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Crosstalk Between Sodium-Glucose Cotransporter Inhibitors and Sodium-Hydrogen Exchanger- 1 and 3 in Cardiometabolic Diseases

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Abstract

The hallmark of type 2 diabetes mellitus (T2DM) is abnormal glucose homeostasis due to hyperglycaemia or insulin resistance. Metabolic abnormalities in T2DM lead to cellular dysfunction and the development of diabetic cardiomyopathy and heart failure. New antihyperglycemic agents, such as glucagon-like peptide-1 receptor agonists and the sodium-glucose cotransporter-2 inhibitors (SGLT2i) have shown to attenuate endothelial dysfunction at the cellular level. In addition, they showed cardiovascular safety and cardioprotective effects. How these drugs exert their cardioprotective effects is unknown, although recent studies show that cardiovascular homeostasis occurs through the interplay of the sodium hydrogen exchangers (NHE), specifically NHE1 and NHE3 with SGLT2i. Another theoretical explanation for the SGLT2i cardioprotective effects is through natriuresis by the kidney. This theory highlights the possible involvement of renal NHE transporters in the management of heart failure. This review outlines possible mechanisms predisposing to diabetic cardiomyopathy and discusses the interaction between NHE and SGLT2i in cardiovascular disease.

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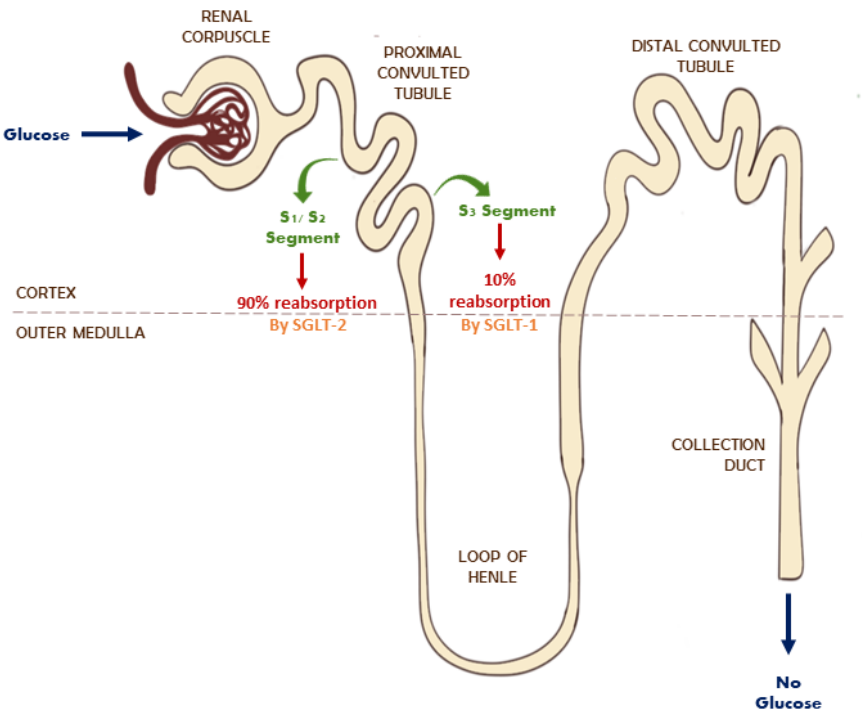

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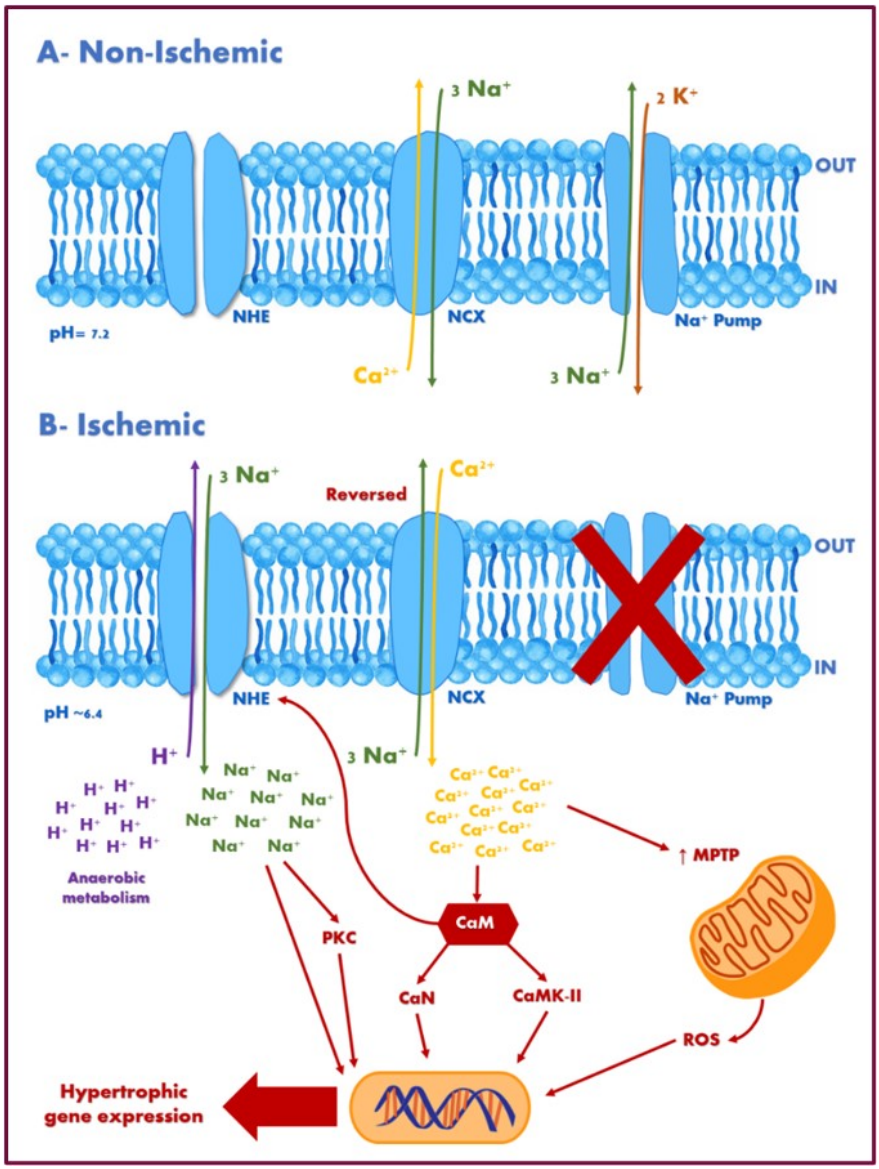

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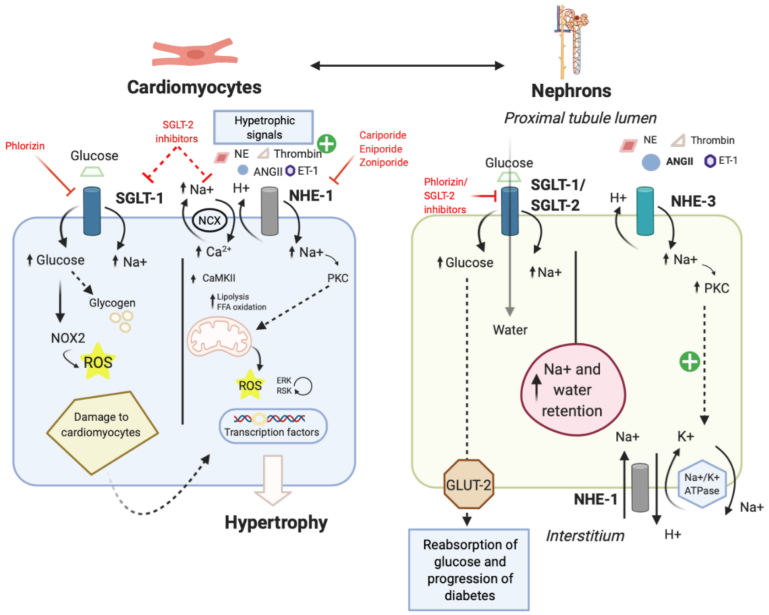

