

Invited review

Pivotal effects of phosphodiesterase inhibitors on myocyte contractility and viability in normal and ischemic hearts

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Phosphodiesterases (PDEs) are enzymes that degrade cellular cAMP and cGMP and are thus essential for regulating the cyclic nucleotides. At least 11 families of PDEs have been identified, each with a distinctive structure, activity, expression, and tissue distribution. The PDE type-3, -4, and -5 (PDE3, PDE4, PDE5) are localized to specific regions of the cardiomyocyte, such as the sarcoplasmic reticulum and Z-disc, where they are likely to influence cAMP/cGMP signaling to the end effectors of contractility. Several PDE inhibitors exhibit remarkable hemodynamic and inotropic properties that may be valuable to clinical practice. In particular, PDE3 inhibitors have potent cardiostimulant effects that can be used for short-term inotropic support, especially in situations where adrenergic stimulation is insufficient. Most relevant to this review, PDE inhibitors have also been found to have cytoprotective effects in the heart. For example, PDE3 inhibitors have been shown to be cardioprotective when given before ischemic attack, whereas PDE5 inhibitors, which include three widely used erectile dysfunction drugs (sildenafil, vardenafil and tadalafil), can induce remarkable cardioprotection when administered either prior to ischemia or upon reperfusion. This article provides an overview of the current laboratory and clinical evidence, as well as the cellular mechanisms by which the inhibitors of PDE3, PDE4 and PDE5 exert their beneficial effects on normal and ischemic hearts. It seems that PDE inhibitors hold great promise as clinically applicable agents that can improve cardiac performance and cell survival under critical situations, such as ischemic heart attack, cardiopulmonary bypass surgery, and heart failure.

Keywords: potassium channels; cyclic AMP; cyclic GMP; ischemia-reperfusion injury; cell survival; cardiac protection; myocardial infarction; signal transduction in cardiovascular system; myocardial contraction; phosphodiesterase inhibitors

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Introduction

The 3',5'-cyclic nucleotide phosphodiesterases (PDE) are a class of enzymes that are capable of cleaving the phosphodiester bond in either cAMP or cGMP to yield 5'-cyclic nucleotides. To date, a large number of gene products forming 11 families of PDE have been classified based upon common characteristics, such as protein sequence, structure, enzymatic properties, and sensitivity to inhibitors^[1]. Although some redundancy in hydrolyzing cAMP and cGMP is apparent among these families, it has become increasingly clear that this complexity has functional relevance and that multiple PDE isoforms are involved in creating heterogeneous cyclic nucleotide signaling within the cell^[2,3].

At least five PDE isoforms (PDE1, PDE2, PDE3, PDE4, and PDE5) have been observed in the heart^[2]. Of the remaining types, PDE6 is solely associated with photoreceptors in the retina while the expression and function of PDE7-PDE11 in the heart have not been studied in depth due to a lack of specific inhibitors or antibodies. The relative expression levels and importance of each PDE within the heart varies by species, stage of development, and level of cardiac stimulation^[4]. Recently, numerous studies have focused on PDE3, PDE4, and PDE5 and their inhibition for treating of a myriad of human diseases, such as acute heart failure syndromes^[5], intermittent claudication^[6], depression^[7], psychosis^[8], chronic obstructive pulmonary disease^[9], pulmonary hypertension^[10], and erectile dysfunction^[11]. There is significant interest in the potential of PDE inhibitors in the treatment of cardiovascular disease. Several recent review articles have discussed the structure and biochemistry^[1,3]

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of PDE, and the crosstalk^[12], compartmentalization^[13], inhibition^[14], and various physiological and pathological functions of individual PDEs^[2,4,15–17]. A monograph dedicated to PDE has also recently been published^[18]. The primary goal of this article is to provide an overview of the effects of PDE3, PDE4, and PDE5 inhibitors on myocyte contractility and survival in normal and ischemic hearts. Table 1 provides a brief summary of the key information on PDE3, PDE4, and PDE5, including the basic characteristics and tissue distribution of these PDE isozymes, as well as the names and relative selectivity of their inhibitors, which will be discussed in greater detail within their respective sections.

Phosphodiesterases and cyclic nucleotide signaling

The secondary messengers cAMP and cGMP regulate a wide variety of cellular functions and morphological processes in the heart, including inotropism, chronotropism, apoptosis, and hypertrophy^[19–22]. The dysfunction of cAMP or cGMP plays a role in heart failure^[19,23] and cardiomyopathy^[24]. The cytosolic levels of cAMP and cGMP are influenced by their production and degradation. The transmembrane protein adenylyl cyclase converts ATP to 3',5'-cyclic AMP (cAMP) and pyrophosphate^[25]. This conversion can be initiated by a G_s mechanism where agonist stimulation of localized receptors, such as the β₁ adrenergic receptor, causes the α subunit of an associated G-protein to bind GTP, dissociate, and bind to adenylyl cyclase thereby activating cAMP production. Since adenylyl cyclase continues to produce cAMP until its stimulation by G_α-GTP

is removed, there is an amplification of the original signal. Adenylyl cyclase can also be inhibited by stimulation of the α-adrenergic receptor through a G_i mechanism^[26]. cAMP binds to the regulatory subunits of cAMP-dependent protein kinase (PKA) leading to activation of the catalytic subunits. Nitric oxide (NO) activates soluble guanylate cyclase (sGC) to produce cGMP and pyrophosphate from GTP^[27]. cGMP may also be produced by membrane-bound particulate fraction guanylate cyclases (pGC), receptor-linked enzymes that are activated by ligands, such as atrial natriuretic factor^[28]. cGMP activates the cGMP-dependent protein kinase (PKG), which phosphorylates downstream targets.

A classic example of the contractile effects of modified cyclic nucleotide signaling is the inotropic and chronotropic response that is due to the elevation of cAMP during β-adrenergic stimulation^[19]. On the other hand, an increase in levels of cGMP is generally thought to reduce contractility^[29], but dose-specific inotropic responses to cGMP activation by NO donors were also reported in isolated cardiomyocytes^[30–32]. In addition, investigators also demonstrated that the NO donor-induced contractile enhancement in frog^[33] and rodent^[34] cardiomyocytes was mediated through a cGMP-independent mechanism. The exact nature of this response remains to be elucidated. The two main factors that contribute to cardiac contractility are calcium transient and myofilament calcium sensitivity. The calcium transient is regulated by many factors, including proteins involved in the excitation-contraction coupling and calcium-induced calcium release, such as L-type voltage gated calcium channel (VGCC)^[35–37] and ryanodine receptor (RyR)^[38,39], as well as

Table 1. Basic characteristics of PDE3, PDE4, PDE5, and their selective chemical inhibitors.

PDE isoform	cGMP K _m (μmol/L)	cAMP K _m (μmol/L)	Tissue distribution	Inhibitors	IC ₅₀	Ref
PDE3A	0.02–0.15	0.18	Heart; vascular smooth muscle; platelets; oocyte; kidney	Milrinone	150 nmol/L	[3,18,254–256]
PDE3B	0.28	0.38	Vascular smooth muscle; adipocytes; hepatocytes; kidney; β cells; developing sperm; T lymphocytes; macrophages	Amrinone Enoximone Pimobendan Cilastazol	16.7 μmol/L 1.8 μmol/L 2.4 μmol/L 200 nmol/L	
PDE4A	–	2.9–10	Ubiquitous	Rolipram	1 μmol/L	[3,18]
PDE4B	–	1.5–4.7	Ubiquitous	Ro 20-1724	2 μmol/L	
PDE4C	–	1.7	Lung; testis; neuronal cells	Cilomilast	120 nmol/L	
PDE4D	–	1.2–5.9	Ubiquitous	Roflumilast	0.8 nmol/L	
PDE5A	2.9–6.2	290	Platelets; vascular smooth muscle; brain; lung; heart; kidney; skeletal muscle	Sildenafil Vardenafil Tadalafil Udenafil	4 nmol/L 0.1 nmol/L 2 nmol/L 6 nmol/L	[3,18]

proteins involved in calcium reuptake, such as sarco/endoplasmic reticulum calcium ATPase (SERCA)^[40], the SERCA regulatory protein, phospholamban (PLB)^[41], Na⁺-Ca²⁺ exchanger (NCX)^[42], and the NCX regulatory protein, phospholemman (PLM)^[43,44]. The sensitivity of the myocardial contractile fibers to Ca²⁺ is regulated by troponin I (cTnI)^[45-51]. The phosphorylation of specific residues on these proteins by PKA and PKG could affect their structure and activity, and thus influence cardiac contractility. In the coronary vasculature, cAMP and cGMP promote vasodilation that may indirectly affect cardiac performance^[52,53]. In addition, the above-mentioned cGMP-independent positive inotropic effects of NO in cardiomyocytes^[33,34] might be achieved through direct S-nitrosylation of the excitation-contraction coupling elements, such as the L-type calcium channels^[34,54] and RyR^[34,55] (Figure 1).

Phosphodiesterase 3

Overview of PDE3 The PDE3 family, consisting of the subfamilies PDE3A and PDE3B, which are expressed in cardiac, vascular smooth muscle, and other tissues, has been well studied as a target of therapeutic drug development^[56]. Members of the PDE3 family are capable of hydrolyzing cAMP and cGMP with high affinity^[14]. However, PDE3 has a V_{max}, the maximum enzyme velocity, for cAMP that is ten times greater than its V_{max} for cGMP, which when taken with its greater affinity for cGMP, means that PDE3 is functionally a cAMP-hydrolyzing enzyme and cGMP is a competitive inhibitor (K_i≈0.6 μmol/L)^[3,57]. Thus, PDE3 is a crucial communicator between the cGMP and cAMP signaling pathways and is likely the point where low concentrations of NO donors are able to exert a positive inotropic effect^[32].

