we reported a net difference in local Ca^{2+} release mechanisms between female and male cardiomyocytes (158). By being less susceptible to Ca^{2+} overload and demonstrating less increased PKC level, female rats have an advantage over the males probably due to their higher estrogen level. In the following experiments, we incubated male cardiomyocytes (C) with 1 μ*M* 17β-oestradiol (C+E) and then analyzed the spatio-temporal parameters of the Ca^{2+} sparks. Although there were no significant differences in Ca^{2+} current of both genders, the amplitude and time course of the Ca^{2+} sparks were bigger than those of the matching males and closely associated with higher protein levels of both RyR2 and FKBP12.6. Therefore, our data demonstrate that estrogen application attenuates the sex differences in Ca^{2+} sparks characteristics, in line with the known phenomena on gender-dependent differences in heart function. *$p < 0.05$ compared to C.

Oxidative stress, antioxidants, and RyR2 in diabetic cardiac dysfunction

As mentioned above, RyR2s are subject to modifications by oxidizing and reducing agents capable of interacting with thiol-containing cysteine residues of the channel protein. In physiological conditions, endogenous oxidizing agents such as ROS are continuously generated in the heart and are controlling tonic influences on intracellular targets, including SR-Ca^{2+} release, but this system turns to induce modification in RyR2 by increased ROS in pathological conditions. The imbalance between oxidant–antioxidant in the cells causes abnormal Ca^{2+} handling, and consequently heart dysfunction in disease states.

Come up documents show that although hyperglycemia, hyperlipidemia, and inflammatory cytokines give rise to the pathogenesis of diabetic cardiovascular dysfunction due to different mechanisms, all these pathogenic effects are related to oxidative stress (21, 36, 56, 97). Diabetes not only causes increased ROS production but also impairs antioxidant capacity in the heart (8, 28, 48, 95, 131). In the late stages of diabetes, the increased xanthine dehydrogenase and decreased selenium GSH peroxidase as well as Cu^{2+}/Zn^{2+} superoxide dismutase activity lead to enhanced oxidative stress in the heart resulting in secondary organ damage associated with the disease (4). Decreased levels of antioxidant enzymes in the diabetic system were found to make the cardiovascular system being susceptible to oxidative damage (8, 21, 95, 131). Moreover, oxidative stress has been shown to increase the activity of transcription factors such as AP-1 and NF-κB (123).

Antioxidants generally function by scavenging ROS or reducing their generation in pathological conditions. Protective role of antioxidants in diabetes-induced cardiac dysfunction has been recently reviewed (133). However, data related to RyR2 are rather scarce.

Enhanced oxidative stress activates matrix metalloproteinases (MMPs) a group of Zn^{2+}-dependent neutral endopeptidases (136, 137). MMPs are widely expressed and MMP-2 is found in all cardiac cells, including cardiomyocytes. The role of pro-oxidant species in MMP-2 activation and subsequent loss of contractile function was shown by the ability of either the peroxynitrite scavenger GSH or inhibitors of MMP activity to prevent contractile dysfunction (25, 136, 137). Doxycycline, the most potent MMP inhibitor of the tetracycline class of antibiotics, was shown to be protective in several models of myocardial oxidative stress injury (25, 109, 136, 137). In cardiomyocytes isolated from doxycycline-treated diabetic rats, the altered kinetic parameters of Ca^{2+} transients, depressed SR-Ca^{2+} loading and basal intracellular Ca^{2+} level, and the spatio-temporal properties of Ca^{2+} sparks were significantly restored (157).

Trace elements such as selenium and zinc might act as antioxidant. Antioxidant micronutrients are one of the body's primary defenses against oxidants (8). Selenium has beneficial effects on glucose metabolism (14). In STZ-induced diabetic rats, sodium selenite restored the altered electrical and mechanical dysfunctions of diabetic rat heart partially by restoring the cell GSH redox cycle (8, 10, 131). We also

demonstrate that sodium selenate administration to the diabetic rats for 4 weeks also reduced the oxidized protein sulfhydryl and nitrite concentrations *via* reducing MMP-2 activation and therefore reducing the degradation of two of its target proteins, troponin I, and α-actinin (10). This treatment induced also a marked normalization in the phosphorylation level of RyR2 (Turan *et al.*, unpublished observations). Similar effects could be foreseen with the application of omega-3E, which, like sodium selenite, improved diabetes-induced changes in myocardial levels of MMP-2 and tissue inhibitor of MMP-4 (TIMP-4) (10). Despite that omega-3E caused significant restorations in diabetes-induced altered activities of antioxidant enzymes without gender-related differences, omega-3E treatment caused more significant recovery in the depressed rates of changes of developed pressure in diabetic male rats compared to female (128).