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Abstract

The hallmark of type 2 diabetes mellitus (T2DM) is abnormal glucose homeostasis due to hyperglycaemia or insulin resistance. Metabolic abnormalities in T2DM lead to cellular dysfunction and the development of diabetic cardiomyopathy and heart failure. New antihyperglycemic agents, such as glucagon-like peptide-1 receptor agonists and the sodium-glucose cotransporter-2 inhibitors (SGLT2i) have shown to attenuate endothelial dysfunction at the cellular level. In addition, they showed cardiovascular safety and cardioprotective effects. How these drugs exert their cardioprotective effects is unknown, although recent studies show that cardiovascular homeostasis occurs through the interplay of the sodium hydrogen exchangers (NHE), specifically NHE1 and NHE3 with SGLT2i. Another theoretical explanation for the SGLT2i cardioprotective effects is through natriuresis by the kidney. This theory highlights the possible involvement of renal NHE transporters in the management of heart failure. This review outlines possible mechanisms predisposing to diabetic cardiomyopathy and discusses the interaction between NHE and SGLT2i in cardiovascular disease.

1. Introduction

Diabetes Mellitus (DM) is a metabolic disorder where the body either does not produce sufficient amount of insulin, has impaired insulin action or a combination of the two. Type 1 (T1DM) which constitutes ~5-10% of diabetes cases has high incidence in children and adolescents and is caused by immune destruction of the β-islets in the pancreas. The majority of cases are Type 2 (T2DM), which results from a combination of beta cell dysfunction and insulin resistance. Insulin resistance and the accompanied hyperinsulinemia are the early detected metabolic abnormality in subjects destined to develop T2DM and precedes the deterioration in glucose homeostasis (American Diabetes, 2017; Kahanovitz et al., 2017). T2DM affects approximately 463 million people worldwide and future estimates suggest that 102 of 1000 people will be diagnosed with diabetes by 2030 (Saeedi et al., 2019). Chronic diabetes without appropriate treatment causes microvascular and macrovascular complications like nephropathy, retinopathy, neuropathy, and atherosclerotic cardiovascular diseases (CVDs).

CVDs are considered the most common cause of morbidity and mortality in diabetic patients. In the US, CVD death rates are 1.7 times higher among adults with DM than those without, which is attributable to the increased risk of stroke, myocardial infarction (MI), and heart failure (HF) (Leon & Maddox, 2015). Patients with T2DM have a two-to-five-folds increased risk of HF, independent of other risk factors like hypertension, coronary artery disease, and dyslipidaemia (Martín-Timón et al., 2014) (Nichols et al., 2004). A rise in glycated haemoglobin by 1% has been associated with an 8% increase in CVD risk (Stratton et al., 2000). Furthermore, the presence of T2DM worsens the prognosis of heart failure. In addition, T1DM patients have a 30% risk of HF with every 1% increase in glycated haemoglobin (Zhao et al., 2014).

Hyperglycaemia and insulin resistance are the major etiological factors promoting cardiomyopathy and HF in diabetic patients (Jia et al., 2016). Ultimately, the progression of HF in DM is linked to pathological changes to the heart muscle and coronary vasculature, which eventually lead to diabetic cardiomyopathy (DCM) (Jia et al., 2018).

Recently discovered antihyperglycemic agents, such as glucagon-like peptide-1 receptor agonists, and the sodium-glucose cotransporter-2 (SGLT2) inhibitors (SGLT2i) have shown cardioprotective effects. SGLT2i were shown to decrease the rates of HF and hospitalization from HF in several clinical trials (Ali et al., 2019). How these new antihyperglycemic drugs

exert cardioprotection is unknown, although some studies show involvement of the sodium hydrogen exchanger (NHE) and sodium-glucose transporter (SGLT) families. This review discusses some of the mechanisms predisposing to diabetic cardiomyopathy and highlights the role of NHE and SGLT transporters in cardiovascular disease.

2. Pathophysiology of Diabetic Cardiomyopathy

DCM is recognized by defects in the structure and performance of the myocardium in individuals with diabetes, independent of other cardiac risk factors. The structural abnormalities in DCM progress through three stages, an early stage characterized by diastolic dysfunction, which gradually develops to systolic dysfunction in the advanced stage, and eventually to HF in the late stage. (Jia et al., 2016).

Early-stage DCM, mainly caused by hyperglycaemia and insulin resistance, presents with impairment in the left ventricle (LV) diastolic filling, compensated by increased LV and atrial filling pressure and left atrial enlargement (Seferović & Paulus, 2015). Hyperglycaemia leads to downregulation of GLUT4, impaired glycolysis, and increased free FA levels (from impaired FA metabolism). Insulin resistance results in increased lipolysis and elevated plasma FFA concentration as well leading to increased influx to myocytes and development of cardiac steatosis. The events result in high levels of ROS, impaired Ca^{2+} homeostasis, mitochondrial dysfunction, endoplasmic reticulum stress, oxidative stress, and activation of the sympathetic nervous system, all of which promote cardiac hypertrophy, fibrosis, and cardiomyocyte apoptosis (Jia et al., 2016). The advanced stage comprises continued cardiac injury and further stimulates the Renin-Angiotensin-Aldosterone System (RAAS) and maladaptive immune responses that culminate in impaired autophagy of cells (Jia et al., 2016). Advanced stage features include LV hypertrophy and cardiac remodelling, with impaired cardiac diastolic function. Consequently, the individual may develop HF with a normal ejection fraction. In late-stage DCM, neurohumoral activation, impaired metabolism, and myocardial fibrosis weaken coronary microcirculation and the diastolic and systolic functions of the heart (Aronow & Ahn, 1999; Jia et al., 2018). Additionally, impaired insulin signalling and oxidative stress both decrease levels of the vasodilator nitric oxide (NO) (Jia et al., 2016).

2.1 The Role of Endothelial Dysfunction in the Development of DCM

Studies on DCM development using animal models have implicated multiple pathophysiologic mechanisms, such as mitochondrial dysfunction, RAAS activation, Ca^{2+} homeostasis impairment, lipotoxicity, myocardial steatosis, glucose toxicity, and most recently, endothelial dysfunction. Hyperglycaemia is one of the main factors triggering endothelial dysfunction by exerting several biochemical changes that damage cardiac and vascular endothelial cells. Some of these changes trigger ROS production and induce oxidative stress levels that overwhelm cells, enhance non-enzymatic glycation, activate protein kinase-C (PKC), and ameliorate the cells' redox potential (Avogaro et al., 2011). Oxidative stress promotes the formation and deposition of AGE products creating elevated interstitial collagen deposition and increased myocardial wall stiffness. If untreated, all of these DCM-related structural changes would result in HF (Jia et al., 2016).

The endothelium is a single-layer cellular lining of the whole vascular system. Endothelial cells have unique functions vital for cardiovascular homeostasis. For example, the endothelium functions as a semi-permeable barrier between blood and body tissues. The endothelium also controls vascular tone by secreting the vasodilators nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors, as well as producing vasoconstrictors like endothelin-1 and thromboxane-A_2. Endothelial dysfunction, characterized by low nitric oxide bioavailability, occurs when endothelial cells lose their barrier property and fail to balance vascular dilatory and constrictive tone, coagulation, and anticoagulation. T1DM and T2DM patients show decreased vasorelaxation by NO (Avogaro et al., 2011; Endemann & Schiffrin, 2004). Reduced NO production is observed in diabetic experimental models (Hink et al., 2001; Shi & Vanhoutte, 2009), and *in-vitro* studies with endothelial cells have shown that high glucose levels lead to less NO production (De Vriese et al., 2000). Endothelial dysfunction is considered the first step in developing atherosclerotic complications in metabolic conditions such as diabetes, pre-diabetes, and obesity (Avogaro et al., 2006).

