Pharmacological Pre- and Post- Conditioning Agents: Reperfusion-Injury of the Heart Revisited

Ioanna Andreadou[1,*], Efstathios K. Iliodromitis[2], Maria Koufaki[3], Dimitrios Th. Kremastinos[2]

[1]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimioupoli-Zografou, 157 71, Athens, Greece,* [2]*Second University Department of Cardiology, Medical School, Attikon General Hospital, University of Athens, 12462, Athens, Greece,* [3]*National Hellenic Research Foundation, Institute of Organic and Pharmaceutical Chemistry, 48, Vas. Constantinou Ave. 11635 Athens, Greece*

Abstract: Ischemic preconditioning (PC) and postconditioning (PostC) are endogenous mechanisms of protection of the ischemic heart. In brief, short cycles of sublethal ischemia separated by brief periods of reperfusion render the heart resistant to infarction from a subsequent lethal episode of prolonged ischemia. Although PC is a powerful form of protection, its clinical application is limited because of ethical and practical reasons. It is of interest that multiple very short periods of ischemia and reperfusion applied at the onset of reperfusion are also capable in limiting the infarct size. In fact, the short ischemic insults in PC have to be applied before the onset of sustained period of ischemia which cannot be precisely anticipated. On the contrary, the very brief insults in postconditioning (PostC) have to be applied immediately after the end of the long ischemia thus making the intervention more easily applicable. Both mechanisms reduce the infarct size by limiting the reperfusion injury. Pharmacological PC and PostC represent ideal alternatives that may substitute the short ischemic insults for pharmaceuticals means. The components of PC share two pathways, one that involves the mitochondrial K_{ATP} channels- free radicals and PKC and another one that involves adenosine and PKC. Reperfusion injury salvage kinases (RISK) prevent the mitochondrial permeability transition pores (mPTP) which destroy the mitochondria and cause cell death. PC *via* PKC and PostC *via* gradual restoration of pH at reperfusion up-regulate RISK and preserve viable part of the ischemic region of the heart. In order to confer pharmacological protection, novel therapeutic strategies, based on the knowledge of the ligands, of the receptors and of the intracellular signaling pathways have emerged. Adenosine, nicorandil and other agents have been already used as pharmacological mimetics of ischemic PC in multicenter trials. Furthermore, agents that increase RISK or directly prevent mPTP are also under investigation as PostC analogues. We summarize recent studies focused on the pharmacological interventions and on the discovery of novel agents that may reduce the infarct size.

Key Words: Reperfusion, preconditioning, postconditioning, mitochondrial permeability transition pore, pharmacological pre and post conditioning agents.

PRECONDITIONING- ENDOGENOUS ADAPTATION

Reperfusion therapy is the gold standard in the treatment of acute myocardial infarction. Restoration of blood flow to ischemic tissue results in a rather paradox phenomenon known as ischemic-reperfusion injury. The potentially detrimental aspect of myocardial reperfusion injury, termed lethal reperfusion injury, is defined as myocardial reperfusion injury related to the restoration of coronary blood. This form of myocardial injury results by itself in cell death. Animal studies have shown that the lethal reperfusion injury is responsible for up to 50% of the final infarct size and a great number of novel strategies focus on the prevention of it [1, 2]. In 1986, Murry and colleagues first described an endogenous protective mechanism called ischemic preconditioning (PC) [3]. In the first study, it was reported that four cycles of 5 min ischemia and reperfusion prior to a prolonged ischemic insult would render the heart more tolerant to infarction in a dog model. The phenomenon of PC has been confirmed in various species including man [4-6] as well as in isolated human myocytes [7] and muscle tissue [8]. Of considerable significance is that it has also been reported in in-vivo human hearts [9-10].

Twenty years of research elucidated the ligands and receptors as well as various pharmacologic agents that are capable of changing the intracellular signal transduction pathways. Furthermore, the natural history of this phenomenon described with two phases of protection, one lasting in 60min approximately and another one which reappears in 24 hours with no additional intervention and lasting in 72hours [11-14]. The mechanistic components underlying the protection mediated by PC have been conventionally classified as "triggers" (factors acting before the index ischemic episode which may activate downstream signaling mechanisms) and "mediators/effectors" (factors acting during the index ischemic episode and mediating the protective effect) [15]. This separation is not rigid, as certain signaling components have been demonstrated to act as both triggers and mediators/effectors.

Receptor dependent triggers of PC include adenosine, which was the first signal transduction element identified as part of the PC mechanism [16]. Other endogenously released

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimioupolis, Zografou, Athens 15771, Greece; Tel:+30 210 7274827; Fax:+30 210 7274747; E-mail: jandread@pharm.uoa.gr

1389-5575/08 $55.00+.00

agents involved in PC include bradykinin, opioids, acetylcholine, free radicals and norepinephrine [17]. Many, but not all, of these triggers couple to Gi/o proteins and PKC that are thought to immediately result in opening of K_{ATP} channels [18].

Receptor independent triggers include nitric oxide (NO) and free radicals [16]. NO has been implicated in this mechanism [19] and it is a requisite cofactor in the preconditioning mediated by the ATP-sensitive potassium channel (K_{ATP}) [20, 21]. NO activates cytosolic guanylate cyclase, which in turn elevates the concentration of cyclic GMP (cGMP), leading to vasodilation [22]. Free radicals also act as triggers of PC. It is noteworthy that free radicals can activate G-proteins [23], protein kinases [24] and ATP-dependent potassium channels [25].

Mediators or triggers in PC are mitochondrial K_{ATP} channels and protein kinase C. Opening of the mitochondrial K_{ATP} channels and subsequent generation of ROS is considered a pivotal step in the initiation of the mechanism of PC during ischemia [26, 27]. The previous mentioned acetylcholine, bradykinin, opioids and phenylephrine appear to have much in common. All four agonists are capable of sparing ischemic myocardium and that protection involves mitochondrial K_{ATP} channels, free radicals and then PKC [28]. Adenosine protects the heart *via* a signal transduction pathway different from that of other agonists such as acetylcholine, bradykinin and opioids [28]. There is thus evidence of two parallel pathways triggering the protective effect of PC. The one pathway involves opening of the mitochondrial K_{ATP} channels and ROS production, whilst the other that involves adenosine appears to be independent of free radicals. PKC plays a pivotal role in the cardioprotective effect of PC. Because PKC can be activated by either ischemia or by extracellular stimuli, it is recognized as a major mediator of PC [29].

Furthermore, it has been shown recently that hypoxia-inducible factor (HIF-1), which is a master gene switch of defensive mechanisms against hypoxia, is necessary for PC and its deficiency was associated with a complete loss of protection against ischemia-reperfusion injury. Cai *et al.*, reported that heterozygotes of HIF deficient mice do not show pre-conditioning, and clinical studies are warranted to investigate whether pharmacological approaches to increase HIF-1 expression may be of therapeutic benefit [30].