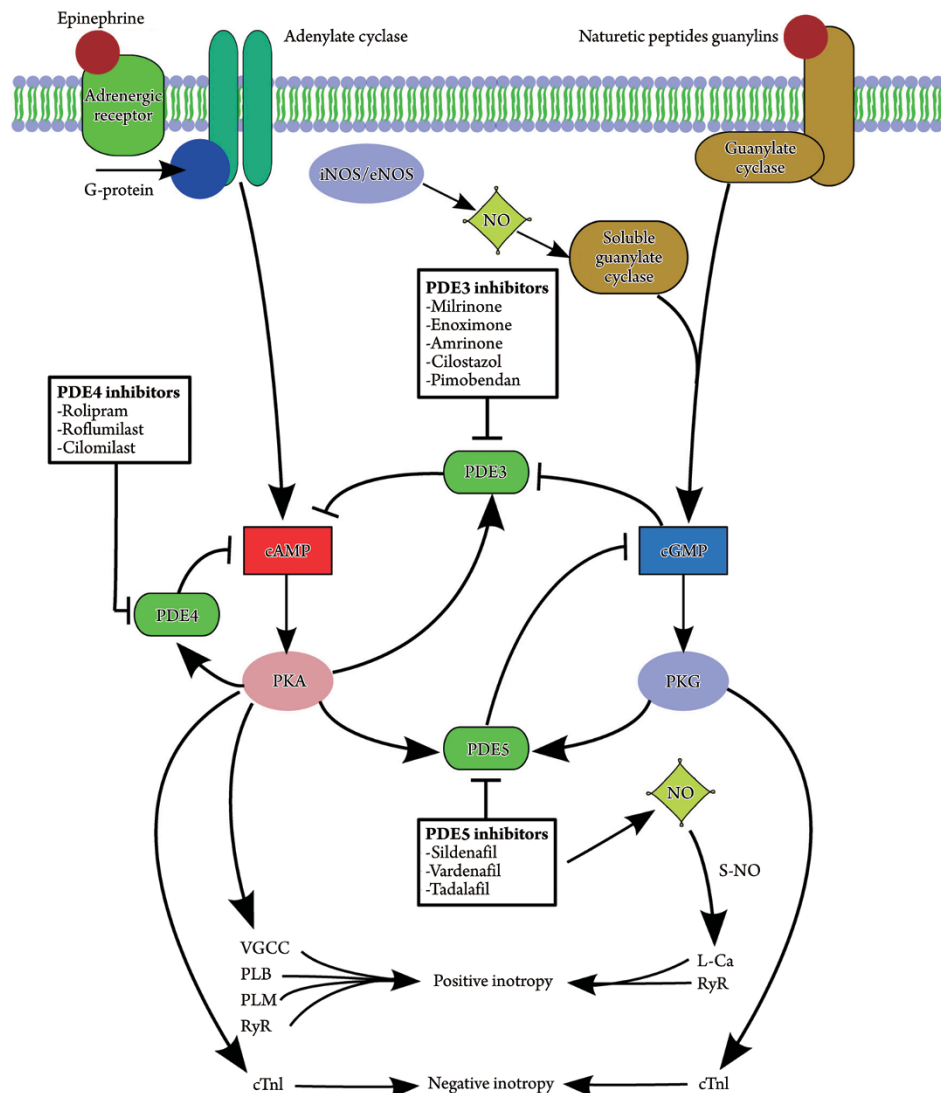


Figure 1. PDE inhibitors and cardiomyocyte contractile signaling crosstalk. PDEs modulate crosstalk and feedback signaling through cAMP, cGMP, and their associated kinases. PDE3, 4, and 5 are phosphorylated and activated by PKA while PDE5 is also phosphorylated by PKG. These PDEs in turn degrade cAMP or cGMP. Ultimately, this signaling cascade influences the activity of PKA and PKG which phosphorylate and modulate end effectors of myocyte contractility. Inhibition of PDE3, 4, and 5 isozymes in this system is thus expected to affect contractility. In particular, PDE3 or PDE4 inhibition may increase cAMP levels and result in enhanced contractility while contractile effects of PDE5 inhibitors may be dose dependent due to crosstalk effects mediated by PDE3. VGCC=Voltage-Gated Calcium Channel; PLB= Phospholamban; PLM=Phospholemman; RyR= Ryanodine Receptor; cTnI= Cardiac Troponin I; S-NO=S-Nitrosylation; L-Ca= L-type Calcium Channel.

Additionally, PDE3 contributes to the majority of the cAMP-hydrolyzing activity in subcellular fractions of human myocardium under basal conditions (not Ca^{2+} stimulated)^[58].

The PDE3A gene gives rise to three isoforms (PDE3A1/2/3) that differ only in the sequence near the N-terminus. PDE3A1 is a 136-kDa protein that is reported to be found only in particulate fractions of human myocardium and contains both NHR1 and NHR2, as well as PKB and PKA phosphorylation sites^[3,12]. PDE3A2 is a 118-kDa protein found in both the particulate and cytosolic fractions that is a truncated version of PDE3A1 lacking the NHR1 region and likely the PKB phosphorylation site^[3,12]. PDE3A3 is a 94-kDa protein found only in the cytosolic fraction that lacks both NHRs and all three phosphorylation sites^[3,12]. The PDE3A and PDE3B isoforms are largely homologous in their amino acid sequence and have similar enzyme kinetics. In the heart, PDE3A and PDE3B are activated by PKA resulting in a negative feedback on the levels of cAMP within the cell^[59]. In fact, it has been suggested that PDE3 may mediate tachyphylaxis in response to long term β -agonist stimulation^[3]. PDE3A1 is not well expressed in cardiomyocytes, but PDE3A2 and PDE3A3 are highly expressed in both cardiomyocytes and vascular smooth muscle^[56]. Multiple PDE3B proteins of differing sizes have been found, but their origin is still unclear^[3]. PDE3B1 has recently been found in the heart tissue of mice and is reported to account for about 30% of the PDE3 activity^[60]. However, it is unknown whether PDE3B1 is found in cardiomyocytes rather than other cell types in the heart tissue.

Regulation of myocardial contractility by PDE3

PDE3 is a crucial modulator of contractility through its control of cAMP signaling. PDE3 inhibitors have long been studied for their positive inotropic effects. Subfamily specific regulation has recently been studied with PDE3A and PDE3B knockout mice^[61]. PDE3A, but not PDE3B, knockout mice showed increased heart rate and contractility compared to wild type mice and these parameters were unresponsive to PDE3 inhibition with cilostamide^[61]. This suggests that the PDE3A isoform is mainly responsible for control of contractility in the heart.

There is increasing evidence that suggests that regulation of many cellular processes results from local modulation and compartmentalization of cyclic nucleotide signaling, rather than global effects^[13]. In neonatal rat cardiomyocytes, β -adrenergic stimulation selectively increases cAMP concentrations in the microdomain near the Z-disc and T-tubules^[62]. Interestingly, studies of the isolated rabbit atria have shown that stimulation of sGC or pGC produced different results on cAMP levels depending on the source of

cGMP, suggesting that PDE3 mediates this compartmentalization of signal^[63]. PDE3 activity in the canine myocardium has been shown to be localized to the sarcoplasmic reticulum (SR), making it possible that PDE3 regulates phosphorylation of PLB^[64]. If so, a decrease in PDE3 activity would increase PLB phosphorylation and result in positive inotropy.

In addition, PDE3 could control cardiac contractility through closely regulating cAMP-dependent phosphorylation of VGCC and thus Ca^{2+} entry into the cardiomyocyte^[65]. cGMP inhibition of PDE3 increases cAMP-stimulated I_{Ca} in isolated human atrial myocytes^[66] and frog ventricular myocytes^[67]. Indeed, it has been suggested that PDE3 plays a role in increasing I_{Ca} in nanomolar concentrations of NO donors while PDE2 decreases I_{Ca} in micromolar concentrations of NO donors^[68]. This suggestion is consistent with a number of studies showing an increased cardiac contractility under conditions with moderate increases in cGMP levels^[30–32]. Glucagon inhibition of PDE3 in frog cardiac myocytes also causes I_{Ca} stimulation^[69]. Finally, it is conceivable that inhibition of PDE3 in noncardiac tissues may also affect cardiac contractility. Inhibition of PDE3 in rat juxtaglomerular cells contributes to the stimulation of renin secretion^[70]. This in turn causes an increase in angiotensin II, which induces both a downregulation of PDE3A expression and an upregulation of the inducible cAMP early repressor (ICER), a pro-apoptotic protein, in cardiac myocytes^[71]. ICER causes further repression of PDE3A expression and the resulting increase in cAMP/PKA signaling increases ICER expression (PDE3A-ICER feedback loop)^[72]. This positive feedback cycle may increase contractility, but likely represents a pathological condition that leads to heart failure where PDE3 expression is also suppressed^[2].

Effects of PDE3 inhibitors on ventricular contractility The PDE3 inhibitory activity of cilostamide was first identified in platelets^[73]. Since then, PDE3 specific inhibitors have been studied for treating chronic heart disease due to the positive inotropic effects of these inhibitors. Clinically relevant PDE3 specific inhibitors include the bipyridine derivatives, milrinone (Primacor[®]) and amrinone (Inocor[®]), as well as the imidazole derivative enoximone (Perfan[®]), all of which are used to treat heart failure. Milrinone is the most well studied and extensively used and is also more potent than amrinone and enoximone. At high concentrations, all three drugs also inhibit PDE4 and PDE5^[14]. A clinically relevant PDE3 inhibitor, cilostazol (Pletal[®]), also inhibits adenosine uptake and is used for the treatment of intermittent claudication^[74]. Another PDE3 inhibitor, pimobendan, which also increases myofilament sensitivity, has been tested

to treat congestive heart failure (CHF) in dogs^[75]. Cilostamide, cilostazol, and amrinone appear to have a greater effect on PDE3A than PDE3B^[14]. A recent study comparing the dose dependent effects of milrinone, amrinone, and enoximone in isolated guinea pig hearts found that milrinone increased contractility at much lower concentrations compared to the other PDE3 inhibitors^[76]. At high concentrations, milrinone also increased coronary flow and thus oxygen supply to a greater extent than the other drugs^[76].

PDE3 inhibitors almost certainly produce their inotropic effects in cardiomyocytes through stimulation of I_{Ca} by regulation of the phosphorylation of VGCC via the cAMP-PKA dependent mechanism described above. This effect is independent of β_1 adrenergic receptor stimulation in isolated rat hearts because PDE3 inhibition by amrinone still produced a significant inotropic response despite β_1 blockade with atenolol^[77]. Milrinone inhibited 70% of the total phosphodiesterase activity in cultured embryonic chick ventricular cells and caused a 43% increase in sarcolemmal uptake of Ca^{2+} ^[78]. In isolated human and rabbit atrial myocytes, pimobendan increased the basal I_{Ca} amplitude^[79]. Milrinone and amrinone also increased calcium influx into calf cardiac Purkinje fiber^[80]. In guinea pig ventricular myocytes, both milrinone and cilostazol increased I_{Ca} with milrinone having a more potent effect^[81]. Evidence also exists that supports a mechanism involving PKA phosphorylation of VGCC. The PKA inhibitor H89 blocked the positive inotropy induced by milrinone^[81]. The positive inotropic effects of milrinone in guinea pig^[82] and dog^[83] can be attenuated by a calcium channel blocker. Interestingly, the effect of PDE3 inhibition may differ from species to species as amrinone reduced I_{Ca} in normal hamster ventricular myocytes and produced no inotropic effects in cardiomyopathic hamsters^[84].

There is also evidence that PDE3 inhibitors cause positive inotropy independently of increases in I_{Ca} and are likely due to increases in cAMP and PKA function in other localized areas resulting in altered calcium handling by SR. For instance, milrinone but not enoximone stimulates Ca^{2+} release from SR by RyR2 in sheep^[85]. This effect may be due to cAMP-PKA mediated phosphorylation of RyR2. Milrinone increases SR Ca^{2+} uptake by Ca^{2+} -ATPase in a cAMP-PKA dependent mechanism^[86], which is also involved in the stimulation of NCX that could conceivably contribute to the inotropic effects of PDE3 inhibitors^[42]. However, high concentrations of milrinone cause a small inhibitory effect on Ca^{2+} uptake due to the NCX in bovine cardiac sarcolemmal membranes, an effect that may limit excessive inotropic responses^[80]. Cilostazol may have effects secondary to its inhibition of adenosine uptake since ryanodine receptor Ca^{2+}

release is also reduced by adenosine receptor stimulation^[87]. Indeed, increases in both the interstitial and circulatory adenosine concentrations by cilostazol seems to antagonize its inotropic effects by PDE3 stimulation in isolated rabbit hearts^[6]. Conversely, milrinone analogues may also be involved in adenosine receptor blockade, which would increase contractility by disinhibition of adenylyl cyclase near A_1 receptors^[88]. Milrinone does not seem to have a calcium sensitizing effect on myofilaments^[81].

Effects of PDE3 inhibitors on myocardial ischemia-reperfusion injury A large number of endogenous and exogenous agents are known to cause preconditioning-like effects and there have been significant interests in determining their mechanism of action and application to clinical cardiology. PDE inhibitors are among these agents and the vasodilatory and contractile effects of some PDE inhibitors, in addition to possible cardioprotection, makes research on these agents an especially promising area of investigation. It is notable that cyclic nucleotide levels and PDE activity have been shown to be altered by ischemic preconditioning in isolated perfused rat hearts^[89].

Early evidence showed that milrinone, when added to the perfusion solution, improved contractile recovery in the isolated perfused rabbit heart following hypoxia/reperfusion injury, suggesting a cardioprotective effect^[90]. Pimobendan, when administered after reperfusion of stunned myocardium in dogs, was able to decrease the contractile dysfunction due to ischemia^[91]. However, pimobendan increased the mortality rate and length of ventricular fibrillation following coronary occlusion in rats^[92]. Amrinone also improved contractile recovery after ischemia-reperfusion (IR) in the isolated rat heart in an extracellular calcium dependent manner^[93].

Furthermore, injection of milrinone or olprinone before IR reduced infarct size in dogs^[94]. Further dissection of the signaling pathways involved in olprinone-mediated protection revealed that it induced cardioprotection through cAMP/PKA and p38-MAPK dependent, but not PKC dependent mechanisms^[94]. The authors proposed that the end-effectors of p38-MAPK may include opening of the mitochondrial ATP-sensitive potassium channel (mitoK_{ATP}) channel or translocation of Hsp27 to the Z-disc to stabilize the myofibril and cytoskeleton. Milrinone, administered before ischemia or at the onset of reperfusion, has recently been shown to attenuate myocardial stunning in pigs^[95]. However, milrinone did not reduce the incidence of arrhythmias due to IR injury in dogs^[96]. The post-myocardial infarction (MI) treatment with milrinone had no significant effect on survival rate in rats^[97]. On the other hand, amrinone preconditioning in isolated perfused rabbit hearts reduced both

the infarct size and incidences of ventricular fibrillation as compared to both control and ischemically preconditioned hearts^[98]. Amrinone also decreased IR injury in isolated rat hearts as shown by decreased levels of creatine kinase and lactate dehydrogenase in the coronary effluent^[99]. The authors attributed the protective effects to the possibility of altered Ca²⁺ handling by amrinone in the myocardium. Pharmacologic inhibition of PDE3 in the post-MI failing heart also provides cardioprotection during subsequent incidences of ischemia, even when ischemic preconditioning was ineffective^[100]. In addition, PDE3 inhibitors are capable of altering protection by other preconditioning modalities. Cilostazol, likely due to inhibition of adenosine uptake, is capable of reducing the transient ischemia duration required for ischemic preconditioning^[101]. However, this study found that cilostazol by itself offered no protection nor was milrinone capable of reducing the time required for ischemic preconditioning^[101]. Cilostazol has recently been shown to potentiate the cardioprotective effects of low-dose atorvastatin in rats by increasing phosphorylation of eNOS^[102]. Indeed, eNOS phosphorylation was about 258% of control values when cilostazol and atorvastatin were given in conjunction. Additionally, cilostazol, but not atorvastatin alone, decreased infarct size while the combination of both drugs provided even greater infarct size reduction^[102].

The differential cardioprotective effects of PDE3 inhibitors may be due to their differences in modulating inflammatory signaling. In rat cardiomyocytes, all tested concentrations (50, 100, 250 µmol/L) of amrinone inhibit NFκB activation by LPS, but it has a biphasic modulatory effect on TNF driven NFκB activation^[103]. In contrast, milrinone does not affect LPS induced NFκB activation and potentiates TNF driven NFκB activation^[103]. Additionally, amrinone reduces LPS and TNF induction of iNOS while milrinone has no effect on LPS induction of iNOS, but has a biphasic response to TNF induction of iNOS^[103]. Amrinone also does not affect production of COX-2 while milrinone increases COX-2 production^[103]. This is notable because NFκB, iNOS, and COX-2 are all known mediators of delayed preconditioning. These findings suggest that many PDE3 inhibitors are capable of causing preconditioning-like effects either by themselves or in conjunction with other approaches. Although the mechanisms still remain to be elucidated through additional studies, the cardioprotective effects that include improvements in both myocyte contractility and viability are indeed very attractive.

Finally, the cardioprotective effects of PDE3 inhibitors may be utilized in cardioplegic preparations for the preservation of myocardium. Amrinone along with St. Thomas

Hospital's solution preserved isolated rat hearts after six hours of global ischemia caused by cardioplegic arrest better than combinations of St Thomas Hospital's solution with milrinone^[104]. This was shown by a decrease in lactate dehydrogenase leakage, representing lower ischemic damage that could result from the cardioprotective effects of amrinone. Enoximone, when added to a cardioplegic solution, has also been shown to improve myocardial performance after reperfusion^[105].

Clinical applications of PDE3 inhibitors The clinical use of PDE3 inhibitors has focused on treating heart failure patients through positive inotropy. In theory, the hemodynamic and vasodilatory effects of PDE3 inhibitors are also attractive. However, clinical trials of PDE3 inhibitors for these purposes have been somewhat disappointing. An early study published in 1989 using milrinone and/or digoxin in patients with heart failure found increased incidence of ventricular arrhythmias^[106]. The Prospective Randomized Milrinone Survival Evaluation (PROMISE) was a double-blind study involving 1,088 patients with New York Heart Association (NYHA) class III or IV advanced heart failure^[107]. Milrinone therapy (40 mg oral daily) was associated with a 28% increase in mortality in the total population and a 53% increase in mortality in NYHA class IV patients compared to placebo. There was no survival benefit for any subgroup and the milrinone treated patients had a higher incidence of hypotension and syncope. More recently, Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure (OPTIME-CHF) looked at milrinone use in 951 patients who were hospitalized with an exacerbation of chronic heart failure (NYHA class III or IV), but did not require inotropic support^[108]. Milrinone (0.5 µg·kg⁻¹·min⁻¹ infusion for 48 h) treated patients had a higher incidences of hypotension and new atrial arrhythmias, but this study did not find a significant increase in mortality. *Post-hoc* analysis of the findings revealed that patients with heart failure resulting from ischemia fared worse with milrinone compared to placebo while patients with heart failure unrelated to ischemia did better with milrinone when compared to placebo^[109].