Several mechanisms contribute to lower NO bioavailability during endothelial dysfunction. Production of ROS through NADPH oxidase, an electron transport chain protein, leads to oxidative stress. ROS reacts with NO to produce a cytotoxic oxidant compound called peroxynitrite. Peroxynitrite increases oxidative stress even further, which in turn lowers NO production through uncoupling of NO synthases (NOS) and mediates low-density lipoprotein oxidation. Peroxynitrite also leads to protein dysfunction via nitration of

proteins. In insulin resistance, the PI3K/Akt pathway involved in NOS activation is inhibited, while endothelin-1 and adhesion molecule production pathways remain intact. Also, the presence of AGE products contributes to oxidative stress and leads to endothelial dysfunction. Endoplasmic reticulum stress, a pro-apoptotic pathway, is another mechanism where the pro-survival unfolded protein response becomes chronically activated (Aviello & Knaus, 2018; Avogaro et al., 2011; Endemann & Schiffrin, 2004; Maamoun et al., 2019; Muniyappa & Sowers, 2013).

Antihyperglycemic medications that target and attenuate endothelial dysfunction such as liraglutide, metformin, pioglitazone, and SGLT2i (empagliflozin; EMPA, canagliflozin, CANA; dapagliflozin, DAPA) are becoming of great interest. (Batzias et al., 2018; Eriksson & Nyström, 2015). In porcine coronary artery cultured endothelial cells, high glucose increased endothelial dysfunction markers, oxidative stress, and VCAM-1, and reduced NOS expression. Treatment with SGLTi exerted a protective effect and prevented endothelial dysfunction (Khemais-Benkhiat et al., 2020). Additionally, in the obese ZSF1 rat model, systolic blood pressure (BP) was higher than the lean control group, NOS was downregulated, and expression of the adhesion molecule VCAM-1 was increased. Chronic treatment of T2DM ZDF rats with empagliflozin (EMPA) prevented oxidative stress, signalling and inflammation, AGE products formation, and attenuated endothelial dysfunction (Steven et al., 2017). In Apo-$E^{-/-}$ streptozotocin (STZ)-induced diabetic mice, treatment with EMPA also attenuated endothelial dysfunction and reduced atherogenesis (Ganbaatar et al., 2019). The EMBLEM trial included 117 patients with T2DM and concurrent CVDs, randomized into a 1:1 ratio to receive either placebo or EMPA over 24 weeks (Tanaka et al., 2019). The primary endpoint was the change in reactive hyperaemia index, an endothelial dysfunction marker, from baseline. Per-protocol analysis did not show an improvement in endothelial dysfunction. However, the study was limited by the small number of patients and unrepresentable population. The mean population systolic BP was 130 mmHg and the BMI 26.4 kg m^{-2}, which are lower than expected values for diabetic patients with concurrent cardiovascular disease. In T2DM mice, treatment with dapagliflozin (DAPA) attenuated endothelial dysfunction, vascular smooth muscle dysfunction, and arterial stiffness (Lee et al., 2018). Gaspari et al. (2018) showed that DAPA attenuated TNFα- and hyperglycaemia-induced endothelial dysfunction *in vitro* with a human endothelial cell line. While *in-vivo*, both adult and aged $ApoE^{-/-}$ mice chronically administered with DAPA showed attenuated endothelial dysfunction and less vascular adhesion molecules.

2.2 The Role of Metabolic Disturbances in the Development of DCM

Myocardial cells are characterized by their metabolic flexibility, which is the ability to utilize several substrates such as glucose, lactate, and fatty acids (FAs) to generate ATP molecules. In a healthy heart, there is a constant supply of ATP by oxidative phosphorylation of FAs in the mitochondria (60 – 90%), while maintaining a balance in using other substrates like glucose and lactate (Stanley & Chandler, 2002).

In diseases such as HF and DM, the metabolic balance is impaired. The failing heart increases the utilization of glucose over FAs to increase energy production. However, in diabetic heart, there may be a metabolic shift toward FA oxidation rather than glucose oxidation. This shift is thought to be due to the chronic hyperglycaemia, insulin deficiency, and insulin resistance. The enhanced FA oxidation observed in a diabetic heart might exceed cardiac utilization capacity and predispose the heart to triacylglycerols (TAGs) and ceramides disposition, which in turn contributes to cardiac hypertrophy and stenosis. Along with the burden created by advanced glycated end products (AGEs), the cardiac metabolic changes promote collagen deposition and induce myocardial fibrosis leading to the damage of the cardiomyocytes present in DCM (Fuentes-Antrás et al., 2015).

Furthermore, in cardiac diseases, ischemia and hypoxia promote a shift to anaerobic respiration. The activity of adenosine monophosphate kinase (AMPK), an energy balancing enzyme that promotes anaerobic ATP production, is allosterically regulated by the ratio of AMP to ATP. When ATP is abundant, it binds to AMPK and inactivates it. Therefore, during pathological low energy states when AMP is abundant, AMPK is activated to provide the heart with ATP. In addition to energy production, AMPK activation protects cells against myocardial injury during ischemia, reduces reactive oxygen species (ROS), and attenuates endoplasmic reticulum stress Additionally, sodium (Na^+) overload, a characteristic of HF, increases calcium (Ca^{2+}) efflux which interferes with the Krebs cycle, that is adding up to the metabolic disturbances (Qi & Young, 2015).

3. Characteristics of NHE & SGLT membrane transporters

3.1 NHE Overview

The sodium hydrogen exchangers (NHE) family of integral membrane protein antiporters consists of 10 isoforms that function by exchanging sodium cations with protons through cell membranes (Packer, 2017). NHE1 and NHE3 are two well-studied isoforms involved in renal

and cardiovascular homeostasis. NHE1, ubiquitous in mammalian cells, is the dominant isoform in the heart where it regulates intracellular pH, cell volume, and proliferation and shows the highest expression in the kidney (Das et al., 1987; Packer, 2017; Parker et al., 2015). NHE3 contributes to regulating extracellular volume and BP by reabsorption of Na^+ in the kidney (Dominguez Rieg et al., 2016; Packer, 2017).

3.1.1 Activity and Regulation of NHE1 and NHE3

NHE1 influxes Na^+ in response to intracellular acidification, where the protein exhibits an allosteric binding site for protons. Regulation of NHE1 can also occur in response to different membrane receptors that can exert conformational changes or C-terminal phosphorylation. Extracellular and hormonal pathways, such as angiotensin II (ANG-II), endothelin-I, and thrombin, control the activity of NHE1 regulators. Receptor regulators of NHE1 include protein kinases, G-coupled receptors, and integrin receptors (Vallés et al., 2015). Tyrosine kinase activation increases NHE1 activity through the Ras-mediated ERK cascade, including Ras downstream effectors such as MEK1/2, Raf-1, and ERK. However, the serine/threonine kinase ERK downstream effector called p90 ribosomal S6 kinase ($p90^{RSK}$) directly phosphorylates NHE1 instead of ERK (Putney et al., 2002). The RSK family of transporters includes four isoforms (RSK1-4). RSK1, also designated as $p90^{RSK}$, sustains regular cardiac function, making this enzyme essential (Lara et al., 2013). $p90^{RSK}$ hyperactivity induces cardiac hypertrophy and HF. In neonates, $p90^{RSK}$ activation increases c-Fos and Egr-1 expression in ventricular myocytes to promote myocytes' development (Takahashi et al., 1999). Furthermore, $p90^{RSK}$ activation reduces glycogen synthase kinase-3β (GSK-3β) activity in mice with defective ryanodine receptor ion channels leading to cardiac hypertrophy progression (Q. He et al., 2010). A study by Takeishi et al. (1999) found aberrant activation of $p90^{RSK}$ in guinea pig pressure-overload-induced hypertrophic myocardium. Moreover, patients with dilated cardiomyopathy had higher levels of activated $p90^{RSK}$ than their healthy peers (Horie et al., 1992; Javadov et al., 2009; Muthusamy et al., 2013; Takeishi et al., 2002; Yamaguchi et al., 2011). These findings highlight the role of $p90^{RSK}$ in inducing cardiac dysfunction, remodelling, and its role in NHE1 activation.

