Effect of Different Potassium Channel Inhibitors on the Outcome of Experimental Coronary Artery Occlusion

PhD thesis

Nasruddin Emhemed El-Reyani, MSc (Pharm)

Department of Pharmacology and Pharmacotherapy

University of Szeged

Szeged, Hungary

2000

Summary

Despite the development of new therapeutic strategies, cardiovascular diseases, principally ischaemic heart disease and myocardial infarction remains among the leading causes of mortality world-wide. Antiarrhythmic therapy has a significant role in the treatment of this disorder. The aim of this study was to investigate the possible role of potassium channels in the prevention of cardiac arrhythmias and in the development of myocardial infarction. Therefore, in coronary artery ligated animals we compared the effect of two ATP-sensitive potassium channel inhibitors, glibenclamide and glimepiride; and studied the efficacy of a newly developed combined potassium and sodium channel inhibitor, GYKI-16638.

The effect of glibenclamide and glimepiride was investigated on the development of reperfusion-induced arrhythmias and it was correlated to their blood glucose lowering action. Myocardial ischaemia-reperfusion induced arrhythmias were produced in anaesthetised, male Sprague-Dawley rats by occlusion of the left main coronary artery for 6 min, followed by 5 min reperfusion. Glimepiride pretreatment (0.001-0.01-0.1-5.0 mg/kg i.p., 30 min before coronary occlusion) significantly decreased the incidence of irreversible ventricular fibrillation and increased the survival rate during reperfusion (64%, 61%, 60%, and 67% vs. 27% in controls). Glibenclamide produced similar effect (81% survival) only in a dose of 5 mg/kg, while smaller doses were ineffective. The minimal hypoglycaemic dose and the dose required to inhibit the hyperglycaemia induced by oral glucose loading were similar after glibenclamide and glimepiride. It is concluded from these experiments that although the blood glucose lowering potency of glibenclamide and glimepiride is rather similar, glimepiride appears to be more potent than glibenclamide in preventing reperfusion induced cardiac arrhythmias.

The effect of glibenclamide and glimepiride was also investigated on the development of myocardial infarction. Permanent coronary artery ligation was performed in rats and the development of infarction was evaluated by a computer-assisted method after nitroblue-terazolium staining. Seven-day coronary ligation produced enlargement of the left ventricular cavity, scar thinning and thickening of the non-infarcted myocardium. Glibenclamide treatment (5 mg/kg b.i.d. intraperitoneally) decreased the infarct volume (29.1±3.5 % vs. 39.1±3.2 % in controls), that occurred primarily as a result of more significant thinning of the scar tissue (1.6±0.04 mm vs. 2.0±0.13 mm in controls).

Glibenclamide also inhibited the thickening of the non-infarcted ventricular septum (2.1±0.10 mm vs. 2.9±0.10 mm in controls). In contrast to the effects of glibenclamide, glimepiride treatment (5 mg/kg b.i.d. intraperitoneally) inhibited the enlargement of the left ventricular cavity (15.2±1.1 % vs. 19.9±1.2 % of the left ventricular volume in controls), it did not precipitate scar thinning and did not influence the development of hypertrophy of the non-infarcted myocardium. These results suggest that glimepiride treatment might inhibit the development of left ventricular dilatation after myocardial infarction. Glibenclamide treatment, however, producing a thinning of the scar tissue may further precipitate morphological changes that can contribute to the development of heart failure.

The effect of GYKI-16638 (*N*-[4-[2-*N*-methyl-*N*-[1-methyl-2-(2,6-dimethylphenoxy) ethylamino]-ethyl]-phenyl]-methanesulfonamide hydrochloride), a novel antiarrhythmic compound, was assessed on arrhythmias induced by 10 min of coronary artery occlusion and 10 min of reperfusion in anaesthetised rabbits. GYKI-16638 (0.03 and 0.1 mg/kg i.v.) significantly increased survival during reperfusion (79 % and 100 %, vs. 33 % in controls, $P<0.05$, respectively). GYKI-16638 in a dose of 0.1 mg/kg significantly increased the number of animals that did not develop arrhythmias during reperfusion (46 % vs. 0 % in controls, $P<0.05$). These results suggest that in rabbits GYKI-16638 has an in vivo antiarrhythmic effect which can be best explained by its combined Class I/B and Class III antiarrhythmic actions.

Our results confirm that equally hypoglycaemic ATP-sensitive potassium channel inhibitors do not possess the same antiarrhythmic efficacy. Further, the combination of sodium- and potassium channel inhibition by a single molecule may provide advantages over highly selective potassium channel blockers in the prevention of cardiac arrhythmias.

Contents

List of full papers related to the thesis

I. **EL-Reyani N**, Bozdogan Ö, Baczkó I, Leprán I, Papp JGy. Comparison of the efficacy of glibenclamide and glimepiride in reperfusion-induced arrhythmias in rats. *Eur J Pharmacol* 1999, **365**: 187-192. Impact factor: 2.047

II. Backó I, **EL-Reyani N**, Farkas A, Virág L, Jost N, Leprán I, Varró A, Papp JGy. Antiarrhythmic and electrophysiological effects of GYKI-16638, a novel N-(phenoxyalkyl)-N-phenylalkylamine in rabbits. *Eur J Pharmacol* 2000, **404**: 181-190. Impact factor: 2.047

III. **EL-Reyani N**, Baczkó I, Leprán I, Papp JGy. Effect of glibenclamide and glimepiride treatment on the development of myocardial infarction in rats. *Acta Physiol Hung* 2000, (***in press***). Impact factor: 0.208

Quotable abstracts at international conferences

IV. **EL-Reyani N**, Bozdogan Ö, Baczkó I, Leprán I, Papp JGy. A comparison for the blood glucose lowering and the anti-arrhythmic effects of glibenclamide and glimepiride in rats. *J Kardiologie* 1997, **2**: 81.

V. **EL-Reyani N**, Leprán I, Papp JGy. Time dependent effect of glibenclamide on the infarct size in rats. *Cardiologia Hungarica* 1998, **98/1**: 31.

VI. **EL-Reyani N**, Leprán I, Papp JGy. Effect of glibenclamide on the development of infarct size in rats. *J Mol Cell Cardiol* 1998, **30**: 539.

VII. **EL-Reyani N**, Leprán I, Papp JGy. Glibenclamide inhibits left ventricular hypertrophy following persistent coronary artery occlusion in rats. *Fund Clin Pharmacol* 1999, **13** (1): PS20.

1. Introduction

Despite growing knowledge in the understanding of cardiovascular physiology and pathophysiology and the development of new therapies in the 20th century, cardiovascular diseases principally ischaemic heart disease (IHD) and myocardial infarction (MI), remain the leading cause of mortality world-wide. In the United States, coronary artery disease results in approximately 1.5 millions myocardial infarction and causes 500,000 fatalities each year (Heart and Stroke Facts, 1997). In developing countries including Libya, a significant number of patients die in similar circumstances. The major cause of sudden death is attributed to the development of ventricular arrhythmias shortly after MI (Josephson *et al.*, 1978; Wit and Janse, 1993; William, 1998). Moreover, when ventricular arrhythmias are frequent in survivors of MI, the relative risk of cardiovascular death is increased 2- to 4-fold (Bigger *et al.*, 1984; Maggioni, 1993). The failure of antiarrhythmic drugs to counteract these arrhythmias may be explained, in part, by lack of specificity and because of several mechanisms involved in the development of arrhythmias.

Patients that survived the acute stage of MI are at high risk of developing necrosis and slowly progressing ventricular hypertrophy (Dawber, 1980; Kannel *et al.*, 1987). It has been consistently shown that left ventricular hypertrophy (LVH) is strongly associated with increased mortality of coronary heart disease in both clinical based studies (Casale *et al.*, 1986; Sullivan *et al.*, 1993; Levy *et al.*, 1994) and population-based investigations (Levy *et al.*, 1989; 1990).

Therefore, strategies relating to particular classes of drugs to prevent the occurrence of early as well as late events of MI, with particular attention to the resulting arrhythmias and progression of left ventricular hypertrophy have been developed. Once this approach is accomplished, both sudden cardiac death due to arrhythmias and heart failure due to ventricular hypertrophy could be decreased.

1. 1. Myocardial ischaemia and progression of myocardial infarction

Sudden interruption of blood supply to the myocardium results in a spectrum of derangements, ranging from transient reversible stunning of the myocardium and arrhythmias to severe irreversible changes i.e. infarction. The alterations in metabolic substances,

particularly an increase in intracellular calcium and formation of oxygen free radicals may contribute largely to the evolvement of ischaemic injury (Blaustein *et al.*, 1986; Basu *et al.*, 1987). Amongst the other metabolic disturbances occurring during ischaemia are the reduction in oxidative phosphorylation (Simon *et al.*, 1997), depletion of gluthatione (Patterson *et al.*, 1988), an increase in cellular free fatty acids (Fiskum *et al.*, 1983), myocardial acidosis (Johnson *et al.*, 1995), polymorphnuclear leukocytes' accumulation (Kevin *et al.*, 1984), high non-physiological levels of catecholamines (Wheatly *et al.*, 1985; Metsuki *et al.*, 1990), accumulation of intracellular unesterified arachidonic acid, release of chemical mediators such as prostaglandins, prostacyclines and thromboxane A_2 (Klein *et al.*, 1987; Vesterqvist, 1988; Yamamoto *et al.*, 1999). All these have additive effects in favour of cellular damage. While no single process has been identified as the critical factor leading to ischaemic injury, the failure of cellular homeostasis characterised by loss of ion gradients across the cell membrane may play a critical factor (McCord, 1985).

Occlusion of a main coronary artery results predominantly in a loss of function of the supplied myocardium (Katz, 1973). The ischaemic cells are deprived of the energy needed to maintain ionic gradients and homeostasis and failure of enzyme systems that finally leads to cell death (Rhodes et al., 1980). Later, this phenomenon results in a restructuring of the geometry of the remaining viable myocardium which is called upon to maintain cardiac performance. This reaction has been found to be characterised by infarct area extension and non-infarct area dilatation, both being considered the main components of left ventricular remodelling (Bassand, 1995). Extension of the infarcted area occurs within hours following the onset of myocardial infarction, whereas, hypertrophy of the non-infarcted myocardium is a more prolonged and durable process (Bassand, 1995). Unless treated, long-term dilatation can eventually result in alteration of the contractile properties of the non-infarcted myocardium and impairment of systolic and diastolic performance of the left ventricle-the hallmark of congestive heart failure (Hochman *et al.*, 1982; Pfeffer *et al.*, 1990).

1. 2. Reperfusion injury

Re-establishing the blood flow to previously ischaemic tissue has a double-edged sword effect. On one hand, supply of oxygen and nutrients is restored and toxic metabolites are removed and recovery from ischaemic injury is established. On the other hand, the return of toxic metabolites to the circulation may have serious metabolic consequences (Haimovici, 1979). Furthermore, upon reperfusion, the myocardial function is often markedly impaired by

what is now recognised as a distinct pathologic process, referred to as "ischaemia/reperfusion injury" (Parks and Granger, 1986; Edward *et al.*, 1997).

Although early reperfusion of the ischaemic myocardium may protect the cells from further damage, it may on the other hand be associated with a host of distinctive pathophysiologic derangement, the most important of which are reperfusion arrhythmias (Sewell *et al.*, 1955). These arrhythmias occur in experimental animals after reperfusion following transient ischaemia, and there is considerable evidence that this derangement also develops in humans following surgical procedures, during thrombolysis therapy or even in spontaneously released coronary artery spasm (Tzivoni *et el.*, 1983; Pogwizd and Corr, 1987; Hearse, 1990; Baxter and Yellon, 1993; Lu *et al*; 1995). Although, the pathogenesis of reperfusion arrhythmias has not been conclusively established, impairment of cellular energy stores and cellular alterations that induce changes in the activity of channels, pumps and exchangers responsible for the potassium and calcium ions homeostasis are essential prerequisites for ischaemia/reperfusion-induced life threatening arrhythmias. It has been suggested that following coronary occlusion the intracellular potassium content declines which results in extracellular potassium accumulation, sometimes to very high levels (Kleber, 1983; Janse and Wit, 1989; Wild *et al.*, 1990). The accumulation of potassium and its heterogeneity are both considered to contribute to the evolution of rhythm disturbances during ischaemia and may well be critical during reperfusion (Hill and Gettes, 1980). As a consequence, the homogeneity in action potential duration is markedly altered in and around the previously ischaemic zone immediately after reperfusion (Coronel *et al.*, 1992). These changes in turn cause a dispersion of refractoriness between ischaemic and normal tissue, which contributes to the electrophysiological substrates for reentrant (Russel and Oliver, 1978, Janse *et al.*, 1986) and non-reentrant arrhythmias (Pogwizd and Corr 1987). Priori *et al.*(1990), showed that more than 70% of the arrhythmias induced by reperfusion in the anaesthetised cat are explained by non-reentrant mechanism, possibly due to enhanced automaticity or triggered arrhythmias. Other factors such as accumulation of cathecolamines (Bralet *et al.*, 1985; Dimassi *et al.*, 1992), production of free radicals (Manning *et al.*, 1984; Woodward and Zakaria, 1985), and generation of phospholipid metabolites (Arnsdorf and Sawacki, 1981; Akita *et al.*, 1986; McHowatt *et al.*, 1993) also play a major role in the development of arrhythmias.

