Trends in Endocrinology & Metabolism

Review

Reactive Carbonyl Species: Diabetic Complication in the Heart and Lungs

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Abnormal chemical reactions in hyperglycemia alter normal metabolic processes in diabetes, which is a key process in the production of reactive carbonyls species (RCS). Increasing the concentration of RCS may result in carbonyl/oxidative stress in both the diabetic heart and lung. Ryanodine receptors (RyRs) not only play a key role in heart contraction, including rhythmic contraction and relaxation of the heart, but they are also important for controlling the airway smooth muscle. RCS modifies RyRs, resulting in RyRs dysfunction, which is involved in important mechanisms in diabetic complications. Very little is known about the mechanistic relationship between the heart and lung in diabetes. This review highlights new findings on the pathophysiological mechanisms and discusses potential approaches to treatment for these complications.

Highlights

Hyperglycemia alters the normal metabolic process, which is also a key process in the production of reactive carbonyls, hyperglycemia-induced levels of carbonyl stress, advanced glycation end products (AGEs), and oxidative stress.

RyR2 plays a critical role in relaying Ca^{2+} signals in many biological processes, not only of cardiac myocytes, but also in the airway smooth muscle.

RCS modifies RyR2 proteins, resulting in dysfunction of RyR2 in both the heart and lung in diabetes.

Understanding the relationship between RCS and modified RyR2 in both the heart and lung in diabetes may be useful in treatment of diabetes complications.

Introduction

Diabetes mellitus (DM) is a metabolic disorder of glucose metabolism in combination with **hyperglycemia** (see Glossary) that results in dysfunction in the cell's ability to transport and utilize glucose. Type 1DM (T1DM) is caused by T lymphocyte-mediated autoimmune destruction of the pancreatic β-cells, resulting in insufficient insulin production and subsequent decrease in glucose utilization. Type 2 DM (T2DM) results from an insulin resistance that instigates hypertrophy of the β-cell to compensate, resulting in hyperinsulinemia leading to eventual insulin resistance. This decreases the amount of insulin produced, resulting in pathophysiological changes that produce elevated blood glucose levels. In chronic hyperglycemia, comorbidities include some long-term complications, such as cardiovascular and **pulmonary complications of DM** [1–5]. DM, both T1DM as well as T2DM, can involve diabetes-associated changes in the structure and function of the myocardium, leading to **heart failure**. Clinically, **diabetic cardiomyopathy** is defined as ventricular dysfunction that occurs independently of coronary artery disease and hypertension (i.e., diabetic cardiomyopathy was initially classified as a dilated cardiomyopathy with prominent left ventricular enlargement and depressed systolic function) [6–8]. Diabetic lung mainly occurs because the alveolar capillary system is characterized by a great microvascular reserve and pulmonary abnormalities, including lung volume, pulmonary diffusing capacity, control of ventilation, bronchomotor tone, and neuroadrenergic bronchial innervation, are commonly subclinical in the diabetic patient. The loss of microvascular reserve in the lung may become clinically important, with increased risk of hypoxia in the case of acute or chronic pathological lung conditions, including pneumonia, chronic obstructive pulmonary disease (COPD), and asthma [9,10]. The lung is rich in microvascular circulation and abundant connective tissue, which raises the possibility of lung affection by microangiopathic process and nonenzymatic glycosylation of tissue proteins [11],

Excessive hyperglycemia stimulates an increase in an imbalance between oxidative and antioxidative statuses within cells and tissues, which leads to an increase in **reactive carbonyl species (RCS)** [12,13]. **Ryanodine receptor** 2 (RyR2) has been a major focus of cardiac complications in diabetes due to RyR2 dysfunction in diabetic cardiomyopathy [8,14]. In addition, RyR2 is

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expressed in **airway smooth muscle (ASM)** cells. Therefore, RyR2 has a key role in both pulmonary complications and diabetic cardiomyopathy in diabetes [15,16]. The pathophysiology of diabetes has highlighted the involvement of glucose metabolism disorders with hyperglycemia, and a consequence of this can be an increased concentration of RCS. Reactive carbonyls are widespread species in living organisms in diabetes; chemical modification of RyR2 proteins by RCS results in mutagenicity [17]. Understanding the relationship between metabolism of RCS and RyR2 in the development of pathological diabetes should help us find an approach to preventing cardiac and pulmonary complications in diabetes. Thus, using RCS and RyR2 as the focal point, we aim to provide an updated discussion of the pathophysiological relationship between the diabetic heart and lung, and how RCS can modulate RyR2 in different biological processes relevant to cardiovascular and pulmonary complications of DM.

Properties of RCS

RCS include a large number of biological compounds with one or more carbonyl groups. They are a widespread species found in living organisms and are mainly known for their damaging effects [18]. The most abundant RCS are derived from oxidation of carbohydrates, lipids, and amino acids. RCS concentrations in the body may have a varying range since the cellular environment constantly changes. An increased level of RCS is a key cause of carbonyl stress caused by factors, such as aging, diabetes, chronic complications associated with diabetes, and other disorders. The most widespread biological reactive carbonyls generated during peroxidation of lipids, nonenzymatic glycation, enzymatic polyol pathway, enzymatic glycation, oxidation of amino acids *in vivo,* and peroxidation of lipids include: malondialdehyde (MDA), 4-hydroxy-trans-2-nonenal (HNE), 4-oxo-trans-2-nonenal, glyoxal, methylglyoxal, acrolein, crotonaldehyde, and hexanal. Nonenzymatic glycation includes glyoxal, methylglyoxal, glucosone, 3-deoxyglucosone, and acrolein. The enzymatic polyol pathway includes 3-deoxyglucosone and 3-deoxyfructose. Enzymatic glycation includes acetaldehyde, glyceraldehyde-3-phosphate, dioxyacetone phosphate, and methylglyoxal. Oxidation of amino acids includes glyoxal, methylglyoxal, acrolein, glycolaldehyde, and 2-hydroxypropanal [19–21]. Figure 1 and Table 1 show the most common saturated and unsaturated RCS detected in living organisms.