Likewise, despite the promising initial results suggesting that amrinone may improve hemodynamics^[110], ejection fraction^[111], and myocardial energy metabolism^[112], the subsequent larger and better controlled studies have found little or no benefit. For instance, a multicenter, double-blinded study published in 1985 had investigated the effects of long-term oral amrinone therapy (started with 1.5 mg/kg t.i.d and increased to a maximum dosage of 200 mg t.i.d.) in 99 patients with NYHA class III or IV heart

failure^[113]. The investigators found no symptomatic improvement or enhanced exercise tolerance after the amrinone therapy. In particular, there were no significant differences in the ejection fraction, cardiothoracic ratio, or incidence of ventricular premature depolarization. Importantly, the adverse side-effects, which are most commonly gastrointestinal complaints, abnormal liver function, thrombocytopenia, and fatigue, occurred in 83% of patients and necessitated withdrawal of amrinone in 34% of the patients. Furthermore, other large studies showed that long-term amrinone treatment did not give sustained benefits after controlled withdrawal from the drug^[114,115]. However, in a study involving 46 patients with refractory heart failure, intravenous treatment with amrinone ($10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was comparable to dobutamine in increasing the cardiac index^[116]. Amrinone also had a higher individualized response rate of pulmonary arterial wedge pressure, more consistent diuresis, and more predictable effective dose as compared to dobutamine. Additionally, in elderly patients (>75 years old) with severe heart failure (NYHA class IV), amrinone (0.75 mg/kg loading followed by 5 or $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion) was found to improve hemodynamics similarly to dobutamine, but resulted in fewer arrhythmic side-effects^[117].

Enoximone (50 or 75, t.i.d, and titrated up to 100 or 150 mg/day as needed), in a double-blinded trial in 1990 involving 102 patients, was initially found to have no benefit in patients with heart failure (NYHA class II or III) and increased mortality rate^[118]. However, a more recent study involving enoximone used at a lower dosage (25 or 50 mg t.i.d.) in 105 patients with NYHA class II or III heart failure found an increase in exercise capacity with no increase in mortality or incidence of arrhythmias^[119]. The Pimobendan in Congestive Heart Failure (PICO) was a randomized, double-blind trial involving 317 patients with NYHA class II or III moderate heart failure, which compared 2.5 mg and 5 mg daily doses of pimobendan on exercise capacity^[120]. This study found that there was indeed enhanced exercise capacity, but the pimobendan treated patients also had a 1.8 times increase in hazard of death compared to patients on placebo. However in 2002, the Effects of Pimobendan on Chronic Heart Failure (EPOCH) study of 306 patients with NYHA class II or III heart failure found no difference between mortality rates at a dosage of 1.25 or 2.5 mg twice daily^[121]. In fact, there was a decrease in adverse cardiac events in the pimobendan treated groups. Although PDE3 inhibitors are commonly used in the treatment of heart failure, in light of these seemingly contradictory findings, a determination of whether the risks of treatment outweigh the benefits remains to be established.

Nevertheless, the strong inotropic effects of PDE3 inhi-

bitors may prove useful in some situations requiring acute support, such as in cardiac surgery. In a study of 99 patients after cardiac surgery who had low cardiac output, milrinone treatment (50 $\mu\text{g}/\text{kg}$ loading dose followed by continuous infusion of 0.375, 0.5, or $0.75 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for at least 12 hours) significantly increased the cardiac index and decreased pulmonary capillary wedge pressure^[122]. Milrinone (50 $\mu\text{g}/\text{kg}$ loading followed by $0.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion) was also effective in weaning surgical patients off bypass in a randomized, double-blind clinical study involving 32 high risk patients with LV dysfunction or pulmonary hypertension^[123]. More recently, a smaller dose of milrinone (25 $\mu\text{g}/\text{kg}$ loading followed by $0.25 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) has also been shown to be effective in weaning patients with mitral valve stenosis or pulmonary hypertension off bypass^[124]. Milrinone ($0.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion, no loading) may also be effective in restoring peripheral circulation after cardiac surgery^[125]. A single bolus (1 or 1.5 mg/kg) of amrinone was sufficient to improve ventricular function^[126] and maintain circulating blood volume^[127] following cardiopulmonary bypass. More recently, a prospective double-blind study involving 234 patients found that amrinone (1.5 mg/kg loading followed by $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion) given upon release of aortic cross-clamp was successful in weaning more patients off of the cardiopulmonary bypass as compared with controls (21% vs 7%)^[128].

PDE3 inhibition is also useful in conditions where standard treatment with β -agonists may not be effective. Whereas downregulation of β -adrenergic receptors suppressed the inotropic reaction to isoproterenol, milrinone and amrinone were still able to exert their contractile effects in a rat model of cardiac hypertrophy^[129]. Because of this feature, PDE3 inhibition may be useful as a pharmacologic bridge to cardiac transplantation in patients with inadequate response to β -agonists. A report of the use of milrinone in ten patients with severe decompensated CHF found that milrinone was an effective treatment, allowing seven patients to survive to transplant^[130]. One patient who was resistant to vasodilators, β -agonists, and balloon counterpulsation was stabilized with milrinone for 21 days and survived to transplant three months later. The other nine patients were stabilized on milrinone between 11 and 51 days. Milrinone therapy was also effective as a bridge to cardiac transplantation in the case of a patient who underwent the removal of a left ventricle (LV) assist device due to sepsis^[131]. Interestingly, the inotropic effects of milrinone may have some applicability in cardiopulmonary resuscitation (CPR). In a pig model of CPR following ligation of the circumflex artery and induction of ventricular fibrillation, the combination of milrinone and vasopressin increased the cardiac index more than other

combinations of vasopressin, milrinone, or epinephrine^[132]. Enoximone was reported to be successful in the CPR protocol of two patients who suffered cardiac arrest from overdose of the β -blocker propranolol where conventional advanced life support was ineffective^[133].

Phosphodiesterase 4

Overview of PDE4 The PDE4 family comprises four genes PDE4A, PDE4B, PDE4C, and PDE4D, which represent over 20 isoforms. In humans, PDE4 expression is ubiquitous, but is highly expressed in brain and heart^[1]. PDE4 activity was discovered early on to be inhibited by rolipram. PDE4 enzymes are cAMP specific with a V_{max} lower than those for PDE1 and PDE2, but similar to those of other PDEs^[3]. The PDE4 family is composed of a very large number of splice variants that are organized based upon variations of the region near the N-terminus. PDE4 isoforms are categorized into long forms, short forms, and super-short forms. Members of the PDE4 family have unique and highly conserved upstream conserved regions (UCRs) near the N-terminus. UCR1, formed by 55 amino acids, is proximal to the N-terminus while UCR2, formed by 76 amino acids, is distal^[15]. UCR1 contains a phosphorylation site that results in about a 60%–250% increase in activity of PDE4 when targeted by PKA^[134,135]. There is an additional phosphorylation site for ERK near the C-terminus that reduces PDE4 activity^[136]. Thus, it is likely that the UCRs help determine the effect of phosphorylation on overall activity of PDE4. PDE4 is involved in numerous cellular processes and is particularly noted for its involvement in cAMP signal compartmentalization and regulation of β -adrenergic signaling. PDE4D3 is localized to the sarcomeric Z-disc^[137].

Regulation of myocyte contractility by PDE4 PDE4D3 has been determined to be a crucial part of the RyR2 complex on the SR membrane^[138]. The RyR2 is the major Ca^{2+} release channel on the SR in cardiomyocytes whose open state probability is affected by a number of factors, including cytosolic $[Ca^{2+}]$ and phosphorylation. PDE4D3, along with protein phosphatases, have the purpose of preventing hyperphosphorylation of RyR2 by PKA at Ser2809^[139]. Hyperphosphorylation increases the likelihood of Ca^{2+} release by the channel in response to stimulation and may cause depletion of the SR resulting in arrhythmia and decreased contractility. These “leaky” RyR2 channels have been observed in human and animal-model failing hearts^[140]. Additionally, PDE4D3 deficient mice demonstrate arrhythmia and cardiomyopathy as well as accelerated heart failure after myocardial infarction^[138]. These effects were ameliorated in mice with

RyR2 that could not be PKA phosphorylated^[138]. This provides strong evidence that hyperphosphorylation of RyR2 by PKA is due to the altered regulation by PDE4 and results in cardiac dysfunction.

All PDE4 isoforms can also be closely associated with β -arrestins, which are scaffold proteins that downregulate β_2 -adrenergic signaling. β -arrestins preferentially bring PDE4D5 to cellular regions that are important for cAMP production under agonal stimulation^[141]. Further evidence demonstrated that PDE4D regulated the β_2 -adrenergic receptor (β_2AR), but not the β_1 -adrenergic receptor, in neonatal rat cardiomyocytes^[142]. This is important because in cardiomyocytes, PKA is localized to β_2AR and PKA phosphorylation of β_2AR decreases coupling to G_s and switches coupling to G_i ^[143]. The recruitment of PDE4D5 to this region creates a localized sink that regulates cAMP production and PKA activity near β_2AR . Indeed, siRNA knockdown of PDE4D5 in human embryonic kidney cells (HEK293) increased phosphorylation of β_2AR ^[144]. Additionally, catalytically inactive PDE4D5 (Asp556Ala) overexpression in HEK293 cells by adenoviral gene delivery amplified PKA activity at the plasma membrane, but not in the cytoplasm^[145]. Catalytically inactive PDE4D5 expression in neonatal rat cardiomyocytes also caused increased G_s to G_i switching of the β_2AR under isoproterenol stimulation, as measured by ERK1/2 activation, an observation that may be the result of increased phosphorylation by PKA^[145]. These findings all suggest that PDE4 regulates PKA phosphorylation of β_2AR , which modifies cAMP production with subsequent downstream effects within the cell, and PDE4 is responsible for modulating the amplitude and duration of cAMP response to β -adrenergic stimulation^[146].