Although numerous pharmacological methods are currently available to investigate the role of different channels, pumps and exchangers, no single approach has proved to be

consistently effective in limiting arrhythmias. However, replenishing of high energy molecules and amelioration of early potassium efflux have been appreciated as promising tools in preventing life-threatening ventricular arrhythmias.

1. 3. Myocardial potassium channels

At present, at least eight distinct potassium channels have been identified in the heart and proposed to play a role in shaping the cardiac electrophysiology. These include both rapidly and slowly activating subtypes of the delayed rectifier potassium channel, the background (inward rectifier) potassium channel, a voltage-dependent transient outward current, a Ca^{2+}-activated transient outward current, a high-conductance plateau channel, and potassium channels regulated by ATP and acetylcholine. In addition, a potassium channel activated by extremely high intracellular sodium ion concentrations has been described, but its physiologic role remains uncertain. These channels can vary markedly in their distribution within different regions of the heart, as well as from species to species. Related to the scope of the present study, emphasis will be focused on myocardial K_{ATP} channels.

1. 3. 1. Myocardial ATP sensitive potassium (K_{ATP}) channels

1. 3. 1. 1. Physiological and pathophysiological roles

Since its first description in cardiac myocytes by Noma (1983), the physiological and pathophysiological roles for potassium channels sensitive to intracellular ATP concentration (K_{ATP}) have been suggested to play a complex role in the cellular strategy in many tissues. These channels were found in pancreatic β-cells (Ashcroft *et al.*, 1984; Rorsman and Trub, 1985), myocardium (Noma, 1983), skeletal muscle cells (Spruce *et al.*, 1987), smooth muscle (Quast and Cook, 1988) and neurons (Bernardi *et al.*, 1988). In the resting pancreatic β-cells, where their physiological role is better understood, the K_{ATP} channels are usually active at low blood glucose, and they set the membrane potential close to the K^+ equilibrium potential, thereby reducing their excitability and inhibition of insulin secretion. However, upon an increase in blood glucose level, the intracellular ATP rises which results in closure of K_{ATP} channels, thereby depolarising the plasma membrane of β-cells. Such depolarisation increases intracellular calcium concentration largely through the activation of voltage-gated Ca^{2+} channel which in turn triggers exocytosis of insulin granules, and hence stimulates the release of insulin (Sturgess *et al.*, 1985; Dunne and Petersen, 1991; Ashcroft and Ashcroft, 1992).

In the heart - where their role is still under debate - K_{ATP} channels have been implicated in the cellular electrophysiological behaviour and ionic homeostasis that occur during various forms of metabolic stress, including ischaemia, hypoxia, and inhibition of glycolysis and/or oxidative phosphorylation (Faivre and Findlay, 1990; Nicholas *et al.*, 1991; Findlay, 1994). Opening of K_{ATP} channels has been proposed as one of the main cardioprotective mechanisms underlying ischaemia-related preconditioning (Downey, 1992; Cole, 1993; Parratt and Kane, 1994). During early ischaemia, opening of K_{ATP} channels and its consequences may lead to conditions that promote the induction of cardiac arrhythmias (Gasser and Vaughan-Jones, 1990; Wild, 1993; Wild and Janse, 1994). There is considerable evidence that K_{ATP} channel activation might also exacerbate the disturbance of the cellular ionic homeostasis and contribute to the failure of cellular electrophysiological state to recover upon reperfusion (Weiss and Venkatesh, 1993).

Recently, the molecular structure of K_{ATP} channels has been clarified. The K_{ATP} channel in pancreatic β-cells is a complex composed of at least two subunits, a member of inwardly rectifying K^+ channels and a sulphonylurea receptor (Inagaki *et al.*, 1996; Miki *et al.*, 1999). Subsequently, two additional homologs of the sulphonylurea receptor, which form cardiac and smooth muscle type K_{ATP} channels, respectively, have been reported (Isomoto *et al.*, 1996; 1997). This study focuses attention on cardiac K_{ATP} channels that can serve as a paradigm with particular emphasis on the better understanding of K_{ATP} channel inhibition.

1. 3. 2. Effects on potassium homeostasis and action potential duration

Myocardial ischaemia disrupts the cellular electrophysiological properties through alterations in the intracellular homeostasis of the involved cells. As a result, the intracellular concentrations of several ions are perturbed that finally culminate in the formation of malignant arrhythmias (Opie *et el.*, 1979). Various chemical substances have been implicated as causative factors in the genesis of these arrhythmias during myocardial ischaemia, extracellular potassium being the predominant among them.

One of the most prominent changes observed during acute ischaemia is the rise in the concentration of K^+ in the extracellular space $[K^+]_o$ as early as within the first 15 seconds and reaching a plateau within 5-10 minutes after coronary occlusion. Two major mechanisms have been emerged to explain the $[K^+]_o$ rise. (1) As a consequence of the inhibition of oxidative

metabolism the cytosolic concentration of lactate and inorganic phosphate increases and these ions subsequently leave the cell. K^+ is thought to be coupled to this anion efflux (via an unknown pathway) to balance the charge movement (Kléber, 1983; Yan *et al.*, 1993); (2) The increase in $[K^+]_o$ also occurs via opening of K^+ channels thereby generating a sufficiently high potassium conductance. K_{ATP} channels are the most likely ones among the various K^+ channels responsible for K^+ loss during ischaemia (Smallwood *et al.*, 1990; Gross *et al.*, 1992; Vanheel and de Hemptinne, 1992). Grover *et al.* (1989) and Spinelli *et al.* (1990) have suggested that by shortening of the action potential duration, K_{ATP} channel opening inhibits voltage dependent Ca^{++} entry into the myocardium, resulting in a decrease of energy consumption and in a decrease of Ca^{++} loading during the ischaemic period. This mechanism could reduce and perhaps limit further damage of the myocardium. This assumption has been supported by Noma (1983), and also by Leprán et al. (1996) and Baczko et al. (1997).

Under conditions where K_{ATP} channels mediate the ischaemic K^+ efflux, glibenclamide, an inhibitor of K_{ATP} channels, has been shown to reduce $[K^+]_o$ accumulation in isolated tissue (Venkatesh *et al.*, 1991; Hicks and Cobbe, 1991) and in regionally or globally ischaemic hearts (Wilde *et al.*, 1990; Kantor *et al.*, 1990; Hamada *et al.*, 1998), whereas nicorandil, a potassium channel opener, significantly accentuated the initial increase in $[K^+]_o$ during global ischaemia in isolated perfused rat hearts (Mitani *et al.* 1991).

In certain conditions during regional ischaemia the administration of potassium channel openers has been shown to be proarrhythmic (Chi *et al.*, 1990). Presumably because of using higher doses of K_{ATP} openers these agents decreased refractoriness of the tissue, whereas glibenclamide has been shown to be markedly antiarrhythmic (Wolleben *et al.*, 1989; Kantor *et al.*, 1990). Thus, although activation of K_{ATP} channels might be expected to oppose spontaneous activity due to increased automaticity (e.g., early or late afterdepolarization), it may exacerbate arrhythmias due to conduction block (e.g., reentrant arrhythmias) (Haverkamp *et al.*, 1995).

1. 4. Potassium channel inhibitors

Potassium channels are ubiquitous and the pharmacology of the different types of potassium channels in various cells has not been fully delineated. It is therefore possible that

some of the potassium channel blockers, developed as potential sub-class specific agents, may exert effects in other tissues as well. There are currently a number of molecules, some of them are in clinical use and others at various stages of clinical and pre-clinical development, that inhibit potassium channels. In the present study, emphasis will be placed on the second generation sulphonylureas, glibenclamide and glimepiride as inhibitors of K_{ATP} channels and on a recently developed antiarrhythmic agent GYKI-16638, that reputed to have novel mechanisms of action or antiarrhythmic profile.

1. 4. 1. K_{ATP} channel inhibitors

Inhibitors of K_{ATP} channels have been invaluable in studying K^+ channel function and to investigate the biochemical properties of this channel. The sulphonylurea class of compounds is the best known and most effective in inhibiting K_{ATP} channels, and their use has been central to the understanding of this channel.

Sulphonylureas are used clinically to treat non-insulin-dependent diabetes mellitus (type-II, NIDDM). The hypoglycaemic effect of these compounds was attributed to their ability to bind with high affinity to a subunit of the K_{ATP} channel, known as the sulphonylurea receptor (SUR) (Isomoto *et al.*, 1996). Occupancy of this receptor by a sulphonylurea inhibits flux of K^+ through the channel pore, depolarizing the plasmalemma and inducing release of insulin. In the heart, Belles *et al.* (1987) and Fosset *et al.* (1988) have shown that sulphonylureas may act, although at higher concentrations, by binding to a specific site on the K_{ATP} channel or to a very closely associated protein. These drugs, among which glibenclamide is the most potent, can inhibit the channel from inside or outside (Zunkler *et al.*, 1989).

During myocardial ischaemia, sulphonylureas, such as glibenclamide, have been reported to block the opening of cardiovascular K_{ATP} channels with high specificity (Sturgess *et al.*, 1985; Fosset *et al.*, 1988). This property significantly modifies the outcome of experimental myocardial infarction. In isolated perfused hearts during regional ischaemia and/ or reperfusion, it has been shown in a number of studies that inhibition of the opening of K_{ATP} channels by glibenclamide is antiarrhythmic and prevents the shortening of action potential duration representing a class III antiarrhythmic effect (Wolleben *et al.*, 1989; Kantor *et al.*, 1990; Zhang *et al.*, 1991; Tosaki *et al.*, 1993; D'Alonzo *et al.*, 1994). In this context, Bril *et al.* (1992) and Rees and Curtis (1995) have shown an increase in the incidence of spontaneous recovery from ventricular fibrillation. Moreover, Linz *et al.* (1994) have reported similar

results and showed glibenclamide to be protective against reperfusion-induced arrhythmias in isolated working rat hearts. In globally ischaemic rat heart, glibenclamide was found to antagonize ventricular fibrillation (Kantor *et al.*, 1987; Wolleben *et al.*, 1989).

There are also in vivo results which coincide with these observations (Ballagi-Pordany *et al.*, 1990; Bekheit *et al.*, 1990; Billman *et al.*, 1993; Kondo *et al.*, 1996). Previously, we demonstrated in in vivo conditions that glibenclamide pretreatment increased the survival rate and decreased the incidence of life-threatening arrhythmias during acute myocardial infarction in conscious rats (Leprán *et al.*, 1996) or during ischaemia/reperfusion in anaesthetised rats (Baczkó *et al.*, 1997; EL-Reyani *et al.*, 1999). However, opposite results have also been presented. For instance, glibenclamide was found to be devoid of an effect on the incidence of ventricular fibrillation developing in response to a secondary insult in anaesthetised dogs with recent myocardial infarction (Chi *et al.*, 1989), or in vitro against reperfusion-induced arrhythmias (Cole *et al.*, 1991; Bernauer, 1997).

Recently, a newly developed sulphonylurea compound, glimepiride has been shown to be more potent than glibenclamide as an antidiabetic agent (Geisen, 1988; Langtry and Balfour, 1998), while producing less adverse effects in the cardiovascular system (Geisen *et al.*, 1996).

The potential harmful effects of K_{ATP} channel inhibitors in inhibiting ischaemic 'preconditioing' and enlarging infarct size have been reported with relatively little attention. Pharmacological doses of glibenclamide have been shown to increase myocardial infarct size (Auchampach *et al.*, 1992; Munch-Ellingsen *et al.*, 1996), and to block the infarct size limiting effect of K_{ATP} openers (Grover *et al.*, 1989; Auchampach *et al.*, 1991), however, these effects were apparent when glibenclamide was given before the ischaemic insult in animals pretreated with K_{ATP} channel activators. Most of these studies involved the use of occlusion-reperfusion induced myocardial cellular damage and the examination of the effect of an intervention after reperfusing the ischaemic myocardium when the process of infarction is still incomplete (Thornton *et al.*, 1993; Munch-Ellingsen *et al.*, 1996). Many investigations showed that K_{ATP} channel inhibitors abolish the protective action of preconditioning on the development of infarct size after coronary artery occlusion and reperfusion (Grover *et al.*, 1992; Rohmann *et al.*, 1994; Miura *et al.*, 1995; Schultz *et al.*, 1997). However, the long-term effects of K_{ATP} channel inhibitors on the development of myocardial infarction have not been investigated.