RCS in DM

DM is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. The metabolic disorder results when abnormal chemical reactions in the hyperglycemic body alter normal metabolic processes, which is also critical in the production of reactive carbonyls. This increases the concentration of RCS, which may result in **carbonyl/oxidative stress** [17,22]. For example, hyperglycemia-induced cell damage is a consequence of increased flux through metabolic pathways [polyol pathway flux, advanced glycation-end product formation, activation of protein kinase C (PKC) isoforms, and increased hexosamine pathway flux] [5]. Other exogenous sources of RCS are products of organic pharmaceutical chemistry, cigarette smoke, food additives, and browned food [20]. This increases the concentration of RCS, which may result in carbonyl/oxidative stress. The most abundant RCS is derived from glucose metabolism disorders in combination with hyperglycemia. Excessive hyperglycemia is a result of an imbalance between oxidative and antioxidative statuses within cells and tissues, which leads to an increase in RCS [23]. The most important class of RCS are derived from peroxidation of polyunsaturated fatty acids in disease pathogenesis [24]. In DM, RCS from lipid peroxidation include mono-aldehydes, alkenals, bifunctional alkenals, 1, 2-dicarbonyls, and 1, 4-dicarbonyls.

As shown in Figure 1 and Table 1 RCS can be classified into three groups: (i) α, β-unsaturated aldehydes (HNE and acrolein), (ii) keto-aldehydes (methylglyoxal), and (iii) dialdehydes (glyoxal and MDA). Furthermore, RCS plays a key role in the pathology of diabetes and its complications,

Glossary

Airway smooth muscle (ASM): an important tissue involved in the regulation of bronchomotor tone; it exists in the trachea and in the bronchial tree, up to the terminal bronchioles.

Atrial fibrillation: one of the most common types of arrhythmias, which are irregular heart rhythms. Atrial fibrillation causes the heart to beat much faster than normal and the upper and lower chambers of the heart do not work together. When this happens, the lower chambers do not fill completely or pump enough blood to the lungs and body. This can make you feel tired or dizzy, or you may notice heart palpitations or chest pain.

Carbonyl/oxidative stress: reactive carbonyls are mainly known for their damaging effects. The most abundant RCS are derived from oxidation of carbohydrates, lipids, and amino acids, and chemical modification of proteins and nucleic acids results in cytotoxicity and mutagenicity.

Catecholaminergic polymorphic ventricular tachycardia (CPVT): an inherited arrhythmia syndrome. It is caused most commonly by abnormal control of calcium movement in cardiac muscle cells. Individuals with CPVT may have symptoms from abnormal heart rhythms (arrhythmias), including palpitations, episodic lightheadedness, or fainting episodes.

Diabetic cardiomyopathy: a disorder of the heart muscle in individuals with diabetes.

Endoplasmic/sarcoplasmic reticulum (ER/SR): an organelle that is the major intracellular Ca^{2+} source.

Excitation-contraction coupling (E-C coupling): the process by which an electrical stimulus triggers the release of calcium by the sarcoplasmic reticulum, the coupling occurs at the sarcolemma–sarcoplasmic reticulum junction. Cardiac excitation-contraction coupling is the process by which the electrical activation of cardiac myocytes leads to the activation of contraction.

Heart failure: a condition in which the heart cannot pump enough blood to meet the body's needs. Heart failure does not mean that your heart has stopped or is about to stop working.

Hyperglycemia: the medical term for high blood sugar (glucose) level. It is a common problem for individuals with diabetes.

and hyperglycemia causes an increase RCS in two ways [17,25,26]: there are two major molecular mechanisms for the formation of RCS. The first way is that in the presence of free iron, hydrogen peroxide can be readily converted via the fenton reaction to hydroxyl radicals, which are derived from hydroxyl radical-mediated oxidation of lipids (e.g., 4-hydroxy trans-2,3-nonenal, MDA, and acrolein). The second way is autoxidation of carbohydrates (e.g., glyoxal and methylglyoxal). Protein carbonylation represents the most common type of nonenzymatic **post-translational modification (PTM)**. The pathway of reactive carbonyls is shown in Figure 2. Scavenging of RCS has shown significant effects in many animal models of disease, which support the notion that RCS generation contributes to the pathogenesis of diabetes and its complications and that scavenging RCS might have clinical benefits.