CARDIOPROTECTION BY POSTCONDITIONING

In 2003, the initial study by Zhao *et al.* [31] in the canine model of coronary artery occlusion established that very short cycles of ischemia/reperfusion applied immediately after the restoration of flow render the heart more tolerant against infarction and delay the development of necrosis. This phenomenon called ischemic postconditioning and the ability to reduce infarct size has been confirmed in different species by many investigators [31-37].

All the above mentioned ligands which act as triggers in preconditioning, together with free radicals are also involved in the cascade of cellular events that upregulate PKC. PKC is upstream of a common intracellular pathway of both protective mechanisms of pre and postconditioning [1] and activates RISK. Under the name RISK (reperfusion injury salvage kinases) is described a series of pro survival kinases which are activated at the time of reperfusion and confer powerful cardioprotection against necrosis and apoptosis [2]. RISK prevent mitochondrial permeability transition pores which permit molecules up to 1500 DA to cross the mitochondrial membrane and then to destroy the cell. RISK are recruited in both preconditioned and postconditioned hearts, and participate in a common signaling pathway that mediates cardioprotection at the time of myocardial reperfusion, irrespective of the initial protective stimulus [38, 39].

High concentrations of endogenously released adenosine trigger the mechanism of heart protection by its interaction with adenosinergic G-protein coupled receptors. The function of adenosine is undoubtedly different in postconditioning than in preconditioning where it may have metabolic sparing and other effects, but is certainly involved only at reperfusion in postconditioning in contrast to its putative actions before and during ischemia in PC [40]. Both cardioprotective methods involve NO as stimulus, but they differ in their cellular pathways. In PC, NO triggers late adaptation through the formation of secondary reactive oxygen species [1]. In postconditioning NO inhibits the opening of the mitochondrial permeability transition pore, which may be an effector targeted by postconditioning. Secondly, NO may be involved as a signaling molecule at the post K_{ATP} channel level where it stimulated cGMP and PKG [40].

Both pre- and postconditioning make use of the mitochondrial K_{ATP} channel to confer cardioprotection. Yang and coworkers reversed the protection of postconditioning with glivenclamide, a nonselective adenosine triphosphate sensitive potassium (K_{ATP}) channel closer and with 5-hydroxydecanoeate (5-HD), a selective mitochondrial K_{ATP} channel antagonist [41].

Both pre- and postconditioning confer their protection to the heart through the attenuation of reperfusion injury and activation of RISK. In the initial description of postconditioning Zhao *et al.* demonstrated attenuated oxidative stress in postconditioned canine hearts as evidenced by reduced plasma malondialdehyde levels 1 hr after the beginning of reperfusion and by attenuated myocardial dihydroethidium fluorescence which is a marker of superoxide production [31]. Additional studies have confirmed these findings [42-45] and they also reported that there is reduced production of hydrogen peroxide and peroxynitrite, as well as mitochondrial peroxide and glutathione depletion in cardiomyocytes treated with hypoxic postconditioning [43, 46, 47]. In the setting of PC it is well established that ROS generated during the preconditioning phase are necessary triggers of the intracellular signal transduction pathway [48, 49]. However, in the setting of PC, the dual role of ROS is separated in time, with the signaling ROS acting during the preconditioning phase and the detrimental one at the time of myocardial reperfusion attenuated by PC [15].

Overall, the mechanism of ischemic preconditioning and postconditioning is common and the whole procedure targets against lethal reperfusion injury by reducing oxidative stress, decreasing intracellular Ca^{2+} overload, improving endothelial function, attenuating apoptotic cardiomyocyte death, and

delaying the restoration of normal pH [2]. All these are obtained by the activation of the specific RISKs, PI3K and ERKs, which prevent the deleterious effects of mitochondrial permeability transition pore (mPTP) opening.

MITOCHONDRIAL PERMEABILITY TRANSITION PORE- A NEW TARGET IN DRUG DESIGN

The mPTP is a nonspecific channel which on opening renders the inner mitochondrial membrane nonspecifically permeable to water and solutes, thereby collapsing the mitochondrial membrane potential and uncoupling oxidative phosphorylation [50, 51]. The mPTP remains closed during ischemia, but opens during the first minute of reperfusion, this event potentially coinciding with the short period of application of postconditioning [52, 53]. The components of lethal reperfusion injury correspond to the known modulators of mPTP opening. Opening of the mPTP during reperfusion is favoured by decreased inner membrane potential, low adenosine nucleotides, matrix Ca^{2+} accumulation, oxidative stress and alkalization and inhibited by matrix cations, rapid restoration of pH and ATP depletion [54-56]. Opening of the mPTP has been associated with onset of cell death by the mechanisms of necrosis whilst reseal of these pores causes apoptosis because of caspases' and cytochrome-c activation. Accordingly, the inhibition of mPTP opening is related to the preservation of normal mitochondrial functioning and has been shown to be cardioprotective [57, 58]. During ischemia, the absence of oxygen forces the heart to provide its ATP through glycolysis causing further inhibition of ATP synthesis. The decrease in pH stimulates Na(+)-H(+) exchange (NHE) activity which represents a major mechanism for H^+ extrusion and pH regulation during ischemia reperfusion [59, 60]. The ATP depletion will result in Ca^{2+} overload in the cytoplasm and increased Ca^{2+} in mitochondria [61]. ATP depletion and high inorganic phosphate concentration induce PTP opening by sensitization to Ca^{2+}. However, the low pH inhibits pore opening, preventing its occurence during ischemia. Reperfusion of the heart is accompanied by re-energisation of mitochondria that can therefore take up the accumulated calcium. There is also a burst of ROS generation in the cytoplasm and mitochondria [62-65]. Together these two factors with the fact that within a few minutes of reperfusion pH returns to normal values, the PTP opens, as it has been reported by many laboratories using different approaches [51, 55, 66, 67].

The pH is involved in protection and its abrupt restoration in normal levels upon reperfusion is an effect that aggravates reperfusion injury and cause irreversible damage, a phenomenon that is called pH paradox [68]. Kim *et al.*, stated that mitochondrial ROS together with the normalization of pH, promote mPTP generation and thus myocyte death following reperfusion. In contrast, Ca^{2+} overload do not seem to promote mPTP upregulation [69].

Several studies so far have shown, as mentioned before, that pre and postconditioning protect the myocardium by inhibiting the mPTP opening which occurs on reperfusing ischemic myocardium [66, 70-75].

Pharmacologic inhibition of mPTP opening during myocardial reperfusion with the use of cyclosporine or sanglifehrin A reduces myocardial infarct size in animals by up to 50% suggesting that mPTP opening contributes to the final infarct size [57-58]. Pharmacologic inhibition of mPTP in human atrial trabeculae subjected to stimulated ischemia reperfusion injury is also cardioprotective [76].

