Myocardial Impact of NHE1 Regulation by Sildenafil

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The cardiac Na^+/H^+ exchanger (NHE1) is a membrane glycoprotein fundamental for proper cell functioning due its multiple housekeeping tasks, including regulation of intracellular pH, Na^+ concentration, and cell volume. In the heart, hyperactivation of NHE1 has been linked to the development of different pathologies. Several studies in animal models that reproduce the deleterious effects of ischemia/reperfusion injury or cardiac hypertrophy have conclusively demonstrated that NHE1 inhibition provides cardioprotection. Unfortunately, NHE1 inhibitors failed to reproduce these effects in the clinical arena. The reasons for those discrepancies are not apparent yet. However, a reasonable clue to consider would be that drugs that completely abolish the exchanger activity, including that its essential housekeeping function may not be the best therapeutic approach. Therefore, interventions tending to specifically reduce its hyperactive state without affecting its basal activity emerge as a novel potential gold standard. In this regard, a promising goal seems to be the modulation of the phosphorylation state of the cytosolic tail of the exchanger. Recent own experiments demonstrated that Sildenafil, a phosphodiesterase 5A inhibitor drug that has been widely used for the treatment of erectile dysfunction is able to decrease NHE1 phosphorylation, and hence reduce its hyperactivity. In connection, growing evidence demonstrates cardioprotective properties of Sildenafil against different cardiac pathologies, with the distinctive characteristic of directly affecting cardiac tissue without altering blood pressure. This mini-review was aimed to focus on the regulation of NHE1 activity by Sildenafil. For this purpose, experimental data reporting Sildenafil effects in different animal models of heart disease will be discussed.

Keywords: NHE1, sildenafil, intracellular pathways, cardiac mechanism, PDE5A

INTRODUCTION

During the last 40 years, the structure, function, and regulation of the sodium/hydrogen exchanger (NHE) have been deeply studied (1–3). This superfamily of transporter proteins comprises 10 isoforms with different cell localization and physiological roles (4, 5). All isoforms share the common fundamental function of protecting cells from intracellular acidification, by means of an electroneutral elimination of one intracellular H^+ in exchange for one extracellular Na^+, and driven by the transmembrane electrochemical Na^+ gradient (3). This mini review focuses on the ubiquitous isoform 1 (NHE1) of this protein family also known as the "cardiac isoform" and its regulation by the cyclic guanosine monophosphate/protein kinase G (cGMP/PKG) signaling route, triggered by inhibition of the phosphodiesterase 5A (PDE5A) by Sildenafil.

THE CARDIAC NA⁺/H⁺ EXCHANGER "NHE1"

The NHE1 was the first cloned isoform, the most characterized (2, 6, 7), and the main expressed variant found in plasma membrane of mammalian cardiac cells (4). The NHE1 is a dimeric transmembrane protein, and as other NHE isoforms, intracellular H^+ extrusion through the NHE1 contributes to maintain intracellular pH (pH_i) of cardiac cells in a healthy narrow range, fulfilling its crucial basal housekeeping function (3). In fact, around normal pH_i values of ∼7.2, the exchanger activity is low but enough to compensate for basal metabolic H^+ production. However, under certain conditions, either physiological or pathological, when pH_i falls far below the normal range, the NHE1, as well as the sodium-bicarbonate cotransporter, become more active being the NHE1 the dominant alkalizing mechanism (8). Interestingly, the recent demonstration that NHE1 is the isoform expressed in mitochondria (9) suggests a potential role of the exchanger in modulating both mitochondrial Na^+ and H^+ gradients (10).

An exacerbated NHE1 activity has been linked to pathological cardiac processes (6). Excessive H^+ efflux through NHE1 leads to a Na^+-dependent Ca^{2+} overload with the consequent activation of deleterious routes, including the calcium/calmodulin dependent protein kinase-histone deacetylase signaling pathway (11), and a mitochondrial permeability transition pore (MPTP) opening (9) followed by the release of reactive oxygen species (ROS) and apoptosis (6, 12). Hyperactivity of NHE1 has been found in several diseased states, such as ischemia/reperfusion injury (6) and postischemic cardiac remodeling (6), as well as in pressure overload (13, 14) and hypertensive (15) cardiac hypertrophy. Reinforcing all these evidences, preclinical studies have demonstrated that blunting NHE1 activity effectively provided cardioprotection in different models of heart failure (16–19). In addition, NHE1 blockade during reperfusion of hearts exposed to an ischemic insult promoted preservation of cardiac function as well as minimization of necrosis and/or apoptosis (20, 21). The latter case is rather paradoxical. NHE1 activation after pH_i fall during ischemia is necessary to preserve cell integrity. However, extracellular pH lowering due to H^+ extrusion under reduced blood flow, together with energetic imbalance due to deficient O_2 supply, rapidly leads to exchanger inhibition. During reperfusion, an immediate washout of extracellular H^+ suddenly restores NHE1 function whose hyperactivity leads to Na^+ and Ca^{2+} overload responsible for the deleterious but distinctive effects of this phase (22). The recovery of pH_i and the subsequent Ca^{+2} overload results in an increased ROS release due to the MPTP opening, uncontrolled myofibrilar hypercontraction, calpain activation-mediated proteolysis, and finally apoptosis (23).

