

# Cardiac ryanodine receptor in metabolic syndrome: is JTV519 (K201) future therapy?

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**Abstract:** Metabolic syndrome is characterized by a combination of obesity, hypertension, insulin resistance, dyslipidemia, and impaired glucose tolerance. This multifaceted syndrome is often accompanied by a hyperdynamic circulatory state characterized by increased blood pressure, total blood volume, cardiac output, and metabolic tissue demand. Experimental, epidemiological, and clinical studies have demonstrated that patients with metabolic syndrome have significantly elevated cardiovascular morbidity and mortality rates. One of the main and frequent complications seen in metabolic syndrome is cardiovascular disease. The primary endpoints of cardiometabolic risk are coronary and peripheral arterial disease, myocardial infarction, congestive heart failure, arrhythmia, and stroke. Alterations in expression and/or functioning of several key proteins involved in regulating and maintaining ionic homeostasis can cause cardiac disturbances. One such group of proteins is known as ryanodine receptors (intracellular calcium release channels), which are the major channels through which  $\text{Ca}^{2+}$  ions leave the sarcoplasmic reticulum, leading to cardiac muscle contraction. The economic cost of metabolic syndrome and its associated complications has a significant effect on health care budgets. Improvements in body weight, blood lipid profile, and hyperglycemia can reduce cardiometabolic risk. However, constant hyperadrenergic stimulation still contributes to the burden of disease. Normalization of the hyperdynamic circulatory state with conventional therapies is the most reasonable therapeutic strategy to date. JTV519 (K201) is a newly developed 1,4-benzothiazepine drug with antiarrhythmic and cardioprotective properties. It appears to be very effective in not only preventing but also in reversing the characteristic myocardial changes and preventing lethal arrhythmias. It is also a unique candidate to improve diastolic heart failure in metabolic syndrome.

**Keywords:** ryanodine receptors, metabolic syndrome, JTV519, K201

## Metabolic syndrome

Metabolic syndrome is characterized by a combination of obesity, hypertension, insulin resistance, dyslipidemia, and impaired glucose tolerance.<sup>1</sup> The mechanisms responsible appear to be multifactorial, and include family history, physical inactivity, and a sedentary lifestyle. Key market players spend millions of dollars developing new therapeutic strategies against components of metabolic syndrome and its related complications. The challenge in this area is that the emerging therapeutic agents seem not to be very effective in treating obesity and insulin resistance or reducing further cardiometabolic risk.<sup>2</sup> This multifaceted syndrome is often accompanied by a hyperdynamic circulatory state characterized by increased blood pressure, total blood volume, cardiac output, and metabolic tissue demand.<sup>3-10</sup> Hypertension generally

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amplifies the high cardiovascular risk if the disease remains uncontrolled for a long time.<sup>11–15</sup>

Experimental, epidemiological, and clinical studies have demonstrated that patients with metabolic syndrome have significantly elevated cardiovascular morbidity and mortality.<sup>10–17</sup> Hypertension and changes in heart rate generally appear early on, with the risk of developing coronary artery disease, arteriosclerosis, and heart failure increasing at a later stage.<sup>3–10,16</sup> In the end, making a decision regarding “which aggravates which first” is complicated. All these assessments aim to generate the best treatment modalities which provide a better health care strategy in a cost-effective manner. The basic therapeutic approach still focuses on decreasing body weight (adipose tissue mass) and hepatic fat deposition. Diet, exercise, and lifestyle modification are out of professional control in most cases, because they depend on the patient’s intellectual capacity and their economic situation. Coping with all these strategies requires good patient monitoring with conventional and/or new therapeutic agents.

Newly identified compounds should aid in the management of body weight with improvement in blood glucose in patients with diabetes.<sup>18</sup> Randomized controlled clinical trials have shown that exenatide, a glucagon-like peptide analog, is effective in reducing glycemic events and assisting with beneficial weight loss.<sup>2,19</sup> Nausea is a known side effect of this drug, along with rare cases of pancreatitis, but its side effect profile still lies within the acceptable range.<sup>19</sup> The other newer agents, ie, the dipeptidyl peptidase-4 (DPP-4) inhibitors (sitagliptin and vildagliptin) combined with metformin, glitazone, and sulfonylurea in clinical trials have been shown to be very effective for blood glucose control.<sup>2,18,20</sup> The trials indicate that these agents are associated with fewer hypoglycemia episodes and less weight gain, and that they are also an effective intervention to decrease obesity. On the other hand, less information is available about whether they have any beneficial effects on cardiovascular complications. However, some knowledge already exists showing that they reduce glycosylated hemoglobin (HbA<sub>1c</sub>) levels by a couple of percentage points and decrease hyperglycemic/hypoglycemic episodes, especially nocturnal ones.<sup>18,20</sup> However, further data are required to assess their long-term efficacy and tolerability in clinical trials to determine their exact cardiovascular benefits in metabolic syndrome.

## Cardiovascular dysfunction in metabolic syndrome

Obesity and metabolic syndrome can cause cardiovascular complications.<sup>1,3–10,13,16,21–24</sup> The underlying molecular

mechanisms responsible could be related to the development of a hyperdynamic circulatory state, which may trigger a variety of cardiac and hemodynamic changes. To date, from metabolic syndrome through diabetes, a new treatment paradigm is emerging. To cope with the complications associated with metabolic syndrome, early diagnosis of the disease is essential. Despite an increase in the diagnosed/undiagnosed ratio in recent years, they have to treat with new updated therapeutic guidelines to protect against complications.<sup>21,23–25</sup> Under these circumstances, a poor diet secondary to a sedentary lifestyle without appropriate treatment increases cardiometabolic risk. The basic treatment strategies still appear as simple as ever, and include control of body weight, blood pressure management, and normalization of blood lipids, with maintenance of normoglycemia. The link between metabolic syndrome and cardiovascular risk is clear, but the underlying molecular mechanisms need to be investigated to assist our treatment decisions.