PHARMACOLOGICAL PRE AND POST CONDITIONING AGENTS

Although preconditioning is a powerful endogenous form of myocardial protection, the application of high grade ischemic stress as a preconditioning stimulus is limited in the clinical setting of myocardial protection since ethical and practical concerns prevent optimal preconditioning protocols being allowed in clinical trials. Pharmacological preconditioning thus represents an ideal alternative to preconditioning and greater effort needs to be exerted to invent pharmacological tools that mimic high-grade preconditioning [77].

In both PC and postconditioning, the elucidation of the signaling pathways which underlie their protective effect, would be expected to reveal novel targets for cardioprotection, which could be manipulated by pharmacological agents to produce benefits for patients undergoing myocardial ischemia reperfusion injury.

Development of novel drugs that mimic ischemic preconditioning may result in increased tolerance to effort angina, may limit the infarct size in acute coronary syndromes and may better preserve the left ventricular function after PCI or open heart surgery. Preconditioning-mimetics may even decrease the incidence of sudden cardiac death by reducing ischaemia/reperfusion ventricular arrhythmias.

K_{ATP} Channel Openers

The use of K_{ATP} channel openers is an attractive approach for cardioprotection in clinical settings [78]. These agents belong to different chemical structures. For more than a decade, the connection between K_{ATP} channels, specifically mitochondrial K_{ATP} channels, and cardiac preconditioning has been known. Potassium channel openers that selectively activate mitoK_{ATP} exhibit also a chemical diversity and comprise pyridyl, benzopyran, thiadiazine derivatives.

Nicorandil, (Fig. **1**) a hybrid of a selective mitochondrial K_{ATP} channel opener and a NO donor, has been extensively used in several multicenter clinical trials, with very promising results [79-81]. The effects of pre-treatment with oral nicorandil on preconditioning during percutaneous transluminal coronary angioplasties (PTCA) were evaluated. Pretreatment with oral nicorandil reduced the severity of myocardial ischemia, suggesting that drug can induce a pharmacological preconditioning effect during PTCA [82].

SG-209, a nicorandil derivative in which the nitrate moiety was replaced by acetate [83] produces activation of potassium channels only, while another derivative in which the nitrate moiety was replaced by alcohol (SG-86) produces no activation of either guanylate cyclase or of potassium channels.

KRN2391 (Fig. **1**) is a vasodilator with a chemical structure very similar to that of nicorandil. The only difference is the substitution of the carbonyl moiety of nicorandil by a N-

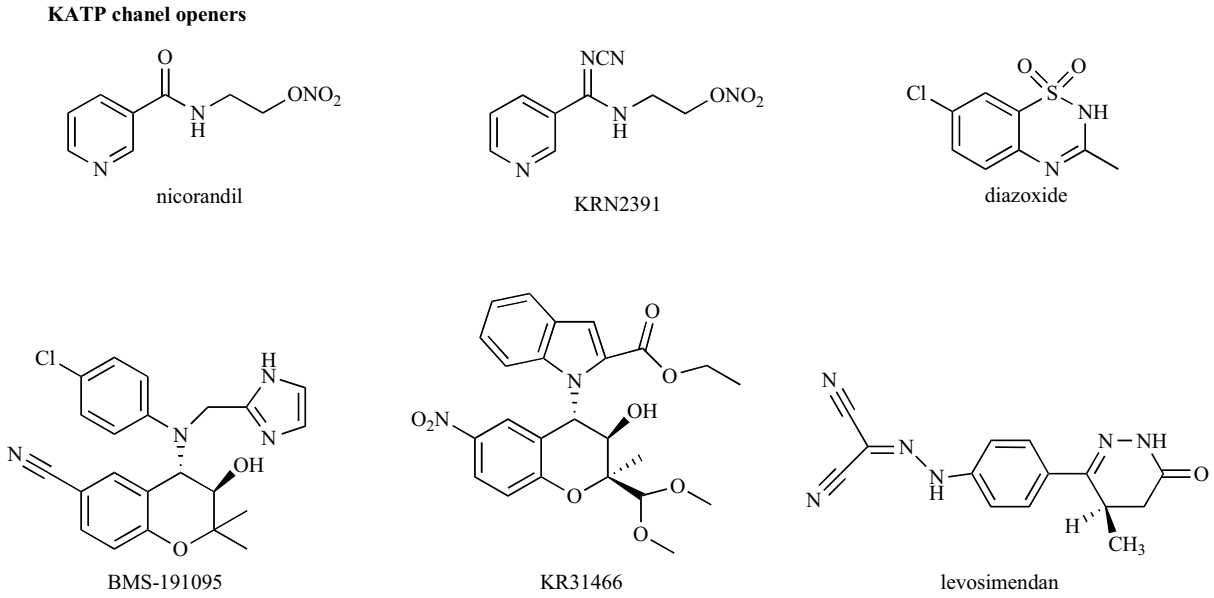


Fig. (1).

cyano-carbonimidoyl moiety. KRN2391 is more potent than nicorandil as a vasorelaxant because the ability to open potassium channels becomes greater through substitution of the carbonyl structure of nicorandil by the N-cyano-carbonimidoyl group. This substitution also enhances the activation of guanylate cyclase. Because the activity of the derivatives in which the nitrate moiety was replaced by hydroxyl or acetate groups is weaker than that of KRN2391, as a potassium channel opener, it may be inferred that the nitrate moiety would be more important not only for the activation of guanylate cyclase but also for the activation of potassium channels [84].

BMS-191095 (Fig. **1**) [85] is a more selective agent for the protection of the heart with virtually no effect on peripheral smooth muscle, compared with the first generation benzopyrans such as cromakalim. BMS-191095 improves postischemic recovery of function and reduces lactate dehydrogenase release in isolated rat hearts. The cardioprotective effects of BMS-191095 are abolished by sodium 5-hydroxydecanoate (5-HD). BMS-191095 does not activate sarcolemmal K_{ATP} current but it opens cardiac mitochondrial K_{ATP} an effect that is prevented by 5-HD. BMS-191095 may owe its cardioprotective selectivity to mitochondrial K_{ATP} activation. However, this drug will not be further developed for clinical use because of significant side effects as the neuronal toxicity [86].

Another benzopyran derivative, KR-31466 (Fig. **1**) which was discovered as anti-ischemic agent, protects heart derived H9c2 cells from hypoxia-induced death through mtK_{ATP} channel opening and PKC activation [87].

Diazoxide (Fig. **1**) a selective $mitoK_{ATP}$ channel opener in cardiac myocytes, is cardioprotective in humans. PC of human blood vessels *in vivo* involves activation of mitochondrial K_{ATP} channels and these channels are involved in the effector limb of the mechanism. Given the similarities between the mechanism of PC in endothelial cells and other tissues, including the myocardium, it is probable that activation of K_{ATP} channels is involved in preconditioning of human tissues in general [88].

