Direct cardiac and endothelial effects of sodium/glucose cotransporter 2 inhibitors and the role of the sodium/hydrogen exchanger 1

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Chapter 1

General introduction

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DIABETES AND CARDIOVASCULAR DISEASE

Type 2 diabetes mellitus (T2DM) is a widespread welfare disease and is accountable for a large proportion of all deaths worldwide. T2DM is typically described as insulin resistance, leading to the reduced ability to control plasma glucose levels. A major morbidity burden that patients with T2DM have is heart failure [1,2], which is characterized by the inadequacy of the heart to pump and/or fill with blood. The cardiac pathologies in heart failure and T2DM have several overlapping metabolic, structural and functional features relevant for the heart [3]. First, impaired cardiac pump (contraction/relaxation) function, mitochondrial function and vascular function are considered as key hallmarks of T2DM and heart failure [8,9]. Second, the structural alterations of the failing and diabetic heart include cardiac stiffness, hypertrophy, fibrosis, cell death and chronic low-grade inflammation [6–8]. Third, metabolic systemic changes covered in both heart failure and T2DM are hyperglycemia, hyperinsulinemia and increased circulating free fatty acids [4,5]. However, the metabolic changes in the myocardium are opposing for heart failure and T2DM. Heart failure signifies by increased glucose oxidation and reduced fatty acid oxidation, while glucose oxidation is decreased and fatty acid oxidation is elevated in T2DM. Since patients with T2DM and heart failure have a severe and worsened prognosis, it is important to understand which contributing factors could be targeted to reduce the pathologies of both diseases.

SGLT2 inhibitors

The sodium/glucose cotransporter 2 (SGLT2) inhibitors are a class of agents currently implemented as second line therapy in the treatment of T2DM. SGLT2 inhibitors, among which empagliflozin, dapagliflozin and canagliflozin figure as the more prominent members, have exhibited marked reductions in heart failure related hospitalization and cardiovascular death in patients with T2DM [10–12]. SGLT2 inhibitors are designed to inhibit the kidney specific SGLT2, which is at best responsible for 90% of the glucose reabsorption from the kidney filtrate. However, it has become apparent that the cardiovascular benefits of SGLT2 inhibitors cannot be explained through the sole palliating of glycaemia. The possibility of direct cardiovascular effects of SGLT2 inhibitors, regardless of renal SGLT2 inhibition, may at least partly explain the clinical benefits [13–15]. These direct effects of SGLT2 inhibitors could target mechanisms underlying the pathogenesis of T2DM and heart failure, including disturbed cardiac sodium/calcium balance via activation of the sodium hydrogen exchanger 1 (NHE1),

chronic low-grade inflammation and metabolic irregularities [13]. These pathologic mechanisms are separately described below.

Cardiac sodium/calcium balance and NHE1 activity

Sodium (Na^+) is an element that is essential for maintenance of membrane potential in excitable cells, intracellular acid-base balance, fluid volume homeostasis and osmotic regulation between cells and extracellular fluid. In the heart, sodium is an important regulator of cardiac cell contraction, by facilitating the transport of numerous solutes and ions across the cell membrane during an action potential, including calcium (Ca^{2+}) and potassium (K^+). This transport is fueled by the potential energy that arises from the influx of Na^+ that is favored by the steady-state electrochemical gradient in a healthy cardiomyocyte [16]. Cardiac contractility highly depends on the cytoplasmatic Ca^{2+} concentration ($[Ca^{2+}]_c$). $[Na^+]_c$ positively regulates $[Ca^{2+}]_c$ via activation of the sodium/calcium exchanger (NCX). The NCX (transmembrane and mitochondrial) exchanges one $[Ca^{2+}]_c$ with three extracellular/mitochondrial Na^+ in its forwards mode to regain low $[Ca^{2+}]_c$ after a contraction. However, in cases where $[Na^+]_c$ is elevated (figure 1) [17–19], NCX becomes less active in forward mode and may even operate in a reversed mode, thereby increasing $[Ca^{2+}]_c$. The elevation in $[Ca^{2+}]_c$ includes systolic and diastolic $[Ca^{2+}]_c$, which means that both the contraction and relaxation status of the heart become disturbed. The depolarizing current of Ca^{2+} release via the NCX can generate delayed afterdepolarizations and arrhythmias [20,21]. In addition, increased $[Na^+]_c$ stimulates the lowering of mitochondrial calcium ($[Ca^{2+}]_m$) through the mitochondrial NCX, possibly leading to decreased mitochondrial energy production and decreased activity of the mitochondrial anti-oxidant system, thereby increasing the net reactive oxygen species (ROS) balance. Thus, by controlling the $[Ca^{2+}]_m$, $[Na^+]_c$ creates a connection between cellular ion homeostasis and metabolism [22,23]. Collectively, the alterations in $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ can cause contractile impairments and mitochondrial dysfunction and may ultimately result in (ir)reversible myocardial injury.