Akt is another kinase known to regulate NHE1 activity. The duration of Akt activation is the determinant of its effect (Takeishi et al., 2002). Short-term Akt activation promotes physiological hypertrophy during postnatal cardiac development characterized by normal or enhanced contractile function (Walsh, 2006), while contractile dysfunction characterizes

long-term Akt activation (Shiojima et al., 2002). A study done on mouse embryo fibroblasts showed that Akt inhibition reduced NHE1 activity by blocking the translocation of NHE1 to the cell membrane. Furthermore, upstreaming of Akt enhances $p90^{RSK}$ activation and thus plays a role in cardiomyopathy (Clement et al., 2013; Kemi et al., 2008).

The role of cardiac AMPK, one of the NHE channel regulatory kinases, in cardiac metabolism is not known. However, studies suggest that activating AMPK by phosphorylation triggers the trafficking of glucose transporters (GLUT1 and GLUT4) to the sarcolemma and increases glucose uptake (Qi & Young, 2015; Rotte et al., 2010).

The G protein-coupled receptor subunits Ga_q and Ga_{13} also activate NHE1. Ga_{13} activates NHE1 through the GTPase RhoA pathway, while Ga_q activates NHE1 through the PKC-dependent mechanism. The suppression of PKC in several Ga_q protein-coupled receptors, namely α1-adrenergic, vasopressin, and endothelin-1, impairs NHE1 activation. However, in some Ca^{2+} mobilizing Ga_q coupled receptors, NHE1 activation can occur independently of PKC. Moreover, integrin receptors can activate NHE1, which may be due to the shared signalling pathway with Ga_{13} that activates NHE1 (Avkiran & Haworth, 2003; Kitamura et al., 1995).

Other than receptor-mediated regulation, NHE1 regulation occurs through the direct binding of regulatory proteins to the C-terminal. Accessory proteins, which take part in the regulation of cardiac NHE1 activity were also investigated, such as carbonic anhydrase-II (CAII), Ca^{2+}-binding proteins (calmodulin and calcineurin B homologous proteins [CHPs]), and phospholipids. Cellular Na^+ concentration, regulated by NHE1, is instrumental for function, playing roles in Ca^{2+} regulation, metabolism, contractility, and heart stability (Lambert et al., 2015).

Several physiological and hormonal modulators regulate NHE3 activity. The majority of the NHE3 regulatory hormones are coupled to protein kinases associated with intracellular signalling cascades. Different mechanisms such as direct phosphorylation, protein trafficking, and interaction with accessory proteins modulate NHE3 activity (Dynia et al., 2010; Pedersen & Counillon, 2019). Moreover, in a normal state, the regulation of NHE3 is dependent on its C-terminal phosphorylation. Various kinases, including casein kinase 2 (CK2), serum glucocorticoid-regulated kinase-1 (SGK1), protein kinase A (PKA), Ca^{2+}/Calmodulin-dependent Protein Kinase-II (CaMKII), cGKII, GSK-3, AKT, ERK and $p90^{RSK}$ mediate NHE3 phosphorylation (Dynia et al., 2010). No et al. (2015) demonstrated that lysophosphatidic acid (LPA) stimulated NHE3 activity by LPA5 receptor and EGF receptor (EGFR) transactivity. This, in turn, activated proline-rich tyrosine kinase 2 (Pyk2) and ERK

specifically in the apical membrane. The authors hypothesized that RSK could be an associated effector of Pyk2 and ERK since RSK is a well-known effector of EGFR and ERK. In contrast, the regulation of RSK by Pyk2 is still not known. The study showed that RSK2, but not RSK1, regulated direct phosphorylation of NHE3 and concluded that RSK2 phosphorylation of NHE3 mediates NHE3 regulation by LPA.

3.2 SGLT Receptors Overview

Sodium-glucose co-transporters (SGLTs) are active symporters that belong to the solute-carrier family-5 (SLC5) of active glucose transportation, and facilitate glucose homeostasis (Wright et al., 2011). The human SLC5 transporter family contains 12 members, with up to six different SGLT receptors identified in human cells. Functional studies showed that all SLC5 family proteins weigh between 60- to 80-kDa (580–718 amino acids). The most-studied isoforms of this family, SGLT1 and SGLT2, are involved in glucose absorption and glucosuria.

3.2.1 Activity and Regulation of SGLT

Several studies have focused on the activity and expression of SGLT under different physiological/pathophysiological settings. SGLT1 expression was in the small intestine, kidneys, liver, lungs, cardiac myocytes, and highly expressed in the human heart. SGLT2 expression was primarily found in the kidney and pancreatic alpha cells. (Kashiwagi et al., 2015). SGLT1 levels are elevated further in cardiac ischemia or hypertrophy disease states. This increase in SGLT1 expression can be linked to the increased phosphorylation of secondary messengers such as ERK 1/2 and the mammalian target of rapamycin (mTOR), involved in the signaling pathways of cardiac ischemia/hypertrophy. However, further studies are required to confirm the proposed mechanism (Di Franco et al., 2017).

The kidney plays a vital role in glucose homeostasis by promoting the reabsorption of filtered glucose. The two isoforms carry out reabsorption across the apical cell membranes (Poulsen et al., 2015). SGLT2 is located on the luminal membrane of the proximal convoluted tubule in S1 and S2 segments, whereas SGLT1 is expressed in the S3 segment (Figure 1) (Chao, 2014). A healthy kidney reabsorbs 90% of filtered glucose from the proximal tubule via SGLT2, whereas a diabetic kidney increases its reabsorption of glucose by 20% more than the normal rate through the overexpression of SGLT2. The active transport of glucose by both isoforms is linked with the transport of Na^+ into the intracellular

fluid (Chao, 2014; Novikov & Vallon, 2016). Inhibition of this process promotes the reduction of intracellular Na^+ levels and excretion of glucose in urine (glucosuria), leading to the correction of hyperglycaemia (Chao, 2014).

4. Role of SGLT & NHE1 and 3 in Diabetes

DM can stimulate the proliferation of vascular smooth muscle cells (VSMCs) to proliferate through insulin and insulin-like growth factor 1 (IGF-1), which is in turn mediated by NHE1. Insulin can stimulate the transcription of NHE1 directly and regulate the activity of NHE1 in nonvascular cells, while IGF-1 regulates NHE1 activity in vascular cells. Moreover, hyperglycaemia affects the activity of NHE1. For example, hyperglycaemia increases the production of diacylglycerol precursors, leading to the PKC activation, consequently activating NHE1. Also, NHE1 in VSMCs can be activated by the oxidized LDL which has been shown to be elevated in DM and hyperlipidaemia. Furthermore, AGEs react with the extracellular matrix, resulting in the thickening of vessel walls. Besides, VSMCs adhesion, which is mediated by cell surface integrins and extracellular matrix proteins, promotes PKC activation and stimulation of NHE1 activity. Interestingly, it was speculated that glycation of the extracellular matrix protein fibronectin inhibited NHE activity and suppressed the growth of VSMCs (Hannan & Little, 1998).

On the other hand, the activity of NHE3 is stimulated as a result of increased levels of insulin, glucose, and specific adipokines in T2DM. The increased activity and upregulation of NHE3 may be instrumental to developing chronic complications in diabetic patients such as: diabetic nephropathy and uric acid nephrolithiasis (Packer, 2017). The early phase of diabetic kidney disease presents changes in eGFR, elevated reabsorption of salt and water, and expanded extracellular volume, all of which advance to hypertension, hyperfiltration, and eventually renal hypertrophy (Girardi & Sole, 2012).