Levosimendan (Fig. **1**), a hydrazono propanedinitrile derivative is a drug used in the treatment of acute heart failure and possess its anti ischemic activity by opening of the K_{ATP} channels in vascular smooth muscle cells. Recently, it has been shown to act on $mitoK_{ATP}$ channels, suggesting a possible application of this drug in clinical situations in which preconditioning would be beneficial [89].

The structures of K_{ATP} channel openers are shown in Fig. **1**.

NO Donors

Nitroglycerin is a pharmacological trigger of the late phase of preconditioning and prophylactic administration of nitrates could be a novel approach to protect the ischemic myocardium in humans [90, 91], (Fig. **2**).

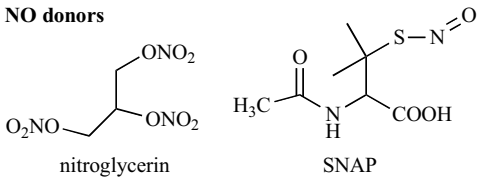


Fig. (2).

A_1 and/or the A_3 Receptor Agonists

Following an enormous amount of research it is clear that the infarct limiting effects of both endogenous adenosine [92] and exogenously applied analogues work *via* the A_1 and/or the A_3 receptor subtypes in isolated cardiac myocytes, animal studies, and human cardiac tissue. Experimental studies have also shown that intermittent administration of low

doses of A1 agonists such as R(-)N6-(2-phenylisapropyl)-adenosine (PIA) (Fig. **3**) confers cardioprotection over long periods [93].

The stimulation of adenosine A1 receptors and the activation of protein kinase C are candidates for the mechanism of PC, as the single use of an adenosine blocker 8-p-sulphophenyltheophylline (8SPT) or a protein kinase C inhibitor polymyxin B or staurosporine (ST) completely blocks the infarct size -reducing effect of PC [94].

The first potent and selective adenosine A_3 receptor agonist, IB-MECA (Fig. **3**) exhibits cardioprotection against ischemia/reperfusion damage when given before the onset of ischemia by triggering pharmacological preconditioning. Moreover, this drug may be useful for the treatment of patients with acute MI, since it is cardioprotective even when administered at the onset of reperfusion. However, it has been reported to have lethal effects at higher concentrations ($\geq$ 10 μM) [95].

Binary conjugates of adenosine A_1 and A_3 receptor agonists (Fig. **3**) were synthesized and tested in a novel cardiac myocyte model of adenosine elicited cardioprotection. Binary drugs having mixed selectivity for both A_1 and A_3 receptors were created through the covalent linking of func-

A1, A3 receptor agonists

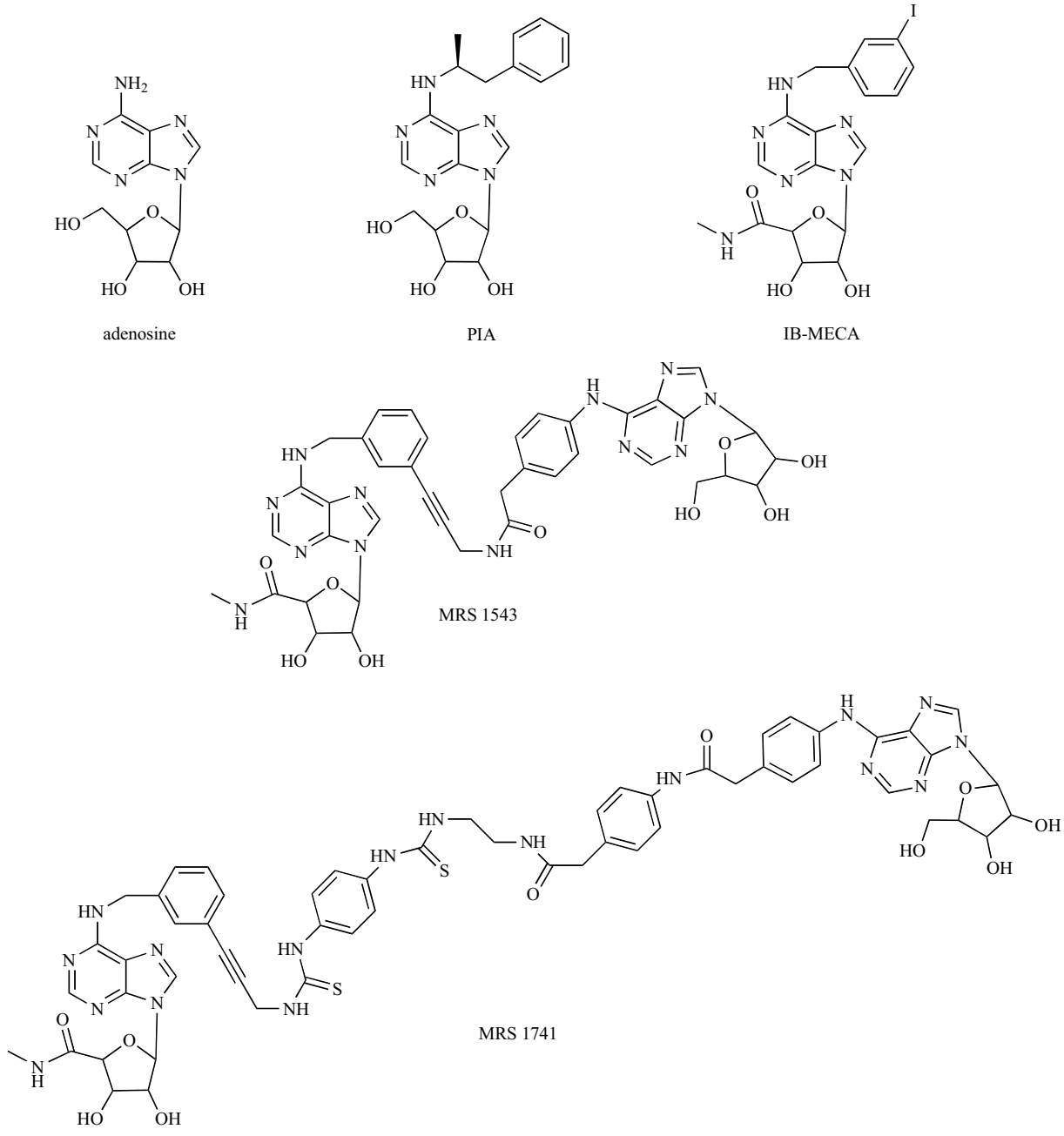


Fig. (3).

tionalized congeners of adenosine agonists, each being selective for either the A_1 or A_3 receptor subtype. The amide linked conjugate MRS 1543 was moderately potent and selective for A3 receptors. The binary agonist MRS 1741 coactivated A_1 and A_3 receptors simultaneously, with full cardioprotection dependent on expression of both receptors [96].