Effects of PDE4 inhibitors on ventricular contractility The PDE4 inhibitor rolipram was first developed in the 1970s as an effective antidepressant. Rolipram analogues also quickly became the focus of development of drugs for treating asthma and chronic obstructive pulmonary disease (COPD). However, clinical development was halted due to significant side effects, including strong stimulation of emesis and nausea^[147]. Recently, new PDE4 inhibitors with fewer side effects have been developed. Clinically relevant examples include cilomilast (Ariflo[®]) and roflumilast (Daxas[®]), which are both under investigation for the treatment of inflammatory airway disease^[148].

In the heart, PDE4 inhibitors seem to affect contractility only when the myocardium is already stimulated. This is consistent with the finding that β -adrenergic stimulation creates microdomains of increased cAMP concentration close to Z-disc and T tubules in neonatal rat cardiac myocytes^[62] and

the observation that PDE4 regulates β_2 AR and is localized to the Z-disc. In isolated human and rabbit atrial myocytes, rolipram was unable to increase basal I_{Ca} ^[79]. Additionally, in isolated frog cardiomyocytes, rolipram was unable to elevate I_{Ca} in the absence of elevated cAMP, but increased I_{Ca} in submaximal levels of elevated cAMP^[65]. In isolated rat ventricular myocytes, Ro 20-1724, a rolipram analogue and PDE4 inhibitor, by itself could not increase I_{Ca} , but was able to produce a significant increase with the PDE3 inhibitor cilostamide^[149]. In rat ventricular myocardium, rolipram by itself did not affect either cAMP levels or basal contractility, but did enhance the contractile responses to glucagon, decreased fade due to tachyphylaxis, and also enhanced the effects of dobutamine^[150]. This was also seen in guinea pig left atria where rolipram and Ro 20-1724 produced negligible or slight increases in contractility at basal conditions, but exerted positive inotropic effects in the presence of forskolin, an adenylyl cyclase activator, and SK&F 94120, a PDE3 inhibitor^[151]. PDE4 inhibition by Ro 20-1724 has been observed to enhance contractility and cAMP accumulation under stimulation by isoproterenol^[152]. In another study, rolipram by itself did not cause increases in contractility in rat papillary muscle, but it markedly potentiated the positive inotropy of milrinone^[153]. Interestingly, the contractile effects of rolipram are conserved in a rat model of hypertrophy where there is downregulation of the β -adrenergic receptor^[129]. However, even under stimulated conditions there is some evidence that PDE4 inhibition may not increase contractility. In rats, Ro 20-1724 did not significantly alter norepinephrine, epinephrine, or dobutamine induced changes but actually significantly attenuated isoproterenol induced increases in the maximum dp/dt , a measure of cardiac contractility^[154]. Additionally, in a case where positive inotropic effects of Ro 20-1724 and rolipram had been found in the dog, these effects were abolished by the β -blockers, propranolol and nadolol, suggesting that at least some level of stimulation is necessary for positive inotropy^[155].

Effects of PDE4 inhibitors on myocyte viability in ischemic myocardium The selective PDE4 inhibitor, rolipram ($1 \text{ mg}\cdot\text{kg}^{-1}$ bolus before ischemia and $1 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ during reperfusion), alone was unable to protect the canine myocardium in one study of IR injury *in vivo*^[156]. Rolipram, administered at the time of reperfusion, reduced inflammation, but did not reduce infarct size in a rat model of myocardial infarction^[157]. However, a much lower nonvasodilatory dose of rolipram ($1 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was able to significantly reduce inflammation and infarct size in another study of IR injury in canine myocardium^[158]. In this study, rolipram also potentiated the cardioprotective effects of the A_{2A} receptor

agonist, ATL-146e^[158]. The authors attributed their results to rolipram-induced reduction of inflammation as well as a possible increased level of endogenous adenosine available due to their preparation and occlusion technique^[158]. Importantly, rolipram has been shown to increase the incidence of arrhythmias following IR injury in rabbits^[159]. It is still unknown whether other PDE4 inhibitors have cardioprotective effects.

Clinical applications of PDE4 inhibitors The two selective PDE4 inhibitors, cilomilast and roflumilast, have been shown to be effective in the treatment of asthma and COPD without the emetic side effect of rolipram^[148]. There is limited information available regarding the cardiovascular effects of PDE4 inhibitors in the clinical setting. In five independent studies investigating the pharmacokinetics of cilomilast in healthy volunteers, the drug appeared to be well tolerated at dosages of $\leq 15 \text{ mg}/\text{d}$ ^[160]. Cilomilast had no significant effects on cardiac function or ECG. In another study in healthy young adults, cilomilast had no significant pharmacokinetic interaction with digoxin^[161]. A study investigating the pharmacokinetics of roflumilast in 19 healthy volunteers found dose dependent adverse effects, such as headache, asthenia, and abdominal discomfort, that were moderate in severity^[162]. It did not find any clinically relevant changes in blood pressure, heart rate, or ECG.

However, in a study of distal human pulmonary artery smooth muscle cells (PASMC), intracellular cAMP was increased by rolipram, cilomilast, and roflumilast^[163]. All three PDE4 inhibitors significantly attenuated DNA production. Additionally, roflumilast attenuated cell proliferation and MMP-2 and MMP-9 production. The authors suggested that PDE4 inhibitors may prove useful in treating pulmonary artery hypertension by reducing proliferation of PASMCs.

Pretreatment of rats with rolipram before administration of LPS prevented the decline in cardiac index and rise in plasma levels of $\text{TNF-}\alpha$ ^[164]. Additionally, Ro 20-1724 increased contractility in endotoxemic rats, indicating the potential use of PDE4 inhibitors in the treatment of sepsis^[165]. In addition, rolipram has been investigated in protecting the rat heart against doxorubicin (DOX) induced cardiomyopathy^[166]. In this study, rolipram, given before DOX administration and every two weeks during the experiment, was able to blunt the cardiotoxic effects of both DOX and LPS. Rolipram also reduced apoptosis under these conditions. The authors attributed the cardioprotection to the $\text{TNF-}\alpha$ inhibiting effect of rolipram.

These findings indicate that PDE4 inhibitors may have some potential use in cardiovascular protection and contractility. However, the overall picture of these drugs remains

vague and additional research is necessary to determine the mechanisms of these actions and clinical applicability.

Phosphodiesterase 5

Overview of PDE5 PDE5 activity was first found in platelets, but it is now known to also be expressed in lung, vascular smooth muscle, heart, and many other tissues. It was originally believed to be similar to another cGMP-specific PDE found in rod and cone photoreceptors (PDE6) but was differentiated by sequence comparison^[167]. The high expression of PDE5 in the corpus cavernosum has been of particular interest due to the ability of PDE5 inhibitors to treat male erectile dysfunction. Recently, inhibition of PDE5 in the lung has also been pursued in the treatment of pulmonary hypertension. There has been controversy over whether PDE5 exists in the cardiomyocyte^[168]. A recent study indicated that PDE5 was not present in normal human left and right ventricular myocardium^[169]. However, there has been strong evidence showing PDE5 expression in the left ventricle and isolated cardiomyocytes from dog^[170] and mouse^[171,172] as well as hypertrophied human left and right ventricles^[169]. PDE5 enzymes are specific in the hydrolysis of cGMP and also contain high affinity binding sites for cGMP^[173]. Interestingly, PDE5 appears to regulate a distinct pool of cGMP that is separate from that produced by atrial natriuretic peptide stimulation of pGC suggesting that it plays a role in compartmentalization of cGMP signaling^[172]. To date, only one family of PDEs, PDE5A, has been described. Three splice variants of PDE5A, PDE5A1/2/3, have been found in humans and differ in their N-terminal region but have similar K_m values^[174]. All variants of PDE5A contain two approximately 120 amino acid GAF domains (GAF-A and GAF-B) near the N-terminus, which bind cGMP. Association of cGMP with the main allosteric binding site, GAF-A, stimulates PDE5 activity 9 to 11 fold^[175-177]. All variants of PDE5 also have a single serine PKA/PKG phosphorylation site, located at Ser102 of human PDE5A1, proximal to the N-terminus from the GAF domains that increases enzyme activity *in vitro*^[178,179]. Binding of cGMP to GAF-A appears to regulate phosphorylation^[178]. Indeed, while PKG is the major enzyme responsible for phosphorylation, PKA can also phosphorylate this site when GAF-A is occupied by cGMP^[179]. Conversely, phosphorylation also stabilizes the binding of cGMP to GAF-A^[179]. Interestingly, only PKG activates PDE5 in smooth muscle cells^[180]. PDE5 is localized to the cytosolic fraction and PDE5A is found near the Z-disc in cardiac myocytes^[170]. NOS activity also appears to be necessary for this localization since cardiomyo-

cytes from eNOS knockout mice and wild-type cardiomyocytes chronically treated with *L*-NAME had diffuse PDE5A distribution^[181]. The diffuse PDE5A distribution has also been seen in isolated cardiomyocytes from a canine model of heart failure^[170]. Furthermore, PDE5 expression is increased in human right ventricle hypertrophy^[169].