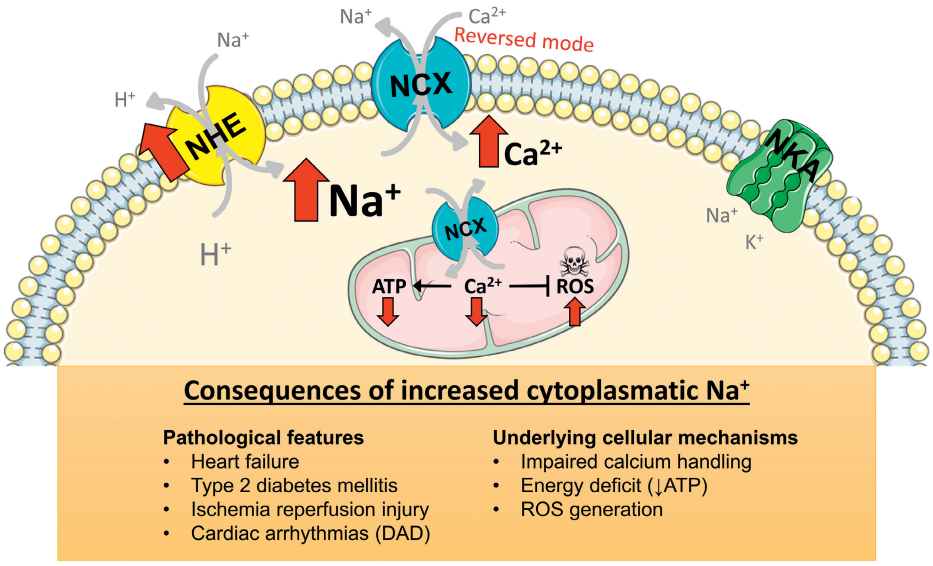

Figure 1 Consequences of increased cytoplasmatic sodium ($[Na^+]_c$) in the cardiac cell. *Increased $[Na^+]_c$ is a prerequisite for elevated $[Ca^{2+}]_c$ and reduced $[Ca^{2+}]_m$, resulting in contractile impairments, energy deficits and enhanced ROS formation. Elevated cardiac $[Na^+]_c$ is seen in T2DM and several cardiac diseases, including heart failure, ischemia reperfusion injury and cardiac arrhythmias. The red marks in the figure show the actions that occur in heart failure and diabetes. NHE Na^+/H^+ exchanger, NCX Na^+/Ca^{2+} exchanger, Na^+/K^+ pump, ATP adenosine triphosphate, ROS reactive oxygen species, DAD delayed afterdepolarizations. Image was made using Servier Medical Art (http://smart.servier.com/).*

Cardiac $[Na^+]_c$ is tightly controlled by membrane transporters, pumps and exchangers [16]. One of those transporters is the sodium/hydrogen exchanger 1 (NHE1). The NHE1 is a ubiquitously expressed membrane protein that enables the exchange of one extracellular Na^+ ion for one intracellular H^+ ion, thereby counteracting intracellular acidification. The activation of NHE1 will result in the increase of $[Na^+]_c$. NHE1 is the major NHE isoform that is expressed in the heart. In healthy conditions, the NHE is minimally active and intracellular pH is primarily regulated via the sodium/bicarbonate cotransporters**.** However, cardiac NHE1 becomes stimulated during pathological conditions such as T2DM, heart failure and ischemia-reperfusion injury [14,24,25]. Moreover, NHE1 gets activated by inflammatory and hyperglycemic insults in vascular endothelial cells [26,27]. While the role of increased $[Na^+]_c$ in the pathogenesis of endothelial dysfunction is not fully understood, inhibition of NHE1 has been shown to block or reverse the impairment attained in immunostimulated or diabetic endothelial cells [26,28]. Compounds that reduce NHE1 activity confer protection against myocardial and vascular injury [26,27,29–33].

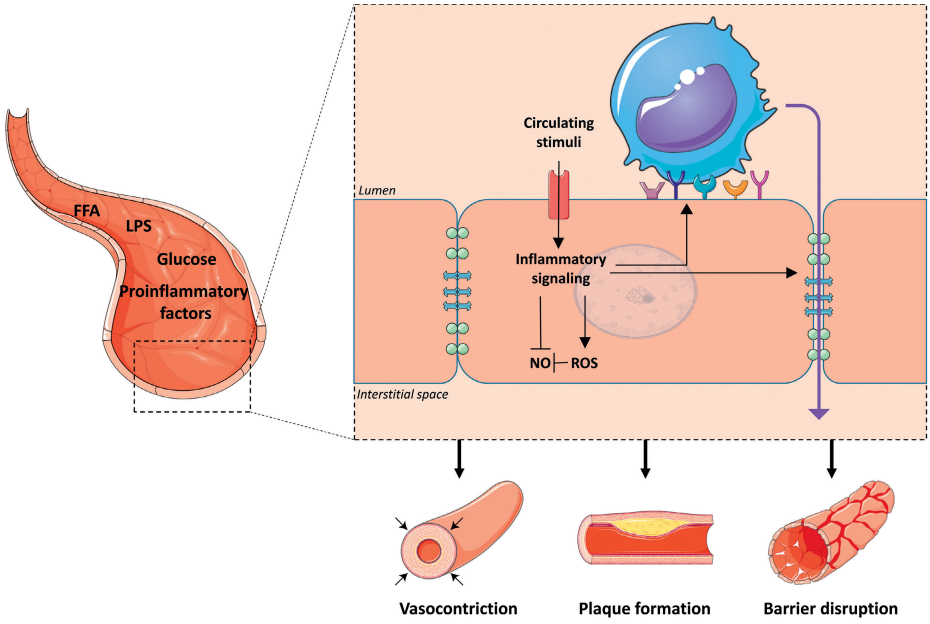

Figure 2 Increased circulatory stimuli in T2DM and heart failure cause endothelial dysfunction via inflammation. *In T2DM and heart failure, high levels of plasma glucose, fatty acids and pro-inflammatory molecules stimulate inflammatory signaling in endothelial cells. This will result in reduced NO bioavailability, increased adhesion of circulating leukocytes and the remodeling of cell-cell junctions causing enhanced endothelial cell permeability. The endothelial dysfunction caused by these processes gives rise to impaired vasodilation, atherosclerotic plaque formation and disrupted endothelial barrier function. LPS lipopolysaccharide, FFA free fatty acids, NO nitric oxide, ROS reactive oxygen species. Image was made using Servier Medical Art (http://smart.servier.com/).*

Vascular inflammation and endothelial dysfunction

Inflammation is a hallmark of T2DM and heart failure appearing in cardiac cells, including endothelial cells [34–36]. The pathogenesis of inflammation in endothelial cells initiates when an extracellular toxin activates pro-inflammatory signaling cascades, leading to endothelial dysfunction (figure 2). Endothelial cells increase their release of pro-inflammatory cytokines, which enhances the expression of leukocyte attracting adhesion molecules and endothelial cell permeability. These actions aggravate the endothelial barrier function and therefore facilitate leukocyte translocation through the vessel wall. There, the leukocytes differentiate into foam cells and form atherosclerotic plaques, induce fibrosis and further stimulate endothelial dysfunction. Endothelial dysfunction also describes a process of impaired vasodilation capacity through reduced nitric oxide (NO) production or availability[7]. A reduction of NO happens when the activity of the enzyme endothelial nitric oxide synthase (eNOS) is hindered, leading to reduced

production of NO. In addition, increased levels of oxidative stress could directly and indirectly reduce NO. First, ROS can react directly with NO resulting in the production of peroxynitrite. Second, ROS can indirectly lower NO when ROS uncouples eNOS during its dimerization process, turning the monomer eNOS into a ROS producing unit, thereby further deteriorating endothelial function. Targeting endothelial inflammation in T2DM and heart failure has been extensively investigated and proposed as a promising strategy to attenuate disease development and progression in T2DM and heart failure [37,38].

Metabolic disturbances

While the healthy heart preferably consumes free fatty acids (FFA, ~60%) as opposed to glucose and other carbohydrates (~20-30%), it is an organ with high metabolic flexibility depending on the availability of substrates [39,40]. In T2DM, the combination of increased FFA and insulin resistance lowers glucose utilization and the heart will predominantly depend on FFA metabolism [41]. In heart failure, an opposite shift occurs, in that now the heart increases its preference for glucose, which is often associated with a buildup of metabolic glucose intermediates in the cytoplasm. The accumulation of glycolytic metabolic intermediates, such as glucose-6-phosphate (G6P) or increased channeling of glucose into aspartate can drive hypertrophy and consequently the development of heart failure [42–44]. In addition, insulin resistance is associated with an increased risk for heart failure even in the absence of T2DM [45]. In both conditions of T2DM and heart failure, cardiac substrate utilization is insufficient, leading to the reduced production of adenosine triphosphate (ATP) while simultaneously increasing adenosine diphosphate (ADP) and adenosine monophosphate (AMP) [46–48]. Increased levels AMP is sensed by adenosine monophosphate-activated protein kinase (AMPK). AMPK activation improves cardiac energy metabolism, enhances vascular function and reduces levels of oxidative stress [49–51]. Therefore, activation of AMPK could be a potential approach to reverse the consequences of the metabolic disarrangements employed in T2DM and heart failure. The metabolic alterations observed in T2DM and heart failure described here are summarized in figure 3.