The number of main Na^+ and water transporters are hypothesized to increase in diabetic kidneys as a compensatory mechanism due to extensive water and Na^+ loss (Song et al., 2003). The study demonstrated that STZ- induced T1DM rats had an increased protein content of Na^+ and water transporters NHE3 (204% of the vehicle mean), thiazide-sensitive Na^+/Cl^- co-transporter, and α, β, and γ subunits of the epithelial sodium channel. According to another study conducted by Klisic et al. (2006) using a similar animal model, brush-border membrane NHE3 activity was significantly higher by 40% after seven days and 37% after 14 days compared to control rats. However, the increased activity of NHE3 was not associated

with changes in NHE3 protein or mRNA. Unlike Song et al. (2003), they selectively used cortical brush-border membrane vesicle for analysis to reflect proximal tubule NHE3 and not the analysis of whole kidney homogenates. An STZ-induced diabetic rat study demonstrated that diabetic kidneys were 67% larger in size, had 22% longer proximal tubules, and 20% longer distal tubules compared to normal rat kidneys Rasch (1984). Since the reabsorption of Na^+ occurs mainly in the proximal tubules, its elongation can easily result in increased activity of NHE3 (Girardi & Sole, 2012). Hyperglycaemia also enhances ANG-II production by stimulation of angiotensinogen and RAAS. This further activates NHE3 via the SGK1 signalling cascade involving phosphatidylinositol 3-kinase (PI3-kinase) and 3-phosphoinositide-dependent protein kinase-1 (PDK1) (Ackermann et al., 2009; Stevens et al., 2008). Another signal cascade of ANG-II induced NHE3 stimulation includes the non-receptor tyrosine kinase (c- Src), PI3-kinase activation, PKC (du Cheyron et al., 2003; Tsuganezawa et al., 1998), and Ca^{2+} and CaMKII (P. He et al., 2010).In the proximal tubule, the uptake of albumin requires the involvement of the megalin/cubilin complex. In diabetic nephropathy, there is decreased endocytosis of albumin due to decreased megalin expression, characterized by microalbuminuria (Tojo et al., 2001). The decreased albumin uptake leads to elevated intratubular albumin concentration, stimulating NHE3 activity and further worsening kidney damage (Girardi & Sole, 2012). In the Opossum kidney cells, high glucose levels resulted in hypertrophy due to increased osmolality (Drumm et al., 2003). Consequently, albumin uptake increased because of NHE3 overactivity.

5. Role of SGLT & NHE1 and 3 in Cardiovascular Diseases

5.1 Ischemia-Reperfusion Injury, Cardiac Remodelling, and Hypertrophy

HF is a syndrome often developed after several remodelling processes in the heart that includes LV hypertrophy, fibrosis, and diastolic dysfunction (Uthman et al., 2018). In diabetes, the heart is in a state of metabolic overload due to cardiac metabolism. Several vital mechanisms were linked to the induction of cardiac impairment and the early development of HF that overlap with other CVDs. NHE and SGLT's potential relevance to the direct effects in the myocardium will be discussed concerning the early stages of HF development.
As NHE 1 is the main plasma membrane isoform in the heart, it takes an essential part in cardiac functioning in normal and disease states. Hormones such as endothelin-1, ANG-II, and α-adrenergic stimulators, contribute to NHE1 activity in cardiac remodelling (Odunewu-Aderibigbe & Fliegel, 2014; Wakabayashi et al., 2013). Overactivity of NHE1 has been

proven to cause several pathological changes in the myocardium, including ischemia-reperfusion injury (IRI), cardiac remodelling, hypertrophy, and apoptosis that eventually can progress to HF, The potential mechanisms underlying the role of NHE1 in the remodelling process can be summarized by the role of both Na^+ accumulation and mitochondrial remodelling (Karmazyn et al., 2008). During the disease state, as an adaptive mechanism, NHE1 activity is increased to correct the reduced intracellular pH. Since the Na^+/K^+ ATPase becomes inactive during ischemia, NHE-mediated Na^+ influx leads to the intracellular accumulation of Na^+ (Cingolani Horacio & Ennis Irene, 2007; Imahashi et al., 2007; Karmazyn et al., 2008). This rise in intracellular Na^+ consequently leads to the two-folds elevation in intracellular Ca^{2+} by direct reversal of Na^+/Ca^{2+} exchanger (Figure 2) (Wakabayashi et al., 2013), leading to an intracellular Ca^{2+} overload which in turn triggers deleterious pathways that lead to myocardial injury, hypertrophy, and subsequent dysfunction (Figure 3). Additionally, impairment of mitochondrial function and structure due to swelling, ATP depletion/dysfunction, ROS production, and opening of the mitochondrial permeability transition pore (MPTP) often accompanies cardiac hypertrophy. On the other hand, NHE1 inhibition and gene ablation attenuates the opening of MPTP and balances the amounts fission and fusion proteins on the mitochondria. Hence, NHE1 inhibition serves as a cardioprotective mechanism to prevent Na^+ and Ca^{2+} accumulation and subsequent activation of intracellular pathways, which in turn may improve mitochondrial function and structure integrity, and the cumulative adverse effects on the myocardium (Odunewu-Aderibigbe & Fliegel, 2014). Various studies have shown that inhibition and genetic ablation of NHE1 of in-vivo models protected the myocardium from ischemia-reperfusion injury (Wang et al., 2003). In another study, although transgenic mice models overexpressing NHE1 had no significant effect on cardiac function, intracellular pH, intracellular Na^+, and ischemia-reperfusion injury, NHE1 inhibition with cariporide prior to the development of ischemia prevented accumulation of Na^+ and Ca^{2+} and decreased ischemia-reperfusion injury, showing that baseline NHE1 activity was not the rate-limiting step (Imahashi et al., 2007).

The effect of SGLT on diabetic hearts has been well researched within the last few years. Although most of the literature reported on the impact of SGLT2i preventing CVD in T2DM, SGLT2 receptors were not detected in the heart. New data confirmed that SGLT1 expression and activity are upregulated in ischemia, hypertrophic, failing, and diabetic hearts in humans with end-stage cardiomyopathy and animal models (García-Ropero et al., 2019; Uthman et al., 2018). Ramratnam et al. (2014) reported that overexpression of SGLT1 in transgenic mice was associated with pathologic cardiac hypertrophy and LV dysfunction.

During ischemia, glucose uptake and utilization increase along with a 2-to-3-fold upregulation of SGLT1. This upregulation was postulated to be an adaptive response to injury and as a response to AMPK and ERK1/2 activation (García-Ropero et al., 2019). How SGLT1 up-regulation makes an impact is not known, and discrepancies between studies leave it uncertain whether SGLT1 receptors exert a protective or deleterious role in cardiac physiology.

During acute injuries, SGLT1 over-expression facilitates glucose uptake and generates ATP molecules for the heart through anaerobic glycolysis. Kashiwagi et al. (2015) provided evidence of the protective role of SGLT1 against IRI. Using the ex-vivo murine langendorff model, they studied the role of SGLT1 inhibition by phlorizin on cardiac function. During IRI, the use of phlorizin resulted in significant impairment in the recovery of LV contractions and increased infarct size (due to increased CPK activity). There was also a reduction in ATP content associated with a decrease in glucose uptake and glycolysis, showing that SGLT1 inhibition during ischemia-reperfusion impairs cardiac metabolism. On the other hand, several studies showed that SGLT1 inhibition leads to the improvement of cardiomyopathy. This is evidenced by several experimental studies in SGLT1 knockdown models. Ramratnam et al. (2014) reported that double-transgenic mice (SGLT1 knockdown with PRKAG2 mutation) have attenuated cardiac glycogen accumulation, cardiac hypertrophy, and LV dysfunction. Similarly, Z. Li et al. (2019) discovered that pharmacological and genetic inhibition of SGLT1 prevented injuries following ischemia-reperfusion (in in-vivo, ex-vivo, and in-vitro models) and reduced ROS, myocardial necrosis, infarct size, along with improved hemodynamic functions. Furthermore, an in-vitro study (Balteau et al., 2011) on adult rat myocytes demonstrated that increased glucose transport through SGLT1 results in NADPH oxidase (NOX2) activation, leading to increased production of ROS and subsequent damage to cardiomyocytes (Figure 3). This effect was counteracted by phlorizin, an SGLT1 inhibitor.