The main consequences of the cardiometabolic risk profile are coronary and peripheral arterial disease, myocardial infarction, congestive heart failure, arrhythmia, and stroke.<sup>10</sup> However, because overweight and obesity are independent risk factors, insulin resistance and lipid disturbances appear to be integral components of the disease.<sup>10,14,18</sup> Reduced HbA<sub>1c</sub>, low-density lipoprotein cholesterol and triglycerides and increased high-density lipoproteins are already well known to lower cardiometabolic risk.

## Cardiac ryanodine receptors

Alterations in the expression and/or function of several key proteins involved in regulating and maintaining ionic homeostasis can cause cardiac disturbances. One such group of proteins is called the ryanodine receptors (RyRs), which are a component of the intracellular Ca<sup>2+</sup> release channels located in the membrane of the sarcoplasmic reticulum.<sup>4,26,27</sup> Three isoforms have been identified in the human heart, and are referred to as type 1, 2, and 3 (RyR<sub>1</sub>, RyR<sub>2</sub>, and RyR<sub>3</sub>, respectively).<sup>27,28</sup> Type 2 ryanodine receptors are the major release channels through which Ca<sup>2+</sup> leaves the sarcoplasmic reticulum and leads to cardiac muscle contraction.<sup>28</sup> Rapid depolarization of the sarcoplasmic reticulum membrane in the heart by Ca<sup>2+</sup> via the RyR<sub>2</sub> receptor is an important step in cardiac contractility. However, the physiological role of the RyR<sub>1</sub> and RyR<sub>3</sub> isoforms also expressed in the human heart remains unclear.<sup>27</sup>

RyR<sub>2</sub> is a homotetramer comprising four 565 kDa monomers, each containing a transmembrane segment.<sup>28</sup> The RyR<sub>2</sub>

receptor normally closes at low cytosolic  $\text{Ca}^{2+}$  levels during diastole. However, submicromolar cytosolic  $\text{Ca}^{2+}$  levels increase the probability of the channel being open with high-affinity binding sites available.<sup>4,28–32</sup> A plant alkaloid ryanodine isolate, *Ryania speciosa*, found in central and South America, locks the  $\text{RyR}_2$  channel in a subconductance state and induces paralysis, and was previously tested as an insecticide.<sup>28</sup> Subsequently, ryanodine was used to purify  $\text{RyR}_2$  from the sarcoplasmic reticulum as a high-affinity ligand.<sup>28,33</sup> Beyond that, no therapeutic indication has been identified as yet which would warrant its development as a pharmaceutical product.

The calcium channel binding protein, FKBP12.6 (12.6 kDa FK506-binding protein) binds to  $\text{RyR}_2$  and stabilizes the closed state of the channel, and has recently been renamed as calstabin 2.<sup>34–36</sup> Dissociation of FKBP12.6 from  $\text{RyR}_2$  increases the open probability of the channel.<sup>28</sup> Furthermore, genetic deletion of calstabin 2 enhances  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum and causes leaky  $\text{RyR}_2$ .<sup>28</sup> Protein kinase A and calcium/calmodulin dependent kinase A (CaMKII) phosphorylate serine 2809 (Ser<sup>2809</sup>) and Ser<sup>2814</sup>, respectively, on  $\text{RyR}_2$ .<sup>37–39</sup> However, many different phosphorylation sites (Ser<sup>2808</sup>, Ser<sup>2809</sup>, Ser<sup>2814</sup>, Ser<sup>2815</sup>, and Ser<sup>2030</sup>) have now been reported for both these enzymes across different species.<sup>37–39</sup> Phosphorylation of  $\text{RyR}_2$  by protein kinase A causes increased sensitivity of  $\text{RyR}_2$  to intracellular  $\text{Ca}^{2+}$ , dissociation of calstabin 2 from the channel, and enhanced  $\text{RyR}_2$  activity,<sup>40</sup> leading to an increase in the open probability of the channel. Recent evidence has demonstrated that nitrosylation and/or oxidation of  $\text{RyR}_2$  also alters the binding affinity of calstabin 2, which affects channel activity.<sup>41,42</sup> CaMKII is also a holoenzyme which sensitizes the channel to cytosolic  $\text{Ca}^{2+}$  and increases the open probability of the channel, but does not dissociate calstabin 2 from the channel.<sup>39</sup>

Other modulatory proteins, ie, phosphodiesterase 4D3 (PDE4D3), calmodulin, protein phosphatase 1 and 2a, and sorcin, modulate the N terminal cytoplasmic domain of  $\text{RyR}_2$ , which includes calstabin 2, protein kinase A, and CaMKII.<sup>28</sup> PDE4D3 binds to kinase-anchoring protein, which is part of the  $\text{RyR}_2$  macromolecular complex and degrades cyclic adenosine monophosphate (cAMP). Protein kinase A also binds kinase-anchoring protein to regulate local cAMP levels near the channel, as does PDE4D3. Protein phosphatase 1 and 2a dephosphorylate phosphorylated channels via spinophilin and PR130, respectively, and indirectly regulate channel activity. Protein phosphatase 1 predominantly dephosphorylates Ser<sup>2808</sup> and Ser<sup>2809</sup>, while protein phosphatase 2a dephosphorylates Ser<sup>2814</sup>. Many of these modulatory proteins

can prevent or ameliorate the probability of the channel being open, thereby contributing directly to cardiac contraction.<sup>28</sup>