1. 4. 2. Class III amiodarone like action

The unfavorable interaction between myocardial ischaemia and Class 1c antiarrhythmic drugs is well established. Experimental data indicate that class 1c drugs exhibit profibrillatory effects in the presence of myocardial ischaemia (Aupetit *et al.*, 1997; Bui-Xuan *et al.*, 1997). Subanalysis of the CAST data also suggest that the interaction between active ischaemia and treatment with flecainide or encainide may have been responsible for the increased mortality in the treatment group (Greenberg *et al.*, 1995).

Class III antiarrhythmic drugs that prolong cardiac repolarization without slowing conduction are now the preferred antiarrhythmic medical treatment for tachyarrhythmias (O'Callaghan and McGovern, 1996; Hohnloser and Woosley, 1994). However, there exists no doubt about the proarrhythmic potential of selective class III drugs. Indeed, in the SWORD trial, D-sotalol given to patients with a diminished left ventricular systolic function and a recent or remote myocardial infarction has been found to increase mortality by 4.6% versus 2.7% in the placebo group (Waldo *et al.*, 1996).

Accordingly, special attention has been paid to antiarrhythmic drugs with complex effects on different ion channels and receptors. These include D,L-sotalol (a delayed rectifier potassium channel and β-adrenergic receptor blocker) and amiodarone (a potassium channel blocker possessing sodium and calcium channel blocking properties and anti-adrenergic activity). Amiodarone has been shown to exert strong antiarrhythmic effect in a number of studies and currently is considered to be one of the most efficacious antiarrhythmic drugs available in the medical practice. The long-term treatment with amiodarone, however, leads to the development of serious extracardiac side effects (Hilleman *et al.*, 1998). Therefore, it seems worthwhile to pursue the development of novel amiodarone-like compounds with marked antiarrhythmic potency but without unwanted extracardiac side effects.

GYKI-16638 (N-[4-[2-N-methyl-N[1[methyl-2-(2,6-dimethylphenoxy)ethylamino]-ethyl]-phenyl]-methanesulfonamide hydrochloride) is a novel N-(phenoxyalkyl)-N-phenylalkylamine that has been developed recently. Although it is not an amiodarone congener, based on its chemical structure the compound was expected to show amiodarone-like electrophysiological effects, i.e. both Class I/B and Class III properties.

1. 5. Purpose of the study

The main aim of the present study was as follows:

(i) To evaluate the possible contributions of K_{ATP} channels to the development of myocardial infarction. For this purpose we intended to use two experimental models: (a) a model of short regional myocardial ischaemia and reperfusion to investigate the developed arrhythmias, (b) a model of experimental myocardial infarction after permanent coronary artery occlusion in the rat, in order to measure directly and compare the effectiveness of two K_{ATP} channel inhibitors, glibenclamide and glimepiride, in inhibiting the development of myocardial infarction at day 1 (when the process of infarction is still incomplete) and day 7 (when the healing process is nearly complete) in metabolically healthy rats.

(ii) To assess the efficacy of a newly developed amiodarone-like compound, GYKI-16638 in coronary artery occlusion and reperfusion induced arrhythmias in anaesthetised rabbits. Because GYKI-16638 combines class IB and class III properties and because of the scarcity of delayed rectifier potassium channels in the rat, we intentionally used rabbits owing to their ubiquitous potassium rectifier currents, all of which are implicated in shaping the cardiac action potential.

2. Materials and Methods

2. 1. Animals

Male Sprague-Dawley CFY rats and male rabbits with an average weight of 300 to 350 g and 2 to 3 kg, respectively, were used. The animals were fed standard laboratory food pellet (Altromin, Gödöllö, Hungary) and were allowed to drink tap water ad libitum. The handling of animals and the investigations conform with the protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Albert Szent-Györgyi Medical Center (Szeged, Hungary).

2. 2. Blood glucose determination in conscious rats

In conscious rats a single drop of blood was taken by cutting the end of the tail. Blood glucose concentration was measured using a med-strip test (One Touch II, Lifescan, Johnson & Johnson, USA). A series of blood samples were taken before treatment, 30 minutes after intraperitoneal drug treatment and 30 min after oral administration of 1 g/kg glucose in 5 ml/kg tap water.

2. 3. Acute myocardial ischaemia and reperfusion in anaesthetised rats

2. 3. 1. Surgical preparation

The animals were anaesthetised with sodium pentobarbitone (60 mg/kg in a volume of 2 ml/kg) intraperitoneally. The skin of the animales was removed with a midline incision from below sternum to neck notch to allow surgery. The carotid artery was gently detached from the nerve and fat and cannulated for measuring the blood pressure using a pressure transducer (Gould-Statham, P23ID, Hugo Sachs Electronik, March-Hugstetten, Germany). Blood pressure was recorded on an oscillographic recorder (Watanabe, WTR 331, Hugo Sachs Electronik). The catheter was filled with saline that contained heparin (500 IU/ml), but the animal was not heparinized. Tracheotomy was performed and a polyethylene tube with a convenient diameter was inserted for artificial respiration later. The left pectoral muscles were separated gently, followed by opening the chest wall in the fourth intercostal space, approximately 2 mm left to the sternum. After incising the pericardium, the heart was eased out of the chest, using gentle pressure on the rib cage. After pushing aside the auricle with a small plastic strip, a 5-0 braided non-absorbable suture (Ethibond 5/0, Ethicon Ltd., United Kingdom) attached to a 16 mm micropoint reverse cutting needle (MERSILK W582, ETHICON) was passed under the left main coronary artery, approximately 2 mm from its

origin, as described by Selye *et al.* (1960) and modified by Baczkó *et al.* (1997). A small plastic button was threaded through the ligature and placed in contact with the heart. The heart was set back in its place and artificial respiration was immediately started using positive pressure ventilation via previously inserted tracheal tube connected to a volume-cycled respirator (Harvard rodent ventilator, model 683, Harvard apparatus, Southnatick, MA, USA). The tidal volume was set for approximately 2.5-2.9 cm^3 (depending on the body weight), and the respiratory rate was maintained at 60 cycles/min. The standard electrocardiogram (lead II, ECG) was recorded using subcutaneous needle electrodes. Both ends of the ligature were led out of the thoracic cavity through a flexible tubing. The artery could then be occluded by applying tension on the tube towards the button and fixed by clamping with artery forceps. The reperfusion was achieved by releasing the clamp with gentle backward pulling of the tube.

2. 3. 2. Experimental protocol

After surgical procedures the animals were allowed to equilibrate for 10 min to stabilise haemodynamic parameters before coronary occlusion. Animals in which this procedure produced arrhythmias or caused a sustained fall in blood pressure to less than 80 mmHg, were excluded from the final evaluation.

Baseline haemodynamic parameters were measured before coronary artery ligation, after 1, 3, and 5 min coronary artery ligation and 1, 3, and 5 min after reperfusion. Arrhythmias were detected and diagnosed in accordance with the Lambeth conventions as ventricular tachycardia, ventricular fibrillation and other types of arrhythmia including single extrasystoles, bigeminy, salvos and bradycardia (Walker et al., 1988).

At the termination of the experiment, heparin (500 IU/kg) was given intravenously via the femoral vein. The heart was rapidly excised and placed in isotonic NaCl solution. The left coronary artery was retightened and the heart was perfused retrogradely with isotonic NaCl solution (10 ml), followed by perfusion with ethanol (2ml) via the aorta for demarcation of occluded (appeared dark red) and the non occluded (white colour) myocardium (Leprán *et al.*, 1983). The non-perfusable area, that remained red coloured, was cut along the epicardially visible border and its weight was measured and expressed in percentage of the wet weight of the ventricles. Hearts showing <10% non-perfusable area were excluded from the final evaluation.

2. 3. 3. Drug administration protocol

Glibenclamide (Sigma-Aldrich, Hungary) or glimepiride (Hoechst, Germany) was dissolved in dimethyl sulfoxide/saline, 1:1 mixture and was administered intraperitoneally 30 min prior to coronary artery ligation in doses ranging 0.001-5 mg/kg. To reduce the influence of the solvent, the volume of injection was 100 µl/kg. Control animals were given the same volume of the solvent.

2. 4. Persistent coronary artery occlusion in rats

2. 4. 1. Surgical preparation

The animals were anaesthetised with ethyl ether and the parasternal area was depilated and disinfected using 70% ethanol. A small parasternal incision was made through the skin and the pectoral muscle layers were gently separated. Coronary artery ligation was performed as described earlier by Selye *et al.* (1960). On the left side at the fourth intercostal space, a small incision was made and the chest was opened in the fourth intercostal space and the ribs were gently spread using a retractor. The exposed heart was quickly pushed out of the thoracic cavity, inverted, and a 5-0 silk suture attached to a 16 mm micropoint reverse cutting needle was passed under the visualised left main coronary artery and ligated with a "double knot" tie, and hence acute regional myocardial infarction was developed. The heart was set back in its place and the chest was closed in layers while the thorax was slightly compressed to evacuate pneumothorax and regain spontaneous ventilation. Sham operated animals were treated in the same manner except that the silk ligature around the coronary artery was left loose. Since in the rat blood to the left ventricle is supplied by the descending and septal arteries (Johns and Olson, 1954), coronary ligation near its origin was expected to affect most of the left ventricle. The animals were then returned to their cages and recovered from the anaesthesia within 1-2 min. In case of developing ventricular fibrillation during the first 2-3 hours after coronary artery ligation, attempt was made to defibrillate the animals by mechanical tapping on the chest wall. After the acute phase of coronary artery ligation, the condition of the animals was periodically followed-up carefully for 1-7 days.

As the aim of this protocol was to investigate the influence of the drugs on the developed infarct size for longer time, no arterial catetherisation or tracheal intubation was performed. Moreover, because of the short time of open-chest surgery (< 40 sec), no ventillatory support was called for.

2. 4. 2. Measurement of infarct size

After one or seven days of coronary artery occlusion, the rats were anaesthetised with pentobarbitone (60 mg/kg i.p.) and their hearts were excised and washed in isotonic NaCl solution. The attached aorta, auricles and right ventricular free wall were carefully removed and by using a series of razor blades, the hearts were sectioned transversely into 1.6 mm thick slices from apex to base. The heart slices were then incubated in 0.1% nitroblue-tetrazolium (Fluka AG, Switzerland) dye for 10 min to allow demarcation of the non-infarcted (stained dark blue), and infarcted (appeared pale) myocardium (Nachlas and Shnitka, 1963) to develop. This differential staining was enhanced by immersing the slices into 1% formalin until further evaluation within 1 day. The slices were subsequently ranked in order from apex to base and digitised using a desktop scanner (ScanJet IIc, Hewlett-Packard) with 400 dpi resolution, and 'million of colours' to delineate the regions of viable and infarcted tissue.

Stored images were pre-processed for enhancing the colour difference using PhotoFinish (Zsoft Corporation) and to increase the signal to noise ratio, then the colour depth was decreased to 256 colours. Further differentiation between the non-infarcted, infarcted and totally necrotic areas and the calculation of the volume of these tissues and the left ventricular cavity were done by using a computer program developed at our Department (Volume 2.0) under Microsoft Windows. Using the cursor pointer a pixel was selected, representing the totally infarcted tissue (not staining with the dye), or partially infarcted tissue (showing some staining), or normal myocardium (well stained), as well as the left ventricular cavity. The colour of other pixels, having similar colour in a prefixed range to the representative one, were automatically redefined by the program. In this way a certain area is repainted to the same colour and the number of pixels representing this colour were counted by the program. Using a 10 mm calibration bar, that was scanned together with the heart slices, and the thickness of slices (1.6 mm), the computer can automatically calculate the volume of the aforementioned tissues (Figure 1).

The volume of the infarcted myocardium (i.e. the volume of the totally infarcted and partially infarcted tissues together) was expressed as a percentage of the volume of the whole myocardium (totally infarcted + partially infarcted + non-infarcted). The volume of the left ventricular cavity was also expressed as a percentage of the total volume of the heart (totally infarcted + partially infarcted + non-infarcted + left ventricular cavity). From the computer stored images we also measure the thickness of the septum (representing the non-infarcted

myocardium, NMIS) and the thinnest part of the infarcted left ventricular myocardium (MILV) were also measured.

All images were processed by the same person using blind technique. The same colour settings were used for differentiating the infarcted and non-infarcted myocardium.