Regulation of myocardial contractility by PDE5 PDE5 is not known to be specifically complexed or associated directly with an end-effector of contractility. Indeed, there is still little knowledge concerning the mechanism of PDE5A localization to the Z-disc. However, this localization may be important since PDE5 activity may influence other structures located near the Z-disc, such as β_2 AR or RyR2. Such an effect would likely be mediated by cGMP, which dampens many stress responses. In isolated guinea pig right atriums, 8-Br-cGMP exerts a small negative inotropic effect, increases the EC₅₀ of isoproterenol, and decreases the cardiotoxic effects of PDE3 inhibition^[182]. Activation of PKG and phosphorylation of troponin I, which results in decreased Ca²⁺ sensitivity, is a possible mechanism for this effect. cGMP may also decrease Ca²⁺ flux and Ca²⁺-dependent action potentials as had been shown in isolated frog ventricles^[183]. A cGMP driven crosstalk effect between PDE5 and PDE3 likely contributes to this regulation as well. In human atrial myocytes, cGMP has been shown to influence Ca²⁺ level in a concentration dependent manner with PDE3 inhibition and Ca²⁺ increase at low concentrations and PDE2 stimulation and Ca²⁺ decrease at high concentrations^[184]. Further evidence for the role PDE3 plays is provided by the observation that in guinea pigs, stimulation of I_{Ca} by 8-Br-cAMP, which is resistant to PDE hydrolysis, was not affected by cGMP, but stimulation by isoproterenol or forskolin was potentiated or inhibited by cGMP in a biphasic, concentration-dependent manner^[185].

Effects of PDE5 inhibitors on ventricular contractility Specific inhibitors of PDE5 have been the most clinically successful of the class of drugs that inhibit PDEs. Originally conceived as a treatment for angina pectoris, sildenafil has been widely used for the treatment of erectile dysfunction (Viagra®) and pulmonary hypertension (Revatio®). However, limitations such as inhibition of PDE6, resulting in visual effects, and a short half life have led to the development of other specific PDE5 inhibitors such as vardenafil (Levitra®) and tadalafil (Cialis®), which either have more potent action, longer half life, and/or lower PDE6 inhibitory activity.

In anesthetized dogs, high doses of sildenafil were capable of producing positive chronotropic and inotropic effects^[186]. However, this is unlikely to be caused by the direct effect of sildenafil on the myocardium. Instead, it can be

explained by reflex sympathetic stimulation caused by vasodilation. Indeed, in humans, sildenafil reduces vagal modulation and increases sympathetic stimulation of the heart^[187] and also slightly increases contractility in healthy human volunteers^[188] although the effect in this study is suspect since the measurements were taken 75 min following dobutamine infusion. Sildenafil-induced sympathetic activation seems to be general, as shown by increases in plasma norepinephrine levels and muscle sympathetic nerve activity^[189].

A number of studies have demonstrated that sildenafil does not seem to have a direct effect on myocardial contractility. Sildenafil (up to 10 $\mu\text{mol/L}$) did not produce a positive inotropic effect in human atrium, ventricle, or failing ventricle^[190]. Even at high concentrations (10 $\mu\text{mol/L}$), sildenafil had no effect on the contractility of dog trabeculae carnea^[191] and this drug did not cause a positive contractile response in human or dog atrial tissue^[192]. In isolated mouse ventricular myocytes, sildenafil slightly enhanced basal shortening, but did not alter calcium transients^[181]. Sildenafil only caused small transient increases in the cardiac index in healthy volunteers^[193] and caused no changes in patients with coronary artery disease^[194].

On the other hand, some *in vitro* or *ex vivo* studies suggest that sildenafil could increase myocardial contractility. For instance, 10 $\mu\text{mol/L}$ sildenafil treatment causes a net increase in cAMP levels in canine ventricular membrane preparations and bovine heart, an effect that would be expected to increase contractility^[195]. The authors attributed the effect to a possible nonselective inhibition of PDE3 by sildenafil. In fact, in human cardiac tissue, sildenafil (0.1–1.0 $\mu\text{mol/L}$) increased cAMP levels almost as much as milrinone^[196].

One possible explanation for the seemingly contradictory observations is that sildenafil may also have negative contractile effects that counteract with any positive inotropic effects. PDE5 inhibition may lead to increased phosphorylation of cTnI by PKG resulting in decreased Ca^{2+} sensitivity. Further evidence for a PKG related mechanism blunting positive inotropy is that in a rat model of right ventricular hypertrophy (RVH), PDE5 inhibition by sildenafil or MY-5445 significantly increases contractility^[169]. In the same study, there were also decreases in contractility of hearts with RVH following treatment with both PDE5 inhibitors and H89, a PKA inhibitor, suggesting that the positive inotropic effects are mediated through cAMP-PKA, and that there is a net negative inotropic effect from the elevation of cGMP-PKG signaling under PKA inhibition^[169].

It is generally accepted that PDE5 inhibitors have their greatest effects when related cellular signaling mechanisms are concurrently stimulated. The prime example of this is

the observation that the therapeutic effects of PDE5 inhibitors in the corpus cavernosum only occurs secondary to the release of NO. Interestingly, PDE5 inhibitors seem to be capable of negatively modulating β -adrenergic stimulation. Dobutamine-mediated enhancement of systolic and diastolic function in dog was blunted by PDE5 inhibition^[170]. This effect was not seen in dogs with pacing-induced heart failure^[170]. In isoproterenol-stimulated mouse hearts and isolated cardiomyocytes, sildenafil suppressed contractility coincident with cGMP elevation and an increase in PKG activity^[181]. This effect was not observed in eNOS knockout mice but was restored with adenoviral transfer of active eNOS^[181]. Sildenafil also slightly attenuated the isoproterenol-induced increase in force of contraction in human myocardial tissue^[190], and contractile response to dobutamine was blunted by sildenafil in healthy human volunteers^[188].

Effects of PDE5 inhibitors on myocardial ischemia-reperfusion injury The PDE5 inhibitors, sildenafil^[197], vardenafil^[197], and tadalafil^[198], have all been shown to have cardioprotective effects against myocardial IR injury. This phenomenon has been discussed in detail in several recent review articles^[199-201]. In summary, sildenafil induces myocardial preconditioning in adult rabbits^[202], infant rabbits^[203], mice^[204], dogs^[205], and rats^[206] by a mechanism that involves NO signaling and the opening of mitoK_{ATP}. A recent study of sildenafil delayed preconditioning in isolated mouse heart has also revealed a role for mitochondrial Ca^{2+} -activated K^{+} channels in cardioprotection^[207]. Vardenafil and tadalafil also reduce myocardial infarct size in rats^[198] and rabbits^[208].

The acute cardioprotective effects of PDE5 inhibitors appear to be dose dependent. In the isolated rat heart, low concentrations of sildenafil (20–50 nmol/L) has been shown to reduce infarct size while higher concentrations had no effect^[209]. This study also showed that sildenafil elevated cGMP and suppressed cAMP levels in the ischemic heart. Sildenafil induces delayed preconditioning 24 h after treatment through a NOS dependent mechanism in the mouse heart^[204]. Sildenafil and vardenafil also have cardioprotective effects when administered at the onset of reperfusion, which depends on the opening of mitoK_{ATP}^[197]. Sildenafil protects cardiac myocytes against necrosis and apoptosis through NO signaling and upregulation of Bcl-2^[171]. Sildenafil has additive cardioprotective effects with low-dose atorvastatin by enhancing expression and phosphorylation of eNOS and iNOS^[210]. This PDE5 inhibitor also reduces the incidence of severe arrhythmias during ischemia in dogs^[211].

Based on numerous studies dissecting the cardioprotective mechanism of action of PDE5 inhibitors, the signaling

cascade likely involves activation of endogenous triggers and mediators of cytoprotection. In particular, phosphorylation or induction of NOS results in an elevation of NO and cGMP production from sGC. cGMP then activates PKG, which opens the mitoK_{ATP} that prevents the loss of ionic gradients and allows for continued ATP production and calcium transport^[199,200]. The protective effects of sildenafil are also associated with PKG-dependent phosphorylation of ERK and GSK3 β ^[212]. The role of mitoK_{ATP} is essential since the cardioprotective effects of PDE5 inhibitors can be abrogated by the putative blocker of mitoK_{ATP}, 5-HD^[197,202]. PKC signaling also appears to be involved since the PKC inhibitor, chelerythrine, abolishes sildenafil-induced cardioprotection against IR injury in rabbits^[213]. Interestingly, a low dose of sildenafil (0.06 mg/kg) given at reperfusion did not alter cGMP levels, but was able to reduce infarct size in the wild-type, eNOS null, and iNOS null mice after IR^[214]. Sildenafil's stimulation of the sympathetic nervous system *in vivo* may also play a role since the autonomic nervous system is involved in remote ischemic preconditioning^[215]. Certain disease states may affect the cardioprotective effect of PDE5 inhibitors. In particular, the infarct size reducing effect of sildenafil in the heart is abolished in diabetic dogs^[216] and mice^[214]. This blunting effect may be a result of abolishment of sildenafil-induced enhancement of PKG expression in the diseased hearts^[216].

Clinical applications of PDE5 inhibitors The hemodynamic profile of PDE5 inhibitors is predominantly characterized by a weak systemic vasodilation, which causes a slight, often transient, drop in systolic and diastolic blood pressure and has been observed in healthy human volunteers treated with sildenafil^[193], vardenafil^[217], and tadalafil^[218]. These vasodilatory effects are potentiated by nitrates, which are contraindicated with PDE5 inhibitors^[193,218]. A hypothetical steal effect of PDE5 inhibitors that may cause detrimental reductions in coronary blood flow has not been observed in studies done on dogs^[219,220].