Nevertheless, calmodulin, a 17 kDa protein, assists in the closing state of  $\text{RyR}_2$ , binding 3583–3603 amino acids after  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum during excitation-contraction coupling, which basically inhibits  $\text{RyR}_2$ .<sup>43–45</sup> Sorcin, a 22 kDa  $\text{Ca}^{2+}$  binding protein, also reduces the open probability of  $\text{RyR}_2$ , which decreases the amplitude of  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum without affecting L-type  $\text{Ca}^{2+}$  current when  $\text{Ca}^{2+}$  levels are elevated.<sup>46,47</sup> Calsequestrin, junctin, and triadin are modulatory proteins related to the C terminus which form a complex with  $\text{RyR}_2$  in the lumen of the sarcoplasmic reticulum.<sup>28,48</sup> Calsequestrin sequesters  $\text{Ca}^{2+}$  in the sarcoplasmic reticulum, causing an increase in the open probability of  $\text{RyR}_2$ .<sup>28</sup> Triadin and junctin link calsequestrin to the channel and normalize the  $\text{Ca}^{2+}$  load in the sarcoplasmic reticulum.<sup>28</sup> Current strategies for the management of cardiac disturbances do not focus directly on  $\text{RyR}_2$  and no relevant molecule has been approved as yet anywhere in the world. Several attempts at developing  $\text{RyR}_2$  as a therapeutic molecule have been made in the past, but were unable to demonstrate efficacy and safety.

## Ryanodine receptor dysfunction in metabolic syndrome

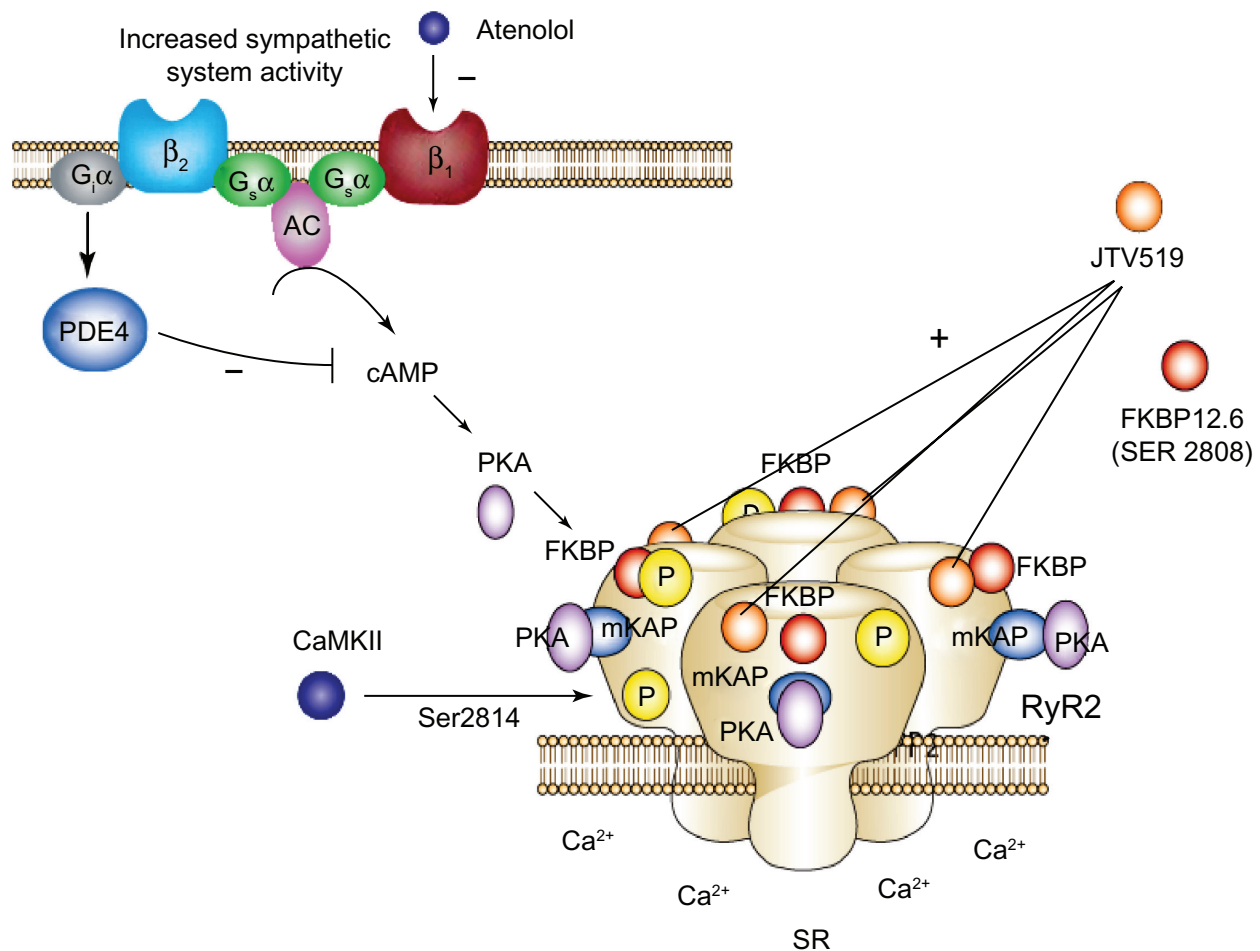
Obesity and its associated comorbidities affect almost one third of the population of the western world, and their prevalence tends to increase each year. The economic cost of metabolic syndrome and its complications have a significant impact on health care budgets worldwide. The most common complications seen in metabolic syndrome are cardiovascular in nature, and include arrhythmias, hypertension, and coronary artery disease. While improvements in body weight, blood lipid profile, and hyperglycemia can reduce the cardiometabolic risk, constant hyperadrenergic stimulation still contributes to the burden of the disease.