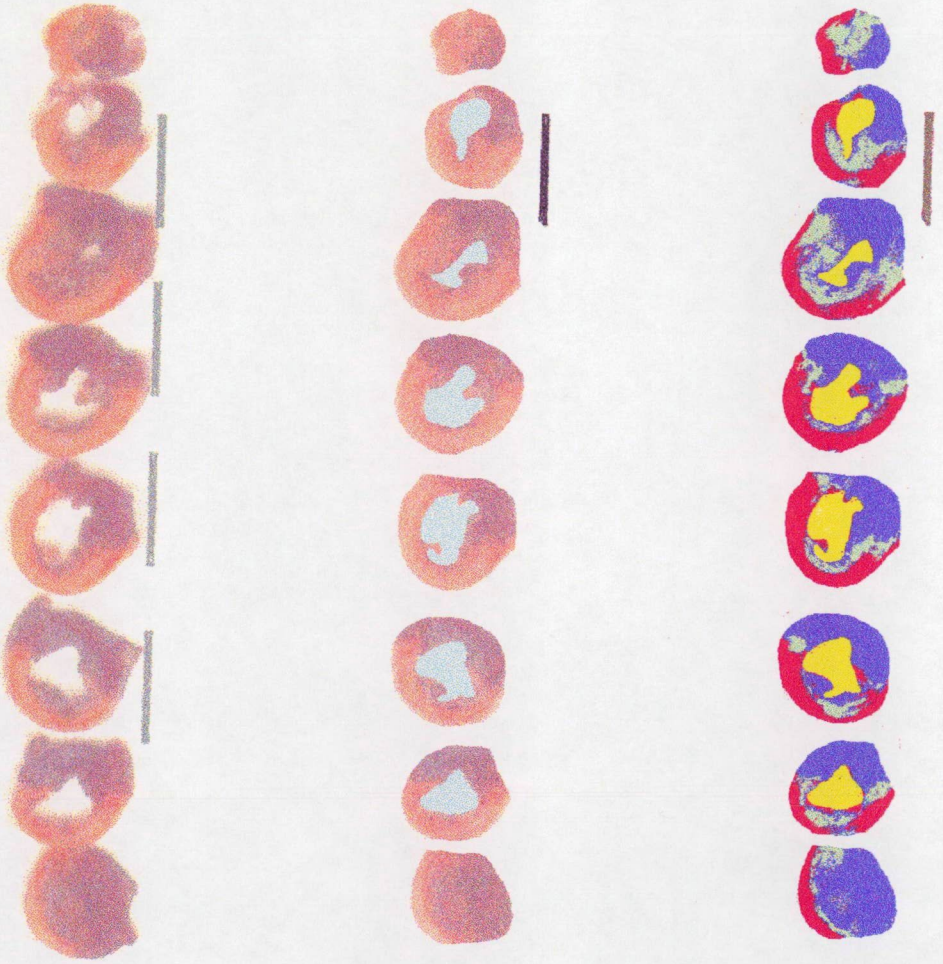


Figure 1. Representative sections from the heart of a control animal at different phases of the evaluation of the infarct volume after 1 day coronary artery occlusion in rats. (A) original scanning with 400 dpi; (B) enhancing the signal to noise ratio and (C) differentiation between totally infarcted (red), partially infarcted (green) and non-infarcted myocardium (blue).

2. 4. 3. Drug administration protocol

Glibenclamide (0.1 or 5 mg/kg), glimepiride (0.001 or 5 mg/kg) obtained from the same sources (see section *2. 3. 4.*) or vehicle (DMSO : EtOH 1:1) were administered i.p. twice a day with a Hamilton syringe in a volume of 100 µl/kg. The first dose of either drug or vehicle was given 30 min prior to occlusion and the treatment was maintained throughout the specified period after coronary artery ligation. These doses were selected on the basis of previous experiments (El-Reyani *et al.*, 1999) i.e. the larger doses decreased basal plasma glucose concentration and inhibited its elevation upon oral glucose loading as well as decreased the incidence of irreversible ventricular fibrillation after transient coronary artery occlusion and reperfusion. The smaller doses either did not influence plasma glucose concentration in metabolically healthy rats or produced only marginal effect on the incidence of arrhythmias. All substances were prepared fresh daily and the animals were allowed to consume food and water ad libitum.

2. 5. Coronary artery ligation and reperfusion in anaesthetised rabbits:

2. 5. 1. Surgical preparation

The animals were anaesthetised with sodium pentobarbitone (30 mg/kg i.v. in a volume of 1 ml/kg) injected into the marginal vein of the right ear. The exposure of carotid artery and trachea was performed as described earlier (see section 2. 3. 1.). For infusion of drug another catheter was introduced into the marginal vein of the left ear. After tracheal cannulation, thoracotomy was performed at the fourth intercostals space and artificial respiration was started immediately (see section 2. 3. 1.). The respiratoy volume and rate was adjusted to keep blood gases and pH within normal range (7 ml/kg/stroke, 40 strokes/min, respectively). Following pericardiotomy, a loose loop of 4-0 atraumatic silk (Ethicon, Edinburg, UK) was placed around the first branch of the left circumflex coronary artery just below its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tube.

2. 5. 2. Experimental protocol

After stabilization of blood pressure and heart rate (approximately 10 min) saline or 0.03 mg/kg or 0.1 mg/kg GYKI-16638 was administered i.v. during a 1 min infusion in a volume of 2 ml/kg 5 min prior to coronary artery occlusion. Coronary artery occlusion and thus local myocardial ischaemia was produced by tightening the loose loop and clamping on

the silk. After 10 min of coronary artery occlusion , the ligature was released to permit reperfusion for 10 min.

The electrocardiogram (lead I, II, III) was registered using a thermographic recorder (ESC 1104 CH, Multiline Ltd, Esztergom, Hungary) with subcutaneous needle electrodes. QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of the TU wave. QT interval corrected for heart rate (QTc) was calculated using the following equation by Carlsson et al., (1993a): QTc = QT- 0.175 x (RR-300).

At the end of the experiment, heparin (500 IU/kg. i.v.) was administered and the animals were sacrificed. The hearts were cut out from the chest in order to determine the size of the occluded zone. After retightening the ligation the hearts were retrogredely perfused via the aorta with 20 ml saline and 10 ml of 96% ethanol as previously described by Leprán *et al.* (1983). The non-denaturated area (occluded zone) was excised and its extent was expressed in percentage of the total wet weight of the ventricles. Four animals with an occluded zone less than 16% or larger than 32% were excluded from the final evaluation.

2. 5. 3. Drug administration protocol

GYKI-16638 (0.03 or 0.1 mg/kg) was dissolved in propylene glycol/saline, 1:1 mixture. Both drugs were applied 5 min prior to coronary artery ligation in a volume of 2 ml/kg. Each dose was prepared on the day of the experiment. Control animals received propylene glycol/saline, 1:1 mixture in a volume of 2 ml/kg.

2. 6. Statistical analysis

All values are expressed as mean ± standard error of the mean (S.E.M.). Analysis of variance (ANOVA) was applied and the results were compared by means of the modified *'t'*-statistical method of Wallenstein *et al.* (1980). The survival rate and the occurrence of arrhythmias were analysed using the χ^2-method.

A P-value less than 0.05 was considered statistically significant for all study protocols applied.

3. Results

3. 1. Effects of sulphonylureas

3. 1. 1. Blood glucose level in conscious rats

During basal conditions, i.e. before any drug treatment, the blood glucose level did not show significant differences among different groups (Table 1). Thirty minutes after the intraperitoneal administration, both glibenclamide and glimepiride significantly reduced the blood glucose concentration when given in a dose of 1 mg/kg (2.6 ± 0.12 and 2.7 ± 0.11 mmol/l, vs. 3.3 ± 0.08 and 3.5 ± 0.06 mmol/l before treatment, respectively, $P < 0.05$). In a dose of 0.1 or 1 mg/kg both compounds inhibited the elevation of plasma glucose concentration after oral glucose loading, while the smallest dose applied (i.e. 0.01 mg/kg) was devoid of such an effect (Table 1).

Table 1. Effect of glibenclamide and glimepiride on the blood glucose level (mmol/l) after oral glucose loading in conscious rats.

Group	Dose (mg/kg)	Basal	Drug	Glucose loading
Control		3,2 ± 0,16	3,7 ± 0,10	5,4 ± 0,26
Glibenclamide	0,01	3,2 ± 0,11	4,3 ± 0,02	5,7 ± 0,17
	0,1	3,0 ± 0,15	3,6 ± 0,10	4,4 ± 0,21*
	1,0	3,3 ± 0,08	2,6 ± 0,12*	3,4 ±0,08*
Glimepiride	0,01	3,1 ± 0,16	3,8 ± 0,42	5,9 ± 0,18
	0,1	3,2 ± 0,04	4,2 ± 0,31	4,0 ± 0,11*
	1,0	3,5 ± 0,06	2,7 ± 0,11*	3,8 ± 0,19*

Results are mean ± S.E. of 6 animals in each group. Blood glucose concentration (mmol/l) was measured before treatment (Basal), 30 min after drug treatment (Drug) and 30 min after oral administration of 1 g/kg glucose (Glucose loading). *$P < 0.05$ compared to the corresponding control value.

3. 1. 2. Myocardial ischaemia-reperfusion induced arrhythmias in anaesthetised rats

3. 1. 2. 1. Haemodynamic parameters

Table 2 shows values for mean arterial blood pressure (MAP) and heart rate (HR). At the doses that did not influence the blood glucose concentration in conscious rats, neither glibenclamide nor glimepiride pretreatment influenced significantly the baseline values of HR, blood pressure or PRI as measured before coronary artery ligation in anaesthetised rats. These haemodynamic parameters did not differ from the control during coronary artery ligation. Larger doses of either glibenclamide or glimepiride (i.e. 5 mg/kg i.p.), however, significantly increased the heart rate before coronary artery ligation which remained high during the occlusion also after glimepiride pretreatment (Table 2). During reperfusion, it was difficult to measure the haemodynamic parameters because of the frequent arrhythmias occuring in this period. Nevertheless, when it was possible to perform statistical analysis, no significant differences were observed after glibenclamide or glimepiride pretreatment concerning the heart rate or blood pressure as compared to the control group.

3. 1. 2. 2. Arrhythmias during ischaemia and reperfusion

In the present experiments, 6 minutes of coronary artery ligation was not long enough to develop severe ischaemia-induced arrhythmias. There were no significant differences among different treatments concerning the incidence of arrhythmias or the survival rate during coronary ligation.

Arrhythmias induced by reperfusion after 6 min myocardial ischaemia started within 10-30 s following the release of the coronary artery ligature. Irreversible ventricular fibrillation occurred in 73% of the control animals and no animal survived without developing arrhythmias during reperfusion. Only 2 of 18 animals recovered spontaneously from ventricular fibrillation in the control group (Table 3).

Pretreatment with either glibenclamide or glimepiride (5 mg/kg, i.p.), significantly increased the survival rate from 27% in the control group to 81% and 67%, respectively during reperfusion after 6 min myocardial ischaemia in anaesthetised rats (Table 3). This protective effect of glimepiride, but not of glibenclamide, was also significant after using smaller doses (0.01 and 0.001 mg/kg, Table 3).

Both glibenclamide and glimepiride decreased significantly the incidence of irreversible ventricular fibrillation occurring during reperfusion after 6 min coronary occlusion. Moreover, this effect was also significant when using smaller doses of glimepiride (i.e., 0.001-0.1 mg/kg) (Table 3). The incidence of ventricular tachycardia and other types of arrhythmias did not show a dose related change after the pretreatments.

The length of arrhythmic attacks was also measured in the animals surviving reperfusion. As compared to the control animals, both glibenclamide and glimepiride at the submaximal doses investigated, i.e. 0.1 mg/kg, significantly decreased the duration of arrhythmias. Moreover, the length of VF was significantly decreased at a wide range of doses i.e. by 0.01-5 mg/kg of glibenclamide and 0.001-0.1 mg/kg of glimepiride (Table 4). However, the total period that was characterised by arrhythmic attacks during reperfusion was not changed by pretreatments (Table 4).

3. 1. 3. Myocardial infarction in rats

3. 1. 3. 1. Survival after coronary artery ligation

Sham operation in rats, i.e. anaesthesia, rapid opening and closing of the chest wall without coronary artery ligation, as expected did not cause death. Out of 146 animals that produced myocardial infarction 16 (11%) died during the first 4 hours after coronary artery ligation in spite of using mechanical tapping on the chest wall to revert ventricular fibrillation to normal rhythm. Further 32 (22%) died after the 4th hour of the 1st day of infarction. No death occurred later until the end of the examination period, i.e. the 7th day. There were no significant differences between different groups concerning the survival rate during the whole run of myocardial infarction.

3. 1. 3. 2. The developed infarct size

Sham operated animals did not show loss of nitroblue-tetrazolium staining in the myocardium and the volume of the calculated 'infarcted' myocardium (measured with low staining of remaining connective tissue or the heart valves), was less than 1% (not shown in tables).

Table 2. Effect of glibenclamide and glimepiride on heart rate (HR), mean arterial blood pressure (BP) in anaesthetised rats.