Zinc supplementation in diabetic mice significantly induced cardiac metallothionein (MT) expression, along with a significant prevention of the development of diabetic cardiomyopathy, suggesting that induction of systemic MT synthesis may be a potential approach to preventing diabetes-induced complications in multiple organs. Thus, cell survival rate was significantly decreased after exposure to high levels of glucose and free fatty acid (palmitate), but did not change for cells pretreated with Zn^{2+}, which induces significant MT synthesis (135). Free Zn^{2+} is greatly increased by thiol-reactive oxidants and may contribute to oxidant-induced alterations of E-C coupling (130). Zinc is also known to induce CaMKII autophosphorylation and to inhibit protein tyrosine phosphatases and might alter RyR2 phosphorylation level. However, a specific effect of Zn^{2+} on RyR2 activity has yet to be investigated.

Hyperglycemia and high serum free fatty acid levels contribute to the pathological adaptations in diabetes and share overproduction of ROS by the mitochondrial electron transport chain. Long-chain saturated fatty acids, such as palmitate, induce dissipation of mitochondrial potential and increased ROS production considered to control apoptosis (74). Similarly, palmitate impairs Ca^{2+} handling; it decreases the Ca^{2+} transient and the SR-Ca^{2+} load in WT cardiomyocytes, although RyR2 activity was not specifically estimated. Many studies reported an increased fatty acid oxidation associated with a marked reduction of glycolysis and glucose oxidation in obesity and type 2 diabetes (18). In contrast to the situation in WT cardiomyocytes, palmitate application did not affect mitochondrial potential or ROS production, whereas it accelerates Ca^{2+} transient in *ob/ob* cells, suggesting enhanced RyR2 activity. Accordingly, antioxidants had no effects on palmitate-induced changes in Ca^{2+}-handling and contraction in *ob/ob* cells (38).

Exercise training has clear beneficial effects over various physiopathological states. Exercise is shown to counteract the adverse effects of diabetes and readapt mitochondrial control of ROS production by increasing the expression of several proteins, including peroxisome proliferator activated receptor-gamma coactivator-1α, and restore the low UCP3 expression in skeletal muscle (79). Interval training in a db/db mouse model of diabetic cardiomyopathy restores contractile function associated with restored SR-Ca^{2+} release synchronicity, twitch amplitude, and SR-Ca^{2+} leak. The latter effect was associated with reduced phosphorylation of CaMKII (122). Exercise training during diabetes normalizes RyR2 function and Ca^{2+} release from the SR, whereas PKA activity was reduced by 75%, but CaMKII activity was increased by 50% (113).

Neurohumoral alterations and RyR2 in diabetic heart

Marked increase in the upregulation of the cardiac RAS has been demonstrated in diabetic heart (42, 81). Activation of the RAS, and subsequent signaling through the AT1 receptors, contribute to the development of diabetic cardiomyopathy in part by increasing oxidative stress (100). Ang II *via* AT1 receptor stimulation is suggested to increase the production of free oxygen radicals (52, 102) and to activate NADPH oxidase enzymes that may generate superoxide anions such as $O_2^{\bullet -}$ in various tissues, including heart (101, 102, 106). AT1 receptors activate protein kinase C (PKC) as was also found in diabetic heart (19, 81). Upregulation of RAS in diabetic heart was further associated with elevated myocyte death (42).

Angiotensin-converting enzyme (ACE) inhibitors and Ang II-receptor blockers improve cardiovascular and all-cause mortality outcomes in patients with diabetes to a greater degree than in nondiabetics (30). In rat cardiomyocytes, AT1 blockade by candesartan restores action potential duration, transient outward K^+ current, I_{to} amplitude, Ca^{2+} homeostasis including Ca^{2+} transients kinetics, SR-Ca^{2+} load, spatio-temporal properties of Ca^{2+} sparks, and basal Ca^{2+} level, as well as the β-adrenergic-mediated enhancement of glucose uptake (96, 156). These effects were associated with a reduction of the Ang II-induced increased PKC level and oxidized protein–thiol level in membrane fraction of diabetic rat heart (156). Of note, incubating diabetic cardiomyocytes with the nonspecific PKC inhibitor bisindolylmaleimide had similar restoring effects (156).

Gassanov *et al.* (46) performed a series of *in vitro* experiments in a human atrial myocyte study by incubation of Ang II and/or AT1 receptor blocker candesartan. They showed that the frequency of Ca^{2+} sparks was markedly increased by Ang II-incubation, as observed in diabetic cardiomyocytes. These changes were normalized following to candesartan application. Although the state of RyR2 was not investigated in these experiments, the results provide evidence that Ang II-induced alterations of Ca^{2+} handling and electrophysiological changes are similar to those previously observed in the pathological diabetic heart.

Recent evidence has indicated the possible importance of the endothelin (ET) system in the pathogenesis of diabetic complications. In hearts from STZ-induced diabetes, mRNA abundance and protein expression for ET1 and ET receptors were elevated (60). In these animals, ET-receptor blockade by bosentan limited the rise in blood pressure and antagonized myocardial contractile depression (134). Basal left ventricular systolic contractility was lower in diabetic compared to non-diabetic hearts and ET-receptor or ACE-I blockers significantly antagonized the decline. Either treatment reduced hydroxyproline (an index of tissue fibrosis) and malondialdehyde (a measure of tissue oxidative stress) to control level (143). However, in this case also, the precise alterations of RyR2 remain to be elucidated.

In cardiac tissue, many of the neurohumoral peptides like Ang II, ET, or norepinephrine bind to receptors that are coupled to the Gq-proteins, $G\alpha_{11}$ and $G\alpha_q$. Recently, Hoyer *et al.* (62) hypothesized also left ventricular hypertrophy and abnormalities in diabetic heart may be mediated by a signaling pathway

involving $G\alpha_{11}$ and/or $G\alpha_q$, which are coexpressed in almost all cell types and are primarily responsible for coupling receptors to β-isoforms of phospholipase C (PLCβ). Their data suggest a signaling pathway *via* the Gq-proteins, $G\alpha_{11}$ and $G\alpha_q$, that could link increased neurohumoral stimulation in STZ-induced diabetes with defective RyR2 channel function by reducing protein expression of FKBP12.6 and annexin A7. In $G\alpha_{11}$ and $G\alpha_q$-knockout animals, SR-Ca^{2+} release and cardiac phenotype remained unchanged upon induction of diabetes.

Concluding Remarks

Improvements in the treatment of noncardiac complications from diabetes have resulted in heart disease becoming a leading cause of death in diabetic patients. Several cardiovascular pathological consequences of diabetes such as hypertension affect the heart to varying degrees. Moreover, hyperglycemia, as an independent risk factor, directly causes cardiac damage and leads to diabetic cardiomyopathy. Most of the therapies are directed to develop a prevention of diabetes-induced organ damage whether or not *via* under controlled hyperglycemia.

In all tissues, unregulated or deficient Ca^{2+} signaling is the most common step leading to deleterious cellular outcomes. The fundamental mechanisms leading to defective RyR2 function have been the matter of numerous studies underlying various cardiac dysfunctions, including diabetic cardiomyopthy. Hypothetical pathways summarizing major underlying mechanisms leading to cardiovascular dysfunction in diabetic subjects are given in Figure 4. A reasonable conclusion seems to be that several factors produce synergistic effects on SR-Ca^{2+} leak or triggered SR-Ca^{2+} release after a change in RyR2 protein conformation. They include hyperphosphorylation, domain unzipping, redox modification, hypernitrosylation, and *S*-glutathionylation. This is generally associated with FKBP12.6 dissociation or a reduced FKBP12.6 content. It is intriguing to note that the 1,4-benzothiazepine derivative drug JTV519/K201, which demonstrates clear beneficial effects in HF and in most studies on defective RyR2-induced arrhythmia, has been used to support each of these hypothetical mechanisms. Nevertheless, the recent identification of the K201-binding site on RyR might help to identifying new therapeutic agents. In diabetic heart dysfunction, ACE inhibitors, β-adrenergic inhibitors, antioxidants,

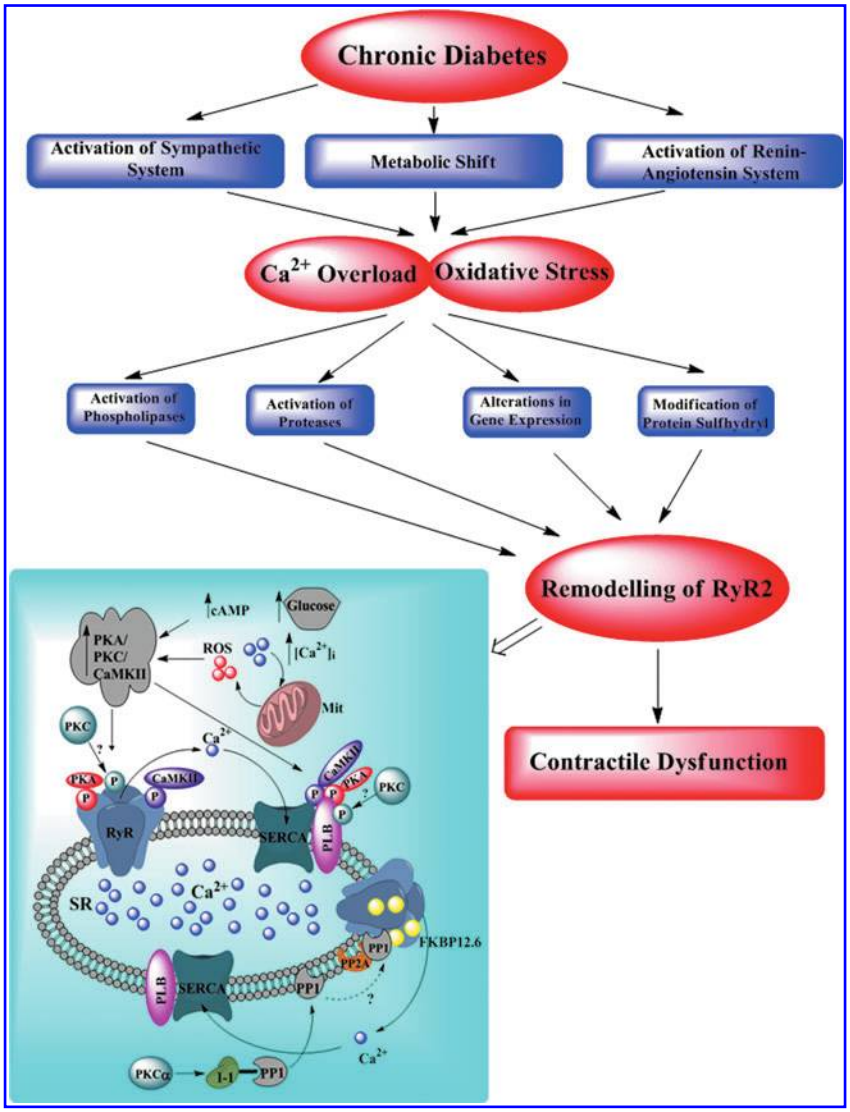

FIG. 4. Hypothetical pathways summarizing some underlying mechanisms leading to cardiovascular dysfunction in diabetic subjects. Several lines of evidence suggest a possible role of oxidative stress in the pathogenesis of diabetic complications. Thus, increased oxidative stress and increased intracellular free Ca^{2+} concentration (Ca^{2+} overload) appear to be overlapping for the initiation of further altered mechanisms into cardiomyocytes. Different signaling molecules that play roles in the remodeling of RyR2 due to hyperglycemia are given in *inset* part of the diagram as hypothetically, in part due to our published data. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

and doxycycline all demonstrate therapeutic effects most generally attributable to better Ca^{2+} handling and normalization of the status of RyR2 complex. Exercise training has various site effects and is currently recommended in HF. Recent studies demonstrate similar beneficial effects of exercise in diabetes. Whatever the blood glucose level in diabetic subjects, whatever also the precise mechanisms leading to alterations in the RyR2 complex, controlling the interactions RyR2-FKBP12.6 is an important candidate target for pharmaceutical prevention of cardiac insufficiency and ventricular arrhythmia during diabetic cardiomyopathy. The site(s) of redox-induced alterations in the RyR2 and its structural consequences should be the matter of important further studies.

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Abbreviations Used

ACE = angiotension-converting enzyme
Ang II = angiotensin II
AT1 = angiotensin II receptor type 1
CaM = calmodulin
CaMKII = Ca^{2+}/calmodulin-dependent protein kinase II
$[Ca^{2+}]_i$ = cytosolic-free calcium ion concentration
DHPR = dihydropyridine receptor
E-C = excitation-contraction
ERK = extracellular-regulated kinase
ET = endothelin
FKBP = FK506-binding immunophilin protein
Glut4 = glucose transporter protein 4
GSH = glutathione
HF = heart failure
H_2O_2 = hydrogen peroxide
I_{Ca} = calcium current
miR- = microRNA subtypes
miRNA = microRNA
MMP = matrix metalloproteinases
MT = metallothionein
NO = nitric oxide
PDE = phosphodiesterase
PKA = protein kinase A
PKC = protein kinase C
PLB = phospholamban
PLC = phospholipase C
PP1 = protein phosphatases 1
PP2 = protein phosphatases 2A
RAS = renin angiotensin system
ROS = reactive oxygen species
RyR = sarcoplasmic reticulum calcium ion release channel
RyR2 = cardiac RyR
SERCA2a = sarco-/endoplasmic reticulum calcium ATPase
SH = thiol group
SR = sarcoplasmic reticulum
STZ = streptozotocin
TIMP = tissue inhibitor of MMPs
WT = wild type

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