To the contrary, PDE5 inhibition for treatment of pulmonary artery hypertension (PAH) has been well explored. Sildenafil attenuated hypoxia-induced PAH in rats when given before and during the chronic hypoxic treatment (10% O₂ for up to 42 days)^[221]. In the clinical setting, a study of 13 patients with PAH found that sildenafil significantly increased cardiac output, decreased pulmonary artery pressure, and decreased pulmonary resistance^[222]. Sildenafil was as effective as inhaled NO for pulmonary vasodilation in children with congenital heart disease^[223] and was superior to inhaled NO in 13 adult patients with PAH because it increased cardiac output without increasing wedge pressure^[224]. In

light of these promising preliminary findings, the Sildenafil Use in Pulmonary HypERTension (SUPER) study included 278 patients with PAH who were randomly assigned to receive placebo, or sildenafil (20, 40, or 80 mg *po* daily) for 12 weeks^[225]. All groups of patients treated with sildenafil experienced increased exercise capacity as measured by 6-minute walking distance as well as decreased mean pulmonary arterial pressure, and increased cardiac index. Patients who completed the 12 week study could elect to enter a 2-year long term extension trial. Those patients who had previously received placebo, but then were titrated to 80 mg of sildenafil, had a significant increase in exercise capacity at week 12 of the extension period. Recently, the United States Food and Drug Administration approved the use of 20 mg dose of sildenafil for the treatment of PAH. Interestingly, sildenafil, vardenafil, and tadalafil have differential effects in a study of 60 patients with PAH^[226]. The authors found that vardenafil had the most rapid effect on pulmonary arterial pressure, sildenafil and tadalafil, but not vardenafil, caused a reduction in the pulmonary to systemic vascular resistance ratio, and only sildenafil significantly improved arterial oxygenation. A new specific PDE5 inhibitor, udenafil (Zydena[®]), has also been shown to attenuate PAH^[227] and the development of compensatory right ventricular hypertrophy due to PAH^[228] in rats. The hemodynamic effects of sildenafil may also be beneficial in cases of acute pulmonary embolism (APE). In a canine study of APE, sildenafil infusion decreased pulmonary arterial pressure as well as pulmonary vascular resistance^[229].

Furthermore, initial clinical data showed that sildenafil improved vasodilation in patients with chronic heart failure as analyzed by ultrasound imaging of the brachial artery^[230]. Sildenafil also dilated epicardial coronary arteries, improved endothelial dysfunction and inhibited platelet activation in a clinical study of 12 patients with coronary artery disease^[231]. Flow-mediated dilation of the brachial artery was also improved by acute and long term treatment with sildenafil in patients with type 2 diabetes^[232]. This is especially interesting because myocardial protection is lost in animal models of diabetes^[205,214]. Very importantly, the sildenafil induced protection of the endothelium from IR injury was demonstrated in a study involving 10 healthy male volunteers^[233]. These subjects were randomly selected to receive placebo or 50 mg oral sildenafil. Two hours later, endothelium-dependent, flow-mediated dilatation of the radial artery was measured before and after IR induced by 15 min of ischemia at the level of the brachial artery followed by 15 min of reperfusion. Seven days later, all subjects were given the other treatment and underwent the same protocol. Sildenafil blunted the impairment of endothelium-dependent vasodilation. This same

study also showed that the protective effect was abolished by glibenclamide, a blocker of K_{ATP} channels. Thus, these studies demonstrate that in humans, PDE5 inhibition by sildenafil improves endothelial function during disease states and after IR, possibly mediated by K_{ATP} channel.

In a dog model of heart failure, researchers found that sildenafil did not affect coronary flow or myocardial oxygen consumption at rest or during exercise^[234]. In fact, PDE5 expression appears to decrease in failing canine hearts^[170]. However, more recent studies showed an augmented PDE5 activity and/or expression in the systemic and pulmonary vasculature and in the kidney in dogs suffering heart failure^[235] and in the hypertrophied human right ventricle^[169]. There are a number of clinical studies showing the beneficial effects of sildenafil in heart failure patients. For example, in a study of 23 patients with chronic heart failure, sildenafil significantly increased exercise capacity^[236]. Another study of 20 patients with chronic heart failure and ejection fractions <35% found that the increased cardiac output and exercise capacity following acute treatment with sildenafil can be largely explained by a decrease in LV load, which is caused by the reduced peripheral resistance, aortic and large artery stiffness, as well as wave reflection from peripheral sites^[237]. Thus, sildenafil is a promising therapy in the treatment of heart failure, especially in the presence of endothelial dysfunction. A recent dog study suggested that oral administration of sildenafil may be an efficient, more cost-effective, and better tolerated alternative to intravenous B-type natriuretic peptide infusion for treating the decompensated heart failure^[235].

Sildenafil may interact with various types of cardiomyopathies. In hypertensive cardiomyopathy induced by L-NAME (20 mg/d, *po*) in rats, chronic sildenafil treatment (45 mg·kg⁻¹·d⁻¹ *po*) over eight weeks significantly reduced arterial blood pressure and peripheral vascular resistance^[238]. Importantly, sildenafil also attenuated the decrease in cardiac output and cardiac remodeling. In a mouse model of doxorubicin (5 mg/kg, *ip*) induced cardiomyopathy, prophylactic treatment with sildenafil (0.7 mg/kg, *ip*) prevented apoptosis and left ventricular dysfunction^[239]. Sildenafil also prevented the ST interval elongation caused by doxorubicin cardiotoxicity. Additionally, sildenafil also prevented cardiomyopathic changes in the hearts of dystrophin knockout mice^[240]. Sildenafil treatment reduced mRNA levels of atrial natriuretic factor, an indicator of cardiac remodeling, and cardiomyocytes from sildenafil-treated mice were protected against isoproterenol-induced injuries^[240]. However, in a report of a hypertrophic cardiomyopathy patient, Holter monitoring for 24 h before treatment with sildenafil revealed 255 ventricular

premature beats and no episode of ventricular tachycardia. After treatment with sildenafil, Holter monitoring revealed 1210 ventricular premature beats and six episodes of unsustained ventricular tachycardia^[241]. This pro-arrhythmic effect of sildenafil needs to be further characterized.

Finally, the cardioprotective and hemodynamic profile of PDE5 inhibitors may have applicability in cardiac surgery. It has been suggested that PDE5 inhibitors could be used: 1) to pharmacologically precondition the heart against ischemia/hypoxia insults caused by aortic cross-clamping and cardiopulmonary bypass; 2) to postcondition the heart with the drug added into the perfusate for reperfusing the ischemic myocardium; and 3) to be used as maintenance therapy to improve coronary flow and perfusion of the myocardium^[242]. However, the true fraction of patients who experience postoperative complications from reperfusion injury appears relatively small and adequately powered trials are necessary to determine effective dosages and protocols for using PDE5 inhibitors as cardioprotectants in the clinical settings^[201].

Regulatory role of other phosphodiesterases in the heart

Although our review article predominantly focuses on the inhibitors of PDE3, PDE4 and PDE5, we should not underestimate the potentially important role played by PDE1 and PDE2 in regulating cyclic nucleotides and cardiac contractility. In brief, PDE1 is a Ca²⁺/calmodulin stimulated cytosolic phosphodiesterase found in human heart and is one of the main contributors to cAMP hydrolyzing activity in the presence of Ca²⁺ in subcellular fractions of human left ventricular myocardium^[58]. It also has been found to be responsible for the majority of cGMP hydrolyzing activity in human ventricular tissue^[191]. However, relevance of PDE1 in human cardiomyocytes is still unclear since PDE1 activity was not identified in isolated rat cardiomyocytes, but instead it was detected in whole myocardium^[12,243]. There are several nonspecific inhibitors of PDE1 that include nimodipine, which also inhibits the L-type Ca²⁺ channel^[244], vinpocetine, which activates the Ca²⁺-activated K⁺ channels^[245], and zaprinast, which also inhibits PDE5^[246]. Many inhibitors that were initially reported to be specific for PDE1 (such as zaprinast, SCH 51866, and IBMX) were later found to inhibit PDE5 as well^[14]. To date, there are no effective and selective PDE1 inhibitors available^[14]. However, the new PDE inhibitor, IC224, holds great promise (selectivity ratio of 127 for PDE1), but it is still unknown whether it is equally selective for the PDE1 subtypes^[247].

Table 2. Cardioprotective and functional effects of PDE3, 4, and 5 inhibitors.

