Involvement of nitric oxide and reactive oxygen species in the early and delayed antiarrhythmic effects of preconditioning

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PhD Thesis

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List of Publications

Related articles (Full papers)

I.
Hajnal Á, Nagy O, Litvai A, Papp JGy, Parratt JR, Végh Á
Nitric oxide involvement in the delayed antiarrhythmic effect of treadmill exercise in dogs
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II.
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CARDIOVASCULAR DRUGS AND THERAPY 18:(6) pp. 449-459. (2004)
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Abstracts in journals with impact factor

1.
Hajnal Á, Csillik A, Litvai Á, Parratt J R, Végh Á
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3.
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A nitrogén-oxid szerepének vizsgálata a fizikai terheléssel kialakított prekondicionálás késői antiarrhythmiás hatásában.
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I.
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Sildenafil (Viagra) reduces arrhythmia severity during ischaemia 24h after oral administration in dogs
BRITISH JOURNAL OF PHARMACOLOGY 141: 549-551 (2004).

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List of Abbreviation

AEST	-	S-(2-aminoethyl)-methyl-isothiourea
AKT	-	Serine/threonine-specific protein kinase
AP	-	action potential
ATP	-	Adenosine Triphosphate
Ca^{2+}	-	calcium ion
$[Ca^{2+}]_{int}$	-	intracellular calcium concentration
CAD	-	coronary artery diseases
CL	-	lucigenin-enhanced chemiluminescence
cNOS	-	constutitive nitrogen-monoxide synthase
DABP	-	diastolic arterial blood pressure
DAG	-	diacylglycerol
DPBS	-	Dulbecco's Phosphate Buffered Saline
EDTA	-	Ethylenediaminetetraacetic acid
G_i	-	inhibitory G-protein
H+	-	hydrogen ion
$[H^+]_{int}$	-	intracellular hydrogen ion concentration
HOE 140	-	bradykinin B2 receptor antagonist
HR	-	Heart rate
IPC	-	ischemic preconditioning
I3P	-	inositol-triposphate
iNOS	-	inducible nitrogen-monoxide synthase
K^+	-	potassium ion
$[K^+]_{int}$	-	intracellular potassium concentration
K_{ATP} channels	-	ATP dependent potassium channel
LAD	-	descending branch of left coronary artery
LCX	-	circumflex branch of the left coronary artery
L-NAME	-	ω-nitro-L-arginine-methyl-ester
LVEDP	-	left ventricular end-diastolic pressure
LVP	-	left ventricular pressure
MABP	-	mean arterial blood pressure
MAP kinase		Mitogen activated protein kinase

MAPKAP 2		MAP kinase-activated protein kinase 2
MBF	-	myocardial blood flow
MnSOD	-	manganese superoxide dismutase
MPG	-	N-2-mercaptopropionylglycine
Na^+	-	Sodium ion
$[Na^+]_{int}$	-	intracellular sodium concentration
NADPH	-	Nicotinamide adenine dinucleotide phosphate
NBT	-	Nitro-blue-tetrazolium
NF-κB		Nuclear transcription factor-κB
$NO^{\cdot}$	-	nitrogen monoxide radical
NOS	-	reactive nitrogen species
O_2	-	Oxygen
$O_2^{\cdot -}$	-	superoxide radicals
$ONOO^{\cdot -}$	-	peroxinitrite
PC	-	precondicioning
PC	-	preconditioning
PI3K	-	phosphatidylinositol-4,5-bisphosphate 3-kinase
PKC	-	phosphokinase C
PLC	-	phospholipase C
PLD	-	phospholipase D
PMA	-	phorbol 12-myristate 13-acetate
PMNs	-	polymorphonuclear leucocytes
ROS	-	reactive oxygen species
s.e.m	-	standard error of mean
SABP	-	systolic arterial blood pressure
SCD	-	sudden cardiac death
SOD	-	superoxide dismutase
STAT		Signal Transducer and Activator of Transcription molecule
VF	-	ventricular fibrillation
VF	-	ventricular fibrillation
VPB's	-	ventricular premature beats
VT	-	ventricular tachicardia

1. Introduction

One of the most important consequences of the reduction in coronary blood flow due to acute myocardial ischemia is the occurrence of severe ventricular tachyarrhythmias, which remain one of the major causes of sudden cardiac death (SCD) in modern societies. Although the underlying mechanisms of these acute arrhythmias are many and varied, it is certain that the ischemia-induced rapid ionic alterations and the subsequent inhomogeneous conduction may play an essential role.

The problem of sudden cardiac death from ventricular fibrillation during coronary artery occlusion or reperfusion, outside the hospital setting is still one of the biggest challenges facing clinical cardiology. Despite all attempts, e.g. the introduction of mobile coronary care units and implantable defibrillators, as well as the training of non-medical personnel in the use of these appliances, more than half of the cardiovascular deaths can be attributed to sudden ventricular fibrillation. Since drug therapy has proved to be largely ineffective except in the clinical setting, understanding the underlying mechanisms that lead to these life-threatening ventricular arrhythmias is crucial for developing novel therapeutic strategies.

In the past few decades many attempts have been made on protecting the myocardium and thus preventing the heart from the severe consequences of ischemia/reperfusion injury. One of these attempts was made by Murry and colleagues[1] in 1986 where they observed that the heart responds to brief ischemia/reperfusion insults with an increased tolerance, rather than with cumulating ischemic damage, during a subsequent, more prolonged episode of ischemia. This phenomenon was termed as „ischemic preconditioning". The fact, that the heart can be protected against severe, often fatal injuries by similar, but sub-lethal ischemic stress has raised considerable interest both in experimental and also in clinical cardiovascular research. The short periods of ischemia are now commonly applied to protect the heart from ischemic injury during human coronary by-bass surgery[2]. There is also evidence that not only the short ischemic episodes but other stimuli, such as rapid cardiac pacing[3] and vigorous physical exercise[2] can also provide protection. Numerous epidemiologic studies have revealed that regular exercise reduces morbidity and mortality in patients with coronary artery diseases

(CAD)[2, 4]. Moreover, it has been shown that exercise attenuates the risk factors for CAD, such as hypertension, hyperlipidemia, diabetes, obesity. However, the exact mechanisms by which the exercise-induced protection attains are still not fully elucidated. The main purpose of the studies, described in the present thesis, was to explore the mechanisms involved in the early and the delayed antiarrhythmic effects of preconditioning induced by brief coronary artery occlusions and by physical exercise, with particular attention of the role of nitric oxide and reactive oxygen species (ROS).

1.1. Consequences of acute myocardial ischemic injury

After occlusion of a coronary artery the blood supply dramatically decreases in the affected tissue, however, the oxygen and energy demand of the working myocardium has not changed. Myocardial energy is formed of ATP, which is produced by the oxidative metabolism of the carbohydrates and fatty acids. There are just two energy stores exist in the myocardium: the glycogen and the high energy phosphates[6-7]. Besides the low oxygen level the metabolic pathways change from aerobic to anaerobic process to produce ATP[6]. However these pathways evoke the drastic decrease of intracellular and extracellular pH, due to the accumulation of lactic acid[8].

During occlusion the ischemia induces the development of ischemic metabolites, such as lactate, platelet activation factor[9], cytokines[10], reactive oxygen species (ROS)[11], $NO^{\cdot}$ and other reactive nitrogen species (NOS)[12] as well as arachidonic acid[13]. These metabolites contribute to the myocardial ischemic injury development, the ionic alteration and dysfunction in contractility. These factors altogether drive the myocardium to arrhythmogenesis.

Electrocardiographically the ischemic event is characterized by marked TQ-interval shortening[14] and ST-segment elevation[15]. This alteration comes from the early changes in transmembrane action potential (AP) and the delayed changes in electrical cell-to-cell coupling[15]. The ionic alteration during myocardial ischemia modifies the AP: i.e. shortens its duration and delays the refractoriness, whereas decreases the amplitude of AP[16]. Moreover the ischemic conditions reduce the resting membrane potential and draw it closer to the threshold potential[17].

1.2. The acute ischemia and reperfusion-induced ventricular arrhythmias

The important consequences of acute ischemic injury are the occurrence of the life-threatening ventricular arrhythmias. The reduced resting membrane potential, slowed conduction, the ionic and metabolic alterations that are present under ischemic conditions, lead to the generation of severe arrhythmias, by provoking spontaneous after-depolarization[16,18]. On the other hand, the unidirectional conduction block and the delay in impulse conduction in the ischemic myocardium favour the occurrence of the re-entry type arrhythmias[17]. The impulse spreading in some part of the tissue is prevented by the conduction blocks. It blocks the retrograde but not the anterograde path (unidirectional block) in the bifurcating conduction pathway. The slowing conduction causes re-entry upset in the refractory tissue[18]. The electrical uncoupling contributes to the conduction slowing and blocking in the initial phase of ischemia through the myocytes gap junctions[19].

After the onset of coronary artery occlusion the ventricular arrhythmias appear in two distinct phases. The phase I arrhythmias appear in one single period in some species (for example rats), but in other animals, like dogs, it is divided into subcomponents as phase Ia and Ib arrhythmias [20, 21]. The phase Ia arrhythmias occur between the 3 and 8 min of the ischemia, whereas the phase Ib arrhythmias appear approximately 15 min after the occlusion and they disappear 30 min after the onset of ischemia. During this 30 min period the ischemic changes can be considered reversible.

1.2.1. Mechanisms of the Ia arrhythmias

Soon after the onset of the coronary artery occlusion, the rapid changes in electrical membrane properties occur, due to the fast metabolic alterations.[23-24]. Thus the shift from aerobic to anaerobic metabolism produces both extracellular and intracellular drop in pH. Since the Na^+/K^+ exchanger requires ATP to maintain the normal ionic gradient the reduction in high energy phosphates causes a loss in intracellular K^+. Because the extracellular K^+ and H^+ accumulation gradually decreases from the centre towards the border of the ischemic region[8-9], the arrhythmias most likely appear in the border zone of the occluded area. Moreover, the intracellular acidosis increases the activity of Na^+/H^+ exchanger, which mechanism leads to the accumulation of intracellular Na^+[26]. The Na^+ influx leads to the influx of Ca^{2+}, due to the reverse mode of Na^+/Ca^{2+} exchanger. In the Ca^{2+}-overloaded ischemic

myocytes uncontrolled activation of the contractile fibres occurs particularly during re-oxygenation[27-29].

Phase Ia arrhythmia is preceded by the rapid evolution of delayed activation of myocytes within the ischemic sub-epicardium. When it exceeds 120-140 ms the localized re-entry and ventricular arrhythmia can be observed[30]. The resulting ventricular tachycardia is formed by large and highly unstable re-entry circuits with several millimetres inner circle length[30]. The re-entry is confirmed to be a source of VF during the phase Ia arrhythmias[31].

1.2.2. Mechanisms of phase Ib arrhythmias

The second phase of early arrhythmias starts around the 15 min, and lasts until the 25-30 min of the occlusion. The main factor of the appearance of Ib arrhythmias is the uncoupling of the cell-to-cell communication through gap junctions[32]. This electrical uncoupling results in the slowing and blocking of conduction caused by the low pH level, the loss of intracellular K^{+30} and ATP as well as the increase in intracellular Ca^{2+} that cause the dephosphorylation of the channel proteins of the gap junctions33. Moreover in smaller degree localized re-entry mechanisms also contribute to the appearance of phase Ib arrhythmias[30].

However, if the coronary occlusion persists over a longer period the ischemia becomes irreversible and approximately 90 min after the occlusion the phase II arrhythmias occur, when a myocardial necrosis has started[22].

1.2.3. Reperfusion injury

The reperfusion results in a rapid restoration of action potential in the ischemic myocardium. However the returning electrical activity is not equally rapid for each cells. The significant inhomogeneity of electrical activation remains during the first 30 seconds of reperfusion within the ischemic area and at the border zone. Persistent membrane depolarization and gap junctional uncoupling could contribute to the occurrence of re-entrant arrhythmias during the early period of reperfusion[37].

Restoration of blood supply to the myocardium results in substantial free radical production[34]. These reactive oxygen and nitrogen species (ROS; NOS) generated during reperfusion play an essential role in the generation of the reperfusion arrhythmias.