In contrast to SGLT1, SGLT2 receptors were not detected in the heart. The mechanism of SGLT2i cardioprotection is still undetermined, but studies have shown that SGLT2i affect cardiomyocytes by directly inhibiting NHE and improving mitochondrial function. Future studies should investigate if there is a link between SGLT2i and SGLT1 in failing hearts and whether dual inhibition may have other beneficial effects on the myocardium. However, Lee et al. (2021) induced MI through left anterior descending artery ligation in non-diabetic mice. Three-days after MI induction, there was a transient expression of SGLT2 in the site of occlusion in the heart showed by immunofluorescence and western

blot. However, the authors could not conclude if SGLT2 is expressed by cardiomyocytes or by the inflammatory cells migrating to the infarct site.

Overall, there is an interplay between the two membrane transporters (SGLT1 and NHE1) in mediating cardiac effects in failing hearts. While the role of NHE1 inhibition is well defined, the cardioprotective mechanism of SGLT2 inhibition is unknown with the exemption of SGLT2i direct NHE1 inhibition. Interestingly, a preclinical in-vitro study in lipopolysaccharide (LPS) stimulated mouse cardio-fibroblasts tested the hypothesis that DAPA can cause NHE1 downregulation AMPK- dependent pathway. Ye et al. (2018) reported that DAPA resulted in elevated levels of the phosphorylated form of AMPK in LPS stimulated cardio-fibroblasts. The results showed that DAPA mitigated the rise in NHE1 mRNA and confirmed the relation between NHE1 and Hap70 through the AMPK dependent pathway. Similarly, Uthman et al. (2018), proved that the three SGLT2i available in the market directly suppressed NHE1 activity in-vitro.

5.2 Diabetic Cardiomyopathy

A plethora of evidence suggests that NHE1 is noticeably involved in mediating cardiac hypertrophic responses in DCM, and therefore a potential therapeutic target (Karmazyn, 2003). Mraiche et al. (2011) used two transgenic mouse models; one expressing wild type NHE1 and another expressing an activated form to investigate the effect of NHE1 activation on cardiac hypertrophy. NHE1 hyperactivation has been linked to elevated glucose levels in DCM induced by PKC-dependent mechanisms. Additionally, an increase in heart weight to body weight, apoptosis, fibrosis, and a decrease in cardiac functionality was recorded. Studies had shown an enhanced mitochondrial NHE1 activity in the hearts of diabetic rats. Allen and Xiao (2003) have illustrated that the main pathway for Na^+ entry during reperfusion of an ischemic diabetic heart is NHE1. Na^+ concentration changes are linked to altered Ca^{2+} influx, production of ROS, and cell damage. Additionally, expression of the activated form of NHE1 increased the sensitivity to neurohormonal stimulation (using phenylephrine). Indeed, patients with DM experience neurohormonal dysregulation. During HF, neurohormonal systems like norepinephrine, ANG-II, aldosterone, and neprilysin are activated, causing impaired insulin sensitivity and microvascular complications (Doliba et al., 2018; Packer, 2017). Reduced insulin sensitivity and adipokine abnormalities are characteristic of DM and pathophysiological for HF.

Compared to NHE1, the NHE3 isoform distribution is mainly limited to the kidney and gastrointestinal epithelial cells. The main role of NHE3 in DCM is related to its regulation of Na^+ reabsorption in the proximal tubules, which regulates sodium uptake following glomerular filtration. NHE3 activity is enhanced with neurohormonal stimulation by norepinephrine, ANG-II, and aldosterone in HF. Additionally, insulin, glucose, and some adipokines, which are elevated in T2DM, stimulate NHE3. In HF, NHE3 activity is elevated in the kidney mediating Na^+ reabsorption, leading to fluid and Na^+ retention, peripheral oedema, and diuretic resistance. NHE3 hyperactivity in DM also leads to kidney mesangial cell proliferation, hyperfiltration, and diabetic nephropathy, contributing to cardiac overload and further worsening of HF (Packer, 2017; Silva dos Santos et al., 2019). Considering all these pathophysiological changes, concluding that the NHE family could link HF and DM is reasonable.

Regarding the SGLT family, evidence shows enhanced SGLT1 expression in end-stage cardiomyopathy in obese mice with T2DM. Controversially, reduced expression of SGLT1 is recorded in T1DM. This suggests that the increase in its expression might be attributed to the hyperinsulinemia state found in T2DM, but not T1DM. SGLT1 expression was linked to cardiac fibrosis and collagen deposition in the heart (Zhou et al., 2015). Hypertrophic cardiomyopathy was induced through a transverse aortic constriction in a titin-truncated mouse model that increases interstitial fibrosis in wild-type mice without affecting SGLT1 deficient mice. Additionally, SGLT1 contributes to the oxidative stress seen in DCM, as its destruction in mouse atrium cardiomyocytes protects the cells against hypoxia and reoxygenation injury (Kuznetsov et al., 2015). Furthermore, mice with cardiomyocyte-specific SGLT1 knockdown were resistant to both in-vivo and ex-vivo myocardial ischemia/reperfusion injury (Yoshii et al., 2019).

SGLT2 is an isoform mainly present in the kidneys, while there is a limited-to-no expression in the heart. However, the cardioprotective effects of SGLT2i suggest that SGLT2 is involved in DCM by expression in the kidney; there is an increased expression of renal SGLT2 and enhanced glucose reabsorption (Vallon & Sharma, 2010). Studies using knockout mice as a negative control have shown an enhanced SGLT2 expression in T2DM and T1DM mice. However, the biological mechanism for SGLT2 upregulation in DM is not understood. A study with human embryonic cells (HEK-293T) showed that insulin phosphorylated the SGLT2 Ser624 residue, which increased ROS production, further damaging kidney cells (Novikov & Vallon, 2016). Interestingly, using hypoinsulinemic T1DM, there was also enhanced expression of SGLT2, which suggests the involvement of other regulatory proteins.

ANG-II can increase SGLT2 expression, and its role in inducing cardiac hypertrophy, heart failure, and DCM is proven. This shows a link between the expression of SGLT2 in the kidneys and DCM (Vallon & Sharma, 2010).

5.3 Hypertension

Hypertension occurs as an autoregulatory response to increased Na^+ concentration due to increased reabsorption. Na^+ reabsorption is mediated by activating the RAAS and the consequent triggering of the ANG-II Type 1 (AT1) receptor, stimulating NHE3-induced Na^+ influx (Dominguez Rieg et al., 2016). Increased Na^+ influx promotes the significant expansion of extracellular volume and cardiac output and mediates a rise in peripheral vascular resistance resulting in elevated BP (Girardi & Sole, 2012). Hypertension signals the body to promote re-establishment of the expanded volume via decreased eGFR followed by pressure natriuresis.

Overexpression of NHE3 in proximal tubules was detected in the spontaneously hypertensive rat (SHR) model of human primary hypertension (X. C. Li et al., 2019). Interestingly, ANG-II leads to the overexpression of NHE3 in cultured cells of the proximal tubules as it stimulates the exocytosis of NHE3. In fact, it was found that along with NHE regulatory factor 1, IRBIT protein forms a complex with NHE3 during exocytosis after ANG-II stimulation. (He et al., 2016). Other anti-natriuretic peptide hormones such as insulin and glucocorticoid caused the activation of NHE3 in proximal tubules (Fuster et al., 2007; X. C. Li et al., 2019; Pao et al., 2010; Wang et al., 2007). Li et al. (2015) studied the role of NHE3 in hypertension using $NHE3^{-/-}$ mice with the transgenic rescue of NHE3 in the small intestine and affirmed their hypothesis that NHE3 is essential for ANG-II induced hypertension. In mice with ANG-II-induced hypertension, the selective genetic deletion of NHE3 of the proximal tubule attenuated the condition (X. C. Li et al., 2019). Studies showed that 50% of hypertensive individuals were insulin resistant. Moreover, hypertensive patients are at an high risk of developing CVDs (Lima et al., 2009). NHE3 participates in Na^+ reabsorption in proximal tubules and plays a critical role in the absorption of dietary Na^+ from the gut. Two studies had investigated the role of gut NHE3 using oral NHE3 inhibitor with low systemic absorption on obese SHR. The treatment had significantly reduced the absorption of Na^+ from the gut and reduced BP (Linz et al., 2016; Linz et al., 2012).