Drug used	Dose, route, and time	Animal model	Reduction in IS	Mechanisms	Functional Effects						
					Pre-ischemia			Post-ischemia			Reference
					HR	MAP	CON	HR	MAP	CON	
PDE3 inhibitors											
Milrinone	30 µg/kg iv, 30 min Pre-I	Dog, BTO	52%	PKA, p38 MAPK	↑	↓	•	-	-	•	[94]
Olprinone	20 µg/kg iv, 30 min Pre-I	Dog, BTO	47%	PKA, p38 MAPK	↑	↓	•	-	-	•	[94]
Olprinone	30 µmol/L, 2 cycles of 5 min IC Pre-I	Isolated failing Rat heart	55%	PKA	•	•	-	•	•	↑	[100]
Amrinone	10 µmol/L, 5 min IC Pre-I	Isolated rabbit heart	41%		•	•	-	•	•	↑	[98]
Cilostazol	20 mg·kg ⁻¹ ·d ⁻¹ po, 3 d Pre-I	Rat, CO	56%	PKA, eNOS, Akt	-	-	•	↑	-	•	[102]
PDE4 inhibitors											
Rolipram	1 ng·kg ⁻¹ ·min ⁻¹ iv, 5 min Pre-I	Dog, CO	33%	Reduced neutrophil infiltration	-	-	-	-	-	-	[158]
PDE5 inhibitors											
Sildenafil	0.7 mg/kg iv, 10 min Pre-I	Dog, CO	48%	PKG	-	-	-	-	-	-	[216]
	0.7 mg/kg iv, 30 min Pre-I	Rabbit, CO	67%	mitoK _{ATP}	-	-	-	-	-	-	[202]
	0.7 mg/kg iv, 24 h Pre-I	Rabbit, CO	41%	mitoK _{ATP}	-	-	-	-	-	-	[202]
	0.71 mg/kg iv, at R	Rabbit, CO	43%	mitoK _{ATP}	-	-	-	-	-	-	[197]
	0.7 mg/kg iv, 30 min Pre-I	Infant rabbit, CO	53%		-	-	-	-	-	-	[203]
	1 mg/kg ip, 18 h Pre-I	Rat, CO	71%	iNOS, eNOS	-	↓	•	-	↓	•	[210]
	0.05 mg/kg iv, 1 h Pre-I	Isolated rat heart	29%	cGMP	-	•	-	-	•	↑	[206]
	50 nmol/L, 10 min IC Pre-I	Isolated rat heart	65%	cAMP, cGMP, mitoK _{ATP}	-	•	-	•	•	↑	[209]
	0.06 mg/kg iv, at R	Mouse, CO	47%	Independent of cGMP, iNOS, eNOS	•	•	•	•	•	•	[214]
	0.7 mg/kg ip, 24 h Pre-I	Isolated mouse heart	75%	iNOS, eNOS	•	•	•	•	•	•	[204]
	0.71 mg/kg ip, 24 h Pre-I	Isolated mouse heart	79%	Adenosine A1 receptor	-	•	-	-	•	-	[257]
	0.71 mg/kg ip, 24 h Pre-I	Isolated mouse heart	57%	mitoK _{Ca} , mitoK _{ATP}	-	•	-	-	•	-	[207]
	Vardenafil	0.014 mg/kg iv, 30 min Pre-I	Rabbit, CO	58%	mitoK _{ATP}	-	↓	-	-	-	-
0.014 mg/kg iv, at R		Rabbit, CO	50%	mitoK _{ATP}	-	-	-	-	-	-	[197]
Tadalafil	10 mg/kg po, 2 h Pre-I	Rat, CO	22%	mitoK _{ATP}	-	↓	•	-	↓	•	[198]

Note: ↑ increase; ↓ decrease; - no change; • not reported; BTO=Bypass Tube Occlusion; CO=*In situ* Coronary Occlusion; IC=Intracoronary Infusion; IS=Infarct Size; HR=Heart Rate; MAP=Mean Arterial Pressure; CON=Contractility (dp/dt_{max}, peak developed pressure, rate force product, rate pressure product, or cardiac output); mitoK_{ATP}=Mitochondrial ATP Sensitive Potassium Channel; mitoK_{Ca}=Mitochondrial Ca²⁺ Activated Potassium Channel; PreI=Pre-Ischemia; R=Reperfusion

PDE2 also hydrolyzes cAMP and cGMP with high affinities and is stimulated by cGMP. Unlike other phosphodiesterases, the regulation of PDE2 by phosphorylation has yet to be described in detail^[3]. PDE2 may serve as a mediator of cyclic nucleotide crosstalk^[12]. PDE2 attenuates the rise in cAMP resulting from β-adrenergic stimulation in rat ventricular myocytes^[248]. cGMP stimulation of PDE2 also inhibits the cAMP-mediated increase of I_{Ca} in amphibian ventricular myocytes^[68] as well as human atrial myocytes^[249]. The first specific inhibitor discovered for PDE2 is erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), but its use has been limited due to its inhibitory effects on adenosine deaminase even at low concentrations^[250]. Four more PDE2 specific inhibitors, IC933^[247], Bay 60-7550^[251], (9-(6-phenyl-2-oxo-

hex-3-yl)-2-(3,4-dimethoxybenzyl)-purin-6-one (PDP)^[252], and oxindole^[253], have recently been described. Nevertheless, since the highly specific inhibitors of PDE1 and PDE2 are still relatively new in experimental studies and far from any clinical use targeting the heart, their potential impact on cardiac contractility cannot be adequately described at the present time.

Summary

This article has extensively reviewed the most updated clinical and laboratory evidence demonstrating the remarkable cardioprotective and/or cytoprotective effects afforded by PDE3 and PDE5 inhibitors, and to the lesser extent, by

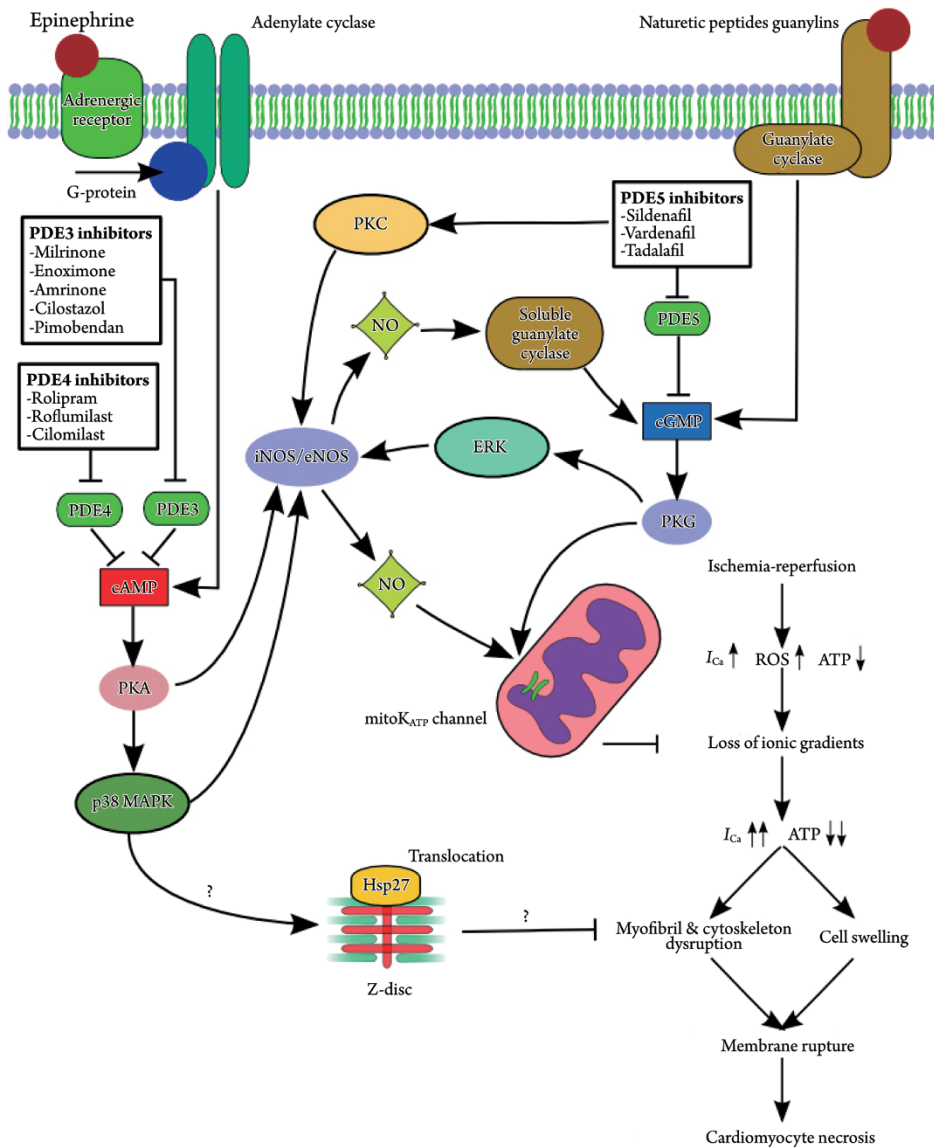


Figure 2. Proposed mechanisms for PDE inhibitor-induced cytoprotection against myocardial ischemia-reperfusion injury. PDE5 inhibitors increase cGMP content, which activates PKG. PKG can subsequently activate $\text{mitoK}_{\text{ATP}}$ channel and prevent the loss of ionic gradients in the necrotic pathway of ischemia/reperfusion injury. PDE5 inhibitors simultaneously initiate a signaling cascade involving PKC and ERK, leading to phosphorylation/induction of nitric oxide synthases (NOS) and release of nitric oxide (NO). This pathway also leads to opening of $\text{mitoK}_{\text{ATP}}$ channel. Alternatively, PDE3, and PDE4 inhibitors may increase the activity of PKA leading to phosphorylation of eNOS and p38 MAPK. p38 MAPK activation may potentially result in translocation of 27 kDa heat shock protein (Hsp27) to the Z-disc, an event that may to stabilize the cytoskeleton and contractile fibers.

PDE4 inhibitors (summarized in Table 2). We have further discussed the putative molecular and cellular mechanisms underlying the modulatory effects of PDE3, PDE4, and PDE5 inhibitors on ventricular contractile function and myocyte viability in the normal and ischemic hearts. The cAMP, cGMP, NOS, $\text{mitoK}_{\text{ATP}}$, and numerous protein kinases are apparently among the principal players forming the signal transduction cascades that result in the positive inotropic (Figure 1) and/or pro-survival (Figure 2) phenotype counteracting myocardial ischemia-reperfusion injury.

Ischemic heart diseases remain the number one cause of morbidity and mortality in Western countries and its prevalence has rapidly increased in many developing countries,

such as China, due to the profound economical, nutritional, social, and environmental changes. There has been a great deal of demand for advanced surgical or interventional procedures as well as new drug therapies to ameliorate or prevent the devastating functional outcomes of ischemic heart diseases, such as congestive heart failure. Therefore, further vigorous investigations in this dynamic area will undoubtedly pave the way for a series of well designed and controlled clinical trials, which may eventually lead to the use of PDE3 and/or PDE5 inhibitors as the primary line of drug therapy in promoting cardiac performance and cell survival under critical situations, such as ischemic heart attack, cardiopulmonary bypass surgery, and heart failure.

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