Ischemic myocardial function and reperfusion-induced arrhythmias: role of ATP sensitive K^+-channel modulation and oxygen free radicals

PhD Thesis

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1995

Contents

List of full papers related to the subject of the Thesis:

-**Ferdinandy P**, Koltai M, Tosaki A, Berthet P, Tarrade T, Esanu A, Braquet P: Cicletanine improves myocardial function deteriorated by ischemia/reperfusion in isolated working rat hearts. *J Cardiovasc Pharmacol* 19: 181-189 (1992)

-Bouma P, **Ferdinandy P**, Sipkema P, Allart CP, Westerhof N: Nitric oxide is an important determinant of coronary flow in the isolated blood perfused rat heart. *Basic Res Cardiol* 87: 570-584 (1992)

-**Ferdinandy P,** Das DK, Tosaki A: Pacing-induced ventricular fibrillation leading to oxygen free radical production in aerobically perfused rat hearts. *J Mol Cell Cardiol* 25: 683-692 (1993)

-Szilvassy Z, Jakab I, **Ferdinandy P**, Koltai M, Lonovics J, Tarrade T, Braquet P: Zaprinast, cicletanine and verapamil attenuate overdrive pacing-induced myocardial ischemia in conscious rabbits. *Life Sci* 53: PL13-18 (1993)

-Szilvassy Z, Koltai M, **Ferdinandy P**, Allard M, Tarrade T, Braquet P: Cromakalim and cicletanine against pacing-induced ST-segment elevation in conscious rabbits. *Life Sci* 54: PL125-130 (1994)

-**Ferdinandy P**, Szilvassy Z, Koltai M, Tarrade T, Braquet P: In vivo infarcted isolated working rat heart: beneficial effect of cicletanine. *Cardiology in the Elderly* 2: 35-42 (1994)

-Szilvassy Z, **Ferdinandy P**, Bor P, Jakab I, Lonovics J, Koltai M: Ventricular overdrive pacing-induced anti-ischemic effect: A conscious rabbit model of preconditioning.
Am J Physiol 266:H2033-H2044 (1994)

-Szilvassy Z, **Ferdinandy P**, Bor P, Jakab I, Szilvassy J, Nagy I, Lonovics J, Koltai M: Preconditioning is lost in rabbits with vascular tolerance to nitroglycerin. *Br J Pharmacol* 112:999-1001 (1994)

-**Ferdinandy P**, Szilvassy Z, Koltai M, Dux L. Ventricular overdrive pacing-induced preconditioning and no-flow ischemia-induced preconditioning in isolated working rat hearts. *J Cardiovasc Pharmacol* 25:97-104 (1995)

1. Introduction

1.1. Ischemia/reperfusion injury

In the modern society ischemia and reperfusion induced arrhythmias and cardiac failure have particular role in mortality of patients suffering from ischemic heart disease as a consequence of sustained arterial hypertension. Therefore, study of endogenous cardioprotective mechanisms and development of therapeutic interventions improving cardiac functions during ischemia and reperfusion are of great importance.

Ischemia causes insufficient cardiac function and arrhythmias, and finally myocardial necrosis. At the cellular level, these phenomena are caused by energy depletion, release of catecholamines, histamine, serotonin, prostaglandins, thromboxanes, leukotriens, PAF, lysophosphatides, endothelins, oxygen free radicals, neuropeptides, opioids etc., which substances than cause membrane damage and pathological ion shifts, especially Ca^{++}-overload, through different second messenger systems. The heterogeneity of developing tissue injury causes mechanical and electrophysiological abnormalities, thereby leading to insufficient cardiac performance and arrhythmias. Reperfusion of the ischemic myocardium is essential for avoiding the development of irreversible tissue damage, however, reperfusion shows some deleterious effect. Reoxygenization and re-energization upon reperfusion give rise to oxygen free radical production ("oxygen paradox") and intracellular Ca^{++} overload ("Ca^{++} paradox"). The additional tissue damage, and the heterogeneity of the recovery of ischemic tissue lead to "myocardial stunning" and development of severe arrhythmias. In general, Ca^{++} overload and the generation of highly reactive oxygen-derived free radicals are thought to be the major cause of ischemia/reperfusion injury[1-6] (Fig.1.).

Ischemia/reperfusion, however, may trigger certain endogenous cardioprotective mechanisms, which include the phenomenon of "ischemic preconditioning",[7] release of nitric oxide,[8] bradykinin,[9] adenosine,[10] accumulation of intracellular cGMP,[11,12] and the opening of the adenosine triphosphate-sensitive K^+-channels (K_{ATP}).[13,14]

The thesis is limited to enlighten some aspects of the role of K_{ATP} modulation in ischemic/reperfused myocardial function and arrhythmias, and the possible role of free radicals in reperfusion-induced ventricular fibrillation.

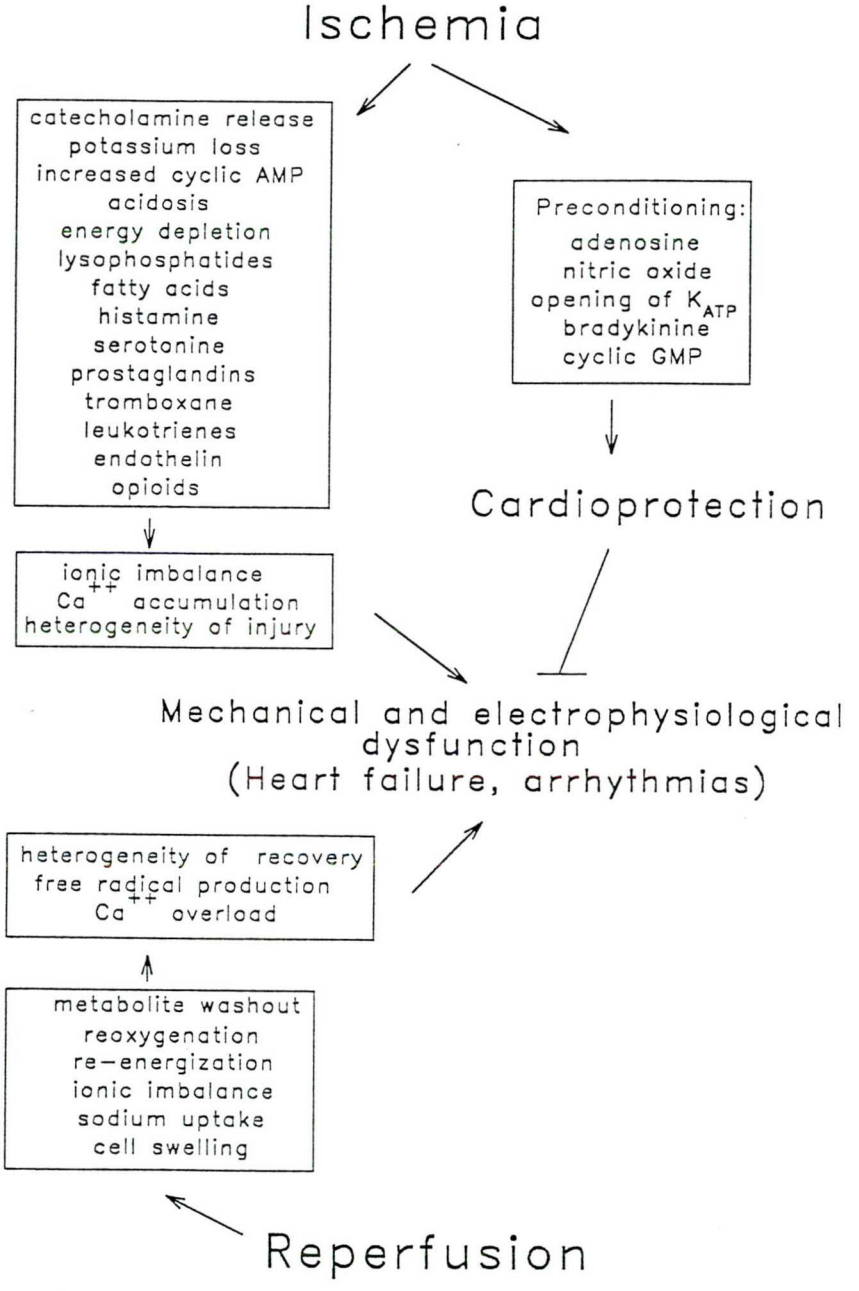

Fig.1. Mechanism of ischemia/reperfusion injury

1.2. ATP sensitive potassium channels and myocardial ischemia

In 1983, Noma[15] using the patch-clamp technique, reported the presence of K^+-channels in guinea pig ventricular myocytes that were regulated primarily by the intracellular concentration of ATP and to a lesser degree by ADP, and he postulated that these channels may play a cardioprotective role during ischemia or hypoxia. These channels were named K_{ATP} and have also been shown to exist in pancreatic beta cells, skeletal muscle, brain and vascular smooth muscle.[16] The significance of K_{ATP} in the control of cardiac and smooth muscle cell function has been one of the major topics in pharmacology for the last few years.[17-21] Potassium channel opener agents developed as antihypertensive drugs relax smooth muscle cells. Opening of potassium channels results in hyperpolarization that inactivates voltage dependent Ca^{++} channels which leads to relaxation of smooth muscle.[22,23] The action site of potassium channel drugs on the myocardium is the K_{ATP} channel, open probability of which is very low during normal conditions. A chemically diverse group of compounds (cromakalim, nicorandil, pinacidil, RP 49356 etc.) called potassium channel openers and the sulphanylurea agents like glibenclamide that antagonise the effect of potassium channel openers has made great contribution to this research field not only as promising drug candidates, but also as major research lancets [24,25]. K_{ATP} has special role during ischemia. Under hypoxic conditions the ATP content decreases and the K_{ATP} channels open. Opening of these channels hyperpolarizes myocytes, lengthens diastolic length of muscle fibres and shortens duration of action potentials. K_{ATP} opening possibly protects myocytes against Ca^{++}-overload via early inactivation of voltage dependent Ca^{++}-channels, and according to Starling law improves cardiac output of the ischemic/reperfused myocardium [20]. Since the development of specific pharmacological modulators of this channel, substantial evidence has accumulated to assume that opening these channels in the heart may play an important cardioprotective role. However, the hemodynamic and electrophysiological consequences of K_{ATP} channel modulation in myocardial ischemia are still controversial, and no data concerning this matter are available in the working rat heart preparation.

Cromakalim, a prototype of K_{ATP} openers, relaxes vascular smooth muscle,[16,26] improves functional recovery and decreases cumulative LDH release of isolated Langendorff rat hearts

with global ischemia and reperfusion.[27] Furthermore, it reduces infarct size in anesthetized dogs subjected to coronary artery occlusion/reperfusion.[28] These effects are antagonized by glibenclamide, a blocker of K_{ATP}, and thus have been attributed to opening of K_{ATP}. Glibenclamide at higher doses was found to worsen ischemic/reperfused myocardial function in isolated Langendorff rat heart[27,29] and in anesthetized open chest dogs,[30] however, it has been shown to be antiarrhythmic in isolated rat heart.[31] Potassium channel opener agents possess arrhythmogenic or antiarrhythmic properties, the discrepancy appears to be related to species, experimental model, and the cellular mechanism of arrhythmias.[20,21,31] K_{ATP} openers have dual effect on cardiac arrhythmias, since shortening of action potential on the one hand can lead to increased incidence of re-entry type arrhythmias, but on the other hand decreases the incidence of early afterdepolarization so theoretically they can be either arrhythmogenic or antiarrhythmic. The potential proarrhythmic effects of K_{ATP} opener compounds limits their therapeutic application as an antihypertensive and/or cardioprotective drug.

Cicletanine [1,3-dihydro-3-(4-chlorophenyl)-7-hydroxy-6-methylfuro (3,4-C) pyridine] a novel antihypertensive agent possesses anti-ischemic and antiarrhythmic properties in the ischemic/reperfused myocardium,[32,33] reduces the extent of myocardial necrosis due to coronary artery ligation in dogs.[34] The mechanism for these effects is unclear, however the drug is known to have several pharmacological actions. It has been shown to inhibit low K_m Ca^{++}-calmodulin dependent cGMP phosphodiesterase (PDE) and cGMP-selective PDE[35,36] and to increase myocardial cGMP-content.[37] It has been also shown to stimulate K^+-efflux in human red blood cell,[38] to open K_{ATP} in human epigastric artery rings,[39] to reverse Na^+ and K^+ imbalance in isolated rat hearts, and to enhance 6-$PGF_{1\alpha}$ release in the ischemic rat heart. [40]

The goals of the present study was to examine the role of K_{ATP} in the anti-ischemic and antiarrhythmic effects of cicletanine, and to investigate the contribution of K_{ATP} to ischemic myocardial function and reperfusion-induced ventricular fibrillation (VF) by using known modulators of K_{ATP}, cromakalim and glibenclamide, during coronary occlusion/reperfusion in isolated working rat hearts. An other purpose of the study was to assess the effect of cicletanine on chronic heart failure induced by experimental myocardial infarction.

1.3. Ischemic preconditioning

Since the original observation by Murry et al.,[41] the ability of the heart to adapt to ischemic stress has been demonstrated both in whole animals and isolated hearts.[42] In a recent study, ventricular overdrive pacing [VOP] was used to produce preconditioning characterized by a decrease in ST-segment elevation and arrhythmia incidence in anesthetized open-chest dogs.[43] We have previously reported that VOP-induced protection resulted in an attenuation of electrophysiological and hemodynamic changes, increase in cardiac cAMP content, and decrease in cardiac cGMP content produced by test ischemia in a conscious rabbit model of preconditioning.[11] Atrial pacing however, did not reduce infarct size in anesthetized rabbits.[44] The involvement of K_{ATP} in the mechanism of preconditioning was proposed by Gross and Auchampach[45] in dogs, whereas others[46,47] could not confirm this assumption in rats and rabbits. In these studies, glibenclamide was used to inhibit opening of K_{ATP}. The conflicting results reported on the involvement of K_{ATP} activation in preconditioning may result largely from differing protocols for inducing repetitive ischemia, differing experimental preparations, and differing measures of ischemic myocardial injury.

Therefore, the aim of this study was to investigate whether VOP induces preconditioning in the isolated rat heart, and to compare the characteristics of protection and the role of activation of K_{ATP} in the mechanism of preconditioning induced by two different protocols, i.e. repeated periods of VOP or no flow ischemia, using the same species and experimental protocol, i.e. isolated working rat heart.

1.4. Free radicals and reperfusion

Recent interest has focused on the suggestion that oxygen free radicals may play an important role in the initiation of reperfusion-induced injury, including development of life threatening arrhythmias, such as VF.[3] VF has been intensively studied, since VF is a major cause of sudden cardiac death, and for some special indication coronary bypass operation is performed during VF instead of cardiopulmonary bypass.[48] The mechanism of myocardial stunning observed after VF is still controversial. Oxygen free radicals, such as superoxide

radical, or hydroxyl radical may initiate lipid peroxydation chain reaction in the membranes, and thereby leading to membrane destruction. The consequent maldistribution of ions causes electrical inhomogeneity and arrhythmias. Support for this hypothesis, and contradictory findings have also been reported.[49,50] Free radicals may possess tissue-protective effects, since nitric oxide is a well know vasculoprotectiv free radical,[51] and superoxide anionradical was shown to stimulate the release an unknown endothelium-derived relaxant factor, the so called superoxide dependent endothelium-derived relaxant factor [52]. Due to the controversies, we decided to approach the question from a quite different aspect. In previous studies, formation of oxygen free radicals were observed only in fibrillating hearts upon reperfusion.[53,54] The aforementioned finding has led us to speculate that the formation of free radicals might be seen in moderately ischemic, electrically fibrillating hearts. Our objective was therefore to examine whether VF could induce the generation of free radicals in the absence of ischemia/reperfusion in aerobically perfused hearts.

1.5. Summary of the purposes of the dissertation

(i) To investigate the contribution of K_{ATP} to ischemic myocardial function and reperfusion-induced VF and to examine the role of K_{ATP} in the anti-ischemic and antiarrhythmic effects of cicletanine.

(ii) To assess the effect of cicletanine in a chronic heart failure model induced by experimental infarction.

(iii) To study whether VOP induces preconditioning in the isolated rat heart, and to assess the role of activation of K_{ATP} in the mechanism of preconditioning induced by two different protocols, i.e. repeated periods of VOP or no flow ischemia, using the same species and experimental protocol, i.e. isolated working rat heart.

(iv) To explore whether VF can elicit oxygen free radical formation in the absence of coronary occlusion and reperfusion in aerobically perfused hearts.

2. Methods

2.1. Animals

Male Wistar or Sprague-Dawley CFY rats (300-370 g, or 350-400 g) were used throughout the studies. All the investigations conform with the *Guide for the care and use of laboratory animals* published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

2.2. Isolated heart preparation

Hearts were excised from rats anesthetized with diethylether, and prepared for working heart perfused at 37°C with Krebs-Henseleit bicarbonate buffer containing (in mM) NaCl 118, KCl 4.3, $CaCl_2$ 2.4, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 11.1, gassed with 95% O_2 and 5% CO_2 as described.[32,55] Heart rate (HR) derived from the left ventricular pressure curve, coronary flow (CF) measured by collecting effluent from the right atrium in a measuring cylinder for a timed period, aortic flow (AF) measured by a calibrated rotameter (KDG Flowmeters, Sussex, England), left ventricular developed pressure (LVDP) counted as peak systolic pressure minus left ventricular end-diastolic pressure (LVEDP), its positive and negative first derivative of left ventricular pressure ($+/-dP/dt_{max}$), and LVEDP were recorded. Ventricular pressure was measured by means of a pressure transducer (B. Braun, Melsungen, Germany) connected to a small polyethylene catheter inserted into the left ventricle through the left atrial cannula. Arrhythmias were monitored by recording epicardial ECG. Data were on-line digitized and recorded on a Z-80 microprocessor based computer.

2.3. Chemicals

Cicletanine racemate (Institut Henri Beaufour, Paris, France), cromakalim (SmithKline Beecham, England), and glibenclamide (Sigma, St. Louis, MO) were dissolved in dimethyl-sulfoxide (DMSO) and added to the perfusion buffer before the onset of each experiment. The

perfusion buffer contained the different compound(s) throughout the experimental protocols. The final concentration of DMSO was $2x10^{-2}$ % (v/v) in each group. Other chemicals used were of analytical grade.

For chronic in vivo treatment, cicletanine racemate was suspended in 1 % methylcellulose. Cicletanine suspension in a volume of 5 ml/kg was perorally gavaged as a single daily dose of 30 mg/kg body weight. Control groups received the vehicle alone. Treatment was started one week before surgery, and maintained throughout 7 days after coronary artery ligation.

2.4. Induction of myocardial infarction in vivo

To assess the effect of cicletanine in a chronic heart failure model, experimental myocardial infarction was induced by the method of Selye et al.[56]. Rats were anaesthetized with diethylether, the thorax was opened at the fourth intercostal space, the heart was exposed, and the left anterior descending coronary artery was ligated at a point close to its origin. The heart was subsequently replaced and the thorax was closed. After the acute phase of coronary artery ligation, the condition of the animals was followed up to 7 days, when hearts were isolated from surviving rats. None of the surviving animals lose body weight more than 5%.

2.5. Induction of ex vivo myocardial ischemia

Test ischemia of 10-min duration was produced by occlusion of the main coronary artery followed by 3 min reperfusion. A suture was placed around the left main coronary artery close to its origin, allowing regional ischemia and reperfusion to be induced.[32] Ten-min test ischemia was chosen for assessing myocardial function, because this short-term regional ischemia induced considerable deterioration of myocardial function, but it did not result in ischemia-induced arrhythmias that might have disturbed measures of myocardial function.[32] However, reperfusion after a 10-min regional ischemia triggered high incidence of ventricular fibrillation [VF], thus allowing us to examine any beneficial effect on arrhythmias. No-flow global ischemia was induced by clamping the aortic and left atrial lines.

2.6. Pacing

Pacing with double threshold square impulses was performed by an electric stimulator (Experimetria, Budapest, Hungary) through silver electrodes attached directly to the surface of the right ventricle. Ventricular tachycardia (VT) was induced by pacing with 10 Hz (600 bpm), VF was induced with 20 Hz frequency.

2.7. Electron spin resonance (ESR) studies

The spin trapping studies were performed by infusing the spin trap 5,5,dimethyl-1-pyrroline N-oxide (DMPO) through a side arm of the perfusion system. The DMPO solution was protected from light-induced degradation, and the spin trap was directly infused into the heart at an infusion rate of 1 ml/min of 50 mmol/liter stock solution. To prevent spin adduct decay, coronary effluent was immediately frozen in liquid nitrogen as it flowed from the heart, with an effluent sampling time of 30 seconds. The ESR spectra were recorded in a flat quartz cell with a Bruker spectrometer operating at X band (9.3 MHz) with a 100 kHz modulation frequency. The microwave power was maintained at 10 mW to avoid saturation. Hyperfine coupling constants were measured directly from the field scan using Mn^{2+} as a marker for calibration.

2.8. Measurement of oxygen consumption and carbon dioxide production

Samples of coronary effluent were taken from the right atrium with glass capillary. The capillaries were immediately closed with rubber caps. Subsequently, pO_2, pCO_2, and pH were determined by an automatic acid-base analyzer (ABL, Radiometer Copenhagen, Copenhagen, Denmark). O_2 consumption and CO_2 production were calculated and expressed as μM/mL/g wet weight.

2.9. Determination of lactate dehydrogenase (LDH) release

Samples of coronary effluent were frozen and analyzed for LDH activity by means of automatic analyzer using Boehringer kits.

2.10. Design of experiments

2.10.1. Design of experiments investigating the contribution of K_{ATP} to myocardial ischemia/reperfusion and examining the role of K_{ATP} in the anti-ischemic effects of cicletanine.

Hearts were divided into 16 groups and perfused (1) with the appropriate dilution of the vehicle DMSO [control, n=16]; (2) 0.1 μM [n=14], (3) 1 μM [n=18], (4) 10 μM [n=13], and (5) 60 μM [n=10] cromakalim; (6) 3 μM [n=10], (7) 15 μM [n=10], and (8) 60 μM [n=16] cicletanine; (9) 0.1 μM [n=9], (10) 1 μM [n=9], and (11) 10 μM [n=9] glibenclamide; the combination of 0.1 μM glibenclamide with (12) 1 μM [n=8], (13) 10 μM [n=10], and (14) 60 μM [n=10] cromakalim; the combination of (15) 0.1 μM glibenclamide with 60 μM cicletanine [n=12], and (16) 1 μM cromakalim with 60 μM cicletanine [n=9]. After a 10-min aerobic working perfusion hearts of all groups were subjected to a 10-min coronary occlusion followed by 2-min reperfusion.

2.10.2. Design of studies on the effect of cicletanine in a chronic heart failure model induced by experimental infarction.

We examined the effect of 2-week peroral cicletanine treatment on functional capacity of working hearts isolated from rats with cardiac failure due to 7-day permanent coronary artery occlusion, the period in which infarct is totally evolved and scar tissue is developed [57,58].

Animals were divided into 3 groups: (1) control, sham-operated, vehicle-treated group (n=11), (2) coronary artery ligated, vehicle-treated group (n=24), and (3) coronary artery ligated, cicletanine-treated group (n=28). Seven days after coronary artery ligation, hearts of survivor rats were subjected to 38 min of drug-free working perfusion (For details, see Cardiol Elderly, 1994;2:35-42 in Annex). Initially, 10 min perfusion was applied at the resting preload and afterload pressure (ST1), then preload pressure was consecutively increased to 20 cm water (2.0 kPa, PL1), 23 cm water (2.3 kPa, PL2), and 26 cm water (2.5 kPa, PL3) for 3 min, respectively. After this preload pressure was returned to the resting level (ST2) for 5 min by means of lifting the venous reservoir to the desired height, while afterload pressure was maintained at the resting level. Subsequently, afterload pressure was consecutively increased to

125 cm water (12.3 kPa, AL1), 150 cm water (14.7 kPa, AL2) and 175 cm water (17.1 kPa, AL3) for 3 min, respectively, it was then decreased to the resting level for 5 min (ST3). Approximately 90 s after changing preload or afterload pressure, cardiac functional parameters reached a new steady state level.

2.10.3. Design of studies on preconditioning

These studies were devoted to investigate whether VOP induces preconditioning in the isolated rat heart, and to compare the role of opening of K_{ATP} in the mechanism of preconditioning induced by repeated periods of VOP or no flow ischemia, by means of perfusion of the hearts in the presence/absence of 10^{-7} M glibenclamide and/or its solvent.

Experimental groups: Non-preconditioned controls with (1) drug free perfusion [NPC, Non-Preconditioned Control], (2) solvent perfusion [NPS, Non-Preconditioned Solvent], and (3) glibenclamide perfusion [NPG, Non-Preconditioned Glibenclamide]; groups preconditioned by 3 intermittent periods of (4) 5 Hz VOP (300 bpm) without drug perfusion [5PC, 5Hz Preconditioning Control], (5) 10 Hz VOP (600 bpm) without drug perfusion [10PC, 10Hz Preconditioning Control], (6) 10 Hz VOP with solvent perfusion [10PS, 10Hz Preconditioning Solvent], (7) 10 Hz VOP with glibenclamide perfusion [10PG, 10Hz Preconditioning Glibenclamide], (8) no-flow global ischemia with drug-free perfusion [NFC, No-Flow Control], (9) no-flow global ischemia with solvent perfusion [NFS, No-Flow Solvent], and (10) no-flow global ischemia with glibenclamide perfusion [NFG, No-Flow Glibenclamide] (n=8 in each group, For details, see Cardiovasc Pharmacol, 1995;25:97-104 in Annex).

Time course of experiments: After a 10-min aerobic working perfusion, all groups of hearts were subjected to 3 intermittent periods of different preconditioning/control stimuli for 5 minutes and 15 seconds separated by a 4-min and 45-s working perfusion performed after each period of preconditioning/control stimuli (For details, see Cardiovasc Pharmacol, 1995;25:97-104 in Annex). Recordings were made at the end of each period of working perfusion and in the 5th and 10th minute of test ischemia. After control and preconditioning protocols test ischemia of 10-min duration was produced by occlusion of the main coronary artery followed by 3-min reperfusion.

2.10.4. Design of experiments on ventricular fibrillation-induced free radicals generation.

Rat hearts (n=8 in each group) isolated from male Wistar rats weighing 300-350 g were subjected to 10 min of 'working' perfusion (preVF period) followed by 10 min of Langendorff perfusion, during which VF or VT was induced by pacing (20 Hz or 10 Hz, respectively). Subsequently 5 min Langendorff perfusion was applied to allow spontaneous defibrillation to occur, then 10 min of working perfusion was again applied (post VF period). Cardiac function was measured in preVF and postVF periods. During VF or VT the spin trap DMPO was infused into the Langendorff perfusion-line reaching 2.5 mM final concentration in the coronary perfusate. Samples of the coronary effluent were immediately cooled in liquid N_2 than ESR spectra of DMPO-OH· adduct were evaluated. Changes of pH in the coronary effluent, O_2 consumption and CO_2 production of the hearts were determined during VF or VT.

2.11. Statistical analysis

Hemodynamic parameters (HR, CF, AF, LVDP, $+/-dP/dt_{max}$, and LVEDP), DMPO-OH signal intensity, LDH activity, O_2 and CO_2 concentration were expressed as means$\pm$standard error of the mean. A one-way analysis of variance was initially carried out to test any difference between the mean values of all groups. All groups were then compared to the vehicle-treated group with a modified t-test using the Bonferroni method for simultaneous multiple comparisons.[59] An analogous procedure was followed for the distribution of binomially distributed variables, such as VF. Each group was compared to the control using Chi-square test, or Fisher's Exact test.

3. Results

3.1. Contribution of K_{ATP} to ischemic myocardial function and reperfusion-induced ventricular fibrillation and to the anti-ischemic and antiarrhythmic effects of cicletanine

Heart rate: Neither concentrations of cicletanine, cromakalim, or 0.1 μM glibenclamide, and their combination influenced HR, however, glibenclamide in doses of 1-10 μM showed concentration dependent bradycardiac effect before ischemia and during coronary occlusion when compared with vehicle-treated group (Table 1).

Table 1. Effects of cromakalim, cicletanine, glibenclamide, and their combination on heart rate (HR) before ischaemia and at the 10th min of coronary occlusion in isolated working rat hearts.

			HR (beats/min)	
Treatment	concentration (μM)	n	before ischaemia	coronary occlusion
Vehicle		16	300±5	283±7
Cromakalim	0.1	14	292±5	282±6
	1	18	294±5	285±6
	10	13	302±6	286±5
	60	10	281±6	274±4
Cicletanine	3	10	294±6	276±7
	15	10	280±8	273±4
	60	16	305±5	296±5
Glibenclamide	0.1	9	283±3	273±7
	1	9	276±7[a]	263±7
	10	9	265±9[b]	240±8[b]
cromakalim+ glibenclamide	1 0.1	8	288±11	286±10
cromakalim+ glibenclamide	10 0.1	10	290±5	283±4
cromakalim+ glibenclamide	60 0.1	10	284±6	282±7
cicletanine+ glibenclamide	60 0.1	12	290±5	281±7
cromakalim+ cicletanine	1 60	9	288±4	279±5

Data are means ± SEM; *a* ($p<0.05$) and *b* ($p<0.01$) show significant reduction of heart rate compared to vehicle-treated hearts.

Coronary flow: A bell shaped dose-response relationship was observed with cromakalim, however, glibenclamide concentration dependently decreased CF before and during coronary occlusion (Fig. 2A-B). Neither concentrations of cicletanine influenced CF significantly before ischemia, but 60 μM cicletanine slightly increased CF during regional ischemia. Glibenclamide at 0.1 μM, the concentration that did not significantly alter CF, abolished the coronary dilator effect of the most effective 1 μM concentration of cromakalim or 60 μM cicletanine. The combination of 1 μM cromakalim with 60 μM cicletanine increased CF before ischemia and during coronary occlusion (31.3 ± 0.5 ml/min [$p < 0.001$], 23.2 ± 1.1 ml/min [$p < 0.001$], respectively, data not shown in Fig. 2), however, the effect of the combination was not significantly different when compared to cromakalim or cicletanine alone. Combinations of 0.1 μM glibenclamide with 10 and 60 μM cromakalim did not alter CF (data not shown).

Aortic flow: AF was markedly deteriorated by coronary occlusion in the vehicle-treated hearts (Fig. 2C-D). Neither concentrations of cromakalim affected AF before ischemia, however, all concentrations of the drug except 60 μM attenuated the deterioration of the parameter during coronary occlusion. Lower doses of cicletanine moderately increased AF before ischemia, and all concentrations of the drug significantly improved AF during coronary occlusion. The highest concentration of glibenclamide decreased AF before and during regional ischemia. Glibenclamide at 0.1 μM, the concentration that did not influence AF, abolished the beneficial effect of 1 μM cromakalim, 10 μM cromakalim (17.2 ± 1.6 ml/min, not shown in Fig. 2), or 60 μM cicletanine, respectively. The combination of 0.1 μM glibenclamide with 60 μM cromakalim did not affect AF (15.2 ± 2.1 ml/min, data not shown in Fig. 2). The combination of 1 μM cromakalim with 60 μM cicletanine substantially improved AF deteriorated by coronary occlusion (29.4 ± 2.4 ml/min, $p < 0.001$, data not shown in Fig. 2), however, the combined effect was not significantly different from cromakalim or cicletanine alone.

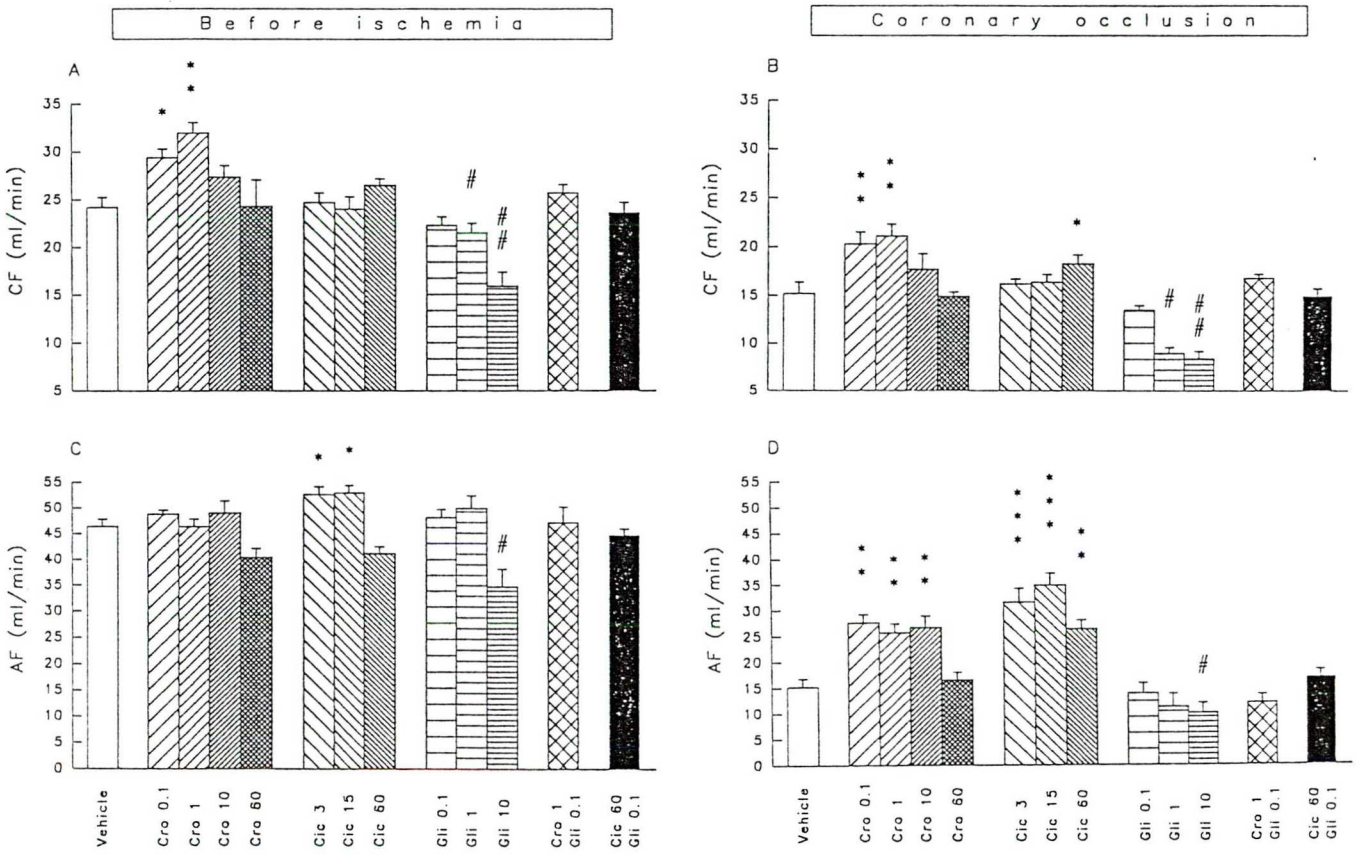

Fig. 2. Effects of cromakalim (CRO, μM), cicletanine (CIC, μM), glibenclamide (GLI, μM), the combination of GLI+CRO, and GLI+CIC on coronary flow (CF), and aortic flow (AF) before ischemia (panels A and C, respectively), and at the 10th min of coronary occlusion (panels B and D, respectively) in isolated working rat hearts. * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) designate significant improvement, # ($p<0.05$) and ## ($p<0.01$) show significant deterioration of the parameters when compared to vehicle-treated hearts.

Left ventricular developed pressure: Compared to vehicle-treated group, neither concentrations of cromakalim or cicletanine influenced significantly LVDP before ischemia (Fig 3A). The highest dose of glibenclamide considerably decreased LVDP. The different combinations of the drugs did not change LVDP significantly before coronary occlusion. Ischemia-induced reduction of LVDP was significantly alleviated by 0.1 μM cromakalim, and a downturn phase was observed in response to increasing concentration of the drug (Fig 3B). Cicletanine treatment resulted in a bell-shaped concentration dependence, and 15 μM cicletanine significantly increased LVDP. Ischemia-induced deterioration of LVDP was concentration dependently aggravated by glibenclamide. The combination of 60 μM cicletanine with 1 μM cromakalim significantly increased LVDP (14.9 ± 0.5 kPa, $p < 0.01$, data not shown in Fig. 3), however, statistically significant additive effect was not observed, when compared to either drug alone. Other combinations of the drugs remained ineffective.

$+dP/dt_{max}$: Before ischemia, cromakalim, cicletanine, and their combination failed to influence $+dP/dt_{max}$ significantly, but glibenclamide reduced it when compared to the vehicle-treated group. The combination of glibenclamide with cromakalim or cicletanine remained also ineffective. Deteriorated $+dP/dt_{max}$ due to coronary occlusion was improved by the higher concentrations of cromakalim or cicletanine. The combinative treatment with 10^{-6} M cromakalim and 6×10^{-5} M cicletanine resulted in a considerable improvement of the parameter. The combination of cromakalim with glibenclamide or cicletanine with glibenclamide did not change $+dP/dt_{max}$ significantly during coronary occlusion.

Left ventricular end-diastolic pressure: Normoxic values of LVEDP obtained with the vehicle-treated group was slightly reduced by 0.1 μM and 1 μM cromakalim, 30 μM and 60 μM cicletanine (Fig 3C), and the combination of 1 μM cromakalim with 60 μM cicletanine (0.39 ± 0.03 kPa, $p < 0.05$, not shown in Fig. 3). Glibenclamide concentration dependently increased LVEDP. Glibenclamide (0.1 μM) abolished cromakalim or cicletanine-induced reduction of LVEDP, respectively. All concentrations of cromakalim or cicletanine, or the combination of cromakalim with cicletanine (1.27 ± 0.12 kPa, $p < 0.001$, not shown in Fig. 3) significantly attenuated the elevation of LVEDP induced by coronary occlusion (Fig 3D). The

protective effect of cicletanine-cromakalim combination was not significantly superior to that of either drug alone. Glibenclamide alone did not influence LVEDP during coronary occlusion, however in the dose of 0.1 μM, it abolished the effect of cromakalim or cicletanine, respectively.

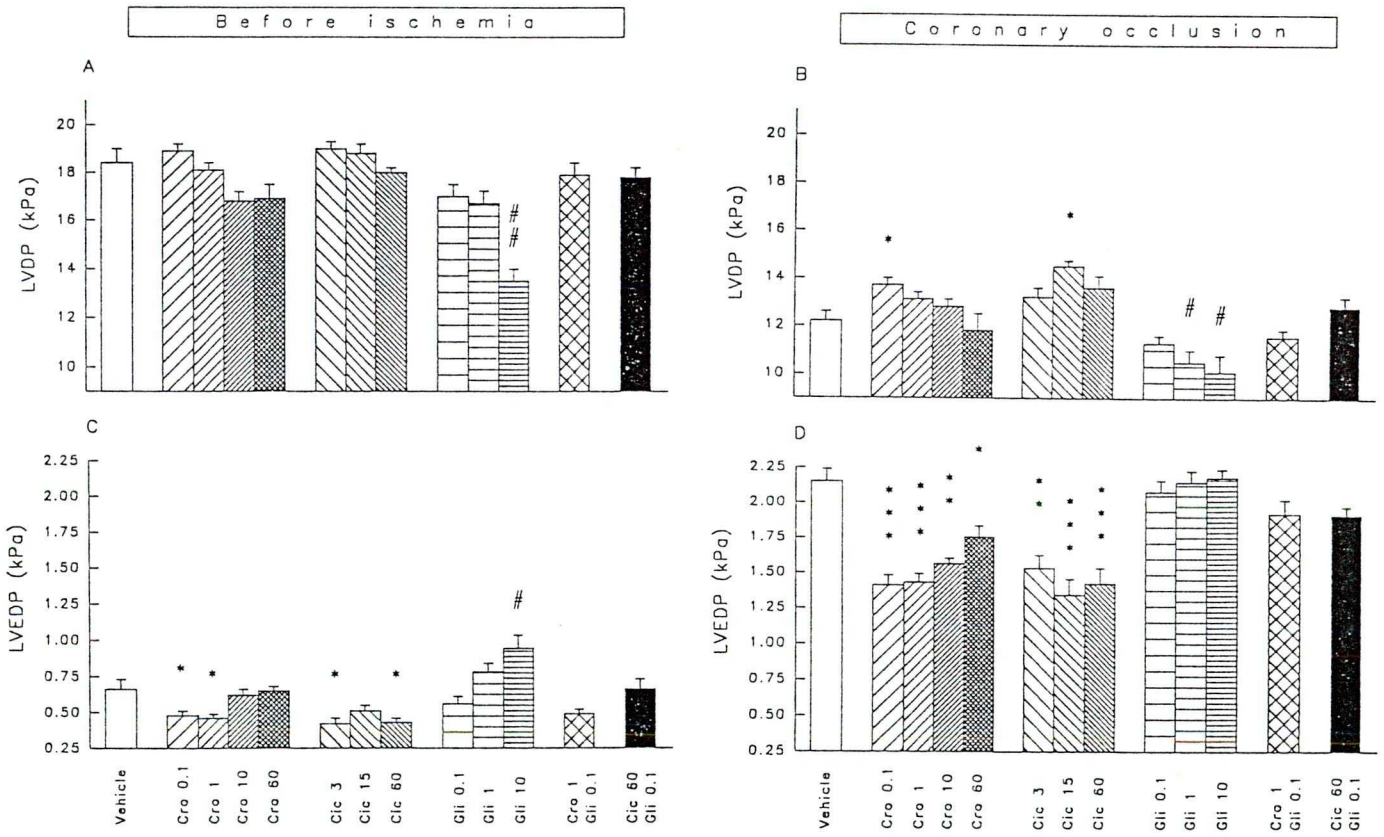

Fig. 3. Effects of cromakalim (CRO, μM), cicletanine (CIC, μM), glibenclamide (GLI, μM), the combination of GLI+CRO and GLI+CIC on left ventricular developed pressure (LVDP) and left ventricular end-diastolic pressure (LVEDP) before ischemia (panels A and C, respectively) and at the 10th min of coronary occlusion (panels B and D, respectively) in isolated working rat hearts. * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) designate significant improvement, # ($p<0.05$) and ## ($p<0.01$) show significant deterioration of the parameters when compared to vehicle-treated hearts.

Ischemia-induced VF: Ten minutes of coronary occlusion did not induce VF in the control group. Sixty μM cromakalim induced reversible VF in 30% ($p<0.01$) of the hearts, which was reversed to 0% by 0.1 μM glibenclamide. There were no ischemia-induced VF in the other groups.

Reperfusion-induced VF: A 2-min reperfusion after a 10-min coronary artery occlusion resulted in 100% incidence of VF in vehicle-treated hearts (Fig. 4). VF occurred less than 20 s after the induction of reflow, and was not terminated spontaneously throughout the reperfusion period. Similarly to cicletanine, lower doses of cromakalim did not affect VF-incidence, however, 10 μM and 60 μM cromakalim decreased arrhythmogeneity, and this effect was abolished by 0.1 μM glibenclamide. Cicletanine (60 μM) significantly decreased the incidence of VF, however, 0.1 μM glibenclamide failed to affect the antiarrhythmic effect of cicletanine. The combination of 60 μM cicletanine with 1 μM cromakalim did not afford protection superior to cicletanine alone (55%, $p<0.01$, not shown in Fig. 4).

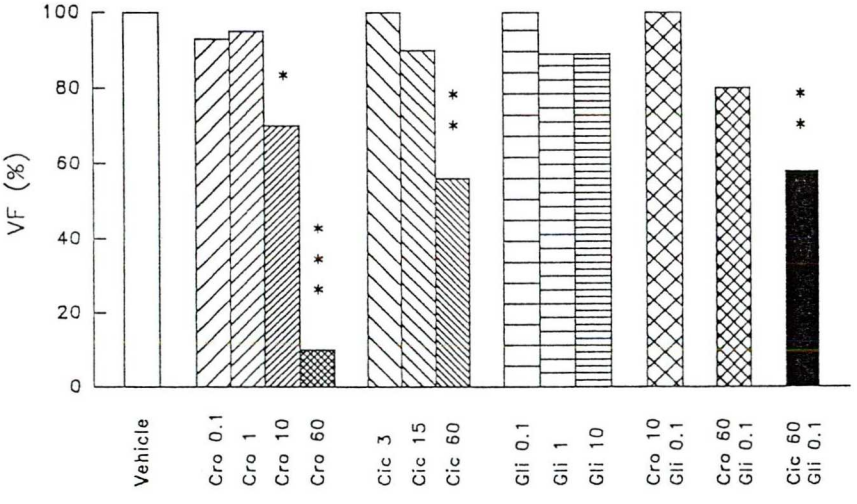

Fig. 4. Effects of cromakalim (CRO, μM), cicletanine (CIC, μM), glibenclamide (GLI, μM), the combination of GLI+CRO and GLI+CIC on the incidence of ventricular fibrillation (VF) induced by reperfusion after a 10-min coronary occlusion in isolated working rat hearts. * ($p<0.05$), ** ($p<0.01$), and *** ($p<0.001$) represent significant difference compared to vehicle-treated hearts.

3.2. Effect of cicletanine in chronic heart failure induced by experimental infarction.

Survival: Survival followed up to 7 days after experimental myocardial infarction was 29% or 32% in the vehicle-treated or the cicletanine-treated group, respectively.

Cardiac function: In hearts obtained from vehicle-treated, sham-operated animals, AF was moderately increased by increasing preload pressure and decreased by increasing afterload pressure (Fig. 5). When preload and afterload were normal (ST1, ST2, ST3), AF did not change significantly. This refers to the stability of this working heart preparation. Different preload and afterload pressure caused similar changes in AF in vehicle-treated coronary artery ligated group, but these values were significantly lower than that measured in the sham-operated group. AF was increased by cicletanine to 39.7 ± 1.9 ml/min ($p < 0.01$) in ST1 period as compared to vehicle treated coronary artery ligated value of 26.1 ± 4.0 ml/min. Chronic cicletanine treatment significantly improved AF in infarcted hearts during all perfusion periods.

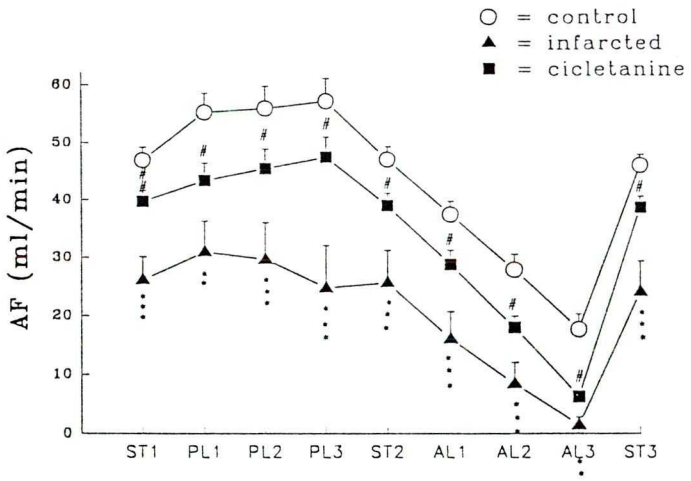

Fig. 5. Effect of myocardial infarction and cicletanine on aortic flow (AF) in isolated working rat hearts. ST1, ST2, and ST3 show perfusion periods using resting preload and afterload pressures, 17 and 100 cm water, respectively. PL1, PL2, PL3 and AL1, AL2, AL3 represent perfusion periods using consecutively increasing preload pressure of 20, 23, 26 cm water and afterload pressure of 125, 150, 175 cm water, respectively. ** $p < 0.01$ and *** $p < 0.001$ show significant difference between vehicle-treated sham-operated (control) and vehicle-treated coronary artery ligated (infarcted) groups; # $p < 0.05$ and ## $p < 0.01$ indicate significant difference between infarcted and cicletanine-treated coronary artery ligated (cicletanine) groups.

In hearts without infarction, LVDP tended to decrease when an increasing preload pressure was applied, and it increased in parallel with increasing afterload pressure (for graphic interpretation, see Cardiol Elderly, 1994;2:35-42 in Annex). LVDP was also stable under resting conditions. LVDP in the vehicle-treated coronary artery ligated group exhibited changes similar to that obtained in the control group under resting conditions and at different preload and afterload pressure. Cicletanine treatment significantly improved tolerance of the infarcted hearts either to preload (from 13.0 ± 0.7 kPa to 14.9 ± 0.6 kPa [$p < 0.05$] in PL3 period) or afterload (from 16.6 ± 1.6 kPa to 20.2 ± 0.6 kPa [$p < 0.05$] in AL3 period).

During different perfusion periods, changes in $+dP/dt_{max}$ were similar to that found with LVDP, the favourable effect of cicletanine could however be seen only after 35 min working perfusion (693 ± 28 kPa/s vs 350 ± 31 kPa/s [$p < 0.05$]). In the vehicle treated coronary artery ligated group, but not in the control and cicletanine treated groups, marked decrease in $+dP/dt_{max}$ at the end of the experiment (ST3 period) might indicate a deteriorating preparation. Coronary artery occlusion considerably decreased $-dP/dt_{max}$ during all perfusion periods, however, cicletanine, although consistently tended to improve, but did not significantly alter this parameter (data not shown).

Increasing preload pressure markedly elevated LVEDP, whereas increase in afterload pressure resulted in a moderate elevation of LVEDP in the control and infarcted groups, however, infarcted hearts exhibited LVEDP values significantly higher than controls (0.63 ± 0.08 kPa vs 1.17 ± 0.09 kPa [$p < 0.001$] in ST1 period, for graphic interpretation, see Cardiol Elderly, 1994;2:35-42 in Annex). Cicletanine markedly attenuated the increased LVEDP under resting conditions (0.78 ± 0.1 kPa [$p < 0.05$] in ST1 period) and at perfusion periods with increased preload pressure.

HR and CF remained uninfluenced either by coronary occlusion or cicletanine treatment (for detailed data, see Cardiol Elderly, 1994;2:35-42 in Annex).

Premature ventricular beats: The total number of PVC, the only type of ventricular arrhythmia occurred throughout the 38 min working perfusion was increased in the vehicle-treated coronary artery ligated group from 1.18 ± 0.5 observed in the sham-operated group to 10.14 ± 1.24

($p<0.001$). Peroral cicletanine treatment significantly decreased PVC to 4.9 ± 0.63 ($p<0.01$) as compared to vehicle-treated coronary artery ligated group.

3.3. K_{ATP} in preconditioning induced by VOP or no-flow ischemia

Cardiac function: When compared to NPC control group, HR, CF, AF, LVDP, and $+/-dP/dt_{max}$ and LVEDP were not significantly changed after the different preconditioning/control stimuli in hearts preconditioned by 3 consecutive periods of VOP (5PC, 10PC, 10PS, and 10PG groups) or no-flow ischemia (NFC, NFS, and NFG groups). Similarly, cardiac functional parameters remained unaltered in NPS and NPG groups (For details and graphyc interpretation see Cardiovasc Pharmacol 1995;25:97-104 in Annex).

In the non-preconditioned control group (NPC), at the 5th minute of test ischemia produced by coronary occlusion a slight decrease in HR, and a marked decrease in CF, AF, LVDP, $+/-dP/dt_{max}$, and an increase in LVEDP occurred when compared to the last measurement prior to coronary artery occlusion. Similar changes were seen in NPS and NPG groups, and in the 10th minute of test ischemia in all non-preconditioned groups. Preconditioning with 3 periods of VOP at 10 Hz significantly attenuated the reduction of AF, LVDP, $+/-dP/dt_{max}$ and the elevation of LVEDP (Fig. 6). These alterations except for $+dP/dt_{max}$ were partially inhibited by 10^{-7} M glibenclamide VOP at 5 Hz did not protect hearts against test ischemia. Preconditioning with 3 periods of no-flow global ischemia significantly improved AF, LVDP, and LVEDP deteriorated by test ischemia, and a slight (not significant) improvement was seen with $+/-dP/dt_{max}$, however, protection remained uninfluenced in the presence of solvent or glibenclamide. VOP at 10 Hz offered protection against LVEDP elevation significantly superior to that produced by no-flow ischemia (Fig. 6).

VF: Reperfusion after test ischemia resulted in 100% incidence of reperfusion-induced VF in NPC, NPS, NPG, 5PC, 10PC, 10PS, and 10PG groups, whereas in NFC, NFS, and NFG groups VF was 62% (not significant [ns]), 75% (ns), and 50% (ns), respectively.

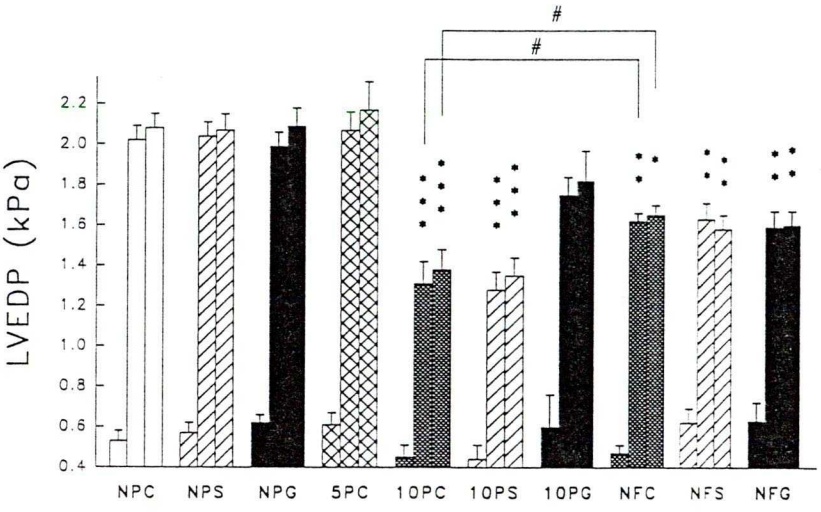

Fig. 6. Bar graph showing the effect of preceding 3 intermittent periods of ventricular overdrive pacing (VOP) or no-flow global ischemia on left ventricular end-diastolic pressure (LVEDP) before coronary occlusion (left part of triple bars) and at the 5th (middle part of triple bars) and 10th (right part of triple bars) minute of subsequent test ischemia produced by coronary artery occlusion. NPC, NPS, and NPG designate non-preconditioned controls with drug free, solvent, and glibenclamide perfusion, respectively. 5PC, 10PC, 10PS, and 10PG show groups of hearts preconditioned with 5 Hz VOP, 10 Hz VOP, 10 Hz VOP perfused with solvent or glibenclamide, respectively. NFC, NFS, and NFG designate groups preconditioned by no-flow global ischemia with drug free, solvent, and glibenclamide perfusion, respectively. Values are means $\pm$SEM (n=8 in each group). * ($p<0.05$), ** ($p<0.01$) *** ($p<0.001$) show significant difference compared to NPC group. # ($p<0.05$) indicates significant difference between 10PC and NFC groups.

3.4. Free radicals in VF

Electron spin resonance studies using the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) demonstrated oxygen free radical formation with peak concentration at the 3rd min of VF, while at the 10th min of VF oxygen free radical generation was not detectable (Fig. 7,8). When hearts were paced for 10 min at 10 Hz to induce VT, formation of oxygen free radical was not observable. Deteriorated postVF AF, LVDP, $+dP/dt_{max}$ and LVEDP were significantly improved from their untreated values of 31.6 ± 6.1 ml/min, 15.0 ± 0.4 kPa, 634 ± 28 kPa/s and 1.05 ± 0.1 kPa to 44.9 ± 2.8 ml/min ($p<0.05$), 18.35 ± 0.5 kPa ($p<0.001$), 914 ± 68 kPa/s ($p<0.01$) and 0.79 ± 0.07 ($p<0.05$) kPa, respectively, when DMPO was infused into the coronary perfusate (2.5 mM/l) during VF (Fig. 9 A-D). Cardiac functions were not depressed after VT of 10 min (data not shown). At the 3rd min of VF and VT, values of pH, pO_2, pCO_2 7.36 ± 0.016, 121.5 ± 14.2 mmHg, 43.5 ± 1.4 mmHg and 7.23 ± 0.071, 122.4 ± 8.7 mmHg, 42.1 ± 0.9 mmHg, respectively, measured in the coronary effluent showed no significant difference (Fig. 10). Fibrillation lasted longer than 5 min after termination of pacing at 20 Hz was considered irreversible VF. The incidence of spontaneous defibrillation was 66% in the control and 89% ($p<0.05$) in the DMPO treated group (Fig.11).

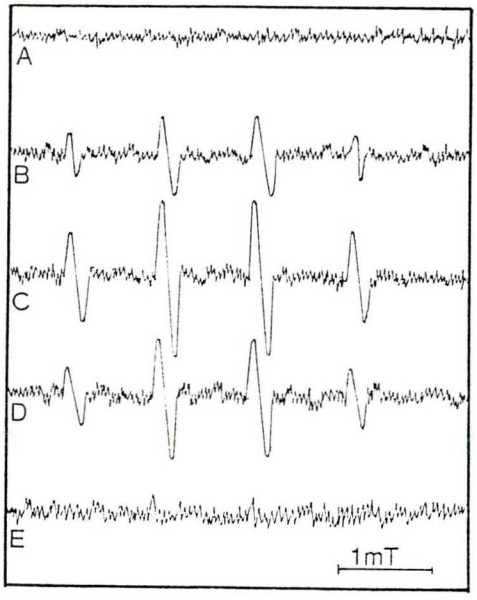

Fig. 7. Representative spectra of oxygen free radical formation during electrically-induced ventricular fibrillation in isolated rat heart. Free radical generation were recorded before fibrillation (A), and after 1 min (B), 3 min (C), 5 min (D), and 10 min (E) of fibrillation.

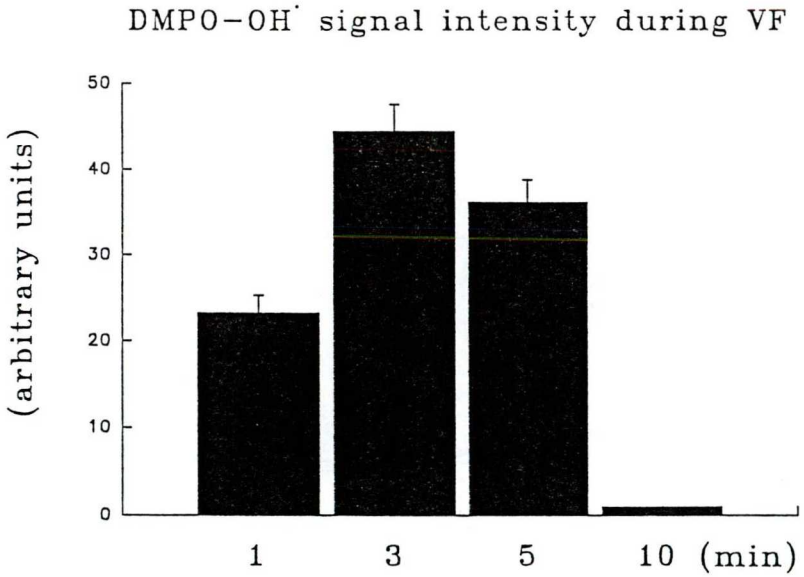

Fig. 8. Time course and quantitative analysis of DMPO-OH· signal intensity (n=8 in each group).

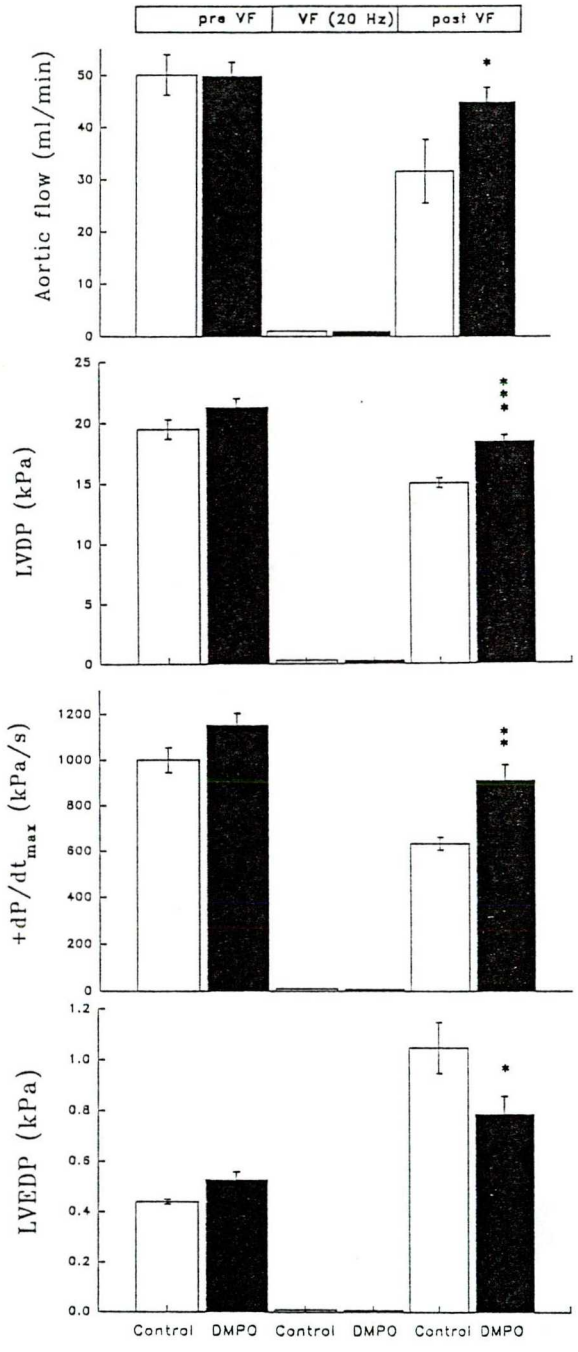

Fig. 9. Parameters of cardiac function [Aortic flow, left ventricular developed pressure (LVDP), $+dP/dt_{max}$, and left ventricular end-diastolic pressure (LVEDP)] before (preVF) and after (postVF) electrically induced ventricular fibrillation (VF, 20 Hz). * ($p<0.05$), ** ($p<0.01$) represent significant difference between DMPO treated (solid bars) and untreated (open bars) groups.

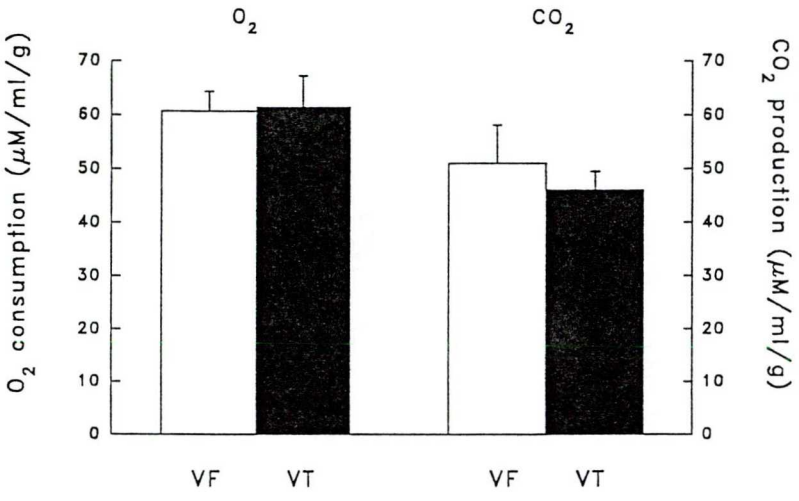

Fig. 10. O_2 consumption and CO_2 production of hearts at the 3rd min of fibrillation (VF, open bars) or 3rd min of tachycardia (VT, solid bars).

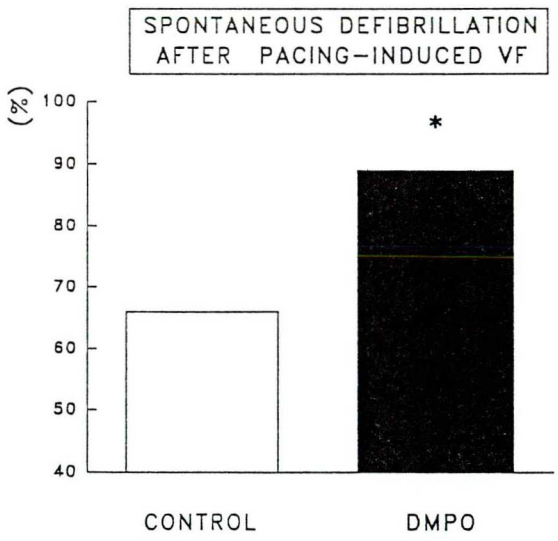

Fig. 11. Incidence of spontaneous defibrillation during the period of 5 min Langendorff perfusion after termination of pacing at 20 Hz. * ($p < 0.05$) signs significant difference between untreated and DMPO treated hearts.

4. Discussion

4.1 New findings

(i) The anti-ischemic but not the antiarrhythmic effect of cicletanine may involve opening of K_{ATP}. Opening of K_{ATP} attenuates, inhibition of the channel exacerbates functional consequences of coronary occlusion, and K_{ATP} opening attenuates reperfusion-induced VF, however, it triggers ischemia-induced VF in isolated working rat heart. K_{ATP} blocking does not affect VF.

(ii) The results reported here indicate that cicletanine treatment improves the ability of the heart with myocardial infarction to adapt to increased preload and afterload, and show the usefulness of this simple model of in vivo infarcted isolated working heart to assess drug action in infarction-induced chronic heart failure.

(iii) VOP as well as no-flow ischemia preconditions heart, however their cardioprotective mechanisms may be different in terms of the activation of K_{ATP} in the rat.

(iv) Early VF produces free radicals, which is not a consequence of relative hypoxia. Anti oxidant therapy increases the incidence of spontaneous defibrillation and improves postfibrillatory cardiac function.

4.2. K_{ATP} and myocardial ischemia and cicletanine

Differences in cardiac performance between Langendorff versus "working" hearts,[60] and the possible mechanosensitive component of K_{ATP} gating in the myocardium[61] may suggest, that the application of left atrially perfused "working" heart may be more advantageous then retrogradely perfused "empty beating" Langendorff hearts for evaluation of the effect of

pharmacological modulation of K_{ATP} on ischemic myocardial function. In correlation with previous findings in Langendorff rat hearts,[16,27] our results indicate that the K_{ATP} opener cromakalim improves ischemic myocardial function, however, in a dose as low as 0.1 μM it exerts significant anti-ischemic effect characterized by improved AF, LVDP, and LVEDP, thus showing the different reactivity of the working rat heart to K_{ATP} modulation. As to the anti-ischemic and the coronary dilator effect of cromakalim, the concentration-response relationship shows a downturn phase at higher doses of the drug. This may be attributed to the increased K^+ efflux that may interfere with the anti-ischemic effect of the drug.[62,63] Cromakalim (10 and 60 μM) produces significant antiarrhythmic effect upon reperfusion. However, 60 μM cromakalim, but not its lower concentrations increases the incidence of VF during coronary occlusion in the present study. Shortening of action potential due to K_{ATP}-opening may lead to an increased susceptibility to re-entry arrhythmias, and may decrease the occurrence of early afterdepolarization. Thus, K_{ATP} openers may exert either arrhythmogenic or antiarrhythmic action depending on the species and the experimental model used.[20,21,31]

The present study confirms our previous observations in isolated working rat hearts[32] and in conscious rabbits[37] that cicletanine attenuates functional consequences of coronary occlusion and pacing-induced global myocardial ischemia, respectively. The present study revealed, however, that the drug in lower concentrations (3 and 15 μM) although exerts favorable anti-ischemic effect, does not produce significant antiarrhythmic action. Similarly to cromakalim in the present study, our previous results prove that the dose-dependence of the anti-ischemic effect of cicletanine (above 90 μM) shows a downturn phase.[32] Therefore, 60 μM cicletanine, the concentration that produced both marked anti-ischemic and antiarrhythmic effect was chosen for studying the interaction with K_{ATP} modulators.

We have found that glibenclamide concentration-dependently deteriorates nonischemic and ischemic myocardial function. Nevertheless, 0.1 μM glibenclamide does not significantly depress myocardial function. In contrast, studies in isolated Langendorff hearts show that 1 μM glibenclamide does not considerably affect the severity of ischemia.[27,29,64] This discrepancy also suggests that the working heart preparation may be more sensitive to blockade of K_{ATP}, than the

conventional Langendorff heart. Dissimilarly to findings of Nielsen-Kudsk and Thirstrup[65] who showed that glibenclamide relaxes isolated rabbit coronary artery rings, and similarly to that found by Grover et al.[27,29] in rat heart, and Samaha et al.[66] in anesthetized dogs, glibenclamide concentration-dependently reduced CF, thus suggesting the essential contribution of K_{ATP} in the maintenance of basal resistance of coronary vasculature in the rat. The cardiodepression induced by glibenclamide, including its bradycardiac effect, may at least in part be attributed to its coronary vasoconstrictor effect. In contrast to the results of others obtained with ischemia-induced arrhythmias in Langendorff rat hearts subjected to low-flow ischemia,[31] or that obtained with arrhythmias induced by reperfusion after global no-flow ischemia,[63] neither concentration of glibenclamide produced antiarrhythmic effect upon reperfusion in our study. The discrepancy may be derived from differences in perfusion technique, induction and duration of ischemia. We have selected 0.1 μM glibenclamide for studying the interaction of K_{ATP} blockade with cicletanine, since this concentration does not considerably influence cardiac performance, but it abolishes the anti-ischemic effect of the optimal concentration (10^{-6} M) of the K_{ATP} opener cromakalim. Although glibenclamide is widely used and accepted as the most specific blocker of K_{ATP}, it should be noted that the effects of the drug are likely not solely related to its actions on K_{ATP}.[67,68] Our study shows that 10^{-7} M glibenclamide abolishes the beneficial effects of cicletanine during ischemia, but it does not affect the antiarrhythmic effect of the drug upon reperfusion. Glibenclamide, however, abolishes the antiarrhythmic effect of cromakalim during reperfusion. This finding indicates that opening of K_{ATP} may be involved in the anti-ischemic, but not in the antiarrhythmic effect of cicletanine. The antiarrhythmic effect of cicletanine has been attributed to its intracellular Ca^{++} antagonistic properties and its prostacyclin releasing effect.[40,69,70] Our results show, that the combination of 1μM cromakalim with 60 μM cicletanine produced marked anti-ischemic and antiarrhythmic effect, however, the combined effects were not significantly different from either drugs alone. Nevertheless, in our previous studies in conscious rabbits with rapid pacing-induced ischemia,[71] a significant additive effect was seen with the combination of the two drugs. The discrepancy may be related to differences in species, mode of ischemia induction, and the doses of the drugs.

The mechanism by which cicletanine may lead to opening of K_{ATP} is not known. The effects of cicletanine on AF, LVDP, and LVEDP are similar to that of cromakalim, and glibenclamide blocks the effect of both drugs, however, in contrast to cromakalim, cicletanine does not increase CF. This might indicate, that the possible K_{ATP}-opener effect of cicletanine may be more specific on myocardial cells than on the coronary smooth muscle cells. Accordingly, opening of K_{ATP} produced by cicletanine on smooth muscle cells is not supported by Noack and Deitmer,[69] since no cicletanine-induced whole-cell K^+ currents were observed in guinea pig portal vein smooth muscle cell, and glibenclamide did not significantly block the cicletanine-induced relaxation in rat, guinea-pig, and rabbit portal vein preparations. Cicletanine may open K_{ATP} channels indirectly through modulation of myocardial cyclic nucleotide metabolism. The drug has been shown to inhibit Ca-calmodulin dependent PDE and cGMP specific PDE,[35,36] thereby leading to an increase in cardiac cGMP level.[37] The cardioprotective effect of elevation of myocardial cGMP concentration is well established,[11,12] however, very few data are available about direct relation between accumulation of cGMP and activation of K_{ATP} currents.[72]

In conclusion, here we suggest that the anti-ischemic but not the anti-arrhythmic effect of cicletanine may involve opening of K_{ATP}. Opening of K_{ATP} by cromakalim attenuates, and blocking the channel by glibenclamide exacerbates functional consequences of coronary occlusion. K_{ATP} opening may protect against reperfusion-induced VF, but it induces VF during coronary occlusion. K_{ATP} blocking with glibenclamide does not influence ischemia-induced or reperfusion-induced arrhythmias in the isolated working rat heart.

4.3. Cicletanine in chronic heart failure

The results obtained in this study indicate that two week peroral cicletanine treatment significantly improves the capability of in vivo infarcted, isolated working rat hearts to adapt to gradually increasing preload and afterload, moreover it reduces the incidence of PVC; nevertheless, survival rate in the acute phase of coronary artery ligation remains uninfluenced

by the drug as compared to vehicle-treated controls.

In experimental cardiology, preclinical screening for a special indication, such as chronic heart failure developing after myocardial infarction, has to involve estimation for putative beneficial effect of the compounds on cardiac function. Chronically implanted catheter to the left ventricular cavity has been applied to follow the time course of deterioration of functional capacity up to 13 weeks after coronary artery occlusion in rats subjected to treadmill exercise test.[73] The expense and labour intensive nature of traditional models performed mainly in large mammals, or chronically instrumented animals restricts possibility to carry out a large number of experiments.[74-78] To overcome such covariances, in this study performed in the rat, we combined in vivo coronary artery occlusion with in vitro working heart exposed to different loading pressure.

Acute treatment with cicletanine has been shown to improve contractile function of the ischemic/reperfused myocardium both in vitro and in vivo.[32,79] Nevertheless, no data have been available as to whether the drug is able to ameliorate functional capacity of heart after myocardial infarction. The results reported here clearly show that peroral treatment with cicletanine for 7 days before permanent coronary artery occlusion followed by 7-day administration after myocardial infarction is able to improve cardiac function significantly as measured by an increase in AF, LVDP, and $+dP/dt_{max}$, and a marked decrease of LVEDP when compared to hearts of vehicle treated rats.

The marked attenuation by cicletanine of resting LVEDP and elevation of LVEDP induced by increasing preload pressure might be explained by a preserved elasticity of muscle fibers in the myocardium. The same mechanism might underlie the less significant effect of the drug on decrease in $-dP/dt_{max,}$ an index of isovolumic relaxation. Similar findings, indicating an increased elasticity of the arterial wall, have been observed after chronic peroral treatment with 3 mg/kg daily cicletanine dose.[80] It has recently been demonstrated that an intravenous bolus of 10 mg/kg cicletanine reduces infarct size and incidence of arrhythmias in anesthetized rabbits subjected to coronary artery occlusion/reperfusion.[34] The implication of this cytoprotective effect, and the K_{ATP} opening properties of cicletanine in the mechanisms through which cicletanine

improves functional capacity of the heart after myocardial infarction is suggested by the results reported here.

The ability of cicletanine to suppress ventricular arrhythmias of whatever origin can be considered as a consequence of a drug-induced prolongation of ventricular effective refractory period.[79] As heart rate remains uninfluenced by cicletanine, the drug may reduce the 'window' for arrhythmias during ventricular repolarization.[81]

In conclusion, this study demonstrates that peroral cicletanine treatment alleviates chronic heart failure induced by myocardial infarction in the rat. Cicletanine may therefore provide an advantageous therapeutic option to confer protection against cardiac failure developing as a consequence of ischemic heart disease induced by sustained arterial hypertension particularly in elderly patients.

4.4. K_{ATP} and preconditioning

The results reported here indicate that preceding three intermittent periods of VOP at 10 Hz or no-flow global ischemia protect heart against functional consequences of a subsequent regional ischemia produced by coronary artery occlusion in isolated working rat hearts. VOP at 5 Hz is unable to induce preconditioning, thus showing that pacing at a frequency close to the normal heart rate of the rat does not exert protective effect. Protection by 10 Hz VOP is more pronounced than that induced by no-flow with respect to alleviation of LVEDP increase and $-dP/dt_{max}$ decrease. In contrast to no-flow, VOP-induced preconditioning is abrogated by 10^{-7} M glibenclamide. The concentration as low as 10^{-7} M of glibenclamide was selected for studying the interaction of K_{ATP} blockade with preconditioning, since this concentration in contrast to 10^{-6} M and 10^{-5} M did not considerably influence cardiac performance, but abolished the anti-ischemic effect of the optimal concentration (10^{-6} M) of the K_{ATP} opener cromakalim, thereby suggesting a specific blockade of K_{ATP} produced by 10^{-7} M glibenclamide in the isolated working rat heart. The incidence of reperfusion-induced VF after a 10-min coronary artery occlusion remained unchanged by preconditioning with 10Hz VOP, while no-flow ischemia-induced

preconditioning resulted in a tendency of a decrease in arrhythmogeneity which was insensitive to glibenclamide.

The marked discrepancy in the results obtained in various studies with glibenclamide on preconditioning suggests that, beyond species differences, cardioprotection induced by a variety of experimental protocols may comprise different mechanisms. There is evidence suggesting that increased tolerance of myocardium to ischemia might be conferred by formation and/or release of endogenous protective substances. Adenosine or nitric oxide (NO) activating K_{ATP}[82] or soluble guanylate cyclase,[51] respectively, and cGMP have been reported as possible mediators of preconditioning.[8,11,83] In this study, attenuation by preconditioning of increase in LVEDP and decrease in AF and $-dP/dt_{max}$ induced by coronary artery occlusion might be due to an inhibition of ischemia-induced end-diastolic contracture. Drugs that activate K_{ATP}[27,29] or promote formation of cGMP may act in a similar manner.[32,37,84] Openers of K_{ATP} do not protect against re-entry arrhythmias and even they may provoke arrhythmias through a shortening of action potential duration and ventricular refractoriness.[85] Our findings that preconditioning induced by VOP at 10Hz does not result in any antiarrhythmic action during reperfusion, and that glibenclamide partially blocks the anti-ischemic effect of preconditioning strongly suggest the involvement of K_{ATP}. On the other hand, glibenclamide does not influence preconditioning produced by no-flow global ischemia, being in accordance with that found by Fralix et al.[86] and Grover et al.[64] who used Langendorff rat hearts preconditioned with 4 intermittent periods of no-flow global ischemia.

The discrepancy in the pharmacological reactivity of preconditioning induced by VOP or no-flow ischemia demonstrated in the present study suggests that opening of K_{ATP} may be involved as an endogenous cardioprotective mechanism only in certain types of ischemic insults. In the isolated working rat heart, VOP may represent stronger stimulus than no-flow ischemia to open K_{ATP} either through an increased activation of the mechanosensitive gating of K_{ATP}[61,87] in cardiomyocytes or through an increased sheer-stress leading to a release of NO[88,89] and cGMP[90] from the endothelium of the coronary vasculature.

We conclude that in the isolated working rat heart preconditioning may be classified as

active and passive preconditioning. Active preconditioning induced by VOP increases myocardial oxygen demand, and its effect may clinically be equivalent to that of physical exercise. In contrast, passive preconditioning induced by decreased oxygen supply, a condition that may remind of ischemic heart disease. Opening of K_{ATP} is likely to contribute only to active preconditioning. In the prevention of myocardial infarction, active preconditioning may deserve higher pharmacological and clinical interest.

4.5. Oxygen free radicals in VF

Our results demonstrate that early VF produces oxygen free radicals in the isolated rat heart, and that VT does not lead to oxygen free radical generation. VF-induced free radical production is not a consequence of relative hypoxia, since VT resulted in a similar degree of relative hypoxia assessed by measurement of the oxygen consumption and carbon-dioxide production of the heart. Postfibrillatory stunning is attenuated by anti-oxidant therapy applied during VF. The anti-oxidant therapy by DMPO increases the incidence of spontaneous defibrillation.

In previous studies, Tosaki et al.[53,54] found, that oxygen free radical generation was observable only in fibrillating hearts upon reperfusion after 30 min global myocardial ischemia in isolated rat hearts, and the same time course of free radical generation was detected as in our present study. The mechanism of fibrillation-induced free radical production is unclear, however, our results excluded the relative hypoxia as a cause of it. It is well known, that VF induces the release of endogenous cathecolamines.[91] The auto-oxidation of cathecolamines forms hydrogen peroxide, and thus can lead to the generation of other oxygen free radicals.[92] Another mechanism explaining VF-induced free radical generation is the intracellular Ca overload observed in ferret hearts with electrically-induced fibrillation.[93] Ca overload activates phospholipase A_2 and thereby leading to free radical production due to the initiation of the arachidonic acid cascade.[94]

In this study, the spin trap DMPO applied during VF improved postfibrillatory cardiac function, and increased the incidence of spontaneous defibrillation after termination of pacing

at 20 Hz. This might suggest, that anti-oxidant therapy inhibits postfibrillatory stunning, and decreases the incidence of sudden cardiac death due to irreversible VF. It might be concluded, that VF-induced free radical generation, may play an important role in the maintenance of sustained VF occurring upon reperfusion.

5. Acknowledgement

This work was supported by grants from OTKA F6396, MHB 709/94, Hungarian Space Research Office, Pro Cultura Hungariae Students for Science Foundation, Budapest, Hungary; IPSEN-Beaufour, Paris, France; and USPHS NIH HL 22559, HL33889, USA.

I greatly acknowledge to Professor László Dux, Professor György Benedek, Professor Nico Westerhof, and Professor László Szekeres, for providing possibility to work at their departments.

I am especially thankful to Mátyás Koltai deceased, and Árpád Tósaki. They were the people, my tutors, whose attitude and scientific thinking mostly determined my carrieer when I was a student. The memory of Mátyás Koltai will never fade in my mind.

I am greatful to Zoltán Szilvássy, my friend and my coworker, for doing our work always with one accord and with enthusiasm, for the common experiments, for the common publications, and for the interesting discussions.

I thank Tamas Csont and Csaba Csonka, my students, for bringing color in our laboratory.

I am thankful to my wife and children, for making everything worthwhile.

6. References

1. Hearse DJ: Stunning: A radical re-view. *Cardiovasc Drugs Ther* 1991;5:853-876

2. Opie LH: Postischemic stunning - The case for calcium as the ultimateculprit. *Cardiovasc Drugs Ther* 1991;5:895-900

3. Bernier M, Hearse DJ, Manning SA: Reperfusion-induced Arrhythmias and Oxigen-derived Free Radicals. *Circ Res* 1986;58:331-340

4. Hearse DJ, Bolli R: Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. *Cardiovasc Res* 1992;26:101-108

5. Curtis MJ, Pugsley MK, Walker MJA: Endogenous chemical mediators of ventricular arrhythmias in ischaemic heart disease. *Cardiovasc Res* 1993;27:703-719

6. Manning AS, Hearse DJ: Reperfusion-induced arrhythmias: mechanisms and prevention. *J Mol Cell Cardiol* 1984;16:497-518

7. Walker DM, Yellon DM: Ischaemic preconditioning: From mechanisms to exploitation. *Cardiovasc Res* 1992;26:734-739

8. Vegh A, Szekeres L, Parratt J: Preconditioning of the ischaemic myocardium; Involvement of the L-arginine nitric oxide pathway. *Br J Pharmacol* 1992;107:648-652

9. Vegh A, Szekeres L, Parratt JR: Local intracoronary infusion of bradykinine profoundly reduce the severity of ischemia-induced arrhythmias in anesthetised dogs. *Br J Pharmacol* 1991;104:294-295

10. Tsuchida A, Miura T, Miki T, Shimamoto K, Iimura O: Role of adenosine receptor activation in myocardial infarct size limitation by ischemic preconditioning. *Cardiovasc Res* 1992;26:456-461

11. Szilvassy Z, Ferdinandy P, Bor P, Jakab I, Lonovics J, Koltai M: Ventricular overdrive pacing-induced anti-ischemic effect: A conscious rabbit model of preconditioning. *Am J Physiol* 1994;266:H2033-H2041

12. Szilvassy Z, Ferdinandy P, Bor P, Jakab I, Szilvassy J, Nagy I, Lonovics J, Koltai M: Loss of preconditioning in rabbits with vascular tolerance to nitroglycerin. *Br J Pharmacol* 1994;112:999-1001

13. Grover GJ, Sleph PG, Dzwonczyk S: Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A_1-receptors. *Circulation* 1992;86:1310-1316

14. Ferdinandy P, Szilvassy Z, Koltai M, Dux L: Ventricular overdrive pacing-induced preconditioning and no-flow ischemia-induced preconditioning in isolated working rat hearts. *J Cardiovasc Pharmacol* 1995;25:97-104

15. Noma A: ATP-regulated K channels in cardiac muscle. *Nature* 1983;305:147-148

16. Gross GJ, Auchampach JA: Role of ATP dependent potassium channels in myocardial ischaemia. *Cardiovasc Res* 1992;26:1011-1016

17. Weston AH, Edwards G: Latest developments in K-channel modulator pharmacology. *Z Kardiol* 1991;80:1-8

18. Erne P, Hermsmeyer K: Modulation of intracellular calcium by potassium channel openers in vascular muscle. *Arch Pharmacol* 1991;344:706-715

19. Nichols CG, Lederer WJ: Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol* 1991;261:H-1675-1686

20. Sanguinetti MC: Modulation of Potassium Channels by Antiarrhythmic and Atihypertensive Drugs. *Hypertension* 1992;19:228-236

21. Padrini R, Bova S, Cargnelli G, Piovan D, Ferrari M: Effects of pinacidil on guinea-pig isolated perfused heart with particular reference to the proarrhythmic effect. *Br J Pharmacol* 1992;105:715-719

22. Mitani A, Sakamoto K, Fukamachi K, Sakamoto M, Kurisu K, Tsuruhara Y, Fukumura F, Nakashima A, Tokunaga K: Effects of glibenclamide and nicorandil on cardiac function during ischemia and reperfusion in isolated perfused rat hearts. *Am J Physiol* 1991;261:H1864-H1871

23. Siegel G, Walter A, Schnalke F, Schmidt A, Buddecke E, Loirand G, Stock G: Potassium channel activation, hyperpolarization, and vascular relaxation. *Z Kardiol* 1991;80:9-24

24. Antzelevitz C, Di Diego JM: Role of K+ Channel Activators in Cardiac Electrophysiology and Arrhythmias. *Circulation* 1992;85:1627-1629

25. Anumonwo JMB, Freeman LC, Kwok WM, Kass RS: Potassium channels in the heart:electrophysiology and pharmacological regulation. *Cardiovascular Drug Reviews* 1991;9:299-316

26. Weston AH, Edwards G: Recent progress in potassium channel opener pharmacology. *Biochem Pharmacol* 1992;43:47-54

27. Grover GJ, McCullough JR, Henry DE, Conder ML, Sleph PG: Anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker gliburide. *J Pharmacol Exp Ther* 1989;251:98-104

28. Grover GJ, Dzwonczyk S, Parham CS, Sleph PG: The protective effects of cromakalim and pinacidil on reperfusion function and infarct size in isolated perfused hearts and anesthetized dogs. *Cardiovasc Drugs Ther* 1990;4:465-474

29. Grover GJ, Dzwonczyk S, Sleph PG: Reduction of ischaemic damage in isolated rat hearts by the potassium channel opener, RP 52891. *Eur J Pharmacol* 1990;191:11-18

30. Auchampach JA, Maruyama M, Cavero I, Gross GJ: Pharmacological evidence for a role of ATP-dependent potassium channels in myocardial stunning. *Circulation* 1992;86:311-319

31. Wolleben CD, Sanguinetti MC, Siegl PKS: Influence of ATP-sensitive potassium channel modulators on ischemia-induced fibrillation in isolated rat hearts. *J Mol Cell Cardiol* 1989;21:783-788

32. Ferdinandy P, Koltai M, Tosaki A, Berthet Ph, Tarrade Th, Esanu A, Braquet P: Cicletanine improves myocardial function deteriorated by ischemia/reperfusion in isolated working rat hearts. *J Cardiovasc Pharmacol* 1992;19:181-189

33. Szilvassy Z, Koltai M, Szekeres L, Tarrade T, Braquet P: Effect of cicletanine on overpacing-induced ST-segment elevation in conscious rabbits. *Eur J Pharmacol* 1991;199:383-386

34. Burton T, Chakrabarty S, Fluck DS, Flores NA, Sheridan DJ: Effects of cicletanine on haemodynamics, arrhythmias and extent of necrosis during coronary ligation in rabbits. *Br J Pharmacol* 1992;107:1135-1139

35. Silver PJ, O'Connor B, Cumiskey WR, Van Aller G, Hamel L T, Bentley RG, Pagani ED: Inhibition of low Km cGMP phosphodiesterases and Ca++-regulated protein kinases and relationship to vasorelaxation by cicletanine. *J Pharmacol Exp Ther* 1991;257:382-391

36. Silver PJ, Buchholz A, Dundore RL, Harris AL, Pagani ED: Inhibition of low Km cyclic GMP phosphodiesterases and potentiation of guanylate cyclase activators by cicletanine. *J Cardiovasc Pharmacol* 1990;16:501-505

37. Szilvassy Z, Jakab I, Bor P, Koltai M, Tarrade T, Esanu A, Braquet PG, Lonovics J: Cicletanine attenuates overdrive pacing-induced global myocardial ischemia in rabbits: Possible role of cardiac cyclic nucleotides. *Coronary Artery Dis* 1993;4:443-452

38. Garay RP, Nazaret C, Diez J, Etienne A, Bourgain R, Braquet P: Stimulation of K+ fluxes by diuretic drugs in human red cells. *Biochem Pharmacol* 1984;33:2013-2020

39. Ebeigbe AB, Cabanie M: In vitro vascular effects of cicletanine in pregnancy-induced hypertension. *Br J Pharmacol* 1991;103:1992-1996

40. Tosaki A, Hellegouarch A, Braquet P: Cicletanine and reperfusion injury: Is there any correlation between arrhythmias, 6-keto-PGF1α, thromboxane B2, and myocardial ion shifts (Na+, K+, Ca++, and Mg++) induced by ischemia/reperfusion in isolated rat heart. *J Cardiovasc Pharmacol* 1991;17:551-559

41. Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-1136

42. Lawson CS, Downey JM: Preconditioning: State of the art myocardial protection. *Cardiovasc Res* 1993;27:542-550

43. Vegh A, Szekeres L, Parratt JR: Tranzient ischemia induced by rapid pacing results in myocardial preconditioning. *Cardiovasc Res* 1991;25:1051-1053

44. Marber MS, Walker DM, Eveson DJ, Walker JM, Yellon DM: A single five minute period of rapid atrial pacing fails to limit infarct size in the in situ rabbit heart. *Cardiovasc Res* 1993;27:597-601

45. Gross GJ, Auchampach JA: Blocade of ATP-sensitive potassium channels prevents myocardial preconditioning. *Circ Res* 1992;70:223-233

46. Liu Y, Downey JM: Ischemic preconditioning protects against infarction in rat heart. *Am J Physiol Heart Circ Physiol* 1992;263:H1107-H1112

47. Thornton JD, Thornton CS, Sterling DL, Downey JM: Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts. *Circ Res* 1993;72:44-49

48. Akins CW: Resection of left ventricular aneurism during hypothermic-fibrillatory arrest without aortic occlusion. *J Thorac Cardiovasc Surg* 1986;91:610-618

49. Tosaki A, Das DK: Reperfusion induced arrhythmias are caused by generation of free radicals. *Cardiovasc Res* 1994;28:422-422

50. Euler DE: Reperfusion induced arrhythmias are *not* caused by generation of free radicals. *Cardiovasc Res* 1994;28:423-423

51. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacological Reviews* 1991;43:109-142

52. Whong K, Yrhim B, Slee W, Rjeong B, Dkim C, Wshin Y: Release of superoxide-dependent relaxing factor(s) from endothelial cells. *Am J Physiol* 1989;257:H1340-H1346

53. Tosaki A, Bagchi D, Pali T, Cordis GA, Das DK: Comparisons of ESR and HPLC methods for the detection of OH radicals in ischemic/reperfused hearts. A relationship between the genesis of free radicals and reperfusion arrhythmias. *Biochem Pharmacol* 1993;45:961-969

54. Tosaki A, Blasig IE, Pali T, Ebert B: Heart protection and radical trapping by DMPO during reperfusion in isolated working rat heart. *Free Radic Biol Med* 1990;8:363-372

55. Ferdinandy P, Das DK, Tosaki A: Pacing-induced ventricular fibrillation leading to oxigen free radical formation in aerobically perfused rat heart. *J Mol Cell Cardiol* 1993;25:683-692

56. Selye H, Bajusz E, Grasso S, Mendell P: Simple techniques fore the surgical occlusion of coronary vessels in the rat. *Angiology* 1960;11:398-407

57. Vleeming W, Wouw PA, Biesebeek JD, Rooij HH, Wemer J, Porsius AJ: Density of beta adrenoceptors in rat heart and lymphocytes 48 hours and 7 days after acute myocardial infarction. *Cardiovasc Res* 1989;23:859-866

58. Fishbein MC, Maclean D, Maroko PR: Experimental myocardial infarction in the rat. *Am J Path* 1978;90:57-70

59. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. *Circ Res* 1980;47:1-9

60. Manning A, Mouton J, Chatelain P: Fantofarone (SR 33557): Effect on post-ischaemic functional recovery in perfused rat hearts. *Eur J Pharmacol* 1992;220:249-258

61. Van Wagoner DR: Mechanosensitive gating of atrial ATP-sensitive potassium channels. *Circ Res* 1993;72:973-983

62. Aksnes G: Why do ischemic and hypoxic myocardium lose potassium?. *J Mol Cell Cardiol* 1992;24:323-331

63. Tosaki A, Szerdahelyi P, Engelman RM, Das DK: Potassium channel openers and blockers: Do they possess proarrhythmic or antiarrhythmic activity in ischemic and reperfused rat hearts. *J Pharmacol Exp Ther* 1993;267:1355-1362

64. Grover GJ, Dzwonczyk S, Sleph PG, Sargent CA: The ATP-sensitive potassium channel blocker glibenclamide (glyburide) does not abolish preconditioning in isolated ischemic rat hearts. *J Pharmacol Exp Ther* 1993;265:559-564

65. Nielsen-Kudsk JE, Thirstrup S: Antidiabetic sulfonylureas relax isolated rabbit coronary arteries. *Eur J Pharmacol* 1991;209:273-275

66. Samaha FF, Heineman FW, Ince C, Fleming J, Balaban RS: ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone in vivo. *Am J Physiol* 1992;262:C1220-C1227

67. Yan G-X, Yamada KA, Kĺber AG, McHowat J, Corr PB: Dissociation between cellular K^+ loss, reduction in repolarization time, and tissue ATP levels during myocardial hypoxia and ischemia. *Circ Res* 1993;72:560-570

68. Quast U: Do the K^+ channel openers relax smooth muscle by opening K^+ channels. *Trends Pharmacol Sci* 1993;14:332-337

69. Noack T, Dietmer P: Effects of cicletanine on whole-cell currents of single smooth muscle cells from the guinea-pig portal vein. *Br J Pharmacol* 1993;109:164-170

70. Chabrier PE, Guinot P, Tarrade T, Auguet M, Cabanie M, Clostre F, Etienne A, Esanu A, Braquet P: Cicletanine, in Scriabine A (ed): Cardiovascular Drug Reviews. New York, Raven Press, 1988;pp 166-179

71. Szilvassy Z, Koltai M, Ferdinandy P, Jakab I, Lonovics J, Tarrade T, Allard M, Braquet PG: Cromakalim and cicletanine against pacing-induced myocardial ischemia in conscious rabbits. *Life Sci* 1994;54:PL125-PL130

72. Sakuta H, Okamoto K, Tandai M: Atrial natriuretic factor potentiates glibenclamide-sensitive K+ currents via activation of receptor guanylate cyclase in follicle-enclosed Xenopus oocytes. *Eur J Pharmacol* 1994;267:281-287

73. Bech MO, Dahr O, Diamant B, Steiness E: Time course of functional deterioration after coronary artery ligation in rats. *Cardiovasc Res* 1989;23:649-654

74. Epstein UJ, Patterson RE: Comparative effects of calcium-channel blocking agents on left ventricular function during acute ischemia in dogs with and without congestive heart failure. *Am J Cardiol* 1985;55:IIB-I6B

75. Jang TL, MacLleod BA, Walker MJA: Effects of halogenated hydrocarbon anasthemics on responses to ligatin of a coronary artery in chronically prepared rats. Anesthesiol. *Anesthesiol* 1983;59:309-15

76. Raya TE, Fonken SJ, Lee RW, Daughtery S, Goldman S, Wong PC, Timmermans PB, Morkin E: Hemodynamic effects of direct angiotensin II blocade compared to converting enzyme inhibition in rat model of heart failure. *American Journal of Hypertension* 1991;4:334s-340s

77. Ware WA, Muir WW, Swanson C: Effects of amiodarone on myocardial performance in normal canine hearts and canine hearts with infarcts. *Am J Vet Res* 1992;52:891-897

78. Feuerstein GZ, Hamburger SA, Smith EFIII, Bril A, Ruffolo RRJr: Myocardial protection with carveridol. *J Cardiovasc Pharmacol* 1992;19:s138-s141

79. Szilvassy Z, Jakab I, Bor P, Koltai M, Tarrade T, Droy-Lefaix MT, Esanu A, Braquet P, Allard M, Lonovics J: Cicletanine-induced anti-ischemic effect in overpaced rabbits: possible relation to changes in cardiac cyclic nucleotide contents. *Coronary Art Dis* 1993;(in press)

80. Levy BI, Curmi P, Poitevin P, Safar ME: Modifications of the arterial mechanical properties of normotensive and hypertensive rats without arterial pressure changes. *J Cardiovasc Pharmacol* 1989;14:253-259

81. Walker MJA, Beatch GN: Electrically induced arrthythmias in the rat. *Proc West Pharmacol Soc* 1988;31:167-178

82. Kirsch GE, Codina J, Birnbaumer L, Brown AM: Coupling of ATP-sensitive K channels to A1 receptors by G proteins in rat ventricular myocytes. *Am J Physiol* 1990;259:H820-H826

83. Mullane K: Myocardial preconditioning. Part of the adenosine revival. *Circulation* 1992;85:845-847

84. Szilvassy Z, Jakab I, Ferdinandy P, Koltai M, Lonovics J, Tarrade T, Braquet PG: Zaprinast, cicletanine, and verapamil attenuate overdrive pacing-induced myocardial ischemia in conscious rabbits. *Life Sci* 1993;53:PL13-PL18

85. Chi L, Uprichard ACG, Lusshesi BR: Profibrillatory actions of pinacidil in a conscious canine model of sudden coronary death. *J Cardiovasc Pharmacol* 1990;15:452-464

86. Fralix TA, Steenbergen C, London RE, Murphy E: Glibenclamide does not abolish the protective effect of preconditioning on stunning in the isolated perfused rat heart. *Cardiovasc Res* 1993;27:630-637

87. Ten Eick RE, Whalley DW, Rasmussen HH: Connections: Heart disease, cellular electrophysiology, and ion channels. *FASEB J* 1992;6:2568-2580

88. Lamonatagne D, Pohl U, Busse R: The Mechanical Activity of the Heart Stimulates Endothelium-Derived Relaxing Factor Release in the Coronary Vascular bed. *J Cardiovasc Pharmacol* 1991;17:S89-S94

89. Cooke JP, Rossitch Jr E, Andon NA, Loscalzo J, Dzau VJ: Flow Activates an Endothelial Potassium Channel to Release an Endogenous Nitrovasodilator. *J Clin Invest* 1991;88:1663-1671

90. Ohno M, Gibbons GH, Dzau VJ, Cooke JP: Shear stress elevates endothelial cGMP: Role of a potassium channel and G protein coupling. *Circulation* 1993;88:193-197

91. Bhagat BD, Pao PS, Dhalla NS: Risk of catecholamines in the genesis of arrhythmias. *Adv Myocardiol* 1980;2:117-121

92. Werns SW, Shen MJ, Lucchesi BR: Free radicals in the ischemic myyocardial injury. *Free Radic Biol Med* 1985;1:103-114

93. Koretsune Y, Marban E: Cell calcium in the pathophysiology of ventricular fibrillation and in the pathogenesis of postarrhythmic contractile dysfunction. *Circulation* 1989;80:369-379

94. Otani H, Engelman RM, Rousou JA, Breyer RH, Das DK: Enhanced prostaglandin synthesis due to phospholipid breakdown in ischemic-reperfused myocardium: control of its production by a phospholipase inhibitor or free radical scavengers. *J Mol Cell Cardiol* 1986;18:953-961

7. Abbreviations

AF	-Aortic flow
CF	-Coronary flow
DMSO	-Dimethyl-sulfoxide
ESR	-Electron spin resonance
HR	-Heart rate
K_{ATP}	-ATP sensitive potassium channel
LVDP	-Left ventricular developed pressure
LVEDP	-Left ventricular end-diastolic pressure
LDH	-Lactate dehydrogenase
PDE	-Phosphodiesterase
PVC	-Premature ventricular contraction
VF	-Ventricular fibrillation
VOP	-Ventricular overdrive pacing
VT	-Ventricular tachycardia