One of the mechanisms underpinning the increased cardiometabolic risk in metabolic syndrome is impaired  $\text{Ca}^{2+}$  storage in the sarcoplasmic reticulum and diastolic  $\text{Ca}^{2+}$  leak during hypersympathetic stimulation.<sup>4,16</sup> It is assumed that treatment should aim to restore the normal hyperphosphorylation state involving excitation-contraction coupling proteins, such as  $\text{RyR}_2$ .<sup>3,4,9,16</sup> Many laboratories have found basal catecholamine levels to be elevated in metabolic syndrome.<sup>3,4,9,10,12,16,25,49</sup> We have also previously demonstrated that induction of metabolic syndrome by chronic high-fat feeding increases circulating plasma epinephrine levels by 55% and norepinephrine levels by 31%.<sup>3</sup>

Chronic activation of the sympathetic nervous system, as occurs in metabolic syndrome, leads to increased protein kinase A-mediated phosphorylation of RyR<sub>2</sub> at Ser<sup>2809</sup> and Ser<sup>2808</sup> (see Figure 1).<sup>3-5,16</sup> Recent experimental evidence suggests that chronic hyperphosphorylation of RyR<sub>2</sub> contributes to impaired contraction, generation of lethal ventricular arrhythmias, and development of heart failure via leaky RyR<sub>2</sub> channels.<sup>50</sup>

We have recently demonstrated that the functional integrity of RyR<sub>2</sub> is compromised by metabolic syndrome, as evidenced by augmented RyR<sub>2</sub> phosphorylation together with diminished RyR<sub>2</sub> binding affinity.<sup>4</sup> RyR<sub>2</sub> Ser<sup>2809</sup> and RyR<sub>2</sub> Ser<sup>2808</sup> phosphorylation sites have been used widely as an index of RyR<sub>2</sub> phosphorylation by protein kinase

A. Other studies have demonstrated that CaMKII also phosphorylates the RyR<sub>2</sub> (Ser<sup>2814</sup> or Ser<sup>2815</sup>) molecule, and that CaMKII can activate RyR<sub>2</sub> gating but not at protein kinase A (Ser<sup>2808</sup> or Ser<sup>2809</sup>) sites, preventing dissociation of FKBP12.6 from RyR<sub>2</sub> (see Figure 1). Protein kinases C and G can also phosphorylate the RyR<sub>2</sub> molecule.<sup>28,39,40,51-53</sup> Protein kinase C-mediated and protein kinase G-mediated pathways are mainly responsible for phosphorylation of the Ser<sup>2030</sup> RyR<sub>2</sub> molecule, which can also be phosphorylated by protein kinase A but is certainly a poor substrate for CaMKII.<sup>28,35,51,54</sup> Increased sympathetic nervous system activity could be a mechanism by which metabolic syndrome leads to impaired RyR<sub>2</sub> function. The possibility of phosphorylation of RyR<sub>2</sub> sites by different pathways in



**Figure 1** Schematic diagram of RyR<sub>2</sub> dysfunction in metabolic syndrome. Cardiac RyR<sub>2</sub> dysfunction seen in metabolic syndrome (MetS) could be related to the cAMP/PKA-dependent pathway under constant hyper adrenergic stimulation. Increased Ser<sup>2809</sup> phosphorylation of cardiac RyR<sub>2</sub> in MetS is possibly mediated by PKA activation. Increase in circulating catecholamine stimulates G-protein-coupled  $\beta$ -ARs thereby activating intracellular cyclic adenosine monophosphate (cAMP) and PKA. JTV-519 (K201) increases binding affinity of FKBP12.6 to RyR<sub>2</sub>, which stabilizes the closes state of RyR<sub>2</sub> channels and prevents Ca<sup>2+</sup> leak which protects from ventricular arrhythmias, contractile dysfunction and reduce Ca<sup>2+</sup> overload.

**Abbreviations:** SR, sarcoplasmic reticulum;  $\beta_1$ -AR, adrenoceptor;  $\beta_2$ -AR, adrenoceptor; G<sub>s</sub> $\alpha$ , stimulatory protein G alpha; G<sub>i</sub> $\alpha$ , Inhibitory protein alpha; AC, adenylyl cyclase; PDE4, phosphodiesterase 4; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; mKAP, a kinase anchoring protein; CaMKII, calcium/calmodulin dependent kinase II; Ser 2808, Serin<sup>2808</sup>; Ser 2814, Serine<sup>2814</sup>.

different species has not been clearly defined as yet, and needs to be investigated.