The promising results of NHE1 inhibition in animal models prompted exploration of the clinical use of NHE1 inhibitors such as cariporide and eniporide in patients. Unfortunately, the results were far from expected, demonstrating lack or insufficient cardioprotection (24, 25) or even worse, severe side effects (26). The reasons for these unexpected discrepancies are not apparent yet. However, the total abolition of the exchanger activity, including both its indispensable function and the pathological

hyperactivation, could be a possible explanation. Despite these unforeseen results, inhibition of NHE1 is still considered the most-effective potential therapeutic approach for preventing pathological remodeling of the myocardium irrespective of its origin (27, 28). Thus, a novel scenario of investigation would be to find interventions oriented to specifically reduce the NHE1 hyperactive state. In this regard, a promising approach seems to be the modulation of its phosphorylation level. It is necessary to be reminded that phosphorylation of the cytosolic tail of the NHE1 may either enhance (residues Ser703, Ser770, Ser771) (12, 29) or reduce (Ser 648) (30) exchanger activity. Different kinases have been linked to the phosphorylation/regulation of the NHE1. Among them, the mitogen-activated protein kinase (MAPK) route or the Ras-Raf-MEK-ERK cascade (31) activation in response to different hormones, growth factors, mechanical muscle stretch, or sustained acidosis are the most prominent ones (12, 31–37).

PDE5A AND CARDIAC PATHOLOGIES: INTRODUCING THE PDE5A INHIBITOR SILDENAFIL

Inhibitors of the cGMP-catabolizing enzyme PDE5A are known by their important vasodilatory properties. Sildenafil, among them, was developed as a promising drug against angina pectoris (38) but subsequently safely employed for the treatment of erectile dysfunction (39), pulmonary hypertension, and high-altitude pulmonary edema (40, 41). The main action of Sildenafil was originally assigned to vasodilation and attributed to the increase in the endothelial nitric oxide (NO)-cGMP pathway (42, 43), even in pulmonary hypertension (40). Basal PDE5A expression in cardiac tissue was initially considered insufficient to be detected (44), but further evidence demonstrated that PDE5A is a ubiquitous enzyme (45) and that the myocardium has baseline PDE5A activity (46). Moreover, recent studies showed that upregulation of cardiac PDE5A expression was tightly associated to failing (47, 48) and hypertrophic (49) hearts, to ischemic left and right ventricles (47), and also to the lethal congenital disease known as failing single ventricle (50). In addition, an increased PDE5A expression has been found in different cardiomyopathies that lead to heart failure, such as Chagas (51), or burn-induced (52) cardiac disease. For the specific objective of our current review, PDE5A overexpression has been linked to oxidative stress and the development of pressure overload-triggered myocardial hypertrophy (53, 54), conditions that clearly resemble NHE1 hyperactivation (6). Taken together, the experimental evidence encourages considering that PDE5A inhibition may conceivably be a suitable target to treat cardiac pathologies.

PDE5A INHIBITION BY SILDENAFIL: EFFECT ON MYOCARDIAL NHE1

General Mechanism

The pharmacological target of Sildenafil is PDE5A, with high isozyme selectivity (43). The three PDE5A splicing variants showed equal activity in terms of cGMP-hydrolytic breakdown,

as well as similar sensitivity to the inhibition by Sildenafil
(45). Due to the structural similarity with cGMP, Sildenafil
competitively interacts and inhibits PDE5A enzyme, with the
consequent increase in cGMP levels (43). This second messenger
binds to PKG, producing an allosteric structural change in
the kinase that leads to thiol oxidation, inducing a disulfide
homodimer, hence activating the kinase (55). A well-known
consequence of PDE5A inhibition is the activation of Ca^{+2}-
dependent K^+ channels (BK_{Ca}) (43). However, novel actions of
Sildenafil will be discussed below.

Effects of Acute Sildenafil Administration on NHE1 Activity in the Myocardium

Sildenafil was found to trigger a direct NHE1 inhibitory
action in rat (56, 57) and cat (58) myocardium following
sustained intracellular acidosis (**Figure 1**), and also in the
setting of ischemia/reperfusion injury in isolated rat hearts
(60). It has been demonstrated that the cGMP/PKG route
also regulates different NHE isoforms in different tissues (61,
62). In our hands, inhibition of the NHE1 hyperactivity by
acute Sildenafil intervention was due to phosphatase-mediated
Ser703 dephosphorylation (57, 58, 60). Importantly, acute NHE1
inhibition by Sildenafil did not affect its basal phosphorylation or
expression, and hence preserved its critical homeostatic function
(56, 57). This posttranslational regulation of the exchanger was
found only after sustained acidosis and was canceled by PKG
inhibitors (56). In apparent contradiction to our results, Richards
et al. (63) reported two different routes for cGMP-triggered
NHE1 regulation according to NO concentration. Using an
aminoacidic sequence that reproduced the C-terminal of the
NHE1, they showed that high NO concentration triggers PKA-
mediated Ser648 phosphorylation, while low NO concentration
leads to PKG-mediated Ser703 phosphorylation (63). Although
speculative, and thinking in terms of dimeric kinases-mediated
phosphorylation (such as PKG and PKA actions), the fact that
the authors used part of the whole NHE1 molecule composes a
completely different experimental scenario that would condition
any possibility of comparison.

We have also demonstrated that the inhibitory effect of
Sildenafil on the NHE1 requires activation of the p38MAPK
(**Figure 1**), since blockade of this kinase canceled the inhibitory
effect of Sildenafil on the exchanger (59). The exact sequence
of events triggered by Sildenafil seems to be as follows:
cGMP/PKG activation increase after PDE5A inhibition;
p38MAPK phosphorylation/activation by PKG (64); p38MAPK-
triggered PP2A assembling (65) and migration to sarcolemmal
membrane (66); NHE1 dephosphorylation at Ser703 by PP2A
(67); inhibition of NHE1 hyperactivity (57). See **Figure 2**
for details.

Another controversial subject is the possible role of acute
Sildenafil on ROS. In this regard, it has been shown that this
drug did not reduce ROS-induced lipid peroxidation in hearts
subjected to regional ischemia/reperfusion (60) but reduced
mitochondrial H_2O_2 production under ischemia (68, 69). Despite
possible differences in the sensitivity of experimental methods,
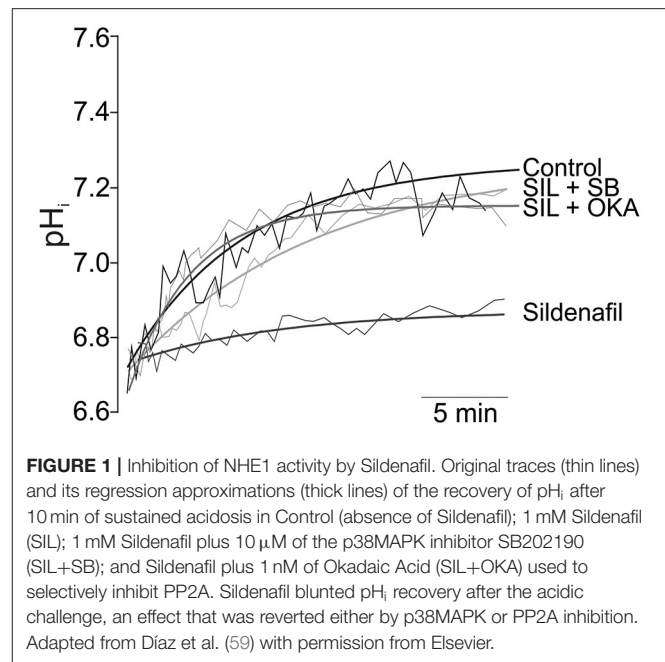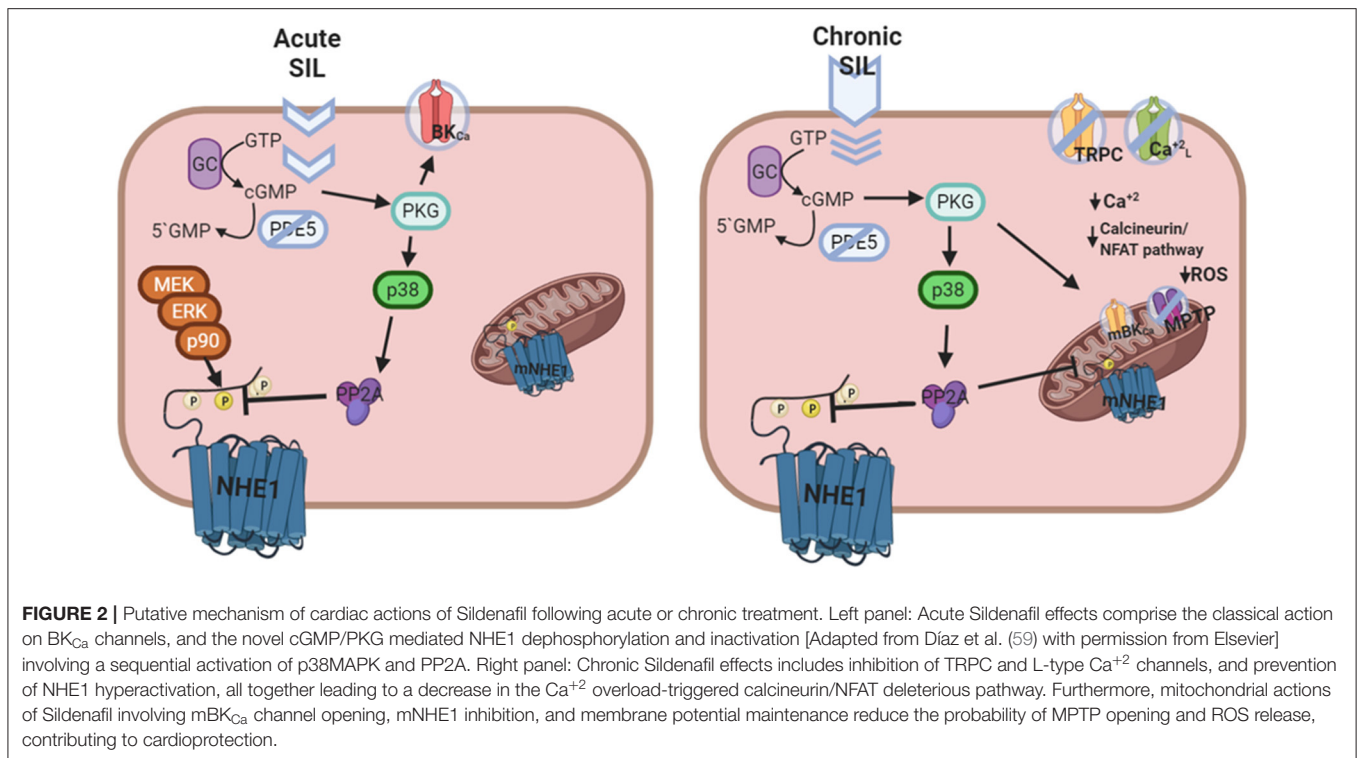ROS-sensitive kinases remained enhanced even after short

FIGURE 1 | Inhibition of NHE1 activity by Sildenafil. Original traces (thin lines)
and its regression approximations (thick lines) of the recovery of pH_i after
10 min of sustained acidosis in Control (absence of Sildenafil); 1 mM Sildenafil
(SIL); 1 mM Sildenafil plus 10 μM of the p38MAPK inhibitor SB202190
(SIL+SB); and Sildenafil plus 1 nM of Okadaic Acid (SIL+OKA) used to
selectively inhibit PP2A. Sildenafil blunted pH_i recovery after the acidic
challenge, an effect that was reverted either by p38MAPK or PP2A inhibition.
Adapted from Díaz et al. (59) with permission from Elsevier.

incubation with Sildenafil, strategy that was enough to effectively
inhibit the NHE1 (60). Longer time periods of Sildenafil
incubation or preischemic application of the drug conducted to a
marked reduction of ROS (68–70).

Cardiac Intracellular Response to Chronic Sildenafil Administration

Different studies were aimed to explain the subcellular basis of
the beneficial actions of chronic PDE5A inhibition by Sildenafil
in the myocardium. Among them, it is interesting to recall
the described inhibitory effect on voltage-operated L-type Ca^{+2}
channels (71), and on the transient receptor potential channels
(TRPC) (72), with the consequent decrease in intracellular Ca^{2+},
therefore eluding the calcium/calmodulin-NFAT pro-apoptotic
pathway (73). The results obtained in our own laboratory add a
new piece of knowledge about Sildenafil anti-hypertrophic and
anti-apoptotic effect, since its previously unknown inhibitory
effect on NHE1 hyperactivity provides an additional clue to
understand how this kind of drug can prevent calcium overload.
Regarding the modulation of NHE1 activity as a potential
therapeutic target, it was reported that complete and chronic
blockade of the exchanger triggers protein upregulation to escape
from inhibition (74). Interestingly, chronic treatment of infarcted
hearts with Sildenafil did not follow this rule by decreasing NHE1
expression (56).

The possibility that chronic effects of Sildenafil could also
involve mitochondrial actions should be considered. Chronic
Sildenafil treatment improved respiratory rate and reduced ROS
production (51). These effects were attributed to restoration of
mitochondrial DNA-encoded gene expression (51), activation
of mitochondrial BK_{Ca} channels (68), prevention of MPTP
opening (75), and/or maintenance of mitochondrial membrane

FIGURE 2 | Putative mechanism of cardiac actions of Sildenafil following acute or chronic treatment. Left panel: Acute Sildenafil effects comprise the classical action on BK_{Ca} channels, and the novel cGMP/PKG mediated NHE1 dephosphorylation and inactivation [Adapted from Díaz et al. (59) with permission from Elsevier] involving a sequential activation of p38MAPK and PP2A. Right panel: Chronic Sildenafil effects includes inhibition of TRPC and L-type Ca^{+2} channels, and prevention of NHE1 hyperactivation, all together leading to a decrease in the Ca^{+2} overload-triggered calcineurin/NFAT deleterious pathway. Furthermore, mitochondrial actions of Sildenafil involving mBK_{Ca} channel opening, mNHE1 inhibition, and membrane potential maintenance reduce the probability of MPTP opening and ROS release, contributing to cardioprotection.

potential (76). In connection, it is important to be reminded that a decrease in mitochondrial NHE1 expression/activity preserves an acidic environment around mitochondria, therefore diminishing Ca^{+2}-induced mitochondrial swelling (9). In addition, it was shown that inhibition of MPTP opening leads to a decrease in ROS production after ischemia (77). Considering these results as a whole, the question as to whether Sildenafil could regulate mitochondrial NHE1 certainly arises. In this regard, recent experiments in the hypertrophied myocardium of SHR suggested that a decreased ROS production after chronic Sildenafil treatment may conceivably result from inhibition of mitochondrial NHE1 through a reduction in its phosphorylation state (78). **Figure 2** summarizes putative signaling pathways triggered by Sildenafil.

Cardiac Pharmacodynamics of Sildenafil

As it was mentioned in a previous section, since the approval and commercialization of Sildenafil more than two decades ago (79), the pharmacodynamics of this drug has been extended to different pathological therapeutic targets. Actually, it has been studied for the treatment of different types of cancers (80), Alzheimer disease (81), vascular dementia (82), and also recently proposed to treat COVID-19 patients (83). Regarding cardiac pathologies, Sildenafil showed the ability to prevent and/or reverse cardiac remodeling induced by acute myocardial infarction (56), chronic mitral regurgitation (84), or pressure overload (85, 86). Additional benefits of Sildenafil were attenuation of sympathetic hyperinnervation (87) and promotion of

an increase in the QT interval (88), therefore reducing the incidence of ischemia-induced arrhythmia. Concerning to other causes of cardiac morbidity, Sildenafil reverted (89), or prevented (52, 85, 90, 91), cardiac fibrosis development regardless of its origin. Furthermore, improvement of heart failure by PDE5 inhibition was shown to involve attenuation of chronotropic response to dobutamin and a T-tubule network restoration (92). Importantly, different studies under diverse experimental conditions demonstrated that the beneficial cardiac effects of Sildenafil are independent of blood pressure (78, 84, 86).

CONCLUDING REMARKS

We have briefly reviewed the role of Sildenafil on NHE1 regulation and its possible pathophysiological relevance. Classical Sildenafil targets were presented, but the main focus of this revision was centered on the novel inhibitory action of this drug on the NHE1. We were particularly interested in stressing the potential benefits of specifically targeting NHE1 hyperactivity, avoiding its complete blockade, which suggests that Sildenafil treatment would be a better therapeutic approach than traditional NHE1 blockers in the field of cardiology.

Finally, considering that Sildenafil has been safely used during more than two decades (39) and that recent results in heart failure patients are certainly promising (93), we suggest that Sildenafil treatment would represent an appropriate opportunity for immediately access to a less expensive and more secure novel therapeutic alternative to treat severe cardiac pathologies characterized by exacerbated NHE1 activity.

AUTHOR CONTRIBUTIONS

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