Numerous endogenous antioxidant mechanisms exist (such as superoxide dismutase – SOD, catalase, glutathione oxidase) in cells and organs that start to eliminate this reactive species during ischemia and reperfusion. However the prolonged ischemia and mainly a

following reperfusion provoke an excess of reactive species that the scavenger system cannot totally neutralize. The ROS may damage the sarcoplasmic reticulum (SR), causing Ca^{2+} release from the SR and an increase in the intracellular Ca^{2+} [35]. Later the high level of Ca^{2+} causes the decreasing of cardiac contractility because of the damage of contractile proteins and because of a reduced sensitivity to Ca^{2+}. These processes might be involved in the contractile dysfunction of ischemic myocardium (myocardial stunning)[29]. Moreover the superoxide radicals ($O_2^{\cdot-}$) interact with NO[36] leading to the reduction of NO bioavailability and the generation of another reactive species, the peroxynitrite ($ONOO^{\cdot-}$). This inactivation of NO by ROS is a key element of the development of the endothelial dysfunction after reperfusion[38].

The neutrophils from the blood contribute to the injury[29], damage the endothelium and also the myocardium. These cells accumulate in the reperfused myocardium and release their toxic agents such as oxidants, proteases and cytokines[39 - 41].

1.3. Preconditioning as a possible mechanism to protect the heart against ischemia and reperfusion injury

The injury of the prolonged coronary artery occlusion and subsequent reperfusion can be prevented by short periods of occlusion and reperfusion insults. This process stimulates those mechanisms, which can protect the myocardium against a subsequent ischemic insult. This adaptation of the heart is termed as ischemic preconditioning (IPC)[1]. The protection induced by PC is independent of the changes in collateral blood flow. A similar adaptation to ischemia and reperfusion can be achieved by other stimuli, like heat stress[41], stretching[43] and also by numerous pharmacological agents (pharmacological preconditioning). The rapid cardiac pacing is another way to create protection[3]. Pacing induced short periods of tachycardia decreases the perfusion, but increases the energy and oxygen consumption. This hypoxic state of the myocardium, the increased heart rate and blood pressure can initiate the protection[44].

The preconditioning stimulus can be evoked by a single period of treadmill exercise as well [45]. The duration and the intensity of the physical exercise are important to induce the protection. The exercise elevates the heart rate, decreases the blood perfusion, increases the energy and oxygen supply, and remote effects may also play a role in this process. The exercise increases the heart vagal activity as well and preserves the baroreflex sensitivity[45].

During exercise there is a large vasodilatation of arterial vasculature in the heart and in the active skeletal muscle. This massive relaxation can be caused by NO. The NO is released from the endothelium from the effect of different hormones such as norepinephrine or the direct effect of shear stress46.

The protective mechanisms start immediately, regardless the preconditioning stimulus. The protection shows a biphasic pattern. The early protection is short lived, usually it disappears 1 or 2 h after the stimulus. However the beneficial effect can be seen again after 12-24 h of the stimulus and remains for the next 2-3 days[47]. In the early phase of preconditioning rapid post translational modifications of pre-existing proteins occur[48]. The PC stimuli release endogenous substances (triggers) to start the PC process[49]. These triggers activate those materials that prolong the processes (mediators). These mediators contribute to the signal transduction process between the triggers and the end-effectors. Parallel to the early protective mechanisms, the PC trigger induces those cellular mechanisms, which has effect on the late phase of PC. The important steps of this process are the *de novo* protein synthesis[48].

1.3.1. Endogenous substrates as a trigger of preconditioning

Numerous endogenous substrates were identified that may contribute as a trigger of the preconditioning, in tests conducted during the beneficial effect of PC. Some of these bind to the cell surface receptors like adenosine[50], bradykinin[51], opioid peptides[52], prostacyclin[53] but the others induce their effect by non-receptor triggered manner such as NO[54] or other free radicals[55]. It is hypothesized that these endogenous triggers act parallel to the early phase of preconditioning[56]. This theory supposes a PC threshold that the triggers have to exceed.

1.3.2. Signal transduction of early preconditioning

The mediated signal of cell surface receptors may couple with inhibitory G-proteins (G_i) such as adenosine, bradykinin and opioid receptors. These proteins are connected to the phospholipase C and D (PLC and D). These enzymes catalyse the process to produce inositol triphosphates (I3P) and diacylglycerol (DAG). The DAG is the key component of the protein kinase C (PKC) activation, which has a central role in the infarct size limitation (as shown in rabbits)[57]. Some authors proposed that the PKC activation and/or translocation might be an effect of oxygen radicals in PC[48]. There is another cellular pathway that acts through the PI3K/AKT molecules to limit the infarct size[58].

The mitogen activated protein kinase (MAP) play a crucial role in the signalling pathways of PC as a mediator. These molecules are rapidly activated after the PC stimulus and they regulate cellular function by phosphorilation of proteins[48]. The MAP kinase-activated protein kinase 2 (MAPKAP 2) was identified to play an important role in the transcription of those proteins, which protect the myocytes in the delay phase of PC[48].

1.3.3. Signal transduction of late preconditioning

After the PC stimuli the cellular signal transduction processes activate gene transcription factors, which induce protein synthesis for the development of late PC. The PC stimulus activates the Nuclear Transcription Factor-κB (NF-κB)[48]. The NF-κB in association with Signal Transducer and Activator of Transcription (STAT) molecules can induce the transcription of the several proteins, such as iNOS, COX-2, aldose reductase, MnSOD and heat shock proteins[59].

1.3.4. End effectors of preconditioning

The final mechanism of PC is still not very clear[48]. Numerous theories were established and mechanisms were identified as the end-effectors of PC. The K_{ATP} channels were suggested as an end-effector in the PC processes. Two types of K_{ATP} channels were identified which are located in the heart: in the sarcolemmal and in the inner mitochondrial membrane. The investigations with their inhibitors show that both channel opening is necessary in the cardioprotection[59, 60].

Opening of the sarcolemmal K_{ATP} channels shortens the AP duration[61] and prevent the Ca^{2+} overload in the myocyte by decreasing the open state probability of the inward Ca^{2+} channels[60]. The L-type Ca^{2+} channel blockers inhibit the PC effect in human myocardium[62]. It was hypostatised that either the mitochondrial Ca^{2+} handling or mitochondrial volume regulation help the preservation of mitochondria or the mitochondrial electron transport chain to preserve the ATP production during the injury by opening the mitochondrial K_{ATP} channels.[57] The mitochondrial protection increases the generation of ATP (protection of K_{ATP} channels), restores the dystrophin in the myocardium inert cell membrane. This restoration protects the sarcolemma and stabilizes the membrane against high permeability.[63]

Some other mechanisms were identified also as end-effectors in PC like the myocytes volume regulation by swelling activated chloride channels[64], actin filaments protection against cytosceletal disruption by activation of small heat shock protein 27 (HSP27)[65].

1.3.5. Possible mechanisms of early and delayed preconditioning against arrhythmias

Numerous participants were identified in the cardioprotection when infarct size or stunning was examined, however only a few articles deal with participants in the antiarrhythmic signal transduction process.

Numerous data were published about the adenosine A1 receptor involvement in early PC protection against infarct size, however the A1 receptor blocker was not effective against arrhythmias in dog models[66]. Perhaps other adenosine receptors may contribute to the antiarrhythmic process[67].

The stable analogue of prostacyclin (7-oxo-PGI_2) can mimic an antiarrhythmic cardioprotective effect in the heart and this protection can be blocked by cyclo-oxigenase inhibitors[68, 69]. Bradykinin B_2 receptor was identified to have a role in the antiarrhythmic effect. The application of selective B_2 antagonist (HOE 140, icatibant) showed that the receptor inhibition completely diminish the antiarrhythmic effect of PC stimulus (cardiac pacing), if the antagonist was given prior to the PC stimulation. However the inhibition just partially abolished the arrhythmias if it was given immediately after the pacing stimulus[70]. This suggests that the bradykinin has a trigger role in this process. In contrast, in rats, it seems that the bradykinin acts in the later phase of the protection against arrhythmias rather than as a trigger[71]. The role of opioid receptors has also been proven in PC protection against ischemia and reperfusion-induced injury, however their involvement in the antiarrhythmic protection seems controversial. The κ receptor has been found to have an antiarrhythmic effect in rats[72], whereas in swine this receptor activation is proarrhythmic[73]. There is possible species specificity in the mechanisms of antiarrhythmic effect of PC[74].

The NO, as a non-receptorial trigger of preconditioning also contributes to the antiarrhythmic effect of PC. Végh et al. (1992) showed, for the first time, that the L-arginine nitric oxide pathway has an involvement in the short periods of coronary artery occlusion induced antiarrhythmic effect in open chest dogs[54].

The application of PKC blockers (satusporine and calphostine C) in rats, modify neither reperfusion arrhythmias nor the antiarrhythmic effect of PC[74]. It seems that the role of PKC in cardioprotection remains controversial in rats, dogs and pigs[57]. It seems that the PI3K/AKT pathways are not involved in the antiarrhythmic effect of ischemic preconditioning in isolated rat heart model[58].

In a dog model the treatment of mitochondrial K_{ATP} channel closer, (5-hydroxydecanoate (5-HD)) inhibits the antiarrhythmic effect of ischemic PC. The K_{ATP} channel opener (diazoxide) inhibits the antiarrhythmic process if it was administrated before and after the PC stimulus, but it just weakens the effect if it was administrated after the PC[75]. This suggests that this channel has a mediator role in the PC protection against arrythmias rather than as an end-effector.

Treatment of selective cyclooxigenase 2 (COX-2) inhibitor (celecoxibe) was ineffective in the antiarrhythmic effect of exercise induced late phase of PC in dogs. It seems that the COX-2 derived prostacycline has no role in the antiarrhythmic effect of late PC[76].

Numerous theories were proposed and molecules identified about the possible end-effectors of PC in the heart. However the role of these molecules in the antiarrhythmic process still remains uncertain.

1.3.6. Non receptorial triggers in cardioprotection

Oxidative phosphorylation and mitochondrial respiration are porogressively uncoupled during hypoxia or ischemia/reperfusion[77]. This uncoupling increases the production of free radicals: reactive oxygen and nitrogen species. The main sources of ROS in the heart are the endothelium, leukocytes and the myocardium itself. In cells, xanthine oxidase, NADPH oxidase and nitric oxide synthase (NOS) are responsible for the production of free radicals[37-39]. The myocardium has defence mechanisms against ROS such as superoxide dismutase, catalase, gluthation perixidase and endogenous antioxidants such as Vitamin E, ascorbin acid, cysteine, tioredoxin[79]. It seems that free radicals not only damage the myocardium cells but also has a role in the PC processes. The ROS and the NO were identified as non receptorial triggers in the protection against the ischemia/reperfusion injury in the PC process[79].

1.3.6.1. Role of ROS in the preconditioning process

Numerous studies showed that ROS have a protective role in the delayed cardioprotection. ROS have a secondary role in the delayed protection, they open mitochondrial K_{ATP} channels[85]. Moreover, some mediators (bradykinin, α-adrenoreceptor agonists, opioid peptides) also generate free radicals by opening these channels[86]. The ROS activate the redox-sensitive molecules such as endogenous antioxidant enzymes like manganese-superoxide dismutase (MnSOD)[87] or thioredoxin. For example the thioredoxin

regulates the reduced state of the proteins, decreases the oxidative stress in cells. This molecule is down-regulated after ischemia and reperfusion, but up-regulated after IPC stimuli. In the PC heart it seems, that the redox-signal induced protein synthesis can contribute to cell survival in the myocites[48].

However, the role of ROS regarding the early protection against arrhythmias still remains unclear.

1.3.6.2. Nitric oxide as a trigger in antiarrhythmic process

NO has a large vasodilatation effect with an extreme short half life. It is released from the endothelium by the effect of several endogenous molecules or physical stimuli. Three different isoforms of NO synthase (NOS) exist in the human body. Two out of three of these are present in the heart: endothelial NOS (eNOS) and inducible form of NOS (iNOS). NOS oxidize the guanidine group of L-arginine by consuming NADPH and molecular oxygen to produce NO.

In dog and in rat *in vivo* models ischemic induced protective antiarrhythmic effect was abolished by using NOS inhibitors in the early phase of PC[54, 88]. However this role of NO was not proved in the protection against infarct size, cell death and post-ischemic dysfunction[89].