The structures of A_1 and/or the A_3 receptor agonists are shown in Fig. (**3**).

Combined Pharmacological Protection

Combined pharmacological protection with a G-protein-coupled receptor agonist, a mitochondrial K_{ATP} channel opener and an NO donor is necessary to confer protection with a mechanism that mimics PC *in vivo*. This synergistic approach is associated with enhanced and sustained activation of PKC-ε.

Isolated and perfused rat hearts were treated with adenosine, diazoxide and S -nitroso-*N*-acetylpenicillamine (SNAP) before ischemia. Although pretreatment with adenosine, diazoxide or SNAP (Fig. **2**) alone was capable of reducing infarct size, pharmacological preconditioning with each drug alone or in a combination of two drugs except of diazoxide plus SNAP failed to reduce infarct size. In contrast, pharmacological preconditioning in combination with adenosine, diazoxide and SNAP (triple combination PPC) conferred significant improvement of LV function and reduction of infarct size that was as effective as PC [97].

Na^+-H^+ Exchange Inhibitors

The Na^+-H^+ exchanger type 1 (NHE-1) is an important acid excluder in cardiomyocytes, and its contribution to ischemic myocardial injury has been indicated by consistent observation that administration of NHE-1 inhibitors before ischemia reduces infarct size, myocardial stunning and arrhythmia. NHE-1 inhibitors are aroyl or heteroaroyl guanidines (Fig. **4**). Inhibition of Na^+/H^+ exchange with amiloride analogues has been shown to provide functional protection during ischemia and reperfusion and to reduce infarct size in isolated rat hearts [98, 99]. Administration of dimethylamiloride in pigs improved reperfusion ion homeostasis [100].

Ethyl-isopropyl-amiloride (EIPA) when administered prior to ischemia, caused a reduction in infarct size in rabbit heart which was similar to that seen with ischemic preconditioning. Unlike the case with ischemic preconditioning, however, protection from EIPA was not blocked by polymyxin B, a PKC inhibitor, or glibenclamide, a K_{ATP} channel blocker [101, 102].

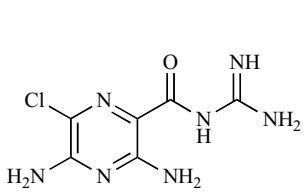
Pretreatment of hearts from Yorkshire-Duroc pigs with cariporide (Fig. **4**) delays myocardial and endothelial injury during ischemia and reperfusion, limits oxygen-derived radical injury, restores function, reduces edema, and preserves endothelin and nitric oxide balance at normal values. The myeloperoxidase changes show that less white blood cell adherence supports reduced reperfusion endothelial damage [103].

In a canine model eniporide (Fig. **4**) (EMD-96785, 3.0 mg/kg) was administered 15 min before a 90-min coronary artery occlusion followed by 3 h of reperfusion. NHE-1 inhibition was found more efficacious than IPC at reducing infarct size /area-at-risk (IS/AAR) and at preserving endothelial cell function in dogs [104].

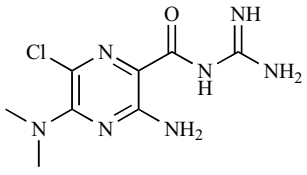
The effects of SMP-300 (Fig. **4**), an orally active, potent, and selective Na^+/H^+ exchange inhibitor, were evaluated on three experimental angina models and on myocardial infarction in rats. SMP-300 (1 mg/kg, p.o.) reduced myocardial infarct size after 40 min of coronary artery occlusion followed by 24 h of reperfusion [105].

T-162559, (Fig. **4**) a non acylguanidine Na^+-H^+ exchange (NHE) inhibitor is more potent than cariporide and eniporide and possesses a cardioprotective effect against ischemia and reperfusion injury in rat and rabbit models [106].

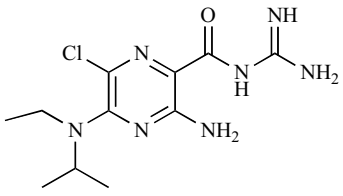
NHE inhibitors



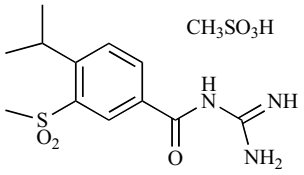
amiloride



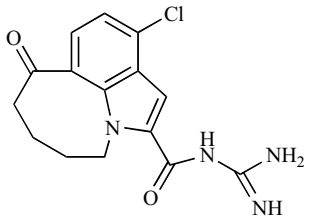
dimethylamiloride



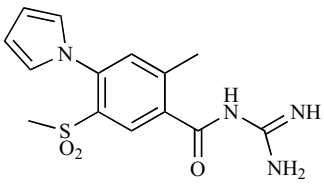
ethylisoprorylamiloride



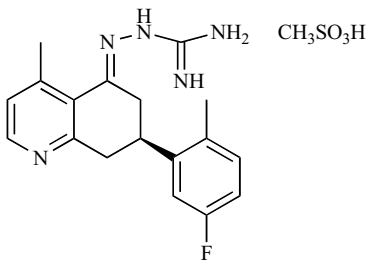
cariporide mesylate



SMP-300



eniporide



T-162559

Fig. (4).

Postconditioning Mimetics

Several studies have demonstrated cardioprotection with pharmacological agents given at time of reperfusion. Cardioprotection elicited at time of myocardial reperfusion by volatile anesthetics, earning them the description as "postconditioning" mimetics [107]. Endogenously released adenosine exerts cardioprotection during reperfusion. The A2 (a and b) receptor rather than the A1 receptor is involved in modulating infarction during reperfusion. Infusion of adenosine or A2a-selective agonists (CGS-21680) at the onset of reperfusion also reduces infarct size. These data suggest that adenosine reduces infarct size when administered at reperfusion by an A2a mechanism [108].

The same signaling components recruited in postconditioning appear to be implicated in anesthetic-induced cardioprotection including the protein kinases Akt [107-109], Erk1/2, p70s6K, e-NOS, [110], GSK-3b, MkATP and mPTP [109, 110], supporting the existence of a common cardioprotective pathway that can be recruited by either, pre, post conditioning or pharmacological agents.

We expect that the progress in research would result in the development of novel agents that may confer their protection to the ischemic and reperfused myocardium.