Group	Dose (mg/kg)	Basal			Occlusion			Reperfusion		
		n1	HR	BP	n2	HR	BP	n3	HR	BP
Control		26	389±7,5	110±3,8	22	374±8,5	80±7,2	6	450±27,6	86±14,7
Glibenclamide	0,01	16	379±9,1	105±5,1	13	364±9,4	73±5,6	4	ND	ND
	0,1	15	392±6,1	104±4,8	15	385±7,9	73±4,7	8	429±21,3	94±9,5
	5	20	432±7,5*	104±5,5	16	398±15,3	49±4,8*	13	432±11,6	89±9,6
Glimepiride	0,0001	9	337±9,0	121±6,7	8	312±10,9	72±11,7	3	ND	ND
	0,001	23	384±12,4	109±3,6	22	369±15,1	78±7,8	14	398±23,5	92±8,4
	0,01	19	386±6,8	107±5,2	18	369±8,4	79±7,6	11	382±12,1	110±9,4
	0,1	15	396±7,1	105±8,1	15	376±12,8	74±10,6	9	374±11,5	92±9,2
	5	22	428±7,7*	108±4,6	18	415±9,8*	87±5,5	12	429±13,2	102±7,5

Results are mean ± S.E. of the animals surviving the given period (n1, n2 and n3 means the number of these animals, respectively). Heart rate (HR), mean arterial blood pressure (BP) and pressure rate index (PRI) was measured before coronary artery ligation (Basal), 5 min after coronary ligation (Occlusion) and 5 min after the release of occlusion (Reperfusion). ND = Not determined because of few surviving animals. * $P < 0.05$ compared to the corresponding control value.

Table 3. Effect of glibenclamide and glimepiride on the survival rate and the incidence of arrhythmias during reperfusion after 6 min coronary artery ligation in anaesthetised rats.

Group	Dose (mg/kg)	*N*	Survived (%)	Incidence of arrhythmias (%)				
				None	RevVF	IrrevVF	VT	Other
Control		22	27	0	9	73	100	100
Glibenclamide	0,01	13	31	0	8	69	100	62*
	0,1	15	53	0	33	47	87	100
	5	16	81*	0	75*	19*	100	94
Glimepiride	0,0001	8	38	0	37	63	100	100
	0,001	22	64*	5	32	36*	91	96
	0,01	18	61*	0	11	39*	100	89
	0,1	15	60*	0	13	40*	93	100
	5	18	67*	0	50*	33*	100	72

N= Total number of animals at the beginning of reperfusion; None= No arrhythmia developed; RevVF= Reversible ventricular fibrillation; IrrevVF= Irreversible ventricular fibrillation; VT= Ventricular tachycardia; Other= Ventricular extrasystoles, bigeminy, and salvos. * $P < 0.05$ compared to the corresponding control value.

Table 4. Effect of glibenclamide and glimepiride on the appearance and length of arrhythmias during coronary reperfusion in anaesthitized rats.

Group	Dose (mg/kg)	n1	Appearance of arrhythmias (min)	n2	Duration of arrhythmias (min)	Length of arrhythmic attacks (sec)			
						VF	VT	Other	Total
Control		22	0.08 ± 0.02	6	4.4 ± 0.5	84 ± 44.8	66 ± 24.1	53 ± 15.5	203 ± 31.9
Glibenclamide	0.01	13	0.26 ± 0.04	4	3.6 ± 0.8	8 ± 7.5*	33 ± 14.4	83 ± 34.2	123 ± 32.0
	0.1	15	0.12 ± 0.03	8	2.7 ± 0.4*	21 ± 12.9*	34 ± 13.5	65 ± 14.6	120 ± 24.8
	5	16	0.16 ± 0.01	13	3.7 ± 0.3	25 ± 6.9*	83 ± 12.3	62 ± 10.2	171 ± 17.1
Glimepiride	0.0001	8	0.07 ± 0.02	3	3.9 ± 0.5	25 ± 15.5	70 ± 4.8	90 ± 6.35	186 ± 16.5
	0.001	22	0.08 ± 0.02	14	4.2 ± 0.4	18 ± 9.1*	53 ± 11.3	73 ± 10.6	145 ± 19.9
	0.01	18	0.18 ± 0.01	11	3.7 ± 0.4	8 ± 5.5*	52 ± 13.3	87 ± 18.2	147 ± 24.2
	0.1	15	0.14 ± 0.01	9	2.2 ± 0.5*	19 ± 15.6*	63 ± 18.4	45 ± 10.6	128 ± 28.1
	5	18	0.17 ± 0.01	12	3.9 ± 0.3	54 ± 14.9	88 ± 16.1	53 ± 11.4	196 ± 17.5

Results are mean ± S.E. n1= total number of animals at the onset of reperfusion, n2= total number of animals surviving coronary reperfusion. * $P<0.05$ compared to the corresponding control value.

Coronary artery ligation resulted in extensive loss of nitroblue-tetrazolium staining that involved the anterior and lateral free wall and extended to 45 ± 2.5 % of the left ventricular myocardium after 1 day infarction in control animals (Table 5). After 7 days the volume of the infarcted myocardium became albeit smaller (39.1 ± 3.2 %) due to the shrinkage of the infarcted tissues. As myocardial infarction progressed the calculated volume of the left ventricular cavity significantly enlarged from 12.1 ± 0.7 % to 19.9 ± 1.2 % 1 day and 7 days after myocardial infarction, respectively.

Glibenclamide treatment in a dose of 5 mg/kg b.i.d. did not influence the development of infarction at 1 day coronary artery occlusion. This treatment, however, considerably decreased the volume of the infarcted myocardium 7 days after coronary artery ligation (Table 5), while the enlargement of the ventricular cavity did not differ from the control group (Table 5). The smaller dose of the compound did not influence the development of myocardial infarction.

Glimepiride, on the other hand, at either high dose i.e. 5 mg/kg or small dose i.e. 0.001 mg/kg did not influence the size of the infarcted myocardium (Table 5). However, at 5 mg/kg, the compound significantly decreased the enlargement of the left ventricular cavity during the evolvement of myocardial infarction..

3. 1. 3. 3. Thickness of myocardium

In the control animals, the progression of myocardial infarction was characterized by thinning of the infarcted left ventricular wall and thickening of the non-infarcted ventricular septum (Table 6).

Glibenclamide at the higher, but not the smaller dose, resulted in more intense thinning of the infarcted myocardium as compared to the control (Table 6). Interestingly, both doses of the compound inhibited the development of hypertrophy of the non-infarcted myocardium, measured as the thickness of the septum 7 days following myocardial infarction (Table 6).

Glimepiride pretreatment (5 mg/kg) decreased the thickness of the infarcted myocardium 1 day after coronary artery ligation. However, following 7 days coronary artery ligation, the compound neither increased the scar thinning nor influenced the thickening of the non-infarcted myocardium (Table 6).

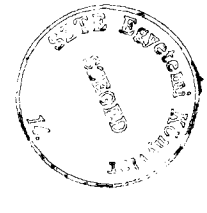


Table 5. Effect of glibenclamide and glimepiride on the volume of the myocardium (Vol) and the percentage volume of the infarcted myocardium (MI) and the left ventricular cavity (LVC) after 1 or 7 days of myocardial infarction in rats

Group	Dose	1 day				7 days			
	mg/kg	n	Vol (mm^3)	MI (%)	LVC (%)	n	Vol (mm^3)	MI (%)	LVC (%)
Control		15	677±19.1	45.3±2.5	12.1±0.7	10	571±47.6†	39.1±3.2	19.9±1.2†
Glibenclamide	0.1	10	653±26.2	37.7±3.4	10.3±0.5	13	540±24.4†	35.1±2.2	18.3±1.4
	5	10	578±25.9*	38.9±2.9	13.5±0.6	8	467±29.7*†	29.1±3.5*	19.0±1.0
Glimepiride	0.001	10	625±29.0	39.0±4.0	11.0±1.0	6	625±36.6	41.1±6.0	15.6±1.5
	5	8	653±26.9	46.1±3.6	8.7±0.6*	8	565±25.9	43.3±2.0	15.2±1.1*

Results are mean ± SE of n animals in which infarct size was measured in each group. Drugs were applied intraperitoneally 30 min before coronary artery occlusion and then twice daily. * $P < 0.05$ compared to the corresponding control value; † $P < 0.05$ compared to 1 day-old myocardial infarction.

Table 6. Effect of glibenclamide and glimepiride on the thickness of the infarcted left ventricle (MILV) and the non-infarcted septum (NIMS) after myocardial infarction in rats

Group	Dose	1 day			7 days		
	mg/kg	n	MILV (mm)	NIMS (mm)	n	MILV (mm)	NIMS (mm)
Control		15	2.5±0.06	2.4±0.08	10	2.0±0.13†	2.9±0.10†
Glibenclamide	0.1	10	2.4±0.09	2.6±0.07	13	1.8±0.09	2.3±0.05*
	5	10	2.2±0.07*	2.4±0.04	8	1.6±0.04*	2.1±0.10*
Glimepiride	0.001	10	2.4±0.09	2.6±0.09	6	2.1±0.14	2.9±0.11
	5	8	2.2±0.09*	2.6±0.08	8	2.0±0.06	2.8±0.11

For details see Table 5.

3. 2. Effects of GYKI-16638

3. 2. 1. Myocardial ischaemia-reperfusion induced arrhythmias in anaesthetised rabbits

3. 2. 1. 1. Haemodynamic parameters

There were no significant differences between the mean arterial blood pressure of control and GYKI-16638 treated animals. Mean arterial blood pressures fell significantly in both groups due to coronary artery occlusion as compared to pre-occlusion values (84±2.6 vs. 95±3.5 mmHg, 69±3.8 vs. 93±3.4 mmHg and 74±3.9 vs. 94±2.7 mmHg in controls, 0.03 and 0.1 mg/kg GYKI-16638 treated animals, respectively, all $P<0.05$).

The infusion of the drug significantly decreased the heart rate at both doses compared to the basal values (Table 7). However, coronary occlusion did not change the heart rate significantly compared to pre-occlusion values nor did reperfusion.

3. 2. 1. 2. QT and QTc intervals

GYKI-16638, in a dose of 0.03 mg/kg, had no effect on QT and QTc intervals, but caused a significant increase of both variables in the dose of 0.1 mg/kg (Table 7). No significant changes occurred in QT or QTc intervals during coronary artery occlusion or reperfusion.

Table 7. Effect of intravenous administration of GYKI-16638 on the mean arterial blood pressure (MBP), heart rate (HR), QT and QTc intervals in anaesthetised rabbits

Group	Dose (mg/kg)	Variable	N	Before infusion	5 min after infusion
Control		MBP	19	101±2.8	100±2.6
		HR		271±7.2	268±6.6
		QT		149±4.0	149±4.4
		QTc		162±3.4	162±3.8
GYKI-16638	0.03	MBP	14	93±3.4	93±2.8
		HR		273±5.2	259±6.3*
		QT		150±4.6	159±7.3
		QTc		164±4.1	171±6.5
	0.1	MBP	17	94±2.7	93±2.5
		HR		270±8.7	253±7.3*
		QT		140±4.4	166±6.4*†
		QTc		153±3.4	173±4.7*

N= number of animals, MBP= mean blood pressure (mmHg), HR= heart rate (1/min), QT= QT interval (msec), QTc= corrected QT interval, *= $P<0.05$ compared to the pre-infusion value of the same group, †= $P<0.05$ compared to the control group.

Table 8. Effect of GYKI-16638 on the incidence of arrhythmias during 10 min coronary artery occlusion in anaesthetised rabbits.

Group	Dose (mg/kg)	N	Survived n (%)	Incidence of arrhythmias, n (%)			
				None	VF	VT	Other
Control		19	12 (63%)	4 (21%)	8 (42%)	2 (11%)	14 (74%)
GYKI-16638	0.03	14	14 (100%)*	3 (21%)	3 (21%)	2 (14%)	11 (79%)
	0.1	17	15 (85%)	5 (29%)	4 (24%)	0 (0%)	2 (71%)

N= total number of animals; n= number of animals exhibiting the given response; %= percentage of the animals exhibiting the given response. VF= ventricular fibrillation; VT=ventricular tachycardia; Other= extrasystoles, salvos, and/or bigeminy. * $P<0.05$ compared to the control group.

Table 9. Effect of GYKI-16638 on the incidence of arrhythmias during 10 min reperfusion following 10 min of coronary artery occlusion in anaesthetised rabbits.

Group	Dose (mg/kg)	N	Survived n (%)	Incidence of arrhythmias, n (%)			
				None	VF	VT	Other
Control		12	4 (33%)	0 (0%)	9 (75%)	7 (58%)	5 (42%)
GYKI-16638	0.03	14	11 (79%)*	3 (21%)	3 (21%)*	4 (29%)	9 (64%)
	0.1	15	15 (100%)*	6 (40%)*	5 (33%)	6 (40%)	8 (53%)

For details see Table 8.