Numerous articles suggested that NO has a role in the late phase of PC protection against myocardial infarction or stunning. Exogenous NO is also sufficient to provoke the protective effect against stunning and infarction in rabbits and rodents[90]. Their protective role against arrhythmias in late phase of protection still remains unclear.

Babai et al. (2004) showed that a single period of treadmill exercise in conscious dogs suppressed the life-threatening ventricular arrhythmias that happened 24h after the treadmill exercise, when a major coronary artery was occluded, and then re-opened[45]. This protection was abolished by the prior administration of aminoguanidine. NO involvement was suggested, since there was evidence of the upregulation of iNOS. The aminoguanidine is not a selective inhibitor of NOS enzymes. It powerfully inhibits a number of other enzymes such as histaminase, mitogene-activated proteine kinase, catalase, SOD and malondialdhyde[91 -94]. Moreover the iNOS upregulation by treadmill exercise does not necessary mean that NO is involved. Furthermore Bolli et al. have suggested the function of NO in the delayed PC: 'NO mediated NO release'[95]. However there wasn't any evidence whether the NO has a trigger and or mediator role in the exercise induced late PC protection against life-threatening arrhythmias[89].

2. Purpose of the research

There were two main objectives of this research.

1. **To examine the role of reactive oxygen species in the early antiarrhythmic effect of ischemic preconditioning**. In this case a highly diffusible and low molecular weight ROS scavenger N-2-mercaptopropionylglycine (MPG)[85-87; 96-97] was used. In this experiment PC was induced by two 5 min coronary artery occlusion 20 min prior to the 25 min occlusion of the LAD.

   In this topic the following questions were further investigated:

   - The demonstration of ROS formation in the ischemia induced preconditioning process, the initial and terminal time of the prolonged 25 min occlusion (test) period were planned. The lucigenin-induced chemiluminescence method was used to investigate the ROS formation in vivo.
   - In non PC dogs the arrhythmic effect of ROS was examined during myocardial ischemia. The possibilities to reduce the arrhythmias with an exogenous ROS scavenger were researched.
   - The MPG scavenging activity on phorbol 12-myristate 13-acetate (PMA) induced ROS generation was examined using the ferro-cytochrome-c reduction in vitro assay. This was implied by the fact that in vitro activation of canine peripheral mononuclear cells by PMA generates ROS formation.

2. **To obtain further evidence that nitric oxide is involved in the delayed phase of preconditioning induced by heavy physical exercise.** In these experiments the role of NO was examined in the delayed antiarrhythmic effect of exercise, using

   - a non-selective NOS inhibitor ω-nitro-L-arginine-methyl-ester (L-NAME) and
   - a relatively selective iNOS inhibitor S-(2-aminoethyl)-methyl-isothiourea (AEST).

   L-NAME was given prior to physical induction. AEST was administered immediately before the 25 minute test occlusion period.

3. Materials & Methods

3.1. Animals

Adult, mongrel dogs of either sex were used, with a body weight between 16 and 41 kg (mean 26.3 ± 1 kg) in the early and 18 and 35 kg (mean 26 ± 1) in the delayed PC experiments. The origin, maintenance, care, handling and treatments of these dogs complied with the requirements of Hungarian law (XXVIII, chapter IV, paragraph 31) and those of the European Commission regarding large experimental animals, which conforms to the *Guide for the Care and Use of Laboratory Animals* (86/609/ECC) published by the US National institutes of Health (NIH Publication no. 85-23; revised 1996).

3.2. Evaluation of the role of ROS in the early protective effects of preconditioning

3.2.1. Surgical preparation

Under light anaesthesia (Sigma, 20 mg kg^{-1} intravenous sodium pentobarbitone) the right femoral artery (for the measurement of systolic, diastolic blood pressure - SABP, DABP, and heart rate - HR) was catheterised. The right femoral vein was also catheterized through which a mixture of chloralose (60 mg kg^{-1}) and urethane (200 mg kg^{-1}) was administered to maintain anaesthesia. The left common carotid artery was prepared and a catheter was inserted into the left ventricle cavity to measure the left ventricle systolic (LVSP) and end-diastolic (LVEDP) pressures.

The dogs were ventilated with room air, and blood gases as well as pH were monitored at various time intervals, and kept within the normal range[96]. Body temperature was recorded from the rectum and was maintained at 37 ± 0.5 ^{o}C.

A thoracotomy was performed at the left fifth intercostal space and the pericardium was excised. The main anterior descending branch of the left coronary artery (LAD) was prepared for occlusion just proximal to the first main diagonal branch. A side branch of this artery was also catheterised for the local, intracoronary administration of saline or of N-2-mercaptopropionylglycine (MPG). The circumflex branch of the left coronary artery (LCX) was prepared and an electromagnetic flow probe (diameter 4 mm) was positioned on the

coronary artery. Myocardial blood flow (MBF) was measured in $ml \cdot min^{-1}$ using a blood flow-meter (Spectramed, Hugo Sacs Electronics, Germany).

A composite electrode[91, 96] was sutured on the surface of the potentially ischemic area. This was used to assess changes in the degree of inhomogeneity of electrical activation. This electrode gives a summarised recording of R-waves from 30 epicardial measuring points. In the normal, adequately perfused myocardium, all sites are activated almost simultaneously resulting in a single large spike. Under conditions of ischemia, fractionation of the summarised R-waves occurs, indicating that adjacent fibres are not simultaneously activated because of conduction inhomogeneity. This is expressed as the greatest delay in activation (in ms) within the ischemic area. This electrode also contains four unipolar leads by which epicardial ST-segment changes were measured. The parameters were recorded on a Graphtec Thermal Array Recorder (Hugo Sachs Electronics, Germany) and data were also analysed off-line by the Advanced CODAS Analysing System

3.2.2. Experimental protocol

The experimental protocol is shown in Figure 1. Four groups of dogs were used. Each animal was subjected to a 25 minute LAD occlusion followed by a rapid reperfusion. Control dogs (C; n = 11) and dogs in the MPG control group (C+MPG; n=9) were given either normal saline or MPG, respectively, by intracoronary infusion for 1h prior to the occlusion The dose of MPG was 0.15 mg $kg^{-1}min^{-1}$ (the total dose: 9 mg kg^{-1}), which roughly equates to an intravenous dose of 90 mg kg^{-1}[1; 99] that has been used in previous studies[83, 96, 97]. Twenty dogs were preconditioned as previously described[98], and 20 min later these dogs were subjected to a 25 minute LAD occlusion, followed by a rapid reperfusion. In one group of these PC dogs MPG (PC+MPG; n = 10)) was administered by the same route and dose as described above. The MPG infusion was started 10 min prior to the first PC occlusion and maintained throughout the whole preconditioning procedure (Figure 1).

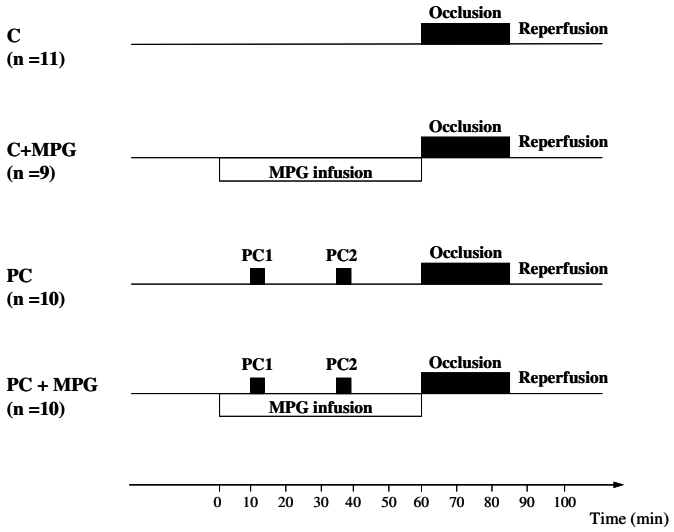

Figure 1. Experimental protocol to examine the role of ROS in the early effect of PC. Before occlusion every group was administered saline (0.5 ml min^{-1}) or MPG (0.15 mg kg^{-1} min^{-1}) by intracoronary infusion over 1 h. The animals then underwent a 25 min LAD occlusion followed by a rapid reperfusion. The groups were preconditioned by two 5 min LAD occlusion. In four preconditioned dogs blood samples (BS) were taken from the coronary sinus for the determination of free radical production at various times of the experiment; as shown in Figure 7.

In order to determine ROS formation during PCs and the effectiveness of MPG to scavenge ROS, four additional dogs (not shown in Figure 1.) were preconditioned in the absence and later in the presence of MPG as shown in Figure 7. Two hours after the second PC stimulus these dogs were treated with MPG. This two hour interval is sufficient enough for the previous preconditioning induced protective effect to disappear[96, 98]. Twenty minutes after the last PC stimulus these dogs were also subjected to a 25 minute occlusion. In this self-control experiment the actual ROS formation was measured at different times (see Figure 7). The ROS were determined from blood samples (BS1-BS9; see details in chapter 3.2.4).

3.2.3. In vitro studies for the determination of superoxide anion generation by canine leucocytes

Superoxide anion generation was assayed by measuring the superoxide-dependent reduction of ferro-cytochrome-c, as described by Guarnieri and colleagues[102]. 2 mLs of blood were collected from the control dogs in tubes containing 100 mM EDTA and 100 mM dextran. PMNs were isolated from blood by Ficoll-Hypaque gradient, then centrifuged at 200 g for 20 min at room temperature. After the centrifugation it was re-suspended in 40 μM ferricytochrome-c containing Dulbecco phosphate-buffered saline (DPBS) solution (1.5×10^6

cells/sample/cuvette). The cells were allowed to equilibrate for 2 minutes at 37 ^{0}C, and then stimulated with 0.3 μM phorbol myristate acetate (PMA). Superoxide radical production was recorded graphically as the increase in absorbance at 550 nm. The effect of increasing doses of MPG (1, 5, 10, 20 mM) was expressed as the percent change in the linear rate of ferricytochrom-c reduction, using a molar absorption coefficient of 19.1×10^{3} M^{-1} cm^{-1}.

3.2.4. Determination of ROS production during preconditioning absence and presence of MPG

In the four dogs which were subjected to preconditioning in the absence and then, 2 hours later, in the presence of MPG, the amount of superoxide was detected from their blood at different times (see Figure 7). The free radical production was examined with the lucigenin-enhanced chemiluminescence (CL) assay[101]. At each time point two ml blood samples were collected from the coronary sinus in EDTA containing micro-tubes. The samples were centrifuged for 5 min, at 200 g at room temperature. Plasma aliquots (100 μl) were added to 250 μM lucigenin in DPBS solution (final volume 1 ml). After a 2 minute equilibration period in darkness, measurements were commenced. CL was measured with a liquid scintillation counter (Packard Tri-Carb 2100 Model) with a single photomultiplier tube positioned in out-of-coincidence mode. CL was detected in the presence of nitro-blue-tetrazolium (NBT; 200 μM). NBT-inhibited CL was assessed as an index of superoxide generation. Results were expressed in counts $min^{-1}mg^{-1}$ protein in 100 μL plasma.

3.3. Examination of the role of NO in delayed preconditioning induced by physical exercise

3.3.1. Surgical preparation

Under light pentobarbitone anaesthesia (sodium pentobarbitone, Sigma, 30 mg kg^{-1} i.v.), heparinised, saline-filled polyethylene catheters were inserted into the left external jugular vein for drug administration, and, in some dogs (group 2; see below) into the left carotid artery for the measurement of arterial blood pressure. Each day the catheters in these dogs were flushed with heparinised saline. The dogs were then allowed to adapt to laboratory conditions for a week. During this period the dogs were transported to the laboratory and were either made to stand on the treadmill or made to exercise.

24 hours later the dogs were given intramuscular ketamine (50 mg kg^{-1}, i.m; Richter) and then anaesthetised with a mixture of α-chloralose and urethane (80 and 200 mg kg^{-1} i.v., respectively; Sigma) and were prepared for the test occlusion. The catheters insertion, thoracotomy and the heart preparation for the experiments were similar than previously described in Chapter 3.2.1.