RCS Modifies RyR2 Function by RyR2 Phosphorylation

Three different isoforms of the RyR have been found in mammalian organisms: RyR1, RyR2, and RyR3. Many of these isoforms are expressed in different tissues but the RyR2 isoform is mainly expressed in the heart and all three isoforms are expressed in smooth muscle. Cryo-electron microscopy (EM) reconstructions show that the RyR has an overall mushroom shape with the stalk crossing the **endoplasmic/sarcoplasmic reticulum (ER/SR)** membrane and the large cap located entirely in the cytosol [27]. The EM maps for RyR2 have been reconstructed to 4.4- and 4.2-Å resolution for the closed and **open state probability**, respectively. Structure comparison of the open and closed RyR2 shows a little armadillo intradomain rearrangement of the containing cytoplasmic domains [28], which can bind Ca^{2+}, Mg^{2+}, and ATP and fine-tune the effect of **intracellular Ca^{2+} homeostasis** [29]. The RyR2 channel consists of four pore-forming subunits, which associate with numerous accessory proteins that have several phosphorylations. The degree of steady state phosphorylation of each site depends on a dynamic balance between multiple protein kinases and phosphatases, allowing precise control of RyR2 phosphorylation and, consequently, channel activity. Alterations in RyR2 phosphorylation play a critical role in various cardiac diseases [30–33]. The RyR2 residue identified Serine-2808, -2814, and -2030 as phosphorylation sites and is thought to be the primary target of PKA phosphorylation [30–32]. RyRs are responsible for the release of Ca^{2+} from intracellular stores during **excitation-contraction coupling (E-C coupling)** in cardiac, skeletal, and smooth muscle. Intracellular Ca^{2+} is an important secondary messenger for signal transduction and is essential for cellular processes [33,34]. More importantly, intracellular Ca^{2+} also tightly controls the membrane voltage and concentration of Ca^{2+} ions on both sides via ion channels and receptors on cell membrane. The Ca^{2+} concentration regulates the mitochondrial matrix by a variety of Ca^{2+} efflux mechanisms, including the mitochondrial Na^+/Ca^{2+} and H^+/Ca^{2+} exchangers. These effects would lead to dysfunction of mitochondrial RyRs [35,36].

What are the molecular mechanisms by which RCS lead to diabetic complications? To date, mechanisms underlying RyR2 dysregulation in diabetes remain poorly defined. RyR2 is crucial for E-C coupling in cardiac muscle [37]. For example, RyR2 channels normally increase the open probability (Po) at low cytosolic diastolic Ca^{2+} concentration (<~10 μM). When the Ca^{2+} concentration is ~10 μM, the Po is maximal and elevating Ca^{2+} concentration beyond this point (1 mM) leads to a reduction in Po [38,39]. The pathological action of RyR2 leads to heart diseases, including **atrial fibrillation**, **catecholaminergic polymorphic ventricular tachycardia (CPVT)**, and heart failure [40,41]. For instance, RyR2 gene mutations underlie the arrhythmogenesis that leads to sudden cardiac death in CPVT [42,43]. In addition, the RyR2 complex plays an essential role in the lungs; dysfunction of RyR2 leads to pulmonary diseases, including diabetic lung [44,45]. What affects the function of RyR2 remains disputed. The primary reason for this is that the potential involvement of increased RyR2 phosphorylation by carbonyl/oxidative stress in the pathogenesis of heart failure, arrhythmias, and diabetic complications is

Intracellular Ca^{2+} homeostasis: regulation of the intracellular concentration of calcium ions in cells results from the integrated function of trans-sarcolemmal Ca^{2+} influx and efflux pathways modulated by membrane.
Mitochondria: a membrane-bound organelle found in the cytoplasm that plays a critical role in the generation of metabolic energy in cells.
Open state probability: a measure of the proportion of the total recording time that an ion channel spends in its open state. Po = open state time/total recording time.
Post-translational modification (PTM): protein synthesis occurs during a process called 'translation.' Post-translational modification of proteins refers to the chemical changes that proteins may undergo after translation. The best characterized post-translational modifications include phosphorylation of proteins involved in regulating signal transduction pathways and glycosylation of membrane proteins.
Pulmonary complications of DM: a disorder of the lung function in people with diabetes, including reduced ventilatory function and gas diffusing capacity.
Reactive carbonyl species (RCS): molecules with highly reactive carbonyl groups, known for their damaging effects on proteins, nucleic acids, and lipids. They are often generated as metabolic products, under oxidative stress or carbonyl stress.
Ryanodine receptor: a class of intracellular calcium channels located in the sarcoplasmic/endoplasmic reticulum membrane and responsible for the release of Ca^{2+} from intracellular stores during excitation-contraction coupling in cardiac, skeletal, and smooth muscle cells.

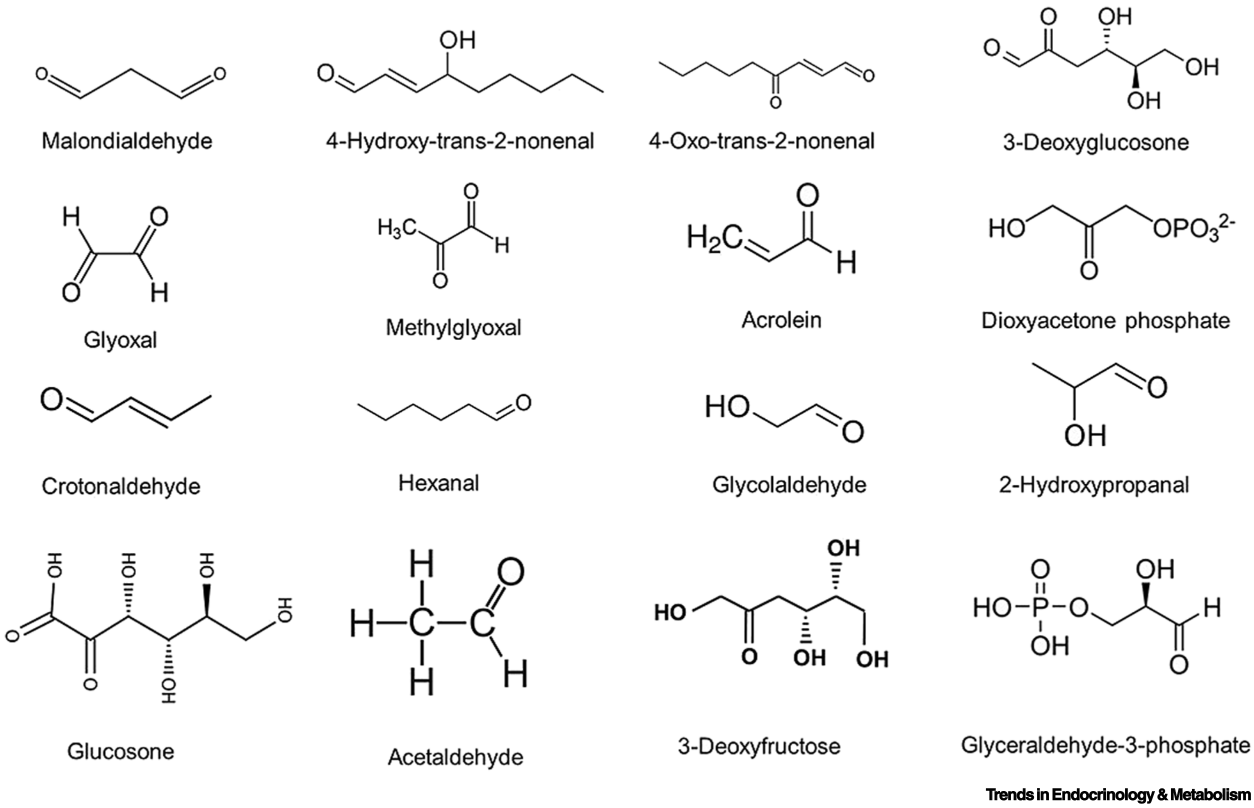

Figure 1. The Chemical Structures of Most Mono- and Di-Carbonyl Species [18–20].

Table 1. The Information of RCS

Reactive carbonyl species	Molecular formula	Molecular weight (g/mol)
Malondialdehyde	$C_3H_4O_2$	72.063
4-Hydroxy-trans-2-nonenal	$C_9H_{16}O_2$	156.225
Acrolein	C_3H_4O	56.064
4-Oxo-trans-2-nonenal	$C_9H_{14}O_2$	154.209
Glyoxal	$C_2H_2O_2$	58.036
Methylglyoxal	$C_3H_4O_2$	72.063
Crotonaldehyde	C_4H_6O	70.091
Hexanal	$C_6H_{12}O$	100.161
Glucosone	$C_6H_{10}O_6$	178.14
Acetaldehyde	C_2H_4O	44.053
3-Deoxyglucosone	$C_6H_{10}O_5$	162.141
3-Deoxyfructose	$C_6H_{12}O_5$	164.16
Glyceraldehyde-3-phosphate	$C_3H_7O_6P$	170.057
Dioxyacetone phosphate	$C_3H_7O_6P$	170.057
Glycolaldehyde	$C_2H_4O_2$	60.052
2-Hydroxypropanal	$C_3H_6O_2$	74.08

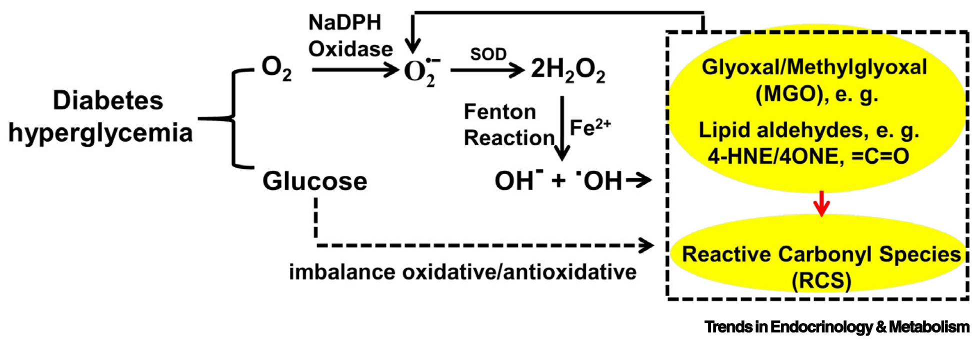

Figure 2. The Pathway of Reactive Carbonyl Processes in Diabetes Mellitus. Hyperglycemia in diabetes mellitus leads to abnormal chemical reactions and an imbalance between oxidative and antioxidative statuses. Metabolic disorders are processes that lead to an increase in reactive carbonyl species [16,24,25].

a multifaceted complex pathophysiological process [41,43]. There is a new focus on the role of RyR2 dysfunction in cardiac diseases, including diabetic cardiomyopathy. In DM, RCS accumulate on select basic residues, which has a physiochemical impact on carbonylation via RyR channels with enhanced or reduced cytoplasmic Ca^{2+} responsiveness. The prototype RCS, methylglyoxal, increases and then subsequently decreases the RyR2 open state probability. Methylglyoxal also increases spontaneous Ca^{2+} release and induces Ca^{2+} waves in healthy myocytes. The potential mechanisms should be: PTM by RCS contributes to the heterogeneity in RyR2 activity that is observed in experimental diabetes [32,46]. These findings identify carbonylation as a novel mechanism that contributes to RyR2 dysregulation in diabetes. However, it is not clear which mechanisms precede PTMs and how RCS are acting as agonists/antagonists at RyR2. It is likely that PTMs at RyR2 function to alter cytoplasmic Ca^{2+} responsiveness of RyR2 with mutations to mimic carbonylation [32,46–48]. As a consequence, Ca^{2+} in the SR leaks via RyR2, causing mitochondrial Ca^{2+} overload and dysfunction. Further consequences include oxidation-antioxidant imbalance and more RCS is produced, which causes PTMs of RyR2. This is a feedback loop between the SR and **mitochondria**, in which SR Ca^{2+} leakage triggers mitochondrial dysfunction and increases the production of RCS, which in turn leads to PTMs of RyR2 and enhances intracellular Ca^{2+} [49]. Some of these key changes are summarized in Figure 3A. RCS and RyR2 should be considered a new therapeutic target to control diabetic cardiomyopathy, diabetic pulmonary complications, and ryanopathy.

RyR2 Protein Is Modulated by RCS in the Diabetic Heart

Diabetic cardiomyopathy as a distinct entity was first recognized by Rubler *et al*. in diabetic patients in 1972 [50]. In the heart, diabetes enhances fatty acid metabolism, suppresses glucose oxidation, and modifies intracellular signaling, leading to impairments in multiple steps of E-C coupling, inefficient energy production, and increased susceptibility to ischemia/reperfusion injury. Loss of normal microvessels and remodeling of the extracellular matrix are also involved in contractile dysfunction of diabetic hearts. Diabetes significantly modifies action potential, Ca^{2+} transient, and Ca^{2+} sensitivity of contractile elements in cardiomyocytes [51–53]. Over nearly 50 years, a large amount of data [40,54–58] has demonstrated that RyR2 plays a key role in heart contraction, including rhythmic contraction and relaxation of the heart. The events that occur in E-C coupling in cardiac muscle are now well established. E-C coupling is a process that governs contractility of the heart through the controlled release of Ca^{2+} from the SR. RyR2 is the route through which Ca^{2+} is released from the SR, providing the necessary driving force for cellular contraction. RyR2 channels play a major role both in contraction and arrhythmogenesis. In arrhythmogenesis, RyR2 channels become abnormally active or leaky,

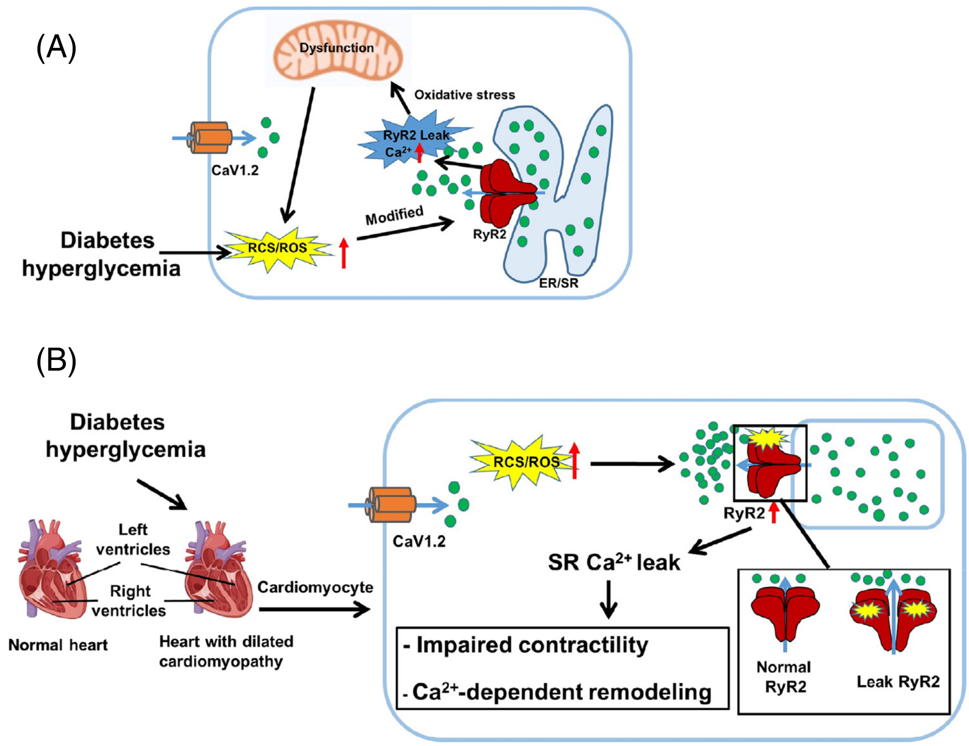

Figure 3. Schematic Representation of the Mechanism of Reacrive Carbonyl Species (RCS) Concentration Modifies Ryanodine Receptor (RyRs) in Cardiomyoctes in Diabetes. (A) Increased RCS concentration modifies RyRs. A possible mechanism is that accumulation of RCS in diabetes leads to modified RyRs, resulting in the activation of RyRs and Ca^{2+} leakage from the sarcoplasmic reticulum (SR). In addition, altered intracellular Ca^{2+} signaling occurs as a consequence of mitochondrial Ca^{2+} uptake. Dysfunction of the mitochondria is a cause or consequence of RCS/reactive oxygen species (ROS) overproduction. This may be a feedback loop between the endoplasmic reticulum (ER)–mitochondrial interface [40,41]. The red arrow denotes an increase in function or concentration. (B) Schematic representation of the mechanisms in cardiomyocytes in diabetes. The key determinants of RCS affect excitation-contraction coupling in cardiomyocytes. Physiologically, Ca^{2+} entry through L-type Ca^{2+} channels (CaV1.2) triggers SR Ca^{2+} release through RyR2. The systolic transient Ca^{2+} activates myofilaments, initiating contraction. In the diabetic heart, RCS alters RyR2 phosphorylation, increasing SR Ca^{2+} leakage, promoting Ca^{2+}-dependent remodeling, and impairing contractility. These are consequences of the diabetic heart [40,46,49]. The red arrow denotes an increase in function or concentration.

thereby increasing dysregulated Ca^{2+} handling in the myocardium. These Ca^{2+}-dependent processes are a detrimental part of cardiomyopathy, CPVT, and even heart failure. In the human heart, disrupted RyR2 Ca^{2+} sensitivity and biochemical modification of the channel are common constituents of a failing heart and RyR2 may underlie the pathological disturbances in intracellular Ca^{2+} signaling [59]. These data establish that the intracellular Ca^{2+} leakage via RyR2 channels plays a role in cardiomyopathy. However, the function of RyR2 in the diabetic heart remains unclear. There is compelling evidence that intracellular Ca^{2+} leakage via RyR2 channels induces glucose intolerance associated with mitochondrial dysfunction [60] and decreased insulin secretion [61]. These data indicate a functional role for RyR2 in diabetic cardiomyopathy. One important reason of intracellular Ca^{2+} leakage via RyR2 channels is that RyR2 acquires a gain-of-function in diabetes. Further research has found that RyR2 in diabetes is more responsive to intrinsic cytoplasmic activators Ca^{2+}, ATP, and cyclic adenosine diphosphate ribose, and less responsive to the cytoplasmic deactivator Mg^{2+}. The threshold for the activation of RyR2 by trans (luminal) Ca^{2+} was also found to be reduced [46,47]. These changes were independent of phosphorylation at Ser-2808 and Ser-2814; RyR2 acquires a gain-of-function phenotype independent of its phosphorylation status during T1D and provides new insights for the enhanced spontaneous Ca^{2+} release in myocytes from T1D rats [62]. These findings on RyR2 PTM by RCS in DM suggest that a high carbonyl/oxidative stress environment leads to RyR2 phosphorylation and thereby contributes to the abnormal SR Ca^{2+} release events. All of these results provide insight into the relationship between RCS and RyR2 in diabetes and heart disease. The pathway of RCS modification of RyR2 in cardiac myocytes is shown in Figure 3B.

RyRs Protein Is Modulated by RCS in the Diabetic Lung

Prakash *et al*. [63] found pulmonary complications in DM, first described in 1989 when they observed an abnormal chest X-ray in a diabetic patient. In recent studies, several groups have confirmed lung dysfunction in diabetes in both clinical and bench research [9,64,65]. In clinical research, a reduction in lung function has been reported in patients with diabetes over the past three decades. Individuals with diabetes have a 1.6-fold higher risk of restrictive lung function impairment and even COPD than those without diabetes [66]. Lung function [percent predicted value of forced vital capacity (%FVC)] is significantly associated with the incidences of DM and prediabetes. Among prediabetic patients, impaired glucose tolerance (IGT) was associated with %FVC. During follow-up, 102 subjects with normal glucose tolerance developed prediabetes. A low %FVC was predictive of an increased risk for development of IGT [67]. This finding, together with the presence of anatomical and biological changes similar to those described in the aging lung, indicates that the diabetic lung is a new target organ, and an underrated complication from restrictive functional pattern [10,66]. DM has been associated

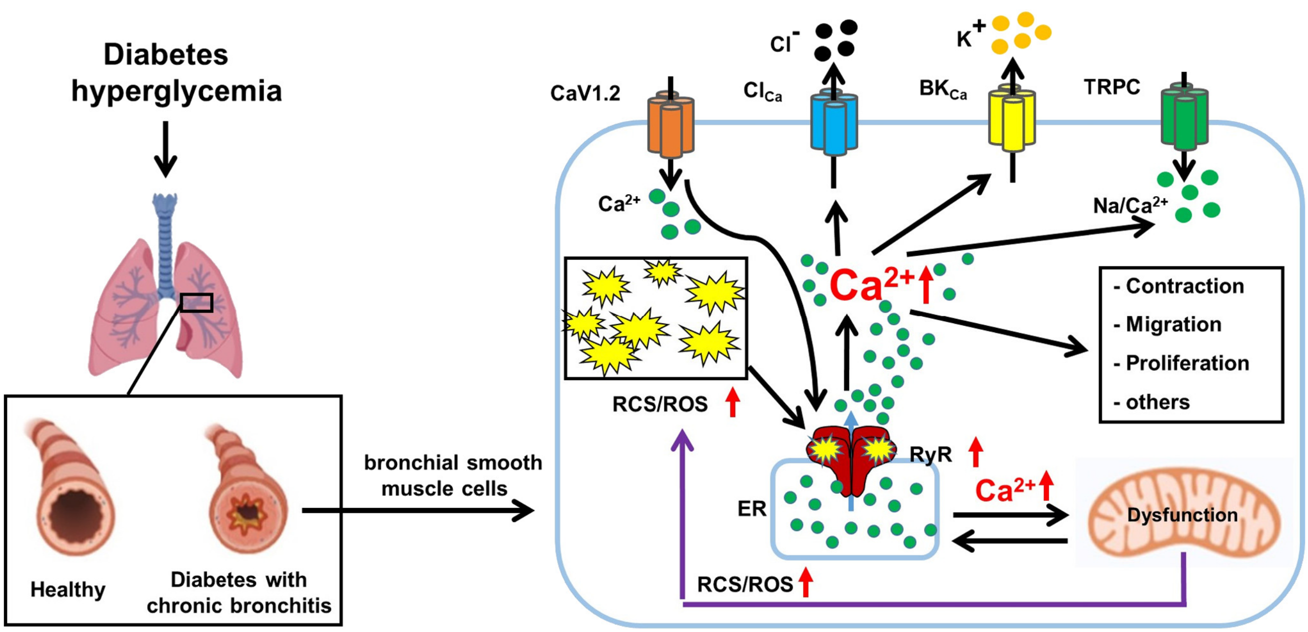

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Figure 4. Schematic Diagram of the Major Pathways Involved in the Model of Reactive Carbonyl Species (RCS) Affect Ca^{2+} Dynamics in Airway Smooth Muscle Cells (ASMCs) in Diabetes. Physiologically, stimulation of cell surface L-type Ca^{2+} channels leads to activation of the ryanodine receptor (RyR) and subsequent release of Ca^{2+} from the sarcoplasmic reticulum (SR) through RyRs. In ASMCs in diabetes, RCS modifies RyR2 proteins which leads to Ca^{2+} removal from the SR to the cytosol. The subsequent increase in Ca^{2+} concentration in ASMCs causes many impairments to bronchitis smooth muscle function, including contraction, migration, and proliferation [14,32,79]. The red arrow denotes an increase in function or concentration. Abbreviations: BK channels, large-conductance calcium-activated potassium channels; ER, endoplasmic reticulum; ROS, reactive oxygen species; TRPC channels, transient receptor potential-canonical channels.

with a reduced level of pulmonary function. However, at the present time, the molecular and cellular mechanisms of the glycemic state and lung function are unclear. Hyperglycemia-upregulated pathways that are potentially involved in lung injury in diabetes include protein glycation [68,69], the PKC pathway [70,71], the NF-KB pathway [72,73], the polyol pathway [74,75], and oxidative stress [76,77]. These pathways may be interrelated. For example, the polyol pathway can also contribute to oxidative stress, and mitochondrial oxidative/antioxidative imbalance has a role in the pathobiology of ASM cells of the diabetic lung, pulmonary function in diabetes, and COPD [78,79]. There is increasing evidence for the presence of autoantibodies in COPD. Chronic oxidative stress is an essential component in COPD pathogenesis and can lead to increased levels of highly reactive carbonyls in the lung, which could result in the formation of RCS. This enhances carbonyl-modified proteins, which arise as a result of oxidative stress, promoting increased RCS. This is a pathological feedback loop [49]. Thus, increased RCS in redox imbalance, oxidative stress, and mitochondrial dysfunction have been implicated in diabetic tissue injury. Furthermore, the ASM, an important tissue involved in the regulation of bronchomotor tone, affects lung function. E-C coupling mechanisms in striated muscles may also broadly transduce diverse

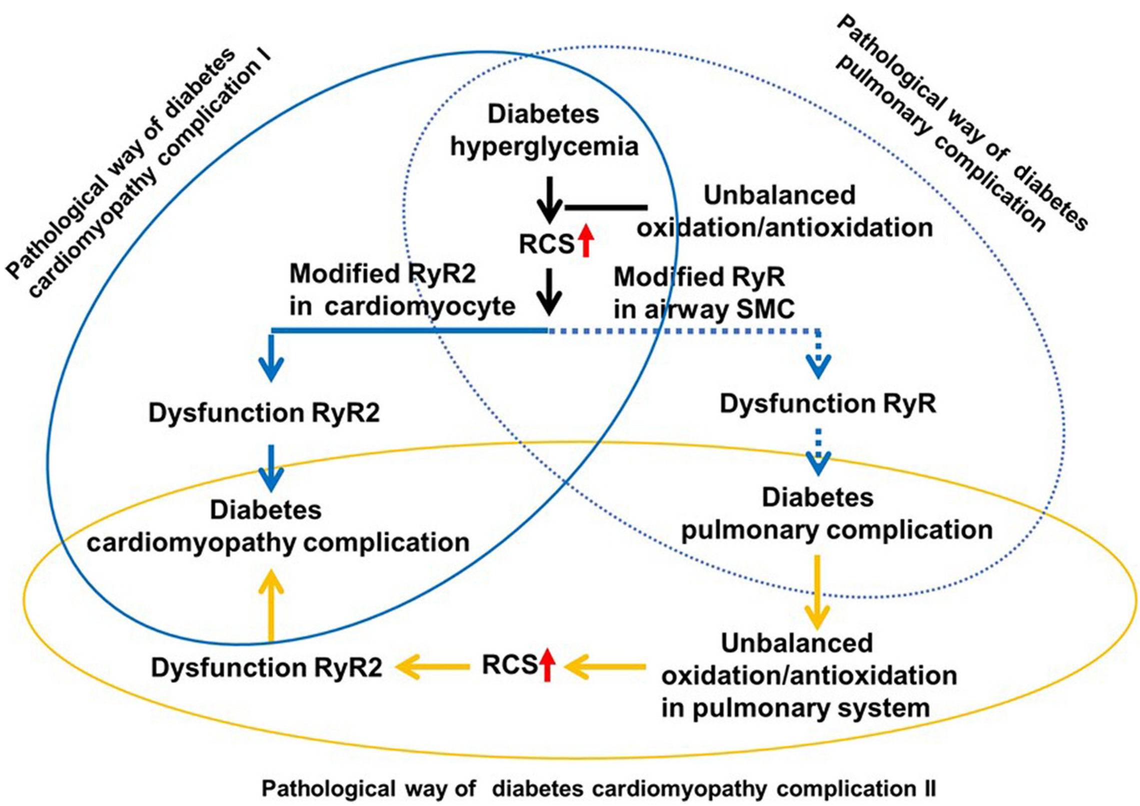

Figure 5. The Pathological Mechanism of Diabetes Pulmonary and Cardiomyopathy Complications and the Relevance of Mechanisms between the Heart and Lung in Diabetes. One pathological mechanism of cardiomyopathy complication is that ryanodine receptor type 2 (RyR2) post-translational modification by reactive carbonyl species (RCS) results in RyR2 dysfunction and dysfunctional Ca^{2+}-signaling pathways in cardiac muscle tissue. This could be the main source of cardiomyopathy. Another pathological mechanism of cardiomyopathy complication is that oxidative stress/carbonyl stress in diabetic pulmonary complications increases the RCS level and this enhances the total RCS concentration in diabetes mellitus and causes an alteration of Ca^{2+} signaling. A pathological mechanism of diabetes in the lung is that RyR2 post-translational modification by RCS results in RyR2 dysfunction and dysfunctional Ca^{2+}-signaling pathways in airway smooth muscle (ASM) cells. The red arrow denotes an increase in function or concentration. Unbroken blue circle lines denote one pathological mechanism of cardiomyopathy complications, dotted blue circle lines denote one pathological mechanism of pulmonary diabetes, and unbroken yellow circle lines denote another pathological mechanism of cardiomyopathy complications.

smooth muscle functions. RyR1 and RyR2 are the predominant RyR isoforms expressed in ASM cells and the characteristics of the calcium sparks in these cells are modulated by activation of the RyR by ryanodine and caffeine, strongly pointing to the role of RyR in the generation of calcium sparks [80,81]. Little is known about how RCS modulates RyRs in ASM, however, we know how RCS modulates RyR2 in cardiomyocytes, as discussed in detail above. Therefore, RCS modification of RyR proteins is a key potential pathophysiological mechanism of the diabetic lung. The detailed mechanisms of RCS and RyR in ASM are summarized in Figure 4.

Relevance to Mechanisms between the Diabetic Heart and Lung

Cardiovascular and pulmonary complications in diabetes are two major contributors to the overall mortality of the disease and their pathophysiological mechanism involves excessive hyperglycemia [11,50]. However, very little is known about the relationship in the mechanisms between the heart and lung in diabetes. Although the role of RyR in arterial smooth muscle cells is not the same as in striated muscles, RCS and RyR2 are likely one key factor that links the dysfunction of these two tissue in diabetes. RyR2 plays a key role in E-C coupling in cardiac and ASM cells. RCS modification of RyR2 proteins results in dysfunction of RyR2 in the heart and lung in diabetes [61,82–84]. This is important to elucidate, as it could provide more insight into how RCS modifies RyR2 proteins in different ways, depending on the disease state, and could help find a new treatment strategy for the two diseases in diabetes. It could also provide more insight into mechanistic hypotheses and avenues of further study [44–47,85]. For example, an increase in the concentration of RCS undoubtedly results in heart and lung complications in diabetes [86,87]. However, which organ is affected first? Do pulmonary complications occur before heart complications in diabetes? This leads us to ask an important question: whether pulmonary complications in diabetes increases RCS, which increases the RCS concentration in the pathophysiology of hyperglycemia, and leads to heart complications in diabetes. All of these discussions conclude that there are three pathological mechanisms for pulmonary and heart complications in diabetes, shown in Figure 5. A pathological mechanism of pulmonary complications in diabetes is that RyR2 PTM by RCS results in RyR2 dysfunction and dysfunctional Ca^{2+}-signaling pathways in ASM. One pathological mechanism of cardiomyopathy complication is that RyR2 PTM by RCS results in RyR2 dysfunction and dysfunctional Ca^{2+}-signaling pathways in cardiac muscle tissue. The changes in RyR2 opening kinetics could be related to hyperphosphorylation of RyR2. This could be a main source of cardiomyopathy. It is generally agreed that oxidative stress/carbonyl stress in diabetic pulmonary complications increases RCS levels and this enhances the total RCS concentration in DM. Alteration of Ca^{2+} signaling could be another main source of cardiomyopathy [88]. Understanding the pathophysiology of diabetic heart and lung will likely improve both clinico-pathology and treatment of these two diabetic complications.

Concluding Remarks and Future Perspectives

Since RCS-modified RyR2 proteins is a major risk factor for diabetic complications, understanding its role in the development of diabetic complications is paramount. A direct link between the diabetic heart and lung and the two major hallmarks of the disease, RCS and RyR2, has been identified but the precise roles that E-C coupling plays in diabetic complications are still debated. RyR2 is expressed in the cardiovascular muscle and ASM, and evidence suggests that RCS-modified RyR2 proteins has significantly contributed to our understanding of the relationship of RCS and RyR2 in the diabetic heart and lung. An understanding of the pathophysiological mechanisms that lead to diabetic complications remains incomplete. Therefore, future studies should focus on RCS-modified RyR2 proteins in the diabetic lung and heart, which will offer invaluable insights into the design of therapeutic approaches and drug targets (see Outstanding Questions).

Outstanding Questions

RCS modifications of RyR2 proteins results in dysfunction of RyR2 in both the heart and lung in diabetes. What is the concentration of RCS that could to lead to RCS-modified RyR2, resulting in diabetic complications? Could scavenging/reduced RCS or RyR2 modulation therapy have beneficial effects in both the diabetic lung and heart?

The diabetic lung is an under-rated complication compared with the diabetic heart. Which is the first target organ? Do pulmonary complications occur before diabetic heart complications in diabetic patients?

What is the relationship between pulmonary complications and heart complications in diabetes? What are the detailed molecular and cellular mechanisms responsible for both these complications? What are the therapeutic strategies that could be used to slow their progression?

Disclaimer Statement

There is no conflict of interest.

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