REFERENCES

[1] Crisostomo, P.R.; Wairiuko, G.M.; Wang, M.; Tsai, B.M.; Morrell, E.D.; Meldrum, D.R. *J. Am. Coll. Surg*., **2006**, *202*, 797.
[2] Yellon, D.M.; Hausenloy, D.J. *New Engl. J. Med*., **2007**, *357*, 1121.
[3] Murry, C.E.; Jennings, R.B.; Reimer, K.A. *Circulation*, **1986**, *74*, 1124.
[4] Liu, Y.; Downey, J.M. *Am. J. Physiol*., **1992**, *263*, H1107.
[5] Vahlhaus, C.; Schulz, R.; Post, H.; Rose, J.; Heusch, G. *J. Mol. Cell. Cardiol*., **1998**, *30*, 197.
[6] Andreadou, I.; Iliodromitis, E. K.; Mikros, E.; Bofilis, E.; Zoga, A.; Constantinou, M.; Tsantili-Kakoulidou, A.; Kremastinos, D.Th. *Free Radical Biol. Med.,* **2004**, *37*, 500.
[7] Ikonomidis, J.S.; Tumiati, L.C.; Weisel, R. D.; Mickle, D. A.; Li, R.K. *Cardiovasc. Res.,* **1994**, *28*, 1285.
[8] Walker, D.M.; Walker, J.M.; Pugsley, W.B.; Pattison, C.W.; Yellon, D.M. *J. Mol. Cell. Cardiol.,* **1995**, *27*, 1349.
[9] Cribier, A.; Korsatz, L.; Koning, R.; Rath, P.; Gamra, H.; Stix, G.; Marchant, S.; Chan, C.; Letac, B. *J. Am. Coll. Cardiol.,* **1992**, *20*, 578.
[10] Yellon, D.M.; Alkulaif, A.M.; Pugsley, W.B. *Lancet,* **1993**, *342*, 276.
[11] Eisen, A.; Fisman, E.Z.; Rubenfire, M.; Freimark, D.; McKechnie, R.; Tenenbaum, A.; Motro, M.; Adler, Y. *Atherosclerosis*, **2004**, *172*, 201.
[12] Liu, G.S.; Thornton, J.; Van Winkle, D.M.; Stanley, A.W.; Olsson, R.A.; Downey, J.M. *Circulation*, **1991**, *84*, 350.
[13] Ytrehus, K.; Liu, Y.; Downey, J.M. *Am. J. Physiol*., **1994**, *266*, H1145.
[14] Meldrum, D.R.; Cleveland, J.C.; Rowland, R.T.; Cain, B.S.; Meng, X.; Gamboni-Robertson, F.; Banerjee, A.; Harken, H. *Am. J. Physiol*., **1997**, *273*, H725.
[15] Hausenloy, D.J.; Yellon, D.M. *Pharmacol. Ther*., **2007**, *116*, 173.
[16] Eisen, A.; Fisman, E.Z.; Rubenfire, M.; Freimark, D.; McKechnie, R.; Tenenbaum, A.; Motro, M.; Adler, Y. *Atherosclerosis*, **2004**, *172*, 201.
[17] Kukreja, R.C. *J. Mol. Cell. Cardiol*., **2001**, *33*, 1395.
[18] Gross, G.J.; Fryer, R.M. *Circ. Res*., **2001**, *84*, 973.
[19] Vegh, A.; Szekeres, L.; Parratt, J. *Br. J. Pharmacol.,* **1992**, *107*, 648.
[20] Horimoto, H.; Gaudette, C.R.; Krukenkamp, I.B. *Surg. Forum,* **1998**, *48*, 202.
[21] Wang, Y.; Ahmad, N,; Wang, B.; Ashraf, M.; *Am. J. Physiol. Heart Circ. Physiol.*, **2007**, *292*, H2300.
[22] Louis, I.J. *Annu. Rev. Pharmacol. Toxicol.,* **1990**, *30*, 535.
[23] Nishida, M.; Maruyama, Y.; Tanaka, R.; Kontani, K.; Nagao, T.; Kurose, H. *Nature*, **2000**, *408*, 492.
[24] Bhatnagar, A.; Srivastava, A.; Szabo, G. *Circ. Res*., **1990**, *67*, 535.
[25] Tokube, K.; Kiyosue, T.; Arita, M. *Am. J. Physiol. Heart Circ. Physiol*., **1996**, *271*, H478.
[26] Schulz, R.; Cohen, M.D.; Behrends, M.; Downey, J.M.; Heusch, G. *Cardiovasc. Res*., **2001**, *52*, 181.
[27] Das, B.; Sarkar, C. *Vascul. Pharmacol*., **2007**, *47*, 248.
[28] Cohen, M. V.; Yang, X. M.; Liu, G. S.; Heusch, G.; Downey J. M. *Circ. Res*., **2001**, *89*, 273.
[29] Sanada, S.; Kitakaze, M. *Intern. J. Cardiol*., **2004**, *97*, 263.
[30] Cai, Z.; Zhong, H.; Bosch-Marce, M.; Fox-Talbot, K.; Wang, L.; Wei, C.; Trush, M.A.; Semenza, G.L. *Cardiovasc. Res*., **2008**, *77*, 463.
[31] Zhao, Z.Q.; Corvera, J.S.; Halkos, M.E.; Kerendi, F.; Wang, N.P.; Guyton, R.A.; Vinten-Johansen, *Am. J. Physiol. Heart Circ. Physiol*., **2003**, *285*, H579.
[32] Kin, H.; Zatta, A.J.; Lofye, D.J.; Amerson, B.S.; Halkos, M.E.; Kerendi, F.; Zhao, Z.Q.; Guyton, R.A.; Headrick, J.P.; Vinten-Johansen, *J. Cardiovasc. Res*., **2005**, *67*, 124.
[33] Tsang, A.; Hausenloy, D.J.; Macanu, M.M.; Yellon, D.M. *Circulation*, **2004**, *11*, 167.
[34] Iliodromitis, E.K.; Zoga, A.; Vrettou, A.; Andreadou, I.; Paraskevaidis, I.A.; Kaklamanis, L.; Kremastinos, D.Th. *Atherosclerosis*, **2006**, *188*, 356.
[35] Iliodromitis, E.K.; Georgiadis, M.; Cohen, M.V.; Downey, J.M.; Bofilis, E.; Kremastinos, D.Th. *Basic Res. Cardiol*., **2006**, *101*, 502.
[36] Yellon, D.M.; Hausenloy, D.J. *Nat. Clin. Pract. Cardiovasc. Med*., **2005**, *2*, 568.
[37] Yellon, D.M.; Opie, L.H. *Lancet*, **2006**, *367*, 456.
[38] Downey, J.M.; Cohen, M.V. *Circulation*, **2005**, *111*, 120.
[39] Hausenloy, D.J.; Tsang, A.; Yellon, D.M. *Trends Cardiovasc. Med*., **2005**, *15*, 69.
[40] Vinten-Johansen, J.; Zhao, Z.Q.; Zatta, A.J.; Kin, H.; Halkos, M.E.; Kerendi, F. *Basic Res. Cardiol*., **2005**, *100*, 295.
[41] Yang, X.M.; Proctor, J.B.; Cui, L.; Krieq, T.; Downey, J.M.; Cohen, M.V. *J. Am. Coll. Cardiol.*, **2004**, *44*, 1103.
[42] Halkos, M.E.; Kerendi, F.; Corvera, J.S.; Wang, N.P.; Kin, H.; Payne, C.S.; Sun, H.Y.; Guyton, R.A.; Vinten-Johansen, J.; Zhao, Z.Q. *Ann. Thorac. Surg*., **2004**, *78*, 961.
[43] Sun, H.Y.; Wang, N.P.; Kerendi, F.; Halkos, M.; Kin, H.; Guyton, R.A.; Vinten-Johansen, J.; Zhao, Z.Q. *Am. J. Physiol. Heart Circ. Physiol*., **2005**, *288*, H1900.
[44] Mykytenko, J.; Kerendi, F.; Reeves, J.G.; Kin, H.; Zatta, A.J.; Jiang, R.; Guyton, R.A.; Vinten-Johansen, J.; Zhao, Z.Q. *Basic Res. Cardiol*., **2006**, *102*, 90.
[45] Sun, H.Y.; Wang, N.P.; Halkos, M.; Kerendi, F.; Kin, H.; Guyton, R.A.; Vinten-Johansen, J.; Zhao, Z.Q. *Apoptosis*, **2006**, *11*, 1583.
[46] Wang, H.C.; Zhang, H.F.; Guo, W.Y.; Su, H.; Zhang, K.R.; Li, Q.X.; Yan, W.; Ma, X.L.; Lopez, B.L.; Christopher, T.A.; Gao, F. *Apoptosis*, **2006**, *11*, 1453.
[47] Serviddio, G.; Di Venosa, N.; Federici, A.D.; D'Agostino, D.; Rollo, T.; Prigigallo, F.; Altomare, E.; Fiore, T.; Vendemiale, G. *FASEB J*., **2005**, *19*, 354.
[48] Baines, C.P.; Goto, M.; Downey, J.M. *J. Mol. Cell. Cardiol*., **1997**, *29*, 207.
[49] Tritto, I.; D'Andrea, D.; Eramo, N.; Scognamiglio, A.; De Simone, C.; Violante, A.; Esposito, A.; Chiariello, M.; Ambrosio, G. *Circ. Res*., **1997**, *80*, 743.
[50] Crompton, M. *J. Physiol*., **2000**, *529*, 11.
[51] Hausenloy, D.J.; Yellon, D.M. *J. Mol. Cell. Cardiol*., **2003**, *35*, 339.
[52] Griffiths, E.J.; Halestrap, A.P. *Biochem. J*., **1995**, *307*, 93.
[53] Qian, T.; Nieminen, A.L.; Herman, B.; Lemasters, J.J. *Am. J. Physiol. (Cell Physiology)*, **1997**, *273*, C1783.
[54] Halestrap, A.P.; Clarke, S.J.; Javadov, S.A. *Cardiovasc. Res*., **2004**, *61*, 372.
[55] DiLisa, F.; Canton, M.; Menabo, R.; Dodoni, G.; Bernadi, P. *Basic Res. Cardiol.,* **2003**, *98*, 235.
[56] Lemasters, J.J. *Cardiovasc. Res*., **1999**, *44*, 470.
[57] Hausenloy, D.J.; Duchen, M.R.; Yellon, D.M. *Cardiovasc. Res*., **2003**, *60*, 617.

[58] Hausenloy, D.J.; Maddock, H.L.; Baxter, G.F.; Yellon, D.M. *Cardiovasc. Res*., **2002**, *55*, 534.
[59] Karmazyn, M.; Gan, X.T.; Humphreys, R.A.; Yoshida, H.; Kusumoto, K. *Circ. Res*., **1999**, *85*, 777.
[60] Fliegel, L.; Karmazyn, M. *Biochem. Cell. Biol*. **2004**, *82*, 626.
[61] Karmazyn, M.; Sostaric, J.V.; Gan, X.T. *Drugs*, **2001**, *61*, 375.
[62] O'Rourke, B.; Cortassa, S.; Aon, M.A. *Physiology*, **2005**, *20*, 303.
[63] Brookes, P.S.; Yoon, Y.; Robotham, J.L.; Anders, M.W.; Sheu, S.S. *Am. J. Physiol. Cell Physiol.*, **2004**, *287*, C817.
[64] Becker, L.B. *Cardiovasc. Res*., **2004**, *61*, 461.
[65] Solaini, G.; Harris, D.A. *Biochem. J*., **2005**, *390*, 377.
[66] Javadov, S.A.; Clarke, S.; Das, M.; Griffiths, E.J.; Lim, K.H.; Halestrap, A.P. *J. Physiol*., **2003**, *549*, 513.
[67] Griffiths, E.J.; Halestrap, A.P. *J. Mol. Cell. Cardiol*., **1993**, *25*, 1461.
[68] Lemasters, J.J.; Bond, J.M.; Chacon, E.; Harper, I.S.; Kaplan, S.H.; Ohata, H.; Trollinger, D.R.; Herman, B.; Cascio, W.E. *EXS*, **1996**, *76*, 99.
[69] Kim, J.S.; Jin, Y.; Lemasters, J.J. *Am. J. Physiol. Heart Circ. Physiol*., **2006**, *290*, H2024.
[70] Hausenloy, D.J.; Yellon, D.M.; Mani-Babu, S.; Duchen, M.R. *Am. J. Physiol. Heart Circ. Physiol.,* **2004**, *287*, H841.
[71] Juhaszova, M.; Zorov, D.B.; Kim, S.; Pepe, S.; Fu, Q.; Fishbein, K.W.; Ziman, B.D.; Wang, S.; Ytehus, K.; Antos, C.L.; Olson, E.N.; Sollot, S.J. *J. Clin. Investig*., **2004**, *113*, 1535.
[72] Sack, M.N. *Circulation*, **2006**, *114*, 1452.
[73] Argaud, L.; Gateau-Roesch, O.; Raisky, O.; Loufouat, J.; Roberd, D.; Ovize, M. *Circulation*, **2005**, *111*, 194.
[74] Bopassa, J.C.; Mischel, P.; Gateau-Roesch, O.; Coutoure-Lepetit, E.; Ovize, M. *Cardiovasc. Res*., **2006**, *69*, 178.
[75] Zhao, Z.Q.; Wang, N.P.; Mykytenko, J.; Reeves, J.; Deneve, J.; Jiang, R. *Circulation*, **2006**, *114*, 261.
[76] Shanmuganathan, S.; Hausenloy, D.J.; Duchen, M.R.; Yellon, D.M. *Am. J. Physiol. Heart Circ. Physiol*., **2005**, *289*, H237.
[77] Uchiyama, Y.; Otani, H.; Okada, T.; Uchiyama, T.; Ninomiya, H.; Kido, M.; Imamura, H.; Nakao, S.; Shingu, K. *J. Thor. Cardiovasc. Surg.,* **2003**, *126*, 148.
[78] Mannhold, R. *Med. Res. Rev*., **2004**, *24*, 213.
[79] Patel, D.J.; Pucell, H.J.; Fox, K.M.; on behalf of the CESAR 2 investigation *Eur. Heart J.,* **1999**, *20*, 51.
[80] The IONA Study Group. *Lancet,* **2002**, *359*, 1269.
[81] Iliodromitis, E.K.; Cokkinos, P.; Zoga, A.; Steliou, I.; Vrettou, A.; Kremastinos, D.Th. *Br. J. Pharmacol.,* **2003**, *138*, 1101.
[82] Kato, T.; Yoshimoto, N. *Drugs,* **2003**, *63*, 133.
[83] Ishibashi, T.; Hamaguchi, M.; Imai, S. *Naunyn- Schmiedeberg's Arch. Pharmacol.,* **1991**, *344*, 235.
[84] Ishibashi, T.; Hamaguchi, M.; Imai, S. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **1992**, *346*, 94.
[85] Rovnyak, G. C.; Ahmed, S. Z.; Ding, C. Z.; Dzwonczyk, S.; Ferrara, F. N.; Humphreys, W. G.; Grover, G. J.; Santafianos, D.; Atwal, K. S.; Baird, A. J.; McLaughlin, L. G.; Normandin, D. E.; Sleph, P. G.; Traeger, S. C. *J. Med. Chem.,* **1997**, *40*, 24.
[86] Grover, G.J.; D'Alonzo, A.J.; Garlid, K.D.; Bajgar, R.; Lodge, N.J.; Sleph, P.G.; Darbenzio, R.B.; Hess, T.A.; Smith, M.A.; Paucek, P.; Atwal, K.S. *J. Pharmacol. Exp. Ther.,* **2001**, *297*, 1184.
[87] Jung, Y-S.; Jung, Y.S.; Kim, M-Y.; Kim, M.H.; Lee, S.; Yi, K.Y.; Yoo, S.E.; Lee, S.H.; Baik, E.J.; Moon, C-H.; Cho, J. P. *J. Pharmacol. Sci.,* **2003**, *92*, 13.
[88] Broadhead, M.W.; Kharbanda, R.K.; Peters, M.J.; MacAllister, R.J. *Circulation*, **2004**, *110*, 2077.
[89] Pollesello, P.; Papp, Z. *J. Cardiovasc. Pharmacol*., **2007**, *50*, 257.
[90] Heusch, G. *Circulation,* **2001**, *103*, 2876.
[91] Iliodromitis, E.K.; Gaitanaki, C.; Lazou, A.; Aggeli, I. K.; Gizas, V.; Bofilis, E.; Zoga, A.; Beis, I.; Kremastinos, D. Th. *Basic Res. Cardiol.,* **2006**, *101*, 327.
[92] Ross, A.M.; Gibbons, R.J.; Stone, G.W.; Kloner, R.A.; Alexander, R.W. Amistad II investigators. *J. Am. Coll. Cardiol.,* **2005**, *45*, 1775.
[93] Mangano, D.T.; Miao, Y.; Tudor, I.C.; Dietzel, C. *J. Am. Coll. Cardiol.,* **2006**, *48*, 206.
[94] Baba, K.; Minatoguchi, S.; Zhang, C.; Kariya, T.; Uno, Y.; Kawai, T.; Takahashi, M.; Takemura, G.; Fujiwara, H. *Clin. Exp. Pharmacol. Physiol.,***2005**, *32*, 263.
[95] Xu, Z.; Jang, Y.; Mueller, R.A.; Norfleet, E.A. *Cardiovasc. Drug Rev.,* **2006**, *24*, 227.
[96] Jacobson, K.A.; Xie, R.; Young, L.; Chang, L.; Liang, B.T. *J. Biol. Chem*., **2000**, *275*, 30272.
[97] Otani, H.; Okada T.; Fujiwara H.; Uchiyama, T.; Sumida T.; Kido, M.; Imamura, H. *Clin. Exp. Pharmacol. Physiol.,* **2003**, *30,* 684.
[98] Duan, J.; Karmazyn, M. *Eur. J. Pharmacol.,* **1992**, *210*, 149.
[99] Meng, H.; Pierce, G. *Am. J. Physiol.,* **1990**, *258*, H1615.
[100] Goldberg, S.P.; Digerness, S.B.; Skinner, J.L.; Killingsworth, C.R.; Katholi, C.R.; Holman, W.L. *Ann. Thorac. Surg.,* **2002**, *73*, 569.
[101] Sato, H.; Miki, T.; Vallabhapurapu, R. P.; Wang, P.; Liu, G. S.; Cohen, M. V.; Downey, J. M. *Basic Res. Cardiol.,* **1997**, *92*, 339.
[102] Munch-Ellingsen, J.; Løkebø, J. E.; Bugge, E.; Ytrehus, K. *Mol. Cell. Biochem.,* **1998**, *186*, 13.
[103] Castellá, M.; Buckberg, G. D.; Tan, Z.; Ignarro, L. J. *J. Thorac. Cardiovasc. Surg.,* **2002**, *124*, 1113.
[104] Gumina, R. J.; Moore, J.; Schelling, P.; Beier, N.; Gross, G. J. *Am J Physiol Heart Circ. Physiol.,* **2001**, *281*, H1260.
[105] Yamamoto, S.; Matsui, K.; Sasabe, M.; Ohashi, N. *J. Cardiovasc. Pharmacol.,* **2002**, *39*, 234.
[106] Kusumoto, K.; Igata, H.; Abe, A.; Ikeda, S.; Tsuboi, A.; Imamiya, E. Fukumoto, S.; Shiraishi, M.; Watanabe T. *Br. J. Pharmacol.,* **2002**, *135*, 1995.
[107] Chiari, P.C.; Bienengracher, M.W.; Pagel, P.S.; Krolikowski, J.G.; Kersten, J.R.; Waltier, D.C. *Anesthesiology*, **2005**, *102*, 102.
[108] Vinten-Johansen, J.; Zhao, Z.-Q.; Jiang, R.; Zatta, A.J.; Dobson, G. P. *J. Appl. Physiol.,* **2007**, *103*, 1441.
[109] Feng, J.; Fischer, G.; Lucchinetti, E.; Zhu, M.; Bestmann, L.; Jegger, D.; Arras, M.; Pasch, J.; Perriard, J.C.; Schaub, M.C.; Zaung, M. *Anesthesiology*, **2006**, *104*, 1004.
[110] Krolikowski, J.G.; Weihrauch, D.; Bienengracher, M.; Kersten, J.R.; Warltier, D.C.; Pagel, P.S. *Can. J. Anaesth.*, **2006**, *53*, 174.

Received: 13 December, 2007 Revised: 14 February, 2008 Accepted: 15 February, 2008