3.3.2. Exercise protocol

The exercise protocol is illustrated in Figure 2. The dogs underwent a surgical procedure as described in Chapter 3.2.1. After one week of adaptation the dogs (n=34) were subjected to a total exercise period of 21 minutes. The slope and the speed of the treadmill were increased every 3 min reaching the maximum during the final 3 min period[45] (see: Figure 2.). Heart rate (HR) was measured during the exercise by a chest lead electrocardiogram. Data were collected by a computer assisted system and analyzed with the Advanced CODAS Analyzing System using the Windaq Waveform Browser playback and analysing software (DATAQ Instruments, USA). 24 hours later these dogs were anaesthetised as described above and underwent a test occlusion (see: Chapter 3.2.1 and 3.3.1.).

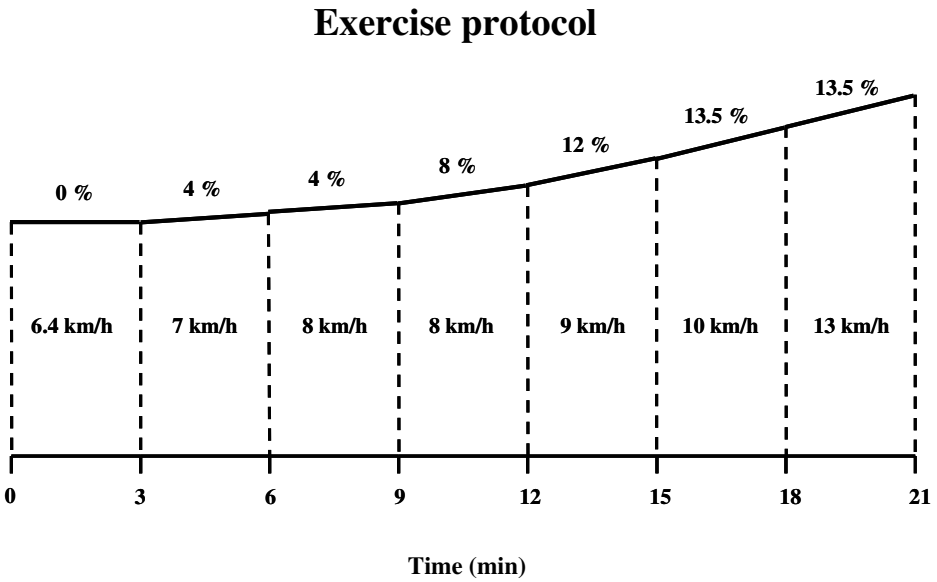

Figure 2. Exercise protocol. The dogs were run for 21 min on treadmill. The slope and the speed was increased every 3 min starting from 6.4 km h^{-1} and a 0% grade during the first 3 min and finishing with a speed of 13 km h^{-1} and a 13.5% slope during the last 3 min period[45].

3.3.3. Experimental protocol

The experimental protocol is illustrated in Figure 3. A total of 65 dogs were used in the study. The dogs were accustomed to the laboratory conditions for 1week by standing on the treadmill. After this adaptation, these dogs were anaesthetised, as described above, and subjected to a 25 minute occlusion of the LAD followed by a rapid re-opening of the occluded artery. Thirteen dogs served as controls; i.e. after surgery and the adaptation period these dogs were subjected to a 25 min occlusion and reperfusion insult (Figure 3). Nine out of the total 65 dogs (group 2; Figure 3) the specific, but non-selective, NOS inhibitor N^{ω}-nitro-L-arginine-methyl-ester (L-NAME, Sigma) was given intravenously in a dose of 10 mg kg^{-1}, 24 h prior to the coronary artery occlusion. In a previous study this dose of L-NAME has been shown to abolish the antiarrhythmic effects of ischemic preconditioning[54]. In another group of nine control dogs (group 3; Figure 3), S-(2-aminoethyl)-isothiourea (AEST, Tocris), a selective inhibitor of the inducible NOS enzyme (iNOS), was administered in intravenous infusion over a 70 minute period (total dose of 2 mg kg^{-1}). The infusion was stopped 20 minutes prior to the coronary artery occlusion. This dose of AEST was similar to that used to inhibit the delayed antiarrhythmic effects of cardiac pacing[103].

Thirty four dogs were subjected to the exercise protocol 24h prior to the coronary artery occlusion. Eleven of these dogs (group 5; Figure 3) were treated with L-NAME (10 mg kg^{-1}) 5 minutes prior to the exercise. Of the remaining exercised dogs ten were given AEST intravenously (group 6; Figure 3) in a dose and time outlined above. The results were compared to those obtained from a group of thirteen exercised dogs given saline intravenously 20 minutes prior to the occlusion (group 4; Figure 3).

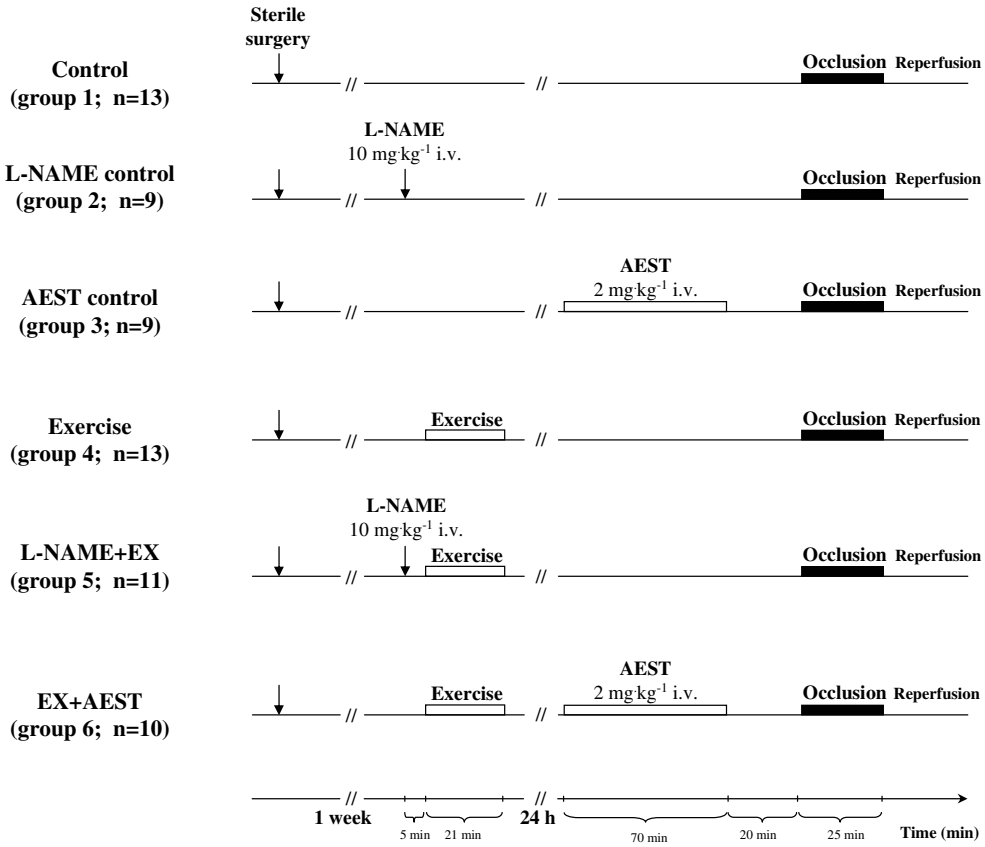

Figure 3. Experimental protocol for the investigation of the role of NO in the delayed antiarrhythmic protection induced by treadmill exercise, using inhibitors of NOS. The number of dogs in each of six groups is given in parenthesis.

3.4. Investigated parameters in both experiment

In the right femoral artery the systolic (SABP), and the diastolic blood pressure (DABP) and also the heart rate (HR) were measured. The mean arterial blood pressure (MABP) was calculated from the SABP and DABP. Left ventricular systolic (LVSP) and end diastolic pressure (LVEDP) were measured from a catheter inserted into the left common carotid artery. The maximum and the minimum of the first derivate of LVP over time (LVdP/dt) were calculated from these parameters. The coronary blood flow was measured in the left circumflex coronary artery using an electromagnetic flow probe (Spectramed, Hugo Sachs Electronics, Germany) to monitor the compensatory coronary blood flow, when a myocardium oxygen consumption increased or when the inhibitors were used. The principle of the measuring is the electro-magnetic force induction in the signal electrode circuit when the flow-meter probe magnet is energized by an alternating current deriving from Faraday's law of electromagnetic induction[104].

Ventricular arrhythmias during a 25 min (LAD) coronary artery occlusion and following reperfusion were assessed as outlined in the 'Lambeth Conventions'[105] and modified as previously described[91, 96]. During occlusion we assessed the total number of ventricular ectopic (premature) beats (VPB's) and the incidence and number of episodes of ventricular tachycardia (VT; defined as a run of four or more VPB's at a rate faster than the resting sinus rate). The incidence of ventricular fibrillation (VF) during both occlusion and reperfusion were also determined. Those dogs were considers survivors whose heart beat was in sinus rhythm for 10 minutes after the combined ischemia-reperfusion insult.

A composite electrode was used to assess changes in the degree of inhomogeneity of electrical activation and recorded epicardial electrocardiograms (for changes ST segment).

Risk area was assessed at the end of each experiment by injecting patent blue V dye into the LAD at a pressure not greater than the systolic arterial pressure in that animal. It was defined as the percentage area of the left ventricular wall together with the septum served by the occluded artery[91, 118].

3.5. Statistical evaluation

All data were expressed as means ± s.e.m. and the differences between groups were compared by Student's t test analysis or ANOVA. A two-way, repeated measured ANOVA was undertaken to determine whether or not there were significant differences of the ST-segment elevation and a degree of inhomogeneity between the groups. The problem of multiplicity was solved by Bonferroni correction. Ventricular premature beats were compared using the Mann-Whitney Rank Sum test and the incidences of VT and VF between the groups using the Fisher Exact test. Differences between the groups were considered significant when $P < 0.05$.

4. Results

4.1. Evaluation of the role of ROS in the early protective effect of preconditioning

4.1.1. Haemodynamic effects of MPG and coronary artery occlusion

These are illustrated in Table 1. Intracoronary infusion of MPG resulted in significant reductions in arterial blood pressure and LVdP/dt, but these values returned to normal by the

time of the onset of the coronary artery occlusion. The infusion of saline for the same period of time had no significant haemodynamic effects.

The coronary occlusion induced a marked fall in arterial blood pressure (SABP and DABP) and increased the LVEDP, without substantially modifying the HR. These haemodynamic changes were similar in the control (C) and in the MPG treated control (C + MPG) dogs. In contrast, the occlusion-induced haemodynamic alterations were less pronounced in dogs subjected to preconditioning (Table 1). Administration of MPG in PC dogs reversed most of these PC-induced haemodynamic changes (Table 1).

When the LAD coronary artery was occluded there was an immediate and sustained increase in blood flow in the adjacent (LCX) coronary artery. This compensatory coronary blood flow increase was not affected by the administration of MPG (Table 2).

4.1.2. Ventricular arrhythmias during occlusion

The distribution of ventricular premature beats over a 25 minute occlusion period in the four groups is illustrated in Figure 4. In control dogs following the occlusion the ectopic activity appeared in two well differentiated phases (1a and 1b). Compared to the controls, in dogs subjected to preconditioning there were only a few VPBs over the entire 25 minute occlusion (377 ± 78 vs 86 ± 34; $P < 0.05$) and this arrhythmia suppressing effect of PC was not significantly modified by MPG (VPBs: 111 ± 39). MPG itself, administered in control animals suppressed the number of the ectopic beats only during phase Ia, but the total number of VPBs was not significantly different from the controls (377 ± 78 vs. 244 ± 56; $p = 0.165$). Similarly, the incidence and the number of episodes of VT were less in the two preconditioned groups, regardless whether MPG was present or not. Thus, VT occurred in 60% of PC dogs and 25% of the PC+MPG treated dogs (2.0±0.7 and 1.2±0.9 episodes of VT) compared to 91% and 89% in the C and C + MPG groups (13.6 ± 4.5 and 8.6 ± 2.5 VT episodes respectively; all $P < 0.05$). MPG itself reduced VPBs during phase Ia, but not in Ib phase arrhythmias. However the total numbers of arrhythmias over the entire occlusion period was not significantly different from the controls ($p = 0.165$).

Table 1. *Haemodynamic changes following saline or MPG infusion and during coronary artery occlusion*

	Baseline	Max change during saline or MPG infusion	Pre occlusion value	Max change during occlusion
Control				
SABP	115 ± 4	-5 ± 0	114 ± 5	-16 ± 4”
DABP	80 ± 4	-3 ± 3	77 ± 3	-10 ± 1”
MABP	92 ± 4	-3 ± 2	89 ± 4	-12 ± 2”
LVEDP	3.5 ± 0.5	0 ± 0	3.8 ± 0.4	9.8 ± 1.1”
+dP/dt	1619 ± 180	-44 ± 44	1610 ± 183	-344 ± 8.3”
-dP/dt	1907 ± 311	169 ± 7	1968 ± 302	-375 ± 5.5”
HR	160 ± 5	2 ± 0	160 ± 5	3 ± 1
C + MPG				
SABP	133 ± 4	-17 ± 3*	129 ± 6	-15 ± 4”
DABP	86 ± 5	-18 ± 3*	81 ± 6	-11 ± 2”
MABP	102 ± 4	-17 ± 3*	97 ± 5	-12 ± 3”
LVEDP	2.5 ± 0.5	0 ± 0.8	3.5 ± 0.7	5.7 ± 1.1” #
+dP/dt	1747 ± 115	-176 ± 68*	1754 ± 134	-261 ± 135
-dP/dt	1743 ± 153	-289 ± 70*	1580 ± 152	-279 ± 47”
HR	142 ± 5	2 ± 3	144 ± 7	1 ± 1
PC				
SABP	121 ± 2	0 ± 2	123 ± 3	-5 ± 1” #
DABP	77 ± 42	1 ± 2	77 ± 5	-9 ± 1
MABP	92 ± 42	2 ± 1	94 ± 4	-8 ± 1”
LVEDP	3.9 ± 0.5	0.3 ± 0.2	4.1 ± 0.6	5.5 ± 1” #
+dP/dt	1406 ± 115	49 ± 36	1646 ± 144	-294 ± 51”
-dP/dt	1747 ± 148	30 ± 30	1757 ± 186	-150 ± 134
HR	142 ± 6	0 ± 0	138 ± 5	1 ± 1
PC+MPG				
SABP	130 ± 5	-17 ± 2*	124 ± 4	-12 ± 2”
DABP	92 ± 6	-16 ± 2*	85 ± 5	-13 ± 3”
MABP	104 ± 5	-16 ± 2*	98 ± 5	-12 ± 3”
LVEDP	4.7 ± 0.4	-0.2 ± 0.4	4.2 ± 0.5	5.3 ± 0.6” #
+dP/dt	1478 ± 84	-118 ± 28*	1465 ± 92	-306 ± 59”
-dP/dt	1640 ± 173	-151 ± 44	1685 ± 167	-400 ± 88”
HR	143 ± 5	1 ± 2	144 ± 6	6 ± 3

Values are mean ± s.e.m. Abbreviations are seen in the methods section. * P<0.05 cp. baseline; “ P<0.05 cp. pre occlusion value; # P<0.05 cp. control group

Table 2. *Changes in LCX artery blood flow (mean; ml·min^{-1}) during occlusions of the LAD coronary artery ('compensatory coronary vasodilatation')*

	Preconditioning				*Prolonged occlusion*	
	Occlusion [1]		*Occlusion* [2]			
	Pre	Post	Pre	Post	Pre	Post
Control (saline)					47.9 ± 5.2	60.2 ± 8.7
Control (MPG)					53.9 ± 2.9	68.7 ± 4.5*
Preconditioned (saline)	51.0 ± 6.5	59.6 ± 6.8*	54.4 ± 5.9	62.7 ± 5.8*	53.6 ± 5.9	64.5 ± 7.4*
Preconditioned (MPG)	54.4 ± 5.5	59.9 ± 6.5*	51.3 ± 5.0	59.9 ± 6.4*	52.6 ± 5.0	66.1 ± 7.2*

Values as mean ± s.e. mean of 10-11 observations. *: P<0.05 compared with baseline.

The incidence of VF during occlusion and reperfusion, as well as survival from the combined ischemia/reperfusion insult is illustrated in Figure 5. In control dogs there was an 82% incidence of VF during occlusion and those dogs that survived the ischemic period fibrillated on reperfusion, thus in this group no dog survived the ischemia/reperfusion insult. In contrast, no preconditioned dog fibrillated during the occlusion period, whether or not they were given MPG, but there was a high incidence of fibrillation on reperfusion. Survival from the combined ischaemia/reperfusion insult was 40% in PC dogs and 29 % in the PC + MPG group.

4.1.3. Severity of ischemia during PC and following coronary artery occlusion

The severity of ischemia was assessed by measuring changes in epicardial ST-segment (Figure 6A) and in the degree of inhomogeneity of electrical activation (Figure 6B). In control dogs following coronary artery occlusion the ST-segment rapidly elevated during the initial 5 minute of the occlusion, and this was maintained throughout the whole ischaemic period (Figure 6/A). MPG given to non-preconditioned dogs did not modify these ST-segment changes. Preconditioning significantly reduced this index of ischemia severity, which effect was not substantially modified by MPG during the early period of the ischemia. However, in the later course of the occlusion (from 8-10 minutes on) the change of the epicardial ST-segment in PC dogs treated with MPG, were similar to the control group. Changes in the electrical activation are shown in Figure 6/B. In control dogs the degree of inhomogeneity within the ischemic myocardium was markedly increased following LAD occlusion, but this

increase was significantly less pronounced in the PC animals. Again, MPG in PC dogs increased this index of ischemia severity only during the later period of the occlusion.

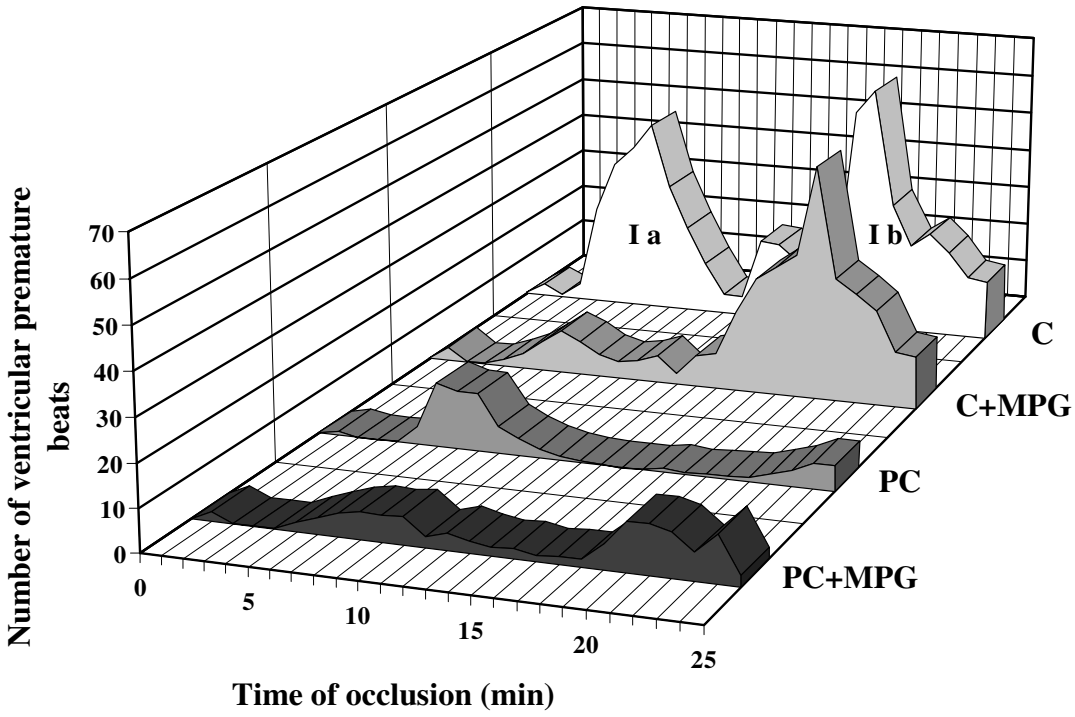

Figure 4. The distribution of VPBs during a 25 min occlusion of the LAD. In the control group there was a great number of VPBs which appeared in two distinct phases (phase Ia and Ib). The PC procedure significantly reduced the number of ectopic beats, and this effect was not substantially modified by the administration of MPG. The ROS scavenger MPG in control dogs attenuated only the phase Ia arrhythmias.

4.1.4. Area at risk

There were no significant differences in the 'area at risk' between the four groups. These were 32.2 ± 1.3 % in the saline controls, 32.4 ± 1.1 % in the controls given MPG, 31.4 ± 1.1 % in the preconditioned group and 34.2 ± 1.4 % in the preconditioned dogs also given MPG.

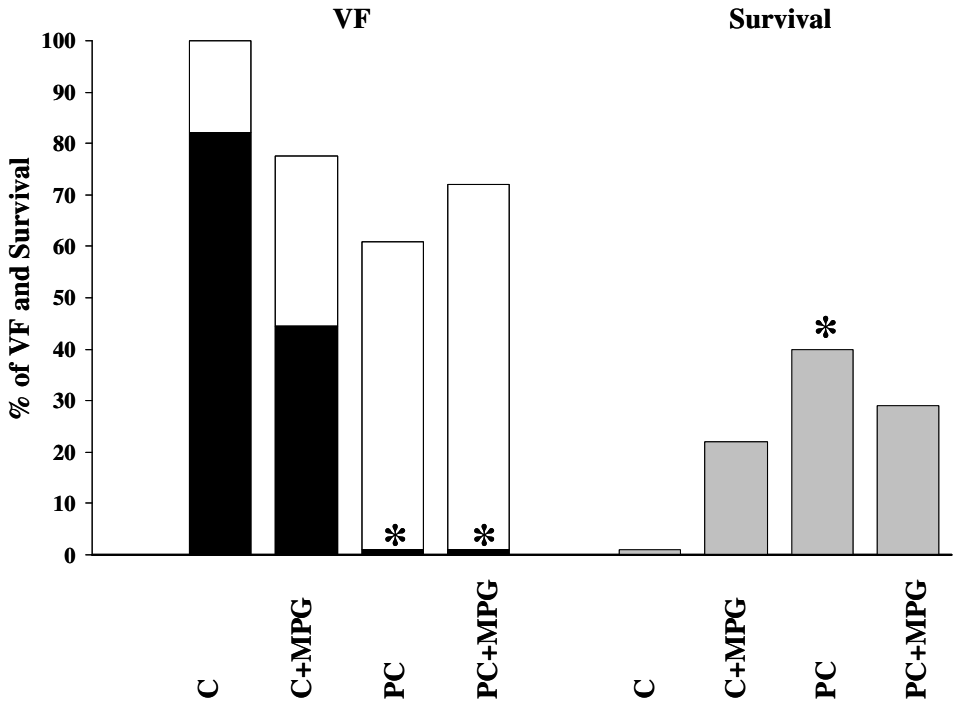

Figure 5. The incidence of ventricular fibrillation and survival from the combined occlusion/reperfusion insult. In the control group there was a high incidence of VF (87 %) during the occlusion period, and no dog survived the reperfusion. In contrast, in the PC dogs no VF occurred during the occlusion and 40% of the animals survived reperfusion. The administration of MPG did not modify the severity of arrhythmias either in the control or the PC groups *:$p<0.05$ vs. control

4.1.5. Effect of MPG on free radical production by canine leucocytes

MPG concentrations smaller than 1 mM did not affect the ferricytochrome-c reduction. The higher concentrations of MPG (5, 10 and 20 mM) significantly decreased this reaction by 13%, 54% and 96 % respectively.

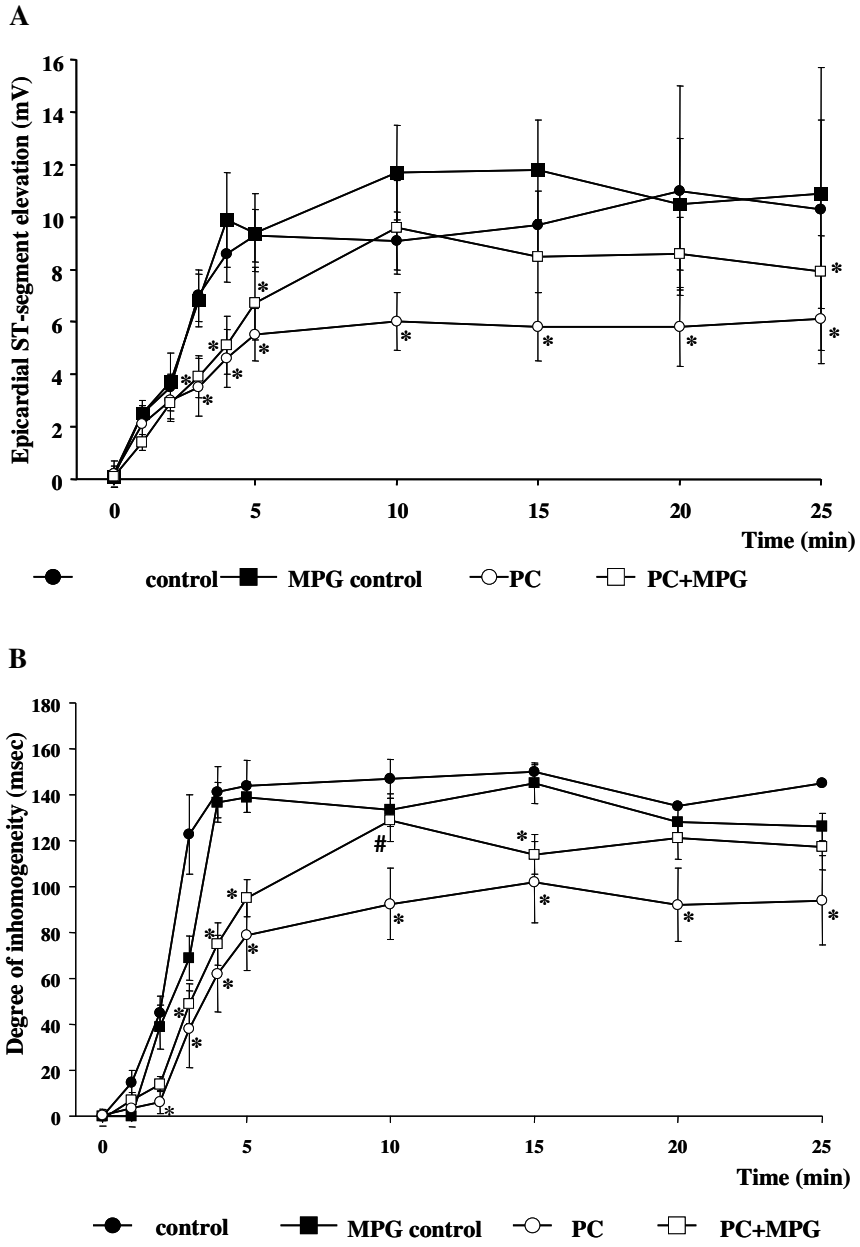

Figure 6. Changes in ischemia severity during a 25 min occlusion of the LAD. Compared to controls, PC significantly reduced both the elevation of epicardial ST-segment (A) and the increase of the degree of inhomogeneity (B). These indices of ischemia severity in the control dogs were not modified by MPG, whereas in the PC dogs MPG reversed the effect of PC during the later course of the occlusion. Values are means ± s.e.m. *:$p<0.05$ vs. control.

3.1.7 Effect of MPG on ROS production before, during and after the PC procedure

In four dogs subjected to preconditioning in the presence and in the absence of MPG, free radical formation was detected by the lucigenin-enhanced chemiluminescence assay. The results are shown in Figure 7. There was no detectable free radical formation following the first preconditioning occlusion. However, 20 minutes later, when the second PC occlusion

was released there was a significant, around three-fold, increase in ROS formation. Two hours later, when the preconditioning procedure was repeated, in the presence of MPG, no detectable ROS production could be observed either after preconditioning or following the prolonged occlusion.

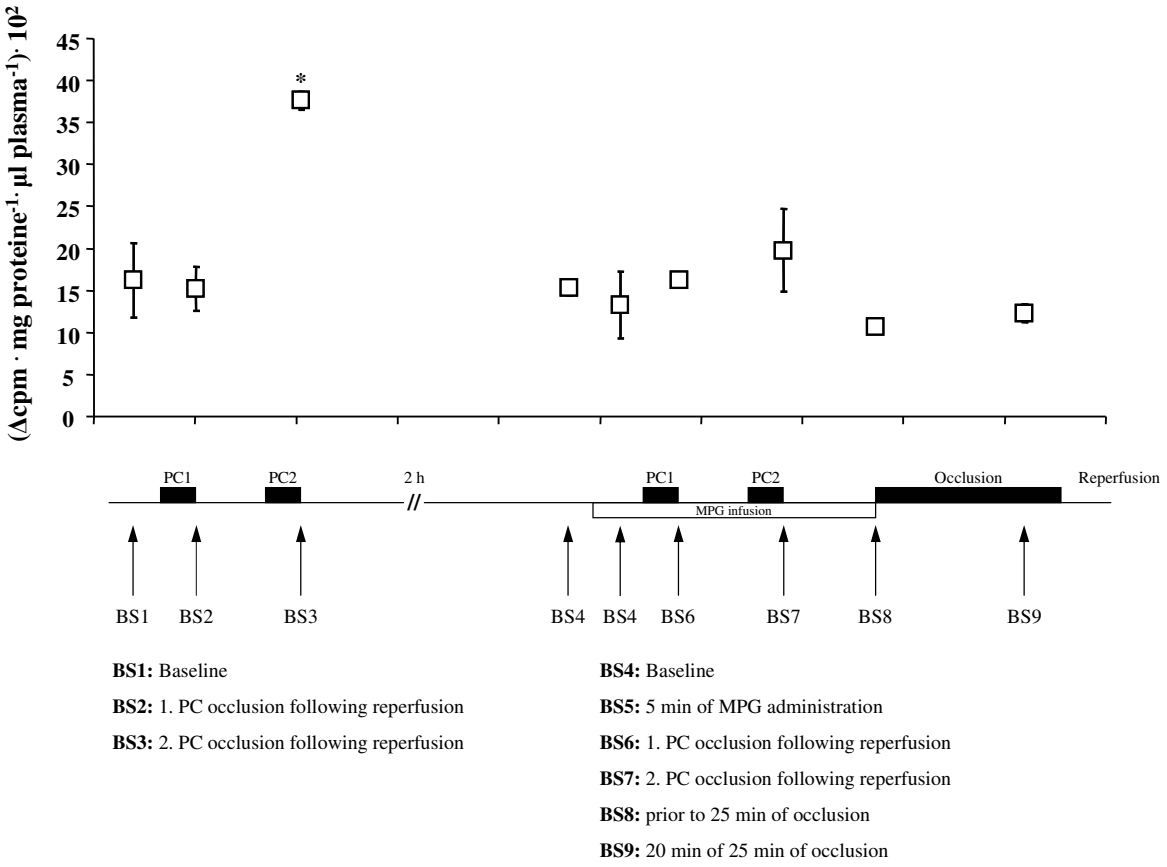

Figure 7 Free radical formation in PC dogs in the presence and in the absence of MPG. The blood samples (BS1-9) were collected at various times from the coronary sinus of the dogs. The burst of chemiluminescence activity were observed only after the second 5 min PC stimulus. This elevation was diminished by the applied MPG. *:p<0.05 vs. baseline (BS1).

4.2. Examination of the role of NO in delayed cardioprotection induced by physical exercise

4.2.1. Heart rate changes during exercise

Exercising dogs on the treadmill for 21 min resulted in sustained increase in heart rate similar to those obtained using the same protocol in the previous study[45] (e.g. from 109±9 to 212±20 beats min^{-1} at the end of the exercise period; P <0.01 of dogs in Exercise group 4). The resting heart rate, before the exercise in those dogs that had been given L-NAME (group

5) was significantly lower (88±4 beats min^{-1}; P <0.01) than the normal HR (119±7 beats min^{-1}). This was presumably because of the marked elevation in arterial blood pressure that resulted from L-NAME administration (increase from 153±9 mmHg (systolic) and 101±3 mmHg (diastolic) to 163±5 mmHg and 122±4 mmHg respectively; P <0.05) evoked a reflex mediated reduction in HR. Despite this the increase in heart rate during exercise was the same in all exercised groups.

4.2.2. Haemodynamic effects of NOS inhibition and of coronary artery occlusion

Administration of L-NAME resulted in the increase in arterial blood pressure (DABP: from 101±3 to 122±4 mmHg, MABP: from 118±4 to 135±4 mmHg; P < 0.05) and a significant decrease in HR (from 107±8 to 78±6 beats min^{-1}; P<0.05) Twenty-four hours later, these acute haemodynamic changes of L-NAME have already disappeared, only the decreased HR remained.

In dogs, exercised 24h previously (but not in non-exercised dogs) the infusion of AEST prior to coronary artery occlusion increased the arterial blood pressure from 119 ± 5 mmHg (systolic) and 80 ± 6 mmHg (diastolic) to 127 ± 7 and 87 ± 4 mmHg respectively. There were no significant changes in any other haemodynamic parameters. This change in arterial blood pressure (+7 ± 3 mmHg) was significantly greater than in non-exercised dogs that were given AEST (-5 ± 5 mmHg; P < 0.05).

4.2.3. Haemodynamic effect of coronary artery occlusion

These are summarised in Table 3. The haemodynamic parameters measured at rest were similar in each of the six groups. The mean arterial blood pressure in the six groups was between 93 ± 3 and 105 ± 11 mmHg, the heart rate was between 145 ± 7 and 154 ± 5 beats min^{-1}, and the LVEDP was between 3.2 ± 0.5 and 4.3 ± 0.4 mmHg. The only significant difference was that the heart rate in those (groups 2 and 5) dogs that had been given L-NAME on the previous day was still lower that in the other groups (118 ± 5 beats min^{-1} in the group 2 dogs and 122 ± 5 beats min^{-1} in the group 5 dogs; P < 0.05 compared to the dogs not given L-NAME).

The haemodynamic changes following coronary artery occlusion were similar to those described in detail previously[91, 98]. There were slight, but significant (P < 0.05) decreases in arterial blood pressure (between 7 to 16 mmHg), and increases in heart rate (less than 10 beats

min^{-1}) in each group following occlusion. The only significant difference between the six groups in the haemodynamic response to coronary artery occlusion was in LVEDP. This was more pronounced in control dogs (group 1; increase from 4.6 ± 0.5 to 16.8 ± 2.1 mmHg at 10 min; $P < 0.05$) than in the exercised dogs (group 4; increase from 3.4 ± 0.7 to 5.3 ± 1.1 mmHg; ns but $P < 0.05$ cp to controls). The increases in LVEDP were again more pronounced in exercised dogs given either NOS inhibitor ((e.g. 3.6 ± 0.5 to 9.9 ± 0.6 mmHg in the L-NAME (group 5) dogs and from 3.2 ± 0.5 to 9.9 ± 1.1 mmHg in the (group 6) dogs that had been given AEST (both $P < 0.05$); see Table 3).

Table 3. *Haemodynamic effect of coronary artery occlusion in control dogs subjected to exercise 24 h previously; effect of L-NAME and AEST.*

	Mean arterial blood pressure (mmHg)		Heart rate (beats · min^{-1})		LVEDP (mmHg)		LV dP/dt_{max} (mmHg · s^{-1})	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Group 1 (n = 13)	97 ± 7	90 ± 3*	151 ±5	154 ± 6	4.6 ± 1	16.8 ± 2.1*	3521 ± 187	3001 ± 213*
Group 2 (n = 9)	94 ± 3	86 ± 3*	118 ± 3^{+}	120 ±5^{+}	3.9 ± 1	15.3 ± 1.4*	2529 ± 157	3110 ± 188*
Group 3 (n = 9)	105 ± 11	90 ± 5*	154 ± 5	158 ± 3	4.3 ± 1	14.6 ± 1.3*	3398 ± 239	2829 ± 162*
Group 4 (n = 13)	105 ± 2	97 ± 3*	152 ± 7	157 ± 5	3.4 ± 1	8.3 ± 1^{*+}	3338 ± 245	2921 ± 177*
Group 5 (n = 11)	93 ± 3	85 ± 3*	123 ± 5^{+}	133 ± 5^{+}	3.6 ± 1	12.5 ± 0.6*	3464 ± 226	3145 ± 98*
Group 6 (n = 10)	97 ± 4	90 ± 3*	145 ± 7	148 ±3	3.2 ± 1	13.4 ± 0.8*	3156 ± 249	2687 ± 162*

Values are means ± s.e.m before (pre) and after (post) coronary artery occlusion. *: $p < 0.05$ compared to pre occlusion value. $^{+}$: $p < 0.05$ compared to control group.

4.2.4. Ventricular arrhythmias during coronary artery occlusion

The results are shown in Figure 8. In control dogs occlusion of the LAD resulted in many ventricular premature beats (VPBs: 311.1 ± 81.9), a high incidence (85%) and number of VT episodes (15.5 ± 6.3 episodes per dog). Six dogs (46%) out of the 13 from the control group fibrillated during occlusion; most of the VF occurred during phase Ib (i.e. between 14 and 18 min of the ischemia), and no control dog survived the reperfusion. In contrast, in the exercised dogs, the number of VPBs (103.1 ± 30.2), the incidence (54%) and number of episodes of VT (2.2 ± 1.0) were significantly reduced compared with control group. Only one of these dogs exhibited VF (8%) during the occlusion (at the 14 min of the ischemic period) and 5 dogs out of 12 fibrillated during reperfusion. Thus 54% of these dogs survived reperfusion ($p < 0.05$ compared to controls). This protection was abolished by both L-NAME

and AEST (Figure 8). Only three of the 21 exercised dogs given a NOS inhibitor survived reperfusion ($P < 0.05$) compared to exercised dogs not given the inhibitor. Neither L-NAME nor AEST significantly influenced occlusion or reperfusion-induced arrhythmias in non-exercised dogs (data are not shown).

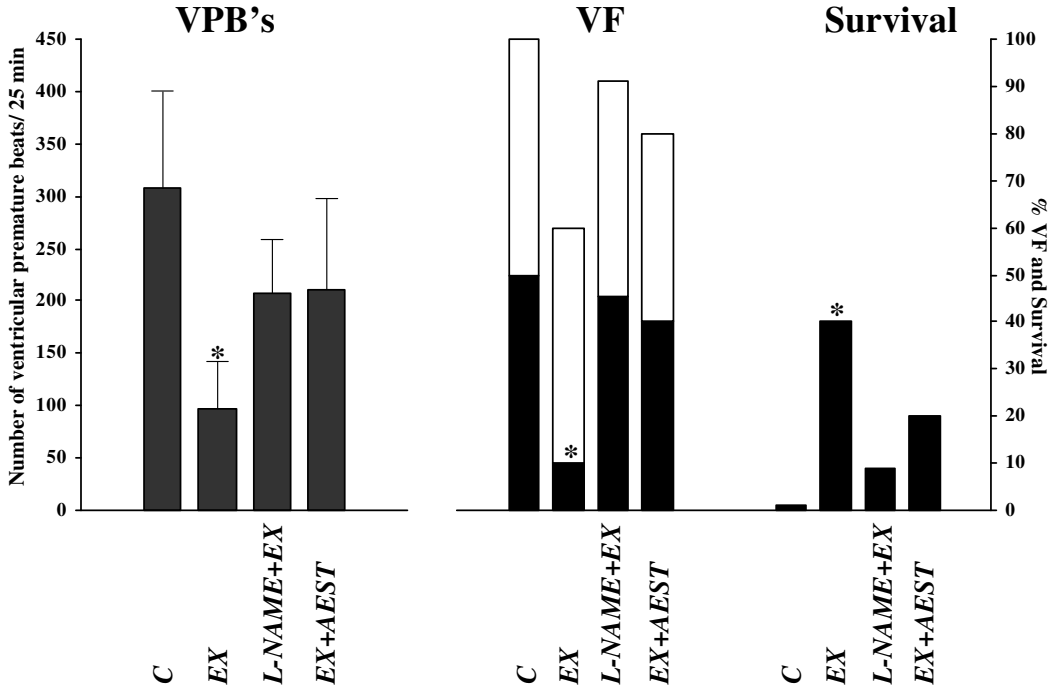

Figure 8. The severity of arrhythmias (VPBs, VF and survival) following a 25 min occlusion and reperfusion insult. Exercise, 24 h prior to coronary artery occlusion and reperfusion significantly reduced the severity of arrhythmia. This effect was largely reversed by NOS inhibitors administered either prior to the exercise stimulus (L-NAME) or, on the following day, before coronary artery occlusion (AEST). *:$p < 0.05$ vs. control.

4.2.5. Changes in the degree of inhomogeneity of electrical activation during LAD occlusion

These are shown in Figure 9. The degree of inhomogeneity of electrical activation was significantly increased during occlusion. This effect was less pronounced in dogs subjected to exercise, 24h previously. The two NOS inhibitors reversed the effect of the exercise; in these dogs the degree of inhomogeneity of electrical activation was similar to that in the control group.

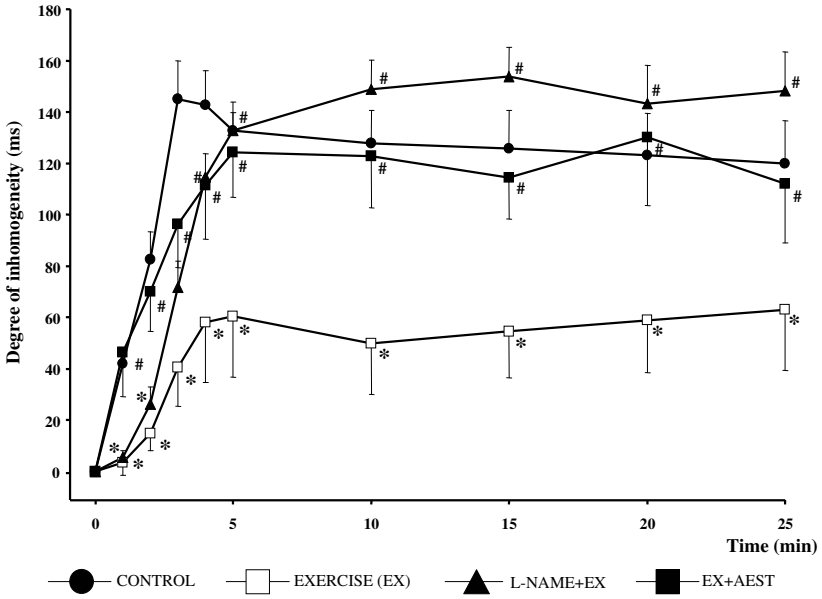

Figure 9. Changes in the degree of inhomogeneity of electrical activation within the ischemic area during a 25 min occlusion of the LAD. In dogs subjected to exercise 24 h previously the inhomogeneity of electrical activation was significantly reduced, but it increased again following inhibition of NO formation. *:$p < 0.05$ vs. control group; #: $p < 0.05$ vs. exercise alone.

4.2.6. Area at risk

There were no significant differences in the area at risk between any of the groups. These were 32 ± 1 % in the controls (group 1), 34 ± 2 % in the exercised dogs (group 4) and 33 ± 1 % and 35 ± 1 % in the exercised dogs given L-NAME and AEST (groups 5 and 6). Neither L-NAME nor AEST modified the area at risk in non-exercised dogs (groups 2 and 3; 31 ± 2 and 34 ± 5 % respectively).

5. Discussion

5.1. The role of ROS in the early antiarrhythmic effect of preconditioning

In canine[69, 107], as in other species[107, 108, 100], the severity of the ventricular arrhythmias is reduced when a prolonged period of occlusion is preceded by one or more brief (preconditioning) ischemia and reperfusion insults. This pronounced antiarrhythmic effect of ischemic preconditioning was clearly demonstrated in this study. None of the preconditioned dogs fibrillated during the occlusion compared with a high incidence (82%) in the control group. The number of VPBs and VT episodes were also much less in dogs that underwent a

PC procedure. Moreover the epicardial ST segment elevation and the degree of inhomogeneity of electrical activation were less pronounced in the PC dogs than in the non-PC controls.

It was demonstrated that the involvement of ROS is unlikely in this marked antiarrhythmic protection. This was proved by the use of MPG, a well known scavenger of ROS. MPG was applied in a dose (equivalent to a total intravenous dose of 90 mg kg−1) similar to that used in previous studies[83, 96, 97]. It was given directly into a side branch of the coronary artery that was later occluded. The results show that such a locally administered MPG failed to modify the antiarrhythmic effects of preconditioning. The incidences of VF and VT, and the distribution of ventricular ectopic beats were similar in the preconditioned dogs irrespective of whether or not the scavenger had been given (Figures 2 and 3). Moreover, MPG given to control dogs (C + MPG) reduced the phase Ia arrhythmias (Figures 2 and 3), a phenomenon that deserves further investigation. Nevertheless, the total number of VPBs in this group was not significantly different from the saline administered controls. Furthermore, the reduction of the two measured parameters of ischemia severity by preconditioning was not significantly modified by MPG, at least, during the first 5 to 8 minutes of ischemia. It can be concluded that in this species under *in vivo* conditions ROS, and in particular superoxide, are not necessary for inducing early protection against life-threatening arrhythmias. The protection is present even when a highly diffusible and effective scavenger had been administered. In contrast, this protection was abolished by blocking the bradykinin B2 receptors[109] or by inhibition of nitric oxide synthase[107] or cyclo-oxygenase[69].

In this study the superoxide production during the PC procedure was also determined both in the absence and in the presence of MPG. The results clearly show that after the first PC occlusion there was no detectable superoxide formation. This could mean two things: either a single 5 minute occlusion is not sufficient to produce superoxide, or the superoxide production induced by the short PC occlusion was effectively scavenged by superoxide dismutase. There is only one free radical that is certainly released during the early phase of ischaemia is nitric oxide (NO). This is not scavenged by MPG, although NO in the presence of superoxide quickly forms peroxynitrite which can be scavenged by MPG. NO triggers the protection, possibly by involving the opening of the mitocondrial K_{ATP} channels and this would generate further free radical[110]. More recently, Juhász and colleagues[111] showed that

scavenging the peroxynitrite by uric acid did not modify the early effect of IPC. However, the exogenously administered peroxynitrite induced similar antiarrhythmic protection, like PC[111].

In an *in vitro* experiment the scavenging activity of MPG was demonstrated in PMCs using a ferricytochrome-c reduction assay. It was proven that the MPG can decrease the ROS formation by a concentration dependent manner. The applied *in vitro* doses of MPG are comparable with an *in vivo* dose of MPG.

The role of ROS has been thoroughly investigated in the cardioprotective effects of preconditioning. In this respect the most relevant studies are those of Richard et al.[100], Tanaka et al.[96] and Baines et al.[55]. Both Tanaka[88, 96] and Baines[55] have found, that in rabbits the administration of MPG, in doses lower than those used in the present study, prior to and during a single PC (5 min) occlusion, completely abolished the infarct size reducing effect of PC. However, Baines and colleagues[55] found that MPG failed to modify the preconditioning effect of four 5 minute occlusions. They proposed that other mediators (adenosine, bradykinin), that are released when multiple occlusions are used, may act together with ROS, thus inhibition of ROS formation only attenuates but not completely abolishes the protection. More ROS were generated by reperfusion after many occlusions compared to just one. Similarly, Richard and colleagues[100], using three preconditioning occlusions in rats, failed to demonstrate any effect of intravenously given MPG (20 mg kg^{-1}). Furthermore, these authors also assessed arrhythmias during and following a 20 minute occlusion period following PC with three 5 minute occlusions, and found that MPG did not modify this protection. However, they did not distinguish between those arrhythmias that developed during the ischemic period and those that resulted from reperfusion. The results in this thesis are in agreement with those of Richard et al[100]; i.e. the administration of MPG failed to modify the protective antiarrhythmic effect of ischemic preconditioning induced by two 5 minute occlusions.

There was also no evidence that ROS are involved in arrhythmia severity when a coronary artery is occluded without preconditioning. The total number of arrhythmias was just as severe after MPG administration. This also accords with previous experience in this species[115]. However the phase Ia arrhythmias disappeared after the MPG infusion. This finding could be a task of a further research.

These results, of course, do not exclude the possibility that ROS are not involved in the early cardioprotection when this is induced by other stimuli or multiple ischemic/reperfusion insults. There is increasing evidence that the intensity of the preconditioning stimulus

determines the signalling pathways that leads to cardioprotection[116,117]. It should not be concluded from our studies that ROS generated during the preconditioning process (ischemia and reperfusion) do not contribute to the delayed effects of PC. However, it should be noted that most studies implicating free radicals as triggers for delayed protection against stunning and infarct size[76-83] have used multiple coronary artery occlusions.

There is one other effect of the coronary artery occlusion that was also not modified by MPG. When a coronary artery is occluded (e.g. the LAD, as in the present study), there is an immediate increase in blood flow in an adjacent main branch (e.g. the LCX). One explanation for this phenomenon might be that the compensatory increase in flow follows the increased oxygen demands from the area of the left ventricular wall. It can be due to the loss of contractility in the ischemic area. Increased oxygen requirements lead to a corresponding, matching increase in blood flow. This observation, [119-121] still lacks a convincing explanation.

5.2. The role of NO in the delayed antiarrhythmic effect of preconditioning induced by physical exercise

The results of this part of the study confirm the previous observations of Babai and colleagues[44] that vigorous treadmill exercise, which is sufficient to increase the heart rate over 200 beats min^{-1} can protect the heart against life-threatening arrhythmias. The first evidence that protection against the ischemia and reperfusion arrhythmias can be achieved by increasing the heart rate was provided by[45] Végh et al. in 1991[44]. This finding was confirmed by several later studies (Kaszala et al., 1996)[118]. It is still not known precisely how cardiac pacing and physical exercise preconditions the heart against the severe consequences of myocardial ischemia. It seems likely that during cardiac pacing and exercise there is an increase in myocardial oxygen demand that results from the increased heart rate and this, together with an elevation of filling pressure within the left ventricle, is sufficient to elicit changes that leads to cardioprotection.

The aim of the present research was to obtain further evidence that nitric oxide is involved in the delayed phase of preconditioning induced by heavy physical exercise. In this case two NOS inhibitors (L-NAME and the AEST) were used. A non-selective NOS inhibitor (L-NAME) was used before the exercise to examine the trigger activity of NO·in the process. A relatively selective iNOS inhibitor AEST was administrated before a prolonged 25 min occlusion period (24 h after the exercise trigger) to examine NO· as a mediator in the antiarrhythmic mechanisms. The single period of exercise induced beneficial effect against

arrhythmias (such as: VPBs and VT reduction, the rise of survival numbers, the reduced ST segment elevation) were abolished by both NOS inhibitors.

The results from the application of two different NOS inhibitors suggest that NO plays both a trigger and a mediator role in this protection. Since the marked antiarrhythmic effects of exercise, as well as the reduced ischemic and haemodynamic changes that were apparent after exercise, were significantly attenuated by these inhibitors. Further it was supposed that NO is most likely derived from the inducible form of NOS enzyme, since the iNOS inhibitor AEST was as effective in reducing the protection as that was L-NAME. The observation revealed that the administration of L-NAME, given prior to the exercise stimulus, abolishes the delayed antiarrhythmic protection. It can be suggested that NO is generated as a result of exercise that acts as a trigger for the subsequent activation of iNOS (NO stimulated NO release). The early generation of NO is necessary for the delayed protection that results from exercise. One practical difficulty with the L-NAME study is that it proved impossible to reach a similar increase in heart rate (190–220 beats min^{-1}) during exercise as that in those dogs which were not treated with L-NAME. This was because the administration of L-NAME resulted in a significant bradycardia.

It can be suggested that it is the generation of NO from the constitutive enzyme during the single exercise period that induces further NO formation from iNOS 24 h later. There is evidence of such NO stimulated NO production; triggering the development of delayed preconditioning when myocardial stunning is used as the endpoint[95]. The subsequent pathways include the activation of PKC[122] and tyrosine kinase[123, 124] resulting from the NO-stimulated generation of cGMP[125] when the arrhythmias were used as the endpoint, although this is still a matter of debate[126]. More recently it was shown in rats[127], that NO produced from cNOS (but not from iNOS) during preconditioning by brief periods of coronary artery occlusion, also triggers delayed preconditioning-induced endothelial protection

Besides NO there are a number of other possible mediators of delayed cardioprotection that are released during exercise. These include catecholamines, which are able to induce late protection (for example against ischemia and reperfusion arrhythmias[128, 129],) through an adrenoceptor mediated PKC activation[130, 131] which then induces iNOS gene expression[132]. Exercise also releases opioid peptides that act on δ-opioid receptors. This induces late preconditioning by a mechanism which involves the formation of prostacyclin and PGE2[133]. However the results of the present study suggest that NO is a particularly important trigger

and mediator for exercise-induced protection against those life-threatening arrhythmias that result from acute coronary artery occlusion.

5.3. Clinical relevance of preconditioning

The highly effective methods of myocardial protection during coronary artery surgery were developed and used in chemical cardioplegia, hypothermia, and cross-clamp ventricular fibrillation. However the increasing number of surgical operation in elder and high risk patients implies that the protection and its methods always have to be improved[110]. The pharmacology preconditioning might induce just one or two cellular pathways which can develop PC effect (e.g. adenosine or A1 receptor selective agonist[134-135]; stable analogue of prostacyclin[136-137]; bradykinin[138]). Some of these have relevance in preconditioning during coronary angioplasty[139] or postoperative recovery of clinical applications[140] if it is used in combination with other PC stimulus. The ischemic preconditioning is utilized generally before some type of cardiac surgery (e.g. coronary artery bypass grafting, coronary stenosis enlarging) to prevent the heart from the following oxygen deficiency. This causes the most powerful protection during the surgical procedure. The rapid cardiac pacing is another mechanism that induces a myocardial preconditioning in clinical aspects. However the duration of this protection is not as long as induced by ischemic PC, the combination with IPC is applicable in the clinical practice[141].

The exercise induced cardioprotection is the most complex mechanism of the protection. In this case the tachycardia, decreasing of blood perfusion, increase of energy and oxygen supply and remote effects may play a role in this process. Moreover the physical activity induces the metabolic pathways, which also has a beneficial effect. Exercise training in patients with elevated cardiovascular risk or established disease can increase the bioavailability of NO and other possible PC mediators, to help the development of secondary prevention[142]. The exercise induced cardio-protection has a more prominent relevance in primary or secondary prevention. This complex mechanism is the simplest cardioprotection that could be produced day by day.

6. Summary and New Scientific Results

The aim of this thesis was to investigate the non-receptor triggers and mediators in the early and late phase of preconditioning against arrhythmias.

(1/a) The role of reactive oxygen species were examined in the early phase of protection induced by two five minute coronary occlusion, by the use of the ROS scavenger MPG. It was found that scavenging of ROS failed to modify the antiarrhythmic effect of PC, indicating that ROS does not play an essential role in the IPC induced early protection against arrhythmias. It can be proposed that this two five minute PC triggers the release of other mediators and stimulates simultaneous protecting pathways which predominate over ROS in the induction of the protection.

(1/b) The ROS formation at different times was investigated during the experiment. It was found that after the second PC stimuli, there is a burst of detectable ROS. In the presence of MPG this burst of ROS disappeared.

(1/c) The MPG treatment blocks the appearance of phase Ia arrhythmias during the 25 minute of test occlusion but not the phase Ib. These were observed from the MPG treated control group (without PC stimuli).

The other part of the research was to obtain further evidence for the involvement of nitric oxide in the delayed phase of the preconditioning-induced protection against arrhythmias. For this purpose two inhibitors of NOS were used. The PC stimulus was induced by physical exercise, 24 h before a 25 min occlusion and reperfusion insult. A single 21 min period of exercise 24 h prior to ischemia resulted in a marked protection against ventricular arrhythmias.

(2/a) It was found that the beneficial, antiarrhythmic effect of treadmill exercise is abolished by the use of the eNOS inhibitor L-NAME before the 21 minutes of exercise.

(2/b) The applied iNOS inhibitor, AEST, can block the protecting effect of the exercise if it is applied immediately before the test occlusion.

This investigation with the applied two NOS inhibitors clearly demonstrates the role of NO as a trigger and a mediator in the exercise induced cardiac preconditioning against ventricular ectopic beats.

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9. Appendix

9.1. Összefoglaló

Az elmúlt néhány évtizedben számos vizsgálat folyt a myocardium védőmechanizmusainak kialakítására, megelőzve a szív károsodását az ischemás/reperfúziós hatásoktól. Murray és kollégái elsőnek írták le a szív adaptációs képességét a hosszan tartó ischemiás állapothoz. A toleranciát rövid, ismétlődő ischemiás/reperfúziós periódusokkal alakították ki. Ezt a jelenséget ischemiás prekondícionálásnak nevezték. Az a tény, hogy a szív megvédhető a végzetes károsodásoktól a saját adaptációs képességének segítségével, a prekondícionálás kiváltásának folyamatát és mechanizmusát mind kísérletes mind klinikai területen a figyelem középpontjába emelte. A rövid, ischemiás periódusokkal kiváltott védőhatás mára általánosan használt klinikai módszer arra, hogy megvédjék a szívet a coronaria by-pass műtétek során. Mára számos bizonyíték szolgál arra, hogy a szívben a védőhatás nem csak a rövid érelzárások által lehet kiváltani, hanem a szívfrekvencia megnövelésével (cardiac pacing) ill. fizikai terheléssel is. Számos epidemiológiai vizsgálat szolgáltat adatokat arra vonatkozóan, hogy a rendszeres mozgás csökkenti a szívbetegségekben szenvedő páciensekben a betegség súlyosbodását és a halálozást. Továbbá a fizikális terhelés csökkenti a szívbetegségek kialakulásának kockázati tényezőit (hipertenzió, hiperlipidémia, diabetes, obezitás). Azonban a fizikális terhelés kiváltotta védőhatás pontos mechanizmusa még tisztázásra vár.

Jelen doktori disszertáció fő témája a korai és késői antiarrhythmiás védőhatások mechanizmusainak vizsgálata volt, különös tekintettel a szabad gyökök és a nitrogén monoxid szerepére a védőhatás antiarrhythmiás folyamataiban.

A disszertáció első fele a szabadgyökök szerepét vizsgálja a rövid érelzárásokkal kiváltott korai antiarrhythmiás folyamatokban, nyitott mellkasú kutya modellben. Szabadgyök fogóként N-2-marcaptopropionyl-glicint (MPG) alkalmaztunk. Az alkalmazott gyökfogó nem szüntette meg a rövid érelzárások kiváltotta antiarrhythmás védőhatást. *In vitro* vizsgálat igazolta, hogy a vérben lévő polimorfonukleáris sejtekben a szabadgyökök kiválthatók ferrocitokróm-c redukciós módszerrel. A kiváltott szabadgyökök gátolhatók MPG-vel. A kísérlet különböző időpontjaiban a sinus coronariusból vett vérminták analízise igazolta az alkalmazott MPG dózis hatásosságát, mely képes volt megkötni a felszabaduló szabadgyök

mennyiségét. A vizsgálatok igazolták, hogy a kontroll csoportban alkalmazott MPG a teljes érelzárás alatt kialakuló arrhythmiák számát ugyan nem csökkentette, de az arrhythmiák Ia fázisát legátolta.

A disszertáció másik fele a nitrogén monoxid szerepével foglalkozik a késői védőhatások antiarrythmiás folyamataiban. A védőhatást egyszeri (21 perces) futópadon történő fizikális terheléssel alakítottuk ki. A nitrogén monoxid szerepén két nitrogén monoxid szintáz gátlóval vizsgáltuk. A folyamatosan expresszálódó cNOS működését L-NAME alkalmazásával gátoltuk, közvetlenül a fizikális terhelés előtt. A kísérlet bebizonyította a NO trigger szerepét a fizikális terhelés kiváltotta antiarrhythmiás folyamatban. Másrészről vizsgáltuk a nitrogén monoxid mediátor szerepét a késői védőhatás kialakításában. E célból az indukálható NOS szelektív gátlóját az AEST-t alkalmaztuk. A vizsgálat igazolta, hogy a NO nem csak trigger de mediátor is a fizikális terhelés kiváltotta antiatthythmiás folyamatokban.

9.2. Articles