3. 2. 1. 3. Arrhythmias during myocardial ischaemia and reperfusion

In all groups, arrhythmias did not develop during 1 min infusion of drugs or vehicle, or between the infusion of drugs and coronary occlusion. In the control group during 10 min ischaemia, 7 animals out of 19 died due to irreversible ventricular fibrillation. All animals survived coronary occlusion in the 0.03 mg/kg GYKI-16638 (n=14) treated groups. In the 0.1 mg/kg of the compound 2 animals out of 17 died because of irreversible ventricular

fibrillation during coronary occlusion (Table 8). However, there were no significant differences in the treated and control groups concerning the incidence of other types of arrhythmias during the coronary occlusion period.

Arrhythmias induced by reperfusion appeared within 10-30 sec following the release of the ligature. Pretreatment with GYKI-16638 at a dose of 0.03 mg/kg significantly reduced the incidence of reperfusion-induced ventricular fibrillation (Table 9). The larger dose (0.1 mg/kg) of the compound reduced the overall occurrence of ventricular fibrillation (33% vs. 75% in controls), and significantly decreased the incidence of irreversible ventricular fibrillation (0% vs. 89% in controls, $P<0.05$). Both doses of the compound significantly increased the number of animals surviving reperfusion (79% and 100% with 0.03 and 0.1 mg/kg GYKI-16638 vs. 33% in controls, $P<0.05$, respectively). The number of animals that did not develop any arrhythmia during reperfusion was significantly higher in the 0.1 mg/kg GYKI-16638 treated group (Table 9). There were no differences in the incidence of other types of arrhythmias.

4. Discussion

4. 1. Cardiac arrhythmias in anaesthetised animals

Transient coronary artery ligation followed by reperfusion in anaesthetised animals, like rats or rabbits, is a widely used and accepted method to investigate the efficacy of antiarrhythmic treatments. In the present experiments, induction of brief i.e. 6 min or 10 min occlusion of the left coronary artery per-se was not long enough to permit significant amount of ischaemia-induced arrhythmias to develop, however, it was purposely chosen to prime the heart to develop severe consistent arrhythmias 10-30 s after reperfusion.

The pathophysiological mechanisms responsible for reperfusion arrhythmias are not fully understood. Although reperfusion is essentially meant for the recovery of ischaemic myocardium, it might be associated with more serious rhythm disturbances including ventricular tachycardia and ventricular fibrillation both experimentally (Curtis and Hearse, 1989; Wolleben et al., 1989; Gelvan *et al.*, 1991; Krumholz and Goldberger, 1991; Liu *et al.*, 1991) and clinically (Kuck *et al.*, 1985; Murohara *et al.*, 1991; Smith *et al.*, 1992)

The genesis and maintenance of reperfusion arrhythmias represent complex phenomena involving both reentrant and non-reentrant mechanisms. Although reentrant arrhythmia is the proposed mechanism during ischaemia, however, some non-reentrant mechanisms may also be involved (Pogwizd and Corr, 1987). It appears that during the reperfusion period both reentrant mechanisms and triggered activities (non-reentrant mechanisms) are important in the initiation and maintenance of reperfusion arrhythmias (Pogwizd and Corr, 1992; Ponce-Zumino *et al.*, 1997; Tachibana *et al.*, 1998). At the cellular level, these arrhythmias may emerge due to the direct effects of abrupt myocardial ischemia which may cause varying metabolic and ionic changes resulting in inhomogeneous refractoriness across and within the ischaemic zone. Dispersion of conduction and refractoriness favour the appearance of reentrant ventricular arrhythmias (Pogwizd and Corr, 1992). The multiple mechanisms, however, seem to provide the best explanation. These could include, in particular, the activation of the ATP-sensitive potassium channels secondary to a reduction in the intracellular ATP content, and an increase in the extracellular potassium concentration, secondary to intracellular potassium loss. These cellular alterations lead to a reduced conduction velocity, a decrease in action potential duration and refractoriness and may also be responsible for the initiation of after-depolarization. In addition, several other factors could be involved in the genesis of ischaemia-induced reperfusion arrhythmias. These

may include alterations of the intercellular coupling at the gap-junction level (Saffitz *et al.*, 1993), as well as the effects of endogenous factors, such as endothelin, angiotensin II, thromboxane A_2 and many others (Curtis *et al.*, 1993).

The multiplicity of the cellular mechanisms involved makes the choice of therapy difficult but suggests that antiarrhythmic agents with a diversity of cellular electrophysiological activities may have the best potential to prevent ischaemia and reperfusion-induced life-threatening arrhythmias.

4. 2. Sulphonylureas and reperfusion arrhythmias in anaesthetised rats

The findings of the present study demonstrate that pre-treatment with two sulphonylureas, glibenclamide or glimepiride (both are K_{ATP} channel inhibitors), significantly decreased the incidence of irreversible ventricular fibrillation during reperfusion following transient myocardial ischaemia in anaesthetised rats. Moreover, this cardioprotective action of glimepiride occured in smaller doses than that producing a blood glucose lowering effect.

The role of K_{ATP} channel blockade in reducing ventricular fibrillation during myocardial ischaemia is well established, but the antiarrhythmic effect may largely depend on the attendant decrease in the loss of intracellular potassium and the prevention of non-uniform shortening of the action potential duration during myocardial ischaemia (MacKenzie *et al.*, 1993; Tweedie *et al.*, 1993). Such an effect by K_{ATP} inhibitors, like glibenclamide or glimepiride, could decrease the development of electric inhomogeneity between the ischaemic and non-ischaemic myocardium and might suppress the substrate for reentrant pathways, resulting in antiarrhythmic, antifibrillatory action. Such an idea is consistent with other investigators using glibenclamide in various in vitro (Pogatsa *et al.*, 1988; Wolleben *et al.*, 1989; Kantor *et al.*, 1990; Tosaki *et al.*, 1993; D'Alonzo *et al.*, 1994), as well as under in vivo experimental conditions (Pogatsa *et al.*, 1988; Bekheit *et al.*, 1990; Billman *et al.*, 1993; Kondo *et al.*, 1996). In agreement with these observations, recent results from our lab have demonstrated in in vivo conditions that inhibition of K_{ATP} channels by glibenclamide provided an effective protection against irreversible ventricular fibrillation and increased the survival rate during acute myocardial infarction in conscious rats (Leprán *et al.*, 1996) or during ischaemia/reperfusion in anaesthetised rats (Baczkó *et al.*, 1997; EL-Reyani *et al.*, 1999). Clinical data seem to corroborate these results. In a randomised crossover study in NIDDM (glibenclamide vs. metformin), glibenclamide significantly reduced the incidence of ventricular premature complexes and ventricular tachycardia during (spontaneous) transient

myocardial ischaemia (Cacciapuoti *et al.*, 1991). Glibenclamide did not alter the ischaemic burden nor did it interfere with non-ischaemia-related arrhythmias (Cacciapuoti *et al.*, 1991). In a study on NIDDM patients suffering from acute MI, ventricular fibrillation occurred significantly less frequently in the glibenclamide-treated group than in NIDDM patients treated otherwise and in non-diabetics (Pogatsa *et al.*, 1992; Lomuscio *et al.*, 1994 and Davis *et al.*, 1996).

Very few data are available on the possible antiarrhythmic effect of glimepiride. Vegh and Papp (1996) have found that only glimepiride but not glibenclamide attenuated the number of episodes and the incidence of ventricular tachycardia during ischaemia after coronary artery ligation in anaesthetised dogs. Our present investigations corroborate these findings and support that, in a wide dose range, glimepiride provides a more potent antiarrhythmic effect than glibenclamide (EL-Reyani *et al.*, 1999).

The reason for the observed difference between glibenclamide and glimepiride, and the divergence of the blood glucose lowering potency and the 'antiarrhythmic' activity have so far not been interpreted. However, some data do describe differences in the various actions of these two compounds. Ozaki *et al.* (1992) found that glimepiride inhibited the cyclooxygenase pathway of isolated human platelets, while the activities of 12-lipoxygenase and phospholipase A_2 were not influenced. On the other hand, glibenclamide inhibited both the cyclooxygenase and 12-lipoxygenase enzymes and also the phospholipase A_2. In diabetic population, Muller *et al.* (1994) have shown that glimepiride was more potent than glibenclamide in lowering blood glucose level, whereas had 2.5-3-fold lower affinity to membranes isolated from rat pancreatic β-cells. Moreover, Bijlstra *et al.* (1996) found that forearm vasodilator response to the administration of the specific K_{ATP} channel opener diazoxide was significantly inhibited by therapeutic concentrations of glibenclamide, while glimepiride was devoid of such an effect. Presumably, differences in the effects on membrane currents or in direct metabolic effects, not related to the increased secretion of insulin, e.g., increased glucogenolysis, decreased fatty acid metabolism (reviewed by Schotborgh and Wilde, 1997), could be related to the more pronounced 'antifibrillatory' action of glimepiride than that of glibenclamide. Moreover, the existence of multiplicity of sulphonylurea receptors that regulate the opening of K_{ATP} channels might largely correlate to the discrepancies of the observed 'pancreatic' and 'cardiac', as well as the 'sarcolemmal' and 'mitochondrial' activity of sulphonylurea compounds. The contribution of these possibilities to the differences between glibenclamide and glimepiride revealed in the present study require further investigation.

Based on the observed differences between the two sulphonylurea compounds, this may suggest the possibility of developing 'cardioselective' or 'ischaemia selective' compounds that inhibit K_{ATP} channels albeit different subtype without decreasing blood glucose level. This conclusion is supported by recent findings that a novel 'cardioselective' K_{ATP} channel antagonist, HMR-1883, reduced the incidence of ventricular fibrillation induced by 2 min coronary artery occlusion during submaximal exercise test in mongrel dogs with healed myocardial infarction (Billman *et al.*, 1998). Such compounds may be the potential candidates for the drug treatment of cardiac arrhythmias with a selective action during myocardial ischaemia.

4. 3. Development of myocardial infarction

In experimental cardiology, regional myocardial infarction by occlusion of the left main coronary artery in the rat is of particular importance because of its similarity to the clinical situation of acute myocardial infarction (Bernauer, 1997). Moreover, it is a widely used method for the evaluation of different phases of myocardial infarction in pharmacological interventions when large number of animals are involved in the experiments. Coronary artery occlusion in this model is characterised by two phases. The first acute phase (i.e. the first hours) after myocardial infarction is characterised by high mortality due to severe arrhythmias (Leprán *et al.*, 1983; Walker *et al.*, 1988), whereas the second late phase is characterised by scar formation and anatomical remodelling of the ventricles (Anversa *et al.*, 1985; Pfeffer *et al.*, 1985).

Preclinical screening for a special indication, such as infarct size developing after myocardial infarction, should involve estimation of putative beneficial effect of compounds on cardiac geometry. Detailed morphologic evaluation of myocardial infarction with classical histologic methods (i.e. serial sectioning, histologic staining, microscopic evaluation, etc.), the expense, labour and intensive nature of traditional methods restricts possibility to carry out a large number of experiments (Jang *et al.*, 1983; Epstein and Patterson, 1985; Ware *et al.*, 1992). There are several techniques that make these large-scale measurements easier and simpler. Among them computer assisted planimetry is emerging to be very helpful to shorten the time of evaluation, but preparation of the samples is still a problem.

Recently, Porzio *et al.* (1995) described a method for simultaneous measurement of the infarct size and left ventricular geometry in the rat. Accordingly, after cryostatic sectioning, nitroblue-tetrazolium staining and mounting on slides, video images were

analysed by a sophisticated computer technique. However, the expenditure and the time consuming nature of the procedure is not solved. In the present study, we developed a much simpler technique using a macroscopic sectioning and staining, no mounting, but a direct digitalization of stained slices of the heart using a flat-bed scanner. Furthermore, during the evaluation of stored images the differentiation of infarcted and non-infarcted tissues were performed automatically by the software using constant settings of the colours representing these area. In this method no manual delineation of the infarcted area is needed as in previous planimetric techniques. Therefore, a subjective component of the differentiation did not influence the evaluation, and myocardial infarction in small patches or the finger type border zone could easily be evaluated.

Using this evaluation method, the present investigations demonstrated that in the control animals the infarct size was smaller by the 7th day after coronary artery ligation as compared to the 1st day and this change was mainly due to the thinning of the scar tissue. The remodelling of the ventricle by the 7th day of infarction was characterised by thickening of the non-infarcted left ventricular myocardium and by the enlargement of the left ventricular cavity. These changes are in accordance with the results of others using classical morphological methods for the evaluation of experimental myocardial infarction in the rat (Fishbein *et al.*, 1978; Roberts *et al.*, 1984; Pfeffer *et al.*, 1992).