NHE1 contributes to pH, salt, and volume regulation, linking it to hypertension. Using NHE1-overexpressing transgenic mice, Kuro-o et al. (1995) showed that NHE1

overexpression caused salt-sensitive BP elevation in mice. Primary hypertensive animal models and peripheral cells of primary hypertensive donors also showed increased NHE1 activation (Orlov et al., 1999). Conversely, NHE1 knockout in mice leads to a reduction in BP and artery tension (Boedtkjer et al., 2012). It is suggested that NHE1 overactivity in VSMCs increases intracellular Na^+, reduces Na^+/Ca^{2+} exchangers, and leads to elevated intracellular Ca^{2+} and increased contraction. With chronic NHE1 overactivation, abnormal cell proliferation can occur in VSMCs (Bobulescu et al., 2005).In proximal tubules, alterations in Na^+ transporters impact the extracellular volume thus changing BP independently from transporters in other renal segments. In hypertension, there is an increase in Na^+ reabsorption that mainly occurs in the proximal tubule and loop of Henle. SGLT2, which is localized in the proximal tubule, is responsible for 60-90% of renal uptake of Na+ and glucose (Cianciolo et al., 2020; Thomas & Cherney, 2018).

The relationship between SGLT2 activity and hypertension is not known yet. When Bautista et al. (2004) compared SGLT2 activity in the proximal tubule of renovascular hypertensive rats with normotensive rats, they saw that Na^+-dependent glucose uptake and SGLT2 expression were higher in the renovascular hypertensive group. In chronically infused ANG-II Wistar rats, the activity and expression of SGLT2 were increased. Using EMPA did not affect the BP; however, losartan, a RAAS inhibitor, reduced BP. In this study, Losartan prevented renal damage, while EMPA produced a minimal protective effect. Nonetheless, EMPA attenuated oxidative stress (Reyes-Pardo et al., 2019). Clinical trials have consistently shown that SGLT2i reduces BP (Sanidas et al., 2020). In the EMPA-REG OUTCOME trial, EMPA was correlated with minimal BP reduction (Zinman et al., 2015). Similarly, CANVAS and CANVAS-R studies showed a reduction in systolic BP by 3.9 mmHg in the canagliflozin (CANA) treated group compared to placebo (Neal et al., 2017). In the DECLARE-TIMI 58 trial, patients treated with DAPA had lower BP by 2.7 mmHg versus placebo (Wiviott et al., 2018). A meta-analysis comprised of 27 RCTs with 12,960 participants concluded that SGLT2i resulted in lower systolic and diastolic BP by 4 mmHg (95%CI, -4.4 to -3.5), and 1.6 mmHg (95%Cl, -1.9 to -1.3), respectively from baseline (Baker et al., 2014).

SGLT2 upregulation could be a partial contributor to hypertension pathogenesis, however several hypotheses explain the role of SGLT2 and its inhibition in BP regulation (Sanidas et al., 2020). Diuresis associated with SGLT2i may cause reduced BP. However, diuresis is a temporary SGLT2i effect, while BP reduction from baseline is a sustained effect (Filippatos et al., 2016; Sanidas et al., 2020).

A direct relationship between SGLT1 and BP has not been established. SGLT1 deficient ($SGLT1^{-/-}$) mice show glucose-galactose malabsorption; however, the absence of SGLT1 did not affect BP compared to wild-type mice (Gorboulev et al., 2012). BP exhibits a diurnal rhythm and SGLT1 expression exhibits a similar rhythm with the highest expression in the morning (Tavakkolizadeh et al., 2001). Remarkably, a hypertensive animal model showed a downregulation in SGLT1 function and expression (Mate et al., 2006). More research is needed to determine how SGLT1 is involved in hypertension pathophysiology (Poulsen et al., 2015).

In summary, SGLT and NHE exhibit different roles in hypertension. Hypertension can worsen the prognosis of DCM where it contributes to the enlargement of the cardiac wall thickness and mass. Increased BP, along with other stimuli, causes vasoconstriction and fluid overload that aggravates cardiac hypertrophy and fibrosis of the myocardium.

6. Available Inhibitors and Their Clinical Outcomes

6.1 Clinical Evaluation of NHE-1 Inhibitors

Substantial evidence supports the protective role of inhibiting NHE1 in reducing IRI development, cardiac hypertrophy, systolic dysfunction, and HF. Several NHE1 inhibitor studies (e.g., cariporide, eniporide, and zoniporide) showed significant protection against CV injuries (Packer, 2017). Despite that, clinical studies in human subjects showed varying results. Therefore, a cardioprotective role of NHE1 inhibition in humans is controversial. The ESCAMI randomized trial investigated eniporide effect on patients (n=1389) with ST-elevation MI (Zeymer et al., 2001) for the primary outcome of the change in infarct size with eniporide as add-on therapy to reperfusion in IRI. However, eniporide did not reduce the infarct size nor improve patients' clinical outcomes. However, the protective effect of cariporide in animal models may have been due to the administration of cariporide during ischemia and not during reperfusion (Klein et al., 2000). Rupprecht et al. (2000) tested the effect of cariporide (40 mg) on 100 patients with acute anterior MI getting direct coronary angioplasty. Compared to placebo, patients who received cariporide had higher ejection fraction (50% vs. 40%; $P<0.05$), lower end-systolic volume (69 vs. 97 mL; $P<0.05$), significant improvement in wall motion abnormalities, and reduced cumulative release of CK-MB ($p= 0.047$). Thus, NHE inhibition by cariporide may prevent reperfusion injury and aid in the recovery from ventricular dysfunction. This study contradicts the ESCAMI study's findings concerning the effects of NHE inhibition, as an adjunct to reperfusion therapy, on

the myocardium. The GUARDIAN study assessed the safety and efficacy of cariporide (20, 80, or 120 mg) in a cohort of patients (n=11,590) at risk for myocardial necrosis (Chaitman, 2003). The cardioprotective effect was only evident in patients who underwent coronary artery bypass graft surgery (CABG) and treated with 120 mg cariporide. The EXPEDITION study was the first phase-3 myocardial protection trial to examine cardioprotective effects of cariporide in high-risk patients (n=5,761) undergoing CABG (Mentzer et al., 2008). The drug resulted in increased mortality rates associated with increased cerebrovascular events (2.2% with cariporide vs. 1.5% with placebo; P=0.02). The incidence of death or MI was significantly reduced from 20.3% in the placebo group to 16.6% in the cariporide-group (P=0.0002). However, due to the increased mortality, the study was early terminated. The findings suggested that NHE1 inhibition could significantly reduce ischemia-reperfusion injuries and that cariporide is unlikely to be used clinically. The mixed findings obtained from the clinical research of NHE inhibitors conflict with the highly favourable evidence from experimental studies and emphasize the challenges facing the translation of potential therapies from the laboratory to the clinic.

6.2 Clinical Evaluation of SGLT Inhibitors

As SGLT1 and SGLT2 are considered the primary transporters involved in glucose homeostasis, several drugs have been developed to inhibit their activity. Inhibiting SGLT1 results in better post-meal blood glucose control by blocking glucose uptake in the intestine, which decreases the glycaemic burden. Furthermore, as most glucose reabsorption processes in the proximal convoluted tubule are mediated by SGLT2, inhibition of this transporter reduces the kidney glucose threshold and excretion of glucose lowers glucose plasma levels. This effect is insulin-independent, and therefore, if this class of inhibitor is used alone, the risk of hypoglycaemia is low. These drugs can also increase weight loss by promoting urinary glucose excretion (Raskin, 2013).

The development of SGLT inhibitors started in 1835 with the discovery of phlorizin, which was speculated to treat malaria and infections until 1886 when it was reported to cause glucosuria and renal effects (Chasis et al., 1933; Dominguez Rieg & Rieg, 2019). Administration of subcutaneous phlorizin to diabetic rats with insulin resistance normalized insulin sensitivity and glucose levels (Rossetti, Shulman, et al., 1987; Rossetti, Smith, et al., 1987). However, the clinical use of phlorizin was limited due to its poor bioavailability, low

solubility, and non-selectivity in SGLT inhibition with increased selectivity to SGLT2 compared to SGLT1 (Crespy et al., 2001; Dominguez Rieg & Rieg, 2019).

Due to the limitations of phlorizin, other compounds were developed, such as T-1095 and its active form T-1095A which are synthetic compounds derived from phlorizin. Oral T-1095 exhibited dose-dependent elevation in urine glucose excretion by inhibiting SGLT2 in the proximal tubule, resulting in reduced blood glucose concentration (Oku et al., 1999). Additionally, T-1095 reduced postprandial blood glucose levels in STZ-induced diabetic rats via inhibition of SGLT1 in the intestine. However, the clinical use of T-1095 was limited due to its non-selectivity.

Currently, several SGLT2 inhibitors are approved for clinical use in the US and worldwide, and others are under development. Sotagliflozin is an example of a dual SGLT1/2 inhibitor with only ~30-folds higher selectivity for SGLT2 over SGLT1, seeking approval by the FDA (Dominguez Rieg & Rieg, 2019). Two randomized controlled trials, SOLOIST-WHF and SCORED, randomized T2DM patients with CKD or recent HF hospitalizations, respectively, to receive either sotagliflozin or placebo and found a statistically significant reduction in death from cardiovascular causes, HF hospitalizations, urgent visits for HF, and all-cause mortality (Bhatt, Szarek, Pitt, et al., 2020; Bhatt, Szarek, Steg, et al., 2020).

Other SGLT inhibitors are still under investigation, such as Mizagliflozin, a selective SGLT1 inhibitor; and licogliflozin, a dual SGLT1/2 inhibitor (Dominguez Rieg & Rieg, 2019).

Recently, several SGLT2i were developed and approved to be used in T2DM patients. In addition to their glucose-lowering effects, CANA DAPA and EMPA showed clinical evidence of improved clinical outcome of HF, chronic kidney disease, and CVD in patients with adequate eGFR.(García-Ropero et al., 2019). The CANVAS program joined the analysis of CANVAS and CANVAS-R, which included patients with T2DM and increased CV risk to assess CANA use compared to placebo (Neal et al., 2017). The CANVAS trial assessed CV risk and major adverse cardiac events, while the CANVAS-R trial assessed the progression of albuminuria in patients using CANA versus placebo. The combined analysis showed CANA lowers CV events and probably attenuates albuminuria progression. However, it increases the risk of metatarsal amputation compared to placebo. EMPA is another example of an SGLT2 inhibitor with cardioprotective evidence. In the EMPA-REG OUTCOME trial (Zinman et al., 2015), EMPA was reported to reduce CV death by 38%, HF hospitalization by 35%, and death from any cause by 32% in T2DM patients at high CV risk. Additionally, the DECLARE-TIMI trial evaluated the effect of DAPA in patients with T2DM and established CVD or CV risk factors (Wiviott et al., 2018). Although DAPA was associated with lower

rates of HF hospitalization or CV death than placebo, there was no difference in major adverse cardiac events between placebo and DAPA. Furthermore, the DAPA-HF and EMPEROR-REDUCED trials found a protective effect of DAPA and EMPA, respectively, against CV death plus HF hospitalizations in HF patients regardless of the presence of diabetes (McMurray et al., 2019; Packer et al., 2020).As per these findings, the American Diabetes Association recommends a combination therapy of metformin and SGLT2 inhibitor for established ASCVD, HF, or chronic kidney disease (ADA, 2021). Other clinical studies pointed to the natriuretic effects of SGLT2i, which impact CV benefits through a reduction in fluid retention and the risk of developing HF. Using immunofluorescence, Pessoa et al. (2014) reported that NHE3 co-localizes with SGLT2, not SGLT1, concluding that SGLT2i causes diuresis via NHE3 inhibition. A recent randomized placebo-controlled crossover study in 20 patients with T2DM and HF treated with EMPA monotherapy showed a significant increase in fractional excretion of Na+ (FENa) compared to placebo (P=0.001). A synergistic effect on the FENa was reported when combined with bumetanide (P=0.001). Moreover, after 14 days of SGLT2 inhibition by EMPA and its persistent natriuretic effect, there was a reduction in blood volume (P=0.035) and plasma volume (P=0.04) without inducing neurohormonal activation, off-target electrolyte wasting, and renal dysfunction. Thus, the benefits of long-term use of EMPA in HF patients may be volume management attributed to the natriuretic effects (Griffin et al., 2020).

7. Conclusion

Diabetes mellitus is highly associated with cardiovascular disease, as hyperglycaemia triggers cardiac metabolic imbalances, endothelial dysfunction, ROS production, RAAS activation, and impaired Ca^{2+} homeostasis, leading to heart failure. There is an increasing evidence supporting the cardioprotective role of SGLT2i. Overall, there is an interplay between SGLT and NHE in mediating cardiac effects seen in the failing hearts. NHE1 and NHE3 are two well-studied isoforms involved in renal and cardiovascular homeostasis. In the heart, NHE1 regulates intracellular pH, cell volume, proliferation, and Na^{+} concentration, which in turn plays a role in Ca^{2+} regulation, metabolism, contractility, and stability of the heart. On the other hand, renal NHE3 contributes to the regulation of extracellular volume and BP. While the role of NHE1 inhibition is well defined, the exact cardioprotective mechanism of SGLT2 inhibition has not been determined, with the exemption of SGLT2i directed NHE1 inhibition. Further studies are needed to investigate the interaction between NHE3 and SGLT2.

Figure Captions

Figure 1. Glucose reabsorption through SGLT1 & SGLT2 in the normal kidney

Figure 2. Potential Pathways Underlying the Hypertrophic Effect of NHE1

(A) During non-ischemic events (normal conditions) NHE is relatively quiescent. The Na+ K+ ATPase (Na^+ pump) utilizes ATP to extrude Na^+, and the bidirectional Na+/Ca^{2+} exchanger works predominantly in the forward (Ca^{2+} efflux) mode. **(B)** During ischemic events $[Na^+]_i$ rises during ischemia concomitant with a fall in pH. NHE becomes activated in response to intracellular acidosis and other hypertrophic stimulatory factors. Since the Na^+/K^+ ATPase becomes inactive during ischemia, NHE-mediated Na^+ influx leads to the intracellular accumulation of Na^+. Increased Na^+ elevates intracellular Ca^{2+} by altering the reversal potential of Na^+/Ca^{2+} exchangers. Elevated Ca^{2+} activates various pro-hypertrophic factors, including CaN and CaMKII, and increases MPTP, contributing to mitochondrial remodelling. Mitochondrial remodelling results in increased ROS production, which in combination with other factors contributes to activating transcriptional factors resulting in cardiac hypertrophy.

Figure 3. The Role of SGLT, NHE, and their inhibitors, in Diabetes and Cardiovascular Diseases. Increased SGLT activity in the proximal tubules leads to decreased natriuresis and increased reabsorption of glucose, worsening heart failure and diabetes, respectively. In the heart, hypertrophic signals such as endothelin-1, ANG-II, thrombin, and norepinephrine increase NHE1 activity, leading to Na^+ accumulation and mitochondrial dysfunction which activates pro-hypertrophic transcription factor. Hyperglycaemia leads to increased glucose transport through SGLT1, leading to increased NOX2 activity, and subsequent damage to the cardiomyocytes through ROS.

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