Aim of this thesis

The general aim of this thesis is to investigate the direct cardiovascular actions of SGLT2 inhibitors in healthy and disease conditions. In **chapter 2**, an extensive literature review of the direct cellular effects of SGLT2 inhibitors is given. In **chapter 3**, we investigated the effects of SGLT2 inhibitors empagliflozin, canagliflozin and dapagliflozin in the

healthy mouse cardiomyocyte on $[Na^+]_c$ and NHE1 activity, and assessed the acute effects of the SGLT2 inhibitors on the physiology of the isolated healthy mouse heart. We also explored the ability of empagliflozin, canagliflozin and dapagliflozin to bind to NHE1 in a computational study. In **chapter 4**, we studied whether acute treatment with empagliflozin can reduce ischemia reperfusion injury in the isolated mouse heart in the presence and absence of insulin, and in relation to NHE inhibition. In **chapter 5**, we studied the direct and acute effects of empagliflozin on cardiac glucose and fatty acid metabolism in type 2 diabetic mouse hearts. In **chapter 6**, we investigated whether empagliflozin is able to reduce $[Na^+]_c$ and NHE1 activity in human endothelial cells. Finally, we explored possible anti-inflammatory effects of empagliflozin and dapagliflozin in tumor necrosis factor α-stimulated endothelial cells (**chapter 7**) and of canagliflozin, empagliflozin and dapagliflozin in lipopolysaccharide-stimulated endothelial cells (**chapter 8**).

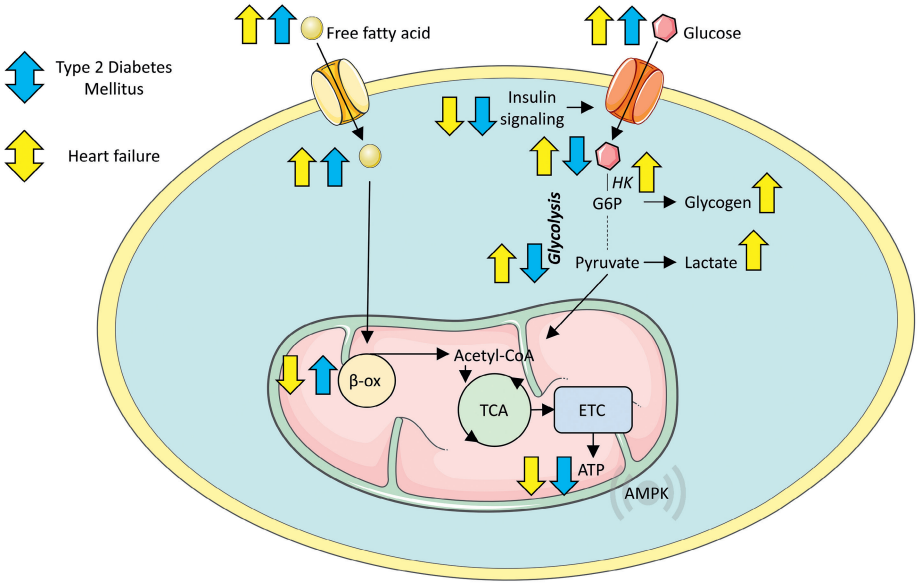

***Figure 3 Changes in glucose and fatty acid uptake and energy production in T2DM and heart failure**. In T2DM, insulin resistance and high levels of circulating FFAs cause reduced glucose uptake, glycolysis and glucose consumption. Heart failure impacts the metabolism by increased cellular uptake of FFA and glucose, but not their uptake into the mitochondria. This will result in the buildup of their intermediates in the cytosol. Both conditions result in energy deprivation (↓ATP) that is sensed by AMPK. β-ox β-oxidation, HK hexokinase, G6P glucose-6-phosphate, TCA tricarboxylic acid cycle, ETC electron transport chain, ATP adenosine triphosphate, AMPK '5 adenosine monophosphate-activated protein kinase. Image was made using Servier Medical Art (http://smart.servier.com/).*

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