4. 4. Sulphonylureas and the development of myocardial infarction

The results in the present study indicate that one week intraperitoneal glibenclamide treatment significantly modified the capability of in vivo infarcted rat hearts to adapt to the gradually progressing compensatory reaction of the left ventricle. This effect was represented by a significant decrease in the volume of the infarcted myocardium at a dose that was previously shown to decrease basal plasma glucose concentration and inhibited its elevation upon oral glucose loading (EL-Reyani *et al.*, 1999). Such an effect appeared to be due to the increased scar thinning 7 days after coronary artery ligation. The smaller dose of the compound, that did not influence significantly the plasma glucose concentration (EL-Reyani *et al.*, 1999), did not influence scar formation, however, it still inhibited the thickening of the non-infarcted myocardium.

The reason(s) why glibenclamide treatment resulted in increased scar thinning and inhibited the hypertrophy of the non-infarcted myocardium is not known. Death of animals having larger myocardial infarction cannot explain the reason for this difference since in our

conditions death was rare after 1 day of coronary artery ligation. K_{ATP} inhibitors may increase the energy requirements of the myocardium by inhibiting the shortening of action potential duration due to decreased intracellular ATP concentration during myocardial ischaemia (Escand and Cavero, 1992; Grover *et al.*, 1994). Such an effect might result in a more intense loss of the myocardium within the infarcted region.

K_{ATP} inhibitors may also decrease vasodilation during hypoxia and ischaemia (Daut *et al.*, 1990; Aversano *et al.*, 1991; Komaru *et al.*, 1991). Inhibition of reactive hyperaemia in the myocardium after coronary artery ligation could also contribute to both the more intense scar thinning and to the inhibition of myocardial hypertrophy.

The hypoglycaemia itself evoked by glibenclamide is not likely to be responsible for scar thinning and inhibition of hypertrophy since glimepiride, in high dose that produced even more intense effects than glibenclamide on the plasma glucose level (Geisen *et al.*, 1988), did not influence scar thinning or the hypertrophy of the non-infarcted myocardium. Moreover, after glimepiride treatment the calculated volume of the left ventricular chamber was smaller than in the vehicle treated controls.

The discrepancies between the effects of the two compounds on the development of myocardial infarction is not only a conjecture. It might be important that glimepiride produces less significant effect on the inhibition of vascular K_{ATP} channels than glibenclamide (Bijlstra *et al.*, 1996; Geisen *et al.*, 1996). Whether this difference also exists in the inhibition of reactive hyperemia, thereby contributing to the observed differences in influencing the development of myocardial infarction, requires further experimentation. It was also found that glimepiride inhibited the cyclooxygenase pathway of isolated human platelets, while not influencing 12-lipoxygenase and phospholipase A_2 (Ozaki *et al.*, 1992). On the other hand, glibenclamide inhibited both the cyclooxygenase and the lipoxygenase pathways, as well as the phospholipase A_2. Such differences in the actions of the two compounds may explain the differences in their influence on the development of myocardial infarction, since phospholipase A_2 inhibition also adversely influences the healing process and results in increased scar thinning and mummification of the infarcted myocardium (Kloner *et al.*, 1978; Mannisi *et al.*, 1987).

In the present study, treatment with glibenclamide has been shown to attenuate the hypertrophy of the non-infarcted myocardium in vivo. Nevertheless, no data have been available as to whether the drug is able to ameliorate the functional capacity of heart after

myocardial infarction. The results reported here clearly show that treatment with glibenclamide for 7 days after permenant coronary artery occlusion tended to improve cardiac geometry as measured by a decrease in the volume of infarction and the marked reduction in the left ventricular hypertrophy when compared to vehicle treated rats.

Inhibition of the development of hypertrophy in the non-infarcted myocardium after permanent coronary occlusion may have two consequences. On one hand it may deteriorate the heart function and promote the shift from a compensated state to a decompensated one after myocardial infarction. On the other hand it may provide protection against cardiac failure developing as a consequence of ischaemic heart disease. Our results corroborate the results of others who have shown that in glibenclamide treated patients the incidence of post-infarction heart failure was significantly lower as compared to other hypoglycaemic agents (Lomuscio *et al.*, 1994). Further experimental and prospective controlled studies will be needed to definitely evaluate the question whether the disparate ability of antidiabetic agents to reduce left ventricular hypertrophy can be translated into better cardiovascular and overall prognosis of susceptible patients.

4. 5. GYKI-16638 and reperfusion arrhythmias in rabbits

The recently developed GYKI-16638 is a member of a new series of N-(phenoxyalkyl)-N-phenylalkylamine compounds. Its structure combines Class I/B and Class III structural elements, i.e. those of sotalol and mexiletine. In the present study, the antiarrhythmic effect of the compound was investigated in anaesthetised rabbits. The compound significantly decreased the number of animals that died due to lethal ventricular arrhythmias during reperfusion after 10 min regional myocardial ischaemia. The antiarrhythmic activity of GYKI-16638 was already observed after the administration of the lower dose that did not influence QT or QTc intervals.

Occurrence of torsades de pointes (TdP) is the classic proarrhythmic effect of selective I_{Kr} blockers that has been causally linked to an increased dispersion of ventricular repolarization both in experimental and clinical studies (Buchanan *et al.*, 1993; Vos *et al.*, 1995; Gottlieb *et al.*, 1995). Such proarrhythmia may be the cause of excess mortality seen in the SWORD trial with D-sotalol (Waldo *et al.*, 1996). These results have shifted the attention towards antiarrhythmic compounds with a combined mechanism of action. Amiodarone, for example, is an antiarrhythmic agent with a complex mode of action that has recently attracted a great deal of interest. It was shown to decrease the ventricular fibrillation vulnerability in

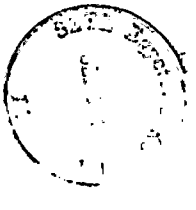
rabbit hearts following long-term pretreatment (Behrens *et al.*, 1997), to be protective against ischaemia-and reperfusion induced arrhythmias (Varro and Rabloczky, 1986; Li and Northover, 1992), and it is effectively used for the treatment of life threatening ventricular arrhythmias in humans (Singh 1999).

Amiodarone was found to have a remarkably low potential for inducing TdP tachyarrhythmias despite its ability to prolong QT interval (Hohnloser *et al.*, 1994). On the other hand, Class I/B antiarrhythmics like, mexiletine and lidocaine were shown to suppress TdP induced by D-sotalol both in animal (Carlsson, 1993b) and human studies (Assimes and Malcom, 1998). Antiarrhythmic drugs with Class I/B action have also been shown to be effective against coronary artery occlusion/reperfusion induced arrhythmias (Bonaduce *et al.*, 1986; He *et al.*, 1992; Komori *et al.*, 1995). These results suggest that an antiarrhythmic compound with combined Class I/B and Class III effects could reduce the incidence of re-entry arrhythmias without a high risk of producing TdP.

When administered chronically, amiodarone exhibits serious extracardiac side effects limiting its use (Hilleman *et al.*, 1998). GYKI-16638 shares some (Class I/B + Class III) but not all of the electrophysiological properties of amiodarone and its chemical structure is also different. Based on its chemical structure (containing no iodine), it can be reasonably expected that this compound, unlike amiodarone, would be relatively free of extracardiac side effects. Due to its Class I/B action it is also expected that the compound lacks the significant inhibitory effect on conduction at normal heart rate. However, further studies are needed to elucidate the possible side effects of GYKI-16638.

The haemodynamic side effects are of particular importance when considering antiarrhythmic drugs. GYKI-16638 did not change the mean arterial blood pressure, but decreased the heart rate of anaesthetised rabbits. A similar heart rate decreasing effect of D-sotalol has been shown by Schwartz et al. (1987), although this compound lacks the antiadrenergic properties of DL-sotalol. A moderate decrease in heart rate may be beneficial, especially in the setting of myocardial ischaemia and reperfusion induced arrhythmias (Bernier et al., 1989).

Based on these results, GYKI-16638 can be regarded as a novel antiarrhythmic drug candidate which provides protection against coronary artery occlusion and reperfusion induced arrhythmias in anaesthetised rabbits. This protection was already noticed at a lower dose that did not lengthen the QTc interval significantly.



5. Conclusion

The present results suggest that potassium channel blockers may show a remarkable diversity which can presumably be utilized therapeutically in the future. Important differences might be expected among these agents in (1) the degree of selectivity for the various potassium channel subtypes, (2) the voltage and time-dependence of channel block, (3) their ability to prolong repolarization at short cycle lengths and (4) the propensity to induce excessive prolongation and early afterdepolarization. Although some properties of the different agents classified can be generalized, one cannot assume that all agents belonging to a certain class will be identical in their electrophysiological profiles or their antifibrillatory and proarrhythmic actions.

Highly potent and selective potassium channel blockers not only provide an extremely useful means of clarifying possible arrhythmogenic mechanisms as expressed in animal models and in humans, but appear to hold considerable promise as an alternate mode of antiarrhythmic therapy. The diversity of potassium channels present in the heart, their regional expression and their possible long-term modulation, present an intriguing number of possibilities for building in selectivity and minimizing the risk of proarrhythmia and other adverse effects.

In the development of new agents a number of issues merit consideration. There is now much evidence that multiple channel and receptor action properties may provide a better antiarrhythmic effect. The development of newer compounds having more complex mechanism of action may provide clinically usefull drugs in the treatment of early and late events of myocardial infarction.

6. References

- Akita H, Creer MH, Yamada KA, Sobel BF, Corr PB. Electrophysiologic effects of intracellular lysophophoglycerides and their accumulation in cardiac lymph with myocardial ischemia in dogs. *J Clin Inves* 1986; **78**: 271-280.
- Amoroso S, Schmide-Antomarchi H, Fosset M, Lazdunski M. Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive potassium channels. *Science* 1990; **247**: 852-854.
- Arnsdorf MF, Sawicki GJ. The effects of lysophosphatidylcholine, a toxic metabolite of ischemia, on the components of cardiac excitabilty in sheep purkinje fibers. *Circ Res* 1981; **49**: 16-30.
- Ashcroft FM, Harrison DE, Ashcroft SJH. Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. *Nature* 1984; **312**: 446-447.
- Ashcroft SJH, Ashcroft FM. The sulfonylurea receptor. *Biochim Biophys Acta* 1992; **1175**: 45-59.
- Ashford MLJ, Boden PR, Treherne JM. Tolbutamide excites rat glucoreceptive ventromedial hypothalamic neurons by indirect inhibition of ATP-K^+ channels. *Br J Pharmacol* 1990; **101**: 531-540.
- Assimes TL, Malcolm I. Torsade de pointes with sotalol treated successfully with lidocaine. *Can J Cardiol* 1998, **14**: 753-756.
- Auchampach JA, Grover GJ, Gross GJ. Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc Res* 1992; **26**:1054-62.
- Auchampach JA, Maruyama M, Cavero I, Gross GJ. The new K^+ channel opener Aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes. *J Pharmacol Exp Ther* 1991; **259**: 961-7.
- Aupetit JF, Loufoua-Moundanga J, Faucon G, Timour Q. Ischemia-induced loss or reversal of the effects of the class I antiarrhythmic drugs on vulnerability to fibrillation. *Br J Pharmacol* 1997, **120**: 523-9.
- Aversano T, Ouyang P, Silverman H. Blockade of the ATP-sensitive potassium channel modulates reactive hyperemia in the canine coronary circulation. *Circ Res* 1991, **69**:618-622.
- Baczkó I, Leprán I, Papp JGy. K_{ATP} channel modulators increase survival rate during coronary occlusion-reperfusion in anaesthetised rats. *Eur J Pharmacol* 1997; **324**: 77-83.
- Ballagi-Pordany G, Koszeghy A, Koltai MZ, Aranui Z, Pogatsa G. Divergent cardiac effects of the first and second generation hypoglycemic sulfonylurea compounds. *Diabetes Res Clin Pract* 1990; **8**: 109-114.
- Bassand JP. Left ventricular remodelling after acute myocardial infarction solved and unsolved issues. *Eur Heart J* 1995; **16** (Suppl I): 58-63.
- Basu DK, Karmazyn M. Injury to rat hearts produced by an exogenous free radical generating system. Study into the role of arachidonic acid and eicosanoids. *J Pharmacol Exp Ther* 1987; **242**: 673-85.