The cardiac RyR<sub>2</sub> dysfunction seen in metabolic syndrome could be related to a cAMP/protein kinase A-dependent pathway under constant hyperadrenergic stimulation (see Figure 1).<sup>2-5,16</sup> Increased Ser<sup>2809</sup> phosphorylation of cardiac RyR<sub>2</sub> in metabolic syndrome is possibly mediated by activation of protein kinase A, whereby there is an increase in circulating catecholamines which stimulate G protein-coupled  $\beta$ -adrenoceptors, thereby activating intracellular cAMP and protein kinase A (see Figure 1). This hypothesis is supported by earlier studies in which chronic sympathetic activation resulted in protein kinase A-mediated hyperphosphorylation of RyR<sub>2</sub>.<sup>28,54,55</sup> Earlier studies indicate that constant sympathetic nervous system activity can switch the predominant cardiac signaling pathway from protein kinase A to CaMKII.<sup>28,37,51,56</sup> Whether metabolic syndrome alters signaling pathway through the protein kinase A or CaMKII is presently unknown; however, Xiao et al recently demonstrated that constant  $\beta$ -adrenoreceptor stimulation switches the signaling pathway from being predominantly protein kinase A-driven to being mainly CaMKII-driven.<sup>50,53</sup>

We have also demonstrated that metabolic syndrome compromises only the functional integrity of the cardiac RyR<sub>2</sub> receptor without downregulation, as evidenced by diminished binding affinity and no change in mRNA or protein expression.<sup>4</sup> Changes in modulatory proteins that could also contribute to impaired functional integrity of RyR<sub>2</sub> include altered dissociation of FKBP12.6 and/or PDE4D3 deficiency. However, whether these modulatory proteins contribute to impaired functional integrity of RyR<sub>2</sub> in metabolic syndrome is unknown.

The majority of patients with metabolic syndrome have absolute or relative insulin deficiency (diabetes), including hyperinsulinemia, dyslipidemia, and dysregulation of the renin-angiotensin system. These parameters compromise the molecular levels of myocytes, including RyR<sub>2</sub> and sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase. Direct and indirect effects of glucotoxicity trigger overproduction of reactive oxygen species, poly(ADP-ribose) polymerase, and advanced glycation end-products, and induce apoptosis.<sup>57</sup> They cause altered expression and function of RyR<sub>2</sub> via post-translational modifications of extracellular matrix components, which contribute to systolic and diastolic dysfunction.<sup>57,58</sup> Hyperglycemia causes two types of post-translation modifications on RyR<sub>2</sub> and sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase; first, it increases production of reactive oxygen species

(superoxide anions, hydroxyl radicals, lipid peroxides, hydrogen peroxide) and, second, it increases reactive nitrogen species (nitrosonium cation, nitroxyl anion, peroxynitrite).<sup>32,59</sup> These free radical and nonradical species alter the tertiary structure of RyR<sub>2</sub>, causing RyR<sub>2</sub> to become sensitive to endogenous ligands, such as Ca<sup>2+</sup> and adenosine triphosphate (ATP).<sup>32,59</sup> We have previously demonstrated that advanced glycation end-products are formed on intracellular RyR<sub>2</sub> during diabetes; however, treatment with insulin minimizes these nonenzymatic products, which attenuate protein activity.<sup>32</sup> RyR<sub>2</sub> is a large transmembrane and long-lived protein (approximately eight days). It localizes in the sarcoplasmic reticulum and plays a critical role in cardiac excitation-contraction coupling. When advanced glycation end-product complexes attenuate the RyR<sub>2</sub> protein, the changes are permanent.<sup>31,32,59</sup> For that reason, an oral hypoglycemic or insulin treatment-focused therapeutic strategy is not able to restore cardiac contraction.

Dyslipidemia is the other major component of metabolic syndrome and characterized by increased circulating fatty acids and triglycerides,<sup>57</sup> causing cardiac lipotoxicity and accumulation of fatty acids in cardiomyocytes which, in turn, leads to increased shortening of the action potential in the K-ATP channel and dysregulation of the open probability in the RyR<sub>2</sub> channel. It also causes diminished cycling of the L-type Ca<sup>2+</sup> channel and reduction of Ca<sup>2+</sup> stores in the sarcoplasmic reticulum.<sup>57</sup> Chronic lipid accumulation in cardiomyocytes also contributes to apoptosis via inhibition of the mitochondrial respiratory chain if fatty acids are not completely metabolized.<sup>57,60</sup> However, intracardiac fatty acid accumulation enhances oxygen demand and generation of reactive oxygen species, and diminishes ATP synthesis because of mitochondrial dysfunction.<sup>60</sup> In the normal subject, the heart obtains energy for cardiac contractility from fatty acid oxidation, but in a hyperinsulinemic state, the need for myocardial glucose decreases significantly and shifts fatty acid synthesis to the  $\beta$ -oxidation pathway, causing secondary dysregulation of open probability of the RyR<sub>2</sub> channel.<sup>61</sup>

The chronic hyperinsulinemia seen in metabolic syndrome also activates the sympathetic nervous system and the renin-angiotensin system indirectly through crosstalk between insulin-dependent signaling and cardiac growth pathways, via similar common elements in their molecules.<sup>57</sup> Insulin activates a signaling cascade with neurohormonal growth agonists, eg, insulin-like growth factor 1 and angiotensin II, which coordinate cell growth and protein synthesis

activated by extracellular signal-regulated kinase, and the phosphoinositol-3 kinase/protein-kinase B cascade associated with physiological cardiac hypertrophy.<sup>62</sup> Both these pathways also activate the sympathetic nervous system and the renin-angiotensin system via trigger adrenergic and AT<sub>1</sub> receptors, as well as the angiotensin II pathway in the hyperinsulinemic state.<sup>57</sup> Constant hyperadrenergic stimulation further activates intracellular cAMP and protein kinase A, thereby causing hyperphosphorylation of RyR<sub>2</sub> and mild to moderate leaky channels (see Figure 1).<sup>28,35,54,55</sup> Decreasing the binding affinity of FKBP12.6 to RyR<sub>2</sub> increases the probability of open RyR<sub>2</sub> channels, and Ca<sup>2+</sup> leak is seen during diastole. Ventricular arrhythmias, contractile dysfunction, and Ca<sup>2+</sup> overload is triggered as part of that pathology.<sup>35,37,54,55,57</sup>

Cardiac hypertrophy and heart failure have been reported in patients with hypertension, diabetes, insulin resistance, and obesity, and increased sympathetic nervous system and renin-angiotensin system activity is well demonstrated in these patients. Indeed, the main complexity in therapeutic disadvantages is the indirect contribution of chronic hyperinsulinemia to already overstimulated adrenergic and angiotensin II pathways, and requires long-term follow-up studies. Intensive glucose control has demonstrated an indirect correlation with decreased cardiovascular complications, including hypertension, myocardial infarction, and stroke. This clearly answers the important question of which comes first, ie, whether overinsulinization triggers the central nervous system indirectly or whether the central nervous system could operate as a main driver of hyperinsulinemia.

Dysregulation of intracellular Ca<sup>2+</sup> homeostasis has been reported previously in situations of abnormal membrane lipid content, glucotoxicity, and hyperinsulinemia, as well as in hyperadrenergic states.<sup>49,57,58</sup> Alterations in the expression and function of sarco(endo)plasmic reticulum Ca<sup>2+</sup>-ATPase, the Na-K-ATPase Na<sup>+</sup>/Ca<sup>2+</sup> exchanger, and RyR<sub>2</sub> have been identified in these patient groups, and associated with hypertension, type 1 and 2 diabetes mellitus, obesity, and dyslipidemia.<sup>28,57,58</sup> Although metabolic syndrome does not always coexist with type 2 diabetes, obesity alone can increase the risk of developing type 2 diabetes and this in turn increases diabetes-associated cardiovascular complications.<sup>49</sup>

## Future therapeutic concepts

Until recent years, normalization of the hyperdynamic circulatory state in metabolic syndrome using conventional

therapies, such as adrenergic receptor blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers, was the most reasonable therapeutic strategy (see Figure 1).<sup>2,4,5</sup> Other important aims were to reduce body weight, increase exercise capacity, restore a normal plasma lipid profile, and improve metabolic biomarkers. However, in spite of normalizing these parameters, cardiometabolic risk remains mostly irreversible if the disease continues in the long term.<sup>2-5</sup> Cardioselective β<sub>1</sub>-adrenoceptor blockers control heart rate and aortic pressure, and decrease the cardiac index, especially during exercise (Figure 1). We recently documented that basal catecholamine levels are elevated in metabolic syndrome.<sup>3</sup> It is very clear that adrenergic receptor blockers can normalize the increased sympathetic nervous system activity, thereby at least being able to protect the RyR<sub>2</sub> channel against Ca<sup>2+</sup> leak during diastole.

The β<sub>1</sub>-adrenoceptor blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers suppress the hyperadrenergic state, thereby restoring the stoichiometry of the RyR<sub>2</sub> channel. Many studies, including CONSENSUS, SOLVD-T, Val-HeFT, and CHARM, have demonstrated that angiotensin receptor blockers are more effective than angiotensin-converting enzyme inhibitors in congestive heart failure.<sup>63-65</sup> In the Val-HeFT trial, angiotensin receptor blockers reduced the heart failure hospitalization rate when added to conventional therapy, including an angiotensin-converting enzyme inhibitor in most patients, but had no effect on mortality.<sup>66,67</sup> The CHARM trial demonstrated that an angiotensin receptor blocker reduced morbidity and mortality as a result of reduced systolic function with or without combination with an angiotensin-converting enzyme inhibitor,<sup>66,67</sup> indicating that this treatment strategy is beneficial and preserves left ventricular systolic function. Adrenergic receptor blockers and angiotensin-converting enzyme inhibitors were compared head-to-head in patients with congestive heart failure in clinical trials such as ELITE I and ELITE II.<sup>66,67</sup> There have been some clinical trials of an angiotensin-converting enzyme inhibitor combined with digoxin, diuretics (SOLD-T), and a β<sub>1</sub>-adrenoceptor blocker (CIBIS-2).<sup>63,64,66,67</sup> One-year mortality-related improvement in survival in patients with congestive heart failure was found to be better in patients who received combination angiotensin receptor blocker and angiotensin-converting enzyme inhibitor therapy, eg, in the CHARM-Added trial (diuretic + digoxin + angiotensin-converting enzyme inhibitor + β<sub>1</sub>-adrenoceptor blocker + angiotensin receptor blocker)

if compared with SOLD-T (diuretic + digoxin + angiotensin-converting enzyme inhibitor) and CIBIS-2 (diuretic + digoxin + angiotensin-converting enzyme inhibitor +  $\beta_1$ -adrenoceptor blocker).<sup>65</sup> Overall, the clinical trials demonstrated that combination of an adrenergic receptor blocker + angiotensin-converting enzyme inhibitor produced better outcomes for patients with congestive heart failure, in terms of cardiovascular mortality risk and hospital admissions.

These treatment regimes can restore sympathovagal balance and can reverse remodeling in the failing heart, as evidenced by a significant reduction in left ventricular volume and an improved contractile response.<sup>63–65,67</sup> The  $\beta_1$ -adrenoceptor blockers failed to meet the necessary safety profile in metabolic syndrome and diabetes because they can affect glycogenolysis, interfere with insulin release, further impair glucose tolerance, and increase serum triglycerides.<sup>68</sup> For these reasons, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors or combinations of these agents fit better in the treatment regime.<sup>66,67</sup> In fact, these algorithms could be used in clinical trials of metabolic syndrome to explore the most beneficial pharmaceutical strategies.

JTV519 (K201) is a promising, newly developed 1,4-benzothiazepine drug and a nonspecific blocker of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  channels, with antiarrhythmic and cardioprotective properties.<sup>69</sup> Like diltiazem, JTV519 blocks the L-type  $\text{Ca}^{2+}$  current, but is not classified as a  $\text{Ca}^{2+}$  channel blocker.<sup>69,70</sup> Rather than acting as a  $\beta$ -adrenoceptor blocker, it blocks  $\alpha_1$ -receptors and intracellular  $\text{Ca}^{2+}$  pathways.<sup>69,70</sup> It is a relatively nonselective blocker of cation currents, including the  $\text{Na}^+$  current ( $I_{\text{NA}}$ ) in a voltage-dependent and frequency-dependent manner.<sup>71,72</sup> The time course of  $\text{Na}^+$  current blockade with JTV519 is slower than with lidocaine, similar to quinidine, and is believed to have intermediate rather than fast kinetics.<sup>70</sup> It also blocks the inward rectifying  $\text{K}^+$  current ( $I_{\text{K1}}$ ) and rapidly activating component of the delayed rectified  $\text{K}^+$  current ( $I_{\text{Kr}}$ ), but not the slowly activating component ( $I_{\text{Ks}}$ ).<sup>73</sup> JTV519 blocks the  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) and muscarinic acetylcholine receptor-operated  $\text{K}^+$  current ( $I_{\text{KAch}}$ ).<sup>70,73</sup>

JTV519 stabilizes the closed state of  $\text{RyR}_2$  and increases the binding affinity of FKBP12.6 for  $\text{RyR}_2$  (see Figure 1), thereby reducing and preventing  $\text{Ca}^{2+}$  leak, and protecting against ventricular arrhythmia, contractile dysfunction, and  $\text{Ca}^{2+}$  overload.<sup>2,16,26,69,74</sup> This beneficial combination of activity dramatically ameliorates the progression of heart failure as a result of myocardial damage resulting from  $\text{Ca}^{2+}$  overload. The most recent evidence shows that JTV519 protects against

$\text{Ca}^{2+}$  leak from the sarcoplasmic reticulum independent of the interaction between FKBP12.6 and  $\text{RyR}_2$ .<sup>75</sup> Spontaneous sarcoplasmic reticulum  $\text{Ca}^{2+}$  release (leak) also occurs when the  $\text{Ca}^{2+}$  content in the sarcoplasmic reticulum reaches a threshold level, ie, overload of  $\text{Ca}^{2+}$  stores in the sarcoplasmic reticulum,<sup>75</sup> and is known as store overload-induced  $\text{Ca}^{2+}$  release (SOICR), which is independent of FKBP12.6 binding to  $\text{RyR}_2$ .<sup>70,75</sup> SOICR causes delayed after depolarizations and, in turn, arrhythmias, such as those seen in catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia type 2.<sup>70,75</sup>

Since 2000, JTV519 has been investigated in Phase II trials for its ability to protect against acute myocardial infarction, and is suggested to reduce reperfusion injury following percutaneous transluminal coronary angioplasty and has been shown to be protective in models of ischemia-reperfusion injury and heart failure.<sup>76</sup> Stabilization of  $\text{RyR}_2$  reduces detrimental intracellular  $\text{Ca}^{2+}$  leak and improves both diastolic and systolic contractile function in the human heart with or without an FKBP12.6- $\text{RyR}_2$  binding interaction.<sup>75</sup> It has also been shown that inhibition of SOICR by JTV519 could be a sophisticated treatment strategy for catecholamine-induced or inherited forms of cardiac arrhythmia (catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia type 2).<sup>77</sup> JTV519 prevents myocardial infarction and sudden cardiac death.<sup>69,70</sup> It provides more effective myocardial protection than calcium channel blockers and  $\beta_1$ -adrenoceptor blockers. JTV519 also has fewer negative inotropic and chronotropic effects,<sup>69,70</sup> and, in particular, prevents myocardial injury caused by ischemia and catecholamines to a greater extent than do nicorandil, prazosin, propranolol, verapamil, and diltiazem.<sup>69,70</sup>

Metabolic syndrome is associated with a hyperdynamic circulatory state characterized by increased blood pressure, total blood volume, cardiac output, and metabolic tissue demand.<sup>78,79</sup> Many laboratories have demonstrated that basal catecholamine levels are elevated in metabolic syndrome.<sup>3,4,9,10,12,16,25,49</sup> We have also previously shown that induction of metabolic syndrome by chronic high-fat feeding increases circulatory plasma epinephrine levels by 55% and norepinephrine levels by 31%.<sup>3</sup> The hyperadrenergic state seen in metabolic syndrome could trigger lethal arrhythmias and myocardial ischemia, especially during exercise.<sup>3–5</sup>

JTV519 is a promising new drug for possible use in the treatment of the cardiovascular disturbances associated with metabolic syndrome. It is a cardioprotective agent against

ischemia and hyperadrenergic states for two important reasons; first of all, it can not only protect against but also reverse myocardial damage, and second, it has fewer inotropic and chronotropic effects than the calcium channel blockers and cardioselective  $\beta$ -adrenoceptor blockers.<sup>70</sup> JTV519 stabilizes the closed state of RyR<sub>2</sub>, is thought to increase the binding affinity of FKBP12.6 for RyR<sub>2</sub>, and protects cardiomyocytes against SOICR, which is independent of the FKBP12.6-RyR<sub>2</sub> interaction.<sup>70,75</sup>

JTV519 blocks cardiac I<sub>NA</sub>, I<sub>K1</sub>, I<sub>Kr</sub>, I<sub>Ca</sub> and I<sub>KAch</sub><sup>70</sup> and inhibits I<sub>KAch</sub> and I<sub>Kr</sub> in atrial muscle cells, which are potential channels for atrial fibrillation.<sup>73,80</sup> It also inhibits I<sub>KAch</sub> in the atrial appendage which generates atrial fibrillation, mostly in the sinus node.<sup>73,80</sup> JTV519 also inhibits ventricular tachycardia and fibrillation,<sup>70</sup> and causes prolongation of the QT intervals. It suppresses torsades de pointes in a dose-dependent manner by blocking  $\alpha_1$ -adrenoreceptors and inhibiting abnormal Ca<sup>2+</sup> release from the sarcoplasmic reticulum.<sup>70</sup> SOICR may also cause delayed after depolarizations and contribute to development of ventricular arrhythmias.<sup>75</sup> Therefore, JTV519 appears to be a very good candidate as an antiarrhythmic agent in the treatment of atrial and ventricular arrhythmias in patients with metabolic syndrome, especially during exercise.

Another clinical application of JTV519 could be in diastolic heart failure. To date, the exact etiology of diastolic heart failure is unknown and there are no drugs available to treat it. JTV519 could be a good choice because it normalizes left ventricular end-diastolic pressure, protects against the effects of catecholamines, and restores aortic valve opening in the diastolic phase.<sup>69,70</sup> Some treatment approaches available for systolic heart failure include digitalis, cyclic nucleotide phosphodiesterase inhibitors (milrinone, inamrinone), angiotensin antagonists (angiotensin-converting inhibitors and receptor blockers),  $\beta$ -adrenoceptor agonists, and  $\alpha$ -adrenoreceptor antagonists.<sup>69,70</sup>

One of the main complications seen in metabolic syndrome is cardiovascular disturbance, ie, hypertension, arrhythmias, coronary artery disease, and heart failure. Metabolic syndrome is associated with a hyperdynamic circulatory state characterized by hypertension and increased cardiac output, with an enhanced cardiometabolic risk profile.<sup>4,5,16</sup> JTV519 stabilizes the closed state of RyR<sub>2</sub> and increases the binding affinity of FKBP12.6 to RyR<sub>2</sub>.<sup>70</sup> It also protects cardiomyocytes against SOICR, which is independent of the FKBP12.6-RyR<sub>2</sub> interaction,<sup>75</sup> protects against stress-induced cardiomyopathy,<sup>69,70</sup> and prevents and restores myocardial injury more effectively than do the  $\beta$ -adrenoceptor blockers,

Ca<sup>2+</sup> channel blockers,  $\alpha$ -adrenoreceptor antagonists, and vasodilator agents.<sup>70</sup> JTV519 appears to be the most effective in not only preventing but also reversing myocardial alterations caused by ischemia and catecholamines, protecting against lethal arrhythmias, and improving diastolic heart failure.<sup>70</sup> It could be a future therapeutic strategy to target the arrhythmias, myocardial ischemia, myocardial infarction, and diastolic heart failure seen in metabolic syndrome.

## Abbreviations

MetS, Metabolic syndrome; CMR, cardio metabolic risk; GLP-I, glucagon like peptide; DPP-4, dipeptidyl peptidase-4 inhibitor; CVS, cardiovascular system; CAD, coronary arterial disease; PAD, peripheral arterial disease; MI, myocardial infarction; CHF, congestive heart failure; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA<sub>1c</sub>, glycated hemoglobin A<sub>1c</sub>; FAs, fatty acids; TGs, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoproteins; RyRs, ryanodine receptors; RyR<sub>1</sub>; RyR<sub>2</sub> and RyR<sub>3</sub>, ryanodine receptor type 1; 2 and 3; SR, sarcoplasmic reticulum; SNS, sympathetic nervous system; RAS, renin angiotensin system;  $\beta_1$ -AR,  $\beta_1$ -Adrenoreceptor;  $\beta_2$ -AR,  $\beta_2$ -Adrenoreceptor;  $\alpha_1$ -AR,  $\alpha_1$ -Adrenoreceptor; G<sub>s</sub> $\alpha$ , stimulatory protein G alpha; G<sub>i</sub> $\alpha$ , inhibitory protein G alpha; AC, adenylyl cyclase; K 201, JTV 519; PDE4, phosphodiesterase 4; FKBP 12.6; calstabin 2, phosphodiesterase 4D3 binding protein 12.6; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; mKAP, kinase anchoring protein; EC, excitation contraction; CaMKII, calcium/calmodulin dependent kinase II; Ser<sub>2808</sub>, serin 2808; Ser<sub>2814</sub>, serine 2814; PDE4D3, phosphodiesterase 4D3; CaM, calmodulin; RAS, renin angiotensin system; PP1 and PP2 respectively, protein phosphatases 1 and 2A; ROS, reactive oxygen species; PARP, Poly ADP-ribose, polymerase; AGEs, advanced glycation end products; SERCA, sarco endo, plasmic reticulum Ca<sup>++</sup>-ATPase; K-ATP, potassium adenosine three phosphate; IGF-1, insulin like growth factor – 1; ERK, extracellular signal regulated kinase; PI3K, phospho inositol three kinase; PKB, protein kinase B; CNS, central nervous system; T2DM, type 2 diabetes; SOICR, store overload induced Ca<sup>++</sup> release; Ang II, angiotensin II; ACE, angiotensin converting enzyme; ACE-I, angiotensin converting enzyme inhibitors; ARB, Angiotensin II receptor blocker; SOICR, store overload induced Ca<sup>++</sup> release; DAD, delayed after depolarization; CPVT, catecholaminergic polymorphic ventricular tachycardia; ARVD2, arrhythmogenic right ventricular dysplasia type 2.



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