- Baxter GF, Yellon DM. Attenuation of reperfusion-induced ventricular fibrillation in the rat isolated hypertrophied heart by preischemic diltiazem treatment. *Cardiovasc Drugs Ther* 1993; 7: 225-231.
- Behrens S, Li C, Franz MR. Effects of long-term amiodarone treatment on ventricular fibrillation vulnerability and defibrillation efficacy in response to monophasic and biphasic shocks. *J Cardiovasc Pharmacol* 1997, **30**: 412-418.
- Bekheit SS, Restivo M, Boutjdir M, Henkin R, Gooyandeh K, Assadi M, Khatib S, Gough WB, EL-Sherif N. Effects of glyburide on ischemia-induced changes in extracellular K^+ and local myocardial activation: a potential new approach to the mangement of ischemia-induced malignant ventricular arrhythmias. *Am Heart J* 1990; **119**: 1025-1033.
- Belles B, Hescheler J, Trub G. Changes of membrane currents in cardiac cells induced by long whole-cells recordings and tolbutamide. *Pflügers Arch* 1987; **409**: 582-58.
- Bernardi H, Fosset M, Lazdunski M. Characterization, purification and affinity labelling of the brain [3H] glibenclamide binding protein, a putative neuronal ATP-regulated K^+ channel. *Proc Natl Acad Sci USA* 1988; **85**: 9816-9820.
- Bernardi H, de Weille JR, Epelbaum J, Mourre C, Amoroso S, Slama A, Fosset M, Lazdunski M. ATP-modulated K^+ channels sensitive to antidiabetic sulfonylureas are present in adenohypophysis and are involved in growth hormone release . *Proc. Natl. Acad. Sci* 1993; **90**: 1340-1344.
- Bernauer W. Concerning the effect of the K^+ channel blocking agent glibenclamide on ischaemic and reperfusion arrhythmias. *Eur J Pharmacol* 1997; **326**: 147-156.
- Bernier M, Curtis MJ, Hearse DJ. Ischaemia-induced and reperfusion-induced arrhythmias: Importance of heart rate. *Am J Physiol* 1989, **235**: 21-31.
- Bigger JT, Fleiss JL, Kleiger R. The relationships among ventricular arrhythmias, left ventricular dysfunction and mortality in the two years after myocardial infarction. *Circulation* 1984; **69**:250-258.
- Billman GE, Avendano CE, Halliwill JR, Burroughs JM. The effect of the ATP-dependent p[otassium channel antagonist, glyburide, on coronary blood flow and susceptibility to ventricular fibrillation in unanesthetized dogs. *J Cardiocvasc Pharmacol* 1993; **21**: 197-204.
- Billman GE, Englert HC, Scholkens BA. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel: Effects on susceptibility to ventricular fibrillatio induced by myocardial ischemia in conscious dogs. *J Pharmacol Exp Ther* 1998, **286**: 1465-1473
- Bijlstra PJ, Lutterman JA, Russel FGM, Thein T, Smith P. Interaction of sulphonylurea derivatives with vascular ATP-sensitive potassium channels in humans. *Diabetologia* 1996; **39**: 1083-1090.
- Blaustein AS, Schine L, Brooks WW, Fanburg BL, Bing OH. Influence of exogenously generated oxidant species on myocardial function. *Am J Physiol* 1986; **250**: H595-9.
- Bralet J, Didier JP, Moreau D, Opie LH, Rochette L. Effect of alpha-adrenoceptor antagonists (phentolamine, nicergoline, and prazosine) on reperfusion arrhythmias and noradrenaline release in perfused rat heart. *Br J Pharmacol* 1985; **84**: 9-18.
- Bril A, Laville MP, Gout B. Effects of glibenclamide on ventricular arrhythmia and cardiac function in ischaemia and reperfusion in isolated rat heart. *Cardiovasc Res* 1992; **26**: 1069-1076.

- Buchanan LV, Kabell G, Brunden MN, Gibson JK. Comparative assessement of ibutilide, D-sotalol, clofilium, E-4031, and UK-68,798 in a rabbit model of proarrhythmia. *J Cardiovasc Pharmacol* 1993, **22**: 540-549.
- Bui-Xuan B, Aupetit JF, Freysz M, Faucon G, Timour Q. Prevention of calcium antagonists of profibrillatory effects of class I antiarrhythnmic drugs in acute myocardial ischemia: a study in pig heart in situ. *Pharmacotherapy* 1997, **17**: 737-45.
- Cacciapuoti F, Spiezia R, Bianchi U. Effectiveness of glibenclamide on myocardial ischemic ventricular arrhythmias in non-insulin-dependent diabetes millitus. *Am J Cardiol* 1991, **67**: 843-847.
- Carlsson L, Abrahamson C, Anderson B, Duker G, Schiller-Linhardt G. Proarrhythmic effects of the class III agent almokolant: importance of infusion rate, QT dispesion, and early afterdepolrisations. *Cardiovasc Res* 1993a, **27**: 2186-2193.
- Carlsson L, Drews L, Duker Gm, Schiller-Linhardt G. Attenuation of proarrhythmias related to delayed repolarization by low-dose lidocaine in the anaesthetised rabbits. *J Pharmacol Exp Therap* 1993b, **267**: 1076-1080.
- Casale PN, Devereux RB, Milner M. Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Ann Intern Med* 1986; **105**:173-178.
- Chi L, Uprichard ACG, Lucchesi BR. Profibrillatory actions of pinacidil in a conscious canine model of sudden coronary death. *J Cardiovasc Pharmacol* 1990 ;**15**: 452-464.
- Chi L, Uprichard ACG, Lucchesi BR. Filure of glibenclamide to protect against ischemic ventricular fibrillation in a canine model of myocardial infarction. *J Mol Cell Cardiol* 1989; **21**: S89.
- Cole W, McPherson CD, Sontag D. ATP regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* 1991; **69**:571-581.
- Coronel R, Wilms-Schopman FJG, Opthof T, Cinca J, Fiolet JWT, Janse MJ. Reperfusion arrhythmias in isolated perfused pig hearts: inhomogeneities in extracellular potassium, ST and TQ potentials and transmembrane action potentials. *Circ Res* 1992; **71**: 1131-42.
- Curtis MJ, Hearse DJ. Reperfusion-induced arrhythmias are critically dependent upon occluded zone size: relevance to the mechanism of arrhythmogenesis. *J Mol Cell Cardiol* 1989; **21**: 624-637.
- Curtis MJ, Pugsley MK, Walker MJA. Endogenous chemical mediators of ventricular arrhythmias in ischaemic heart disease. *Cardiovasc Res* 1993; **27**: 703-719.
- D'Alonzo AJ, Darbenzio RB, Hess TA, Sewter JC, Sleph PG, Grover GJ. Effetc of potassium on the action of KATP modulators cromakalim, pinacidil, or glibenclamide on arrhythmias in isolated perfused rat heart subjected to regional ischaemia. *Cardiovasc Res* 1994; **28**: 881-887.
- Dart C, Standen NB. Adenosine-activated potassium current in smooth muscle cells isolated from the pig coronary artery. *J Physiol Lond* 1994; **471**: 767-786.
- Daut J, Maier-Rudolph W, von Beckerath N, Mehrke G, Gunther K, Goedel-Meinen L. Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* 1990; **247**: 1341-4.

- Daut J, Klieber HG, Cyrys S, Noak T. KATP channels and basal coronary vascular tone. *Cardivasc Res* 1994; **28**: 811-817.
- Davies NW, Standen NB, Stanfield PR. ATP-depedent potassium channels of muscle cells: their properties, regulation, and possible functions. *J Bioenerg Biomembr* 1991; **23**: 509-535.
- Davies NW, Spruce AE, Stanfield PR. Multiple blocking mechanisms of ATP-sensitive potassium channels of frog skeletal muscle by tetraethylammonium ions. *J Physiol Lond* 1989; **413**: 31-48.
- Davis TME, Parsons RW, Broadhorst R, Hobbs M, Jamrozik K. Arrhythmias and mortality after myocardial infarction in diabetic patients: relationship to diabetes treatment. *Diabetologia* 1996, **39** (Suppl 1): A51 (Abstract).
- Dawber TR. The Framingham Study: The Epidemiology of Atherosclerotic Disease. *Cambridge*, (MA) (1980): Harvard University Press.
- Dimassi N, Bril A, Autissier N, Bralet J, Rochette L. Relations between reperfusion arrhythmias and myocardial norepinephrine and accumulation of calcium in the rat. *Cardioscience* 1992; **3**: 7-12.
- Downey JM. Ischemic preconditioning: nature's own cardioprotective intervention. *Trends Cardiovasc Pharmacol* 1992; **2**: 170-176.
- Edward M, Boyle Jr, Timothy H, Carol J, Cornejo and Edward D. Verrier. Ischemia-Reperfusion Injury. *Ann Thorac Surg* 1997; **64**, (Suppl 4): S24-S30.
- EL-Reyani N, Bozdogan Ő, Baczkó I, Leprán I, Papp JGy. Comparison of the efficacy of glibenclamide and glimepiride in reperfusion-induced arrhythmias in rats. *Eur J Pharmacol* 1999; **365**: 187-192.
- Faivre JF, Findlay I. Action potential duration and activation of the ATP-sensitive potassium current in isolated guinea pig ventricular myocytes. *Biochim Biophys Acta* 1990; **1029**: 167-172.
- Findlay I. Inhibition of ATP-sensitive K^+ channels in cardiac muscle by the sulphonylurea drug glibenclamide. *J Pharmacol Exp Ther* 1992, **261**:540-545.
- Findlay I. The ATP-sensitive K^+ channel of cardiac muscle and action potential shortening during metabolic stress. *Cardiovsc Res* 1994; **28**: 760-761.
- Findlay I. ATP4- and ATP·Mg inhibit the ATP-sensitive K^+ channel of rat ventricular myocytes. *Pflug Arch Eur J Physiol* 1988; **412**: 37-41.
- Fishbein MD, Macs Clean D, Maroko P. Experimental myocardial infarction in the rat. Am *J Pathol* 1978: 57-70.
- Fiskum G. Involvement of mitochondria in ischaemic injury and in regulation of intracellular calcium. *Am J Emerg Med* 1983; **1** (2): 147-153.
- Fosset M, de Weille JR, Green RD, Schmid-Antomarchi H, Lazdunski M. Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *J Biol Chem* 1988; **263**: 7933-8.
- Gasser RNA, Vaughan-Jones RD. Mechanism of potassium efflux and action potential shortening during ischemia in isolated mammlian cardiac muscle. *J Physiol Lond* 1990; **431**: 713-741.

- Geisen K, Vegh A, Krause E, Papp JGy. Cardiovascular effects of conventional sulfonylureas and glimepiride. *Horm Metab Res* 1996; **28**: 496-507.
- Geisen K. Special Pharmacology of the new sulfonylurea glimepiride. *Arzneim-Forsch (Drug Res)* 1988; **38**: 1120-1130.
- Gottlieb SS, Cines M, Marshall J. Torsade de points with administration of high-dose intravenous d-sotalol to a patient with congestive heart failure. *Pharmacotherapy* 1997, **17**: 830-831.
- Gelvan D, Saltman P, Powell SR. Cardiac reperfusion damage prevented by a nitroxide free radical. *Proct Natl Acad Sci USA* 1991; **88**: 4680-4684.
- Greenberg HM, Dwyer EM, Jr. Hochman JS, Stienberg JS, Echt DS, Peters RW. Interaction of ischemia and encainide/flecainide treatment: a proposed mechanism for the increased mortality in CAST 1. *Br Heart J* 1995, **74**: 631-5.
- Gross GJ, Auchampach JA. Role of ATP-dependent potassium channels in myocardial ischaemia. *Cardiovasc Res* 1992; **26**: 1011-1016.
- Grover GJ, Sleph PG, Dzwonczyk. 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.
- Grover GJ, McCullough JR, Henry DE, Conder ML, Sleph PG. The anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with potassium channel blocker glyburide. *J Pharmacol Exp Ther* 1989; **251**: 98-110.
- Grover GJ. Protective effects of ATP sensitive potassium channel openers in models of myoardial ischaemia. *Cardiovasc Res* 1994; **28**: 778-782.
- Haimovici M. Muscular, renal and metablic complications of acute arterial occlusions. Myonephrotic-metabolic syndrome. *Surgery* 1979; **85**: 461-468.
- Haworth RA, Goknur AB, Berkoff HA. Inhibition of ATP-sensitive potassium channels of adult rat heart cells by antiarrhythmic drugs. *Circ Res* 1989; **65**: 1157-1160.
- Hearse DJ. Ischemia, reperfusion and determinants of tissue injury. *Cardiovasc Drugs Ther* 1990; **4**: 767-76.
- Heart and Stroke Facts: Statistical Supplement. Dallas,TX: *American Heart Association* 1997.
- Hill JL, Gettes LS. Effect of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine. *Circulation* 1980; **61**: 768-772.
- HillemanD, Miller MA, Parker R, Doering P, Pieper JA. Optimal management of amiodarone therapy: efficacy and side effects. *Pharmacotherapy* 1998, **18**: 138S-145S.
- Hochman JS, Bulkley BH. Expansion of acute myocardial infarction: an experimental study. *Circulation* 1982; **65**: 1446-50.
- Hohnloser SH, Woosley RL. Sotalol. *N Engl J Med* 1994, **331**: 31-8.
- Imanishi S, Arita M, Kiyosue T, Aomine M. Effects of SG-75 (nicorandil) on electrical activity of canine cardiac Purkinje fibers: possible increase in potassium conductance. *J Pharmacol Exp Ther* 1983; **225**: 198-205.

- Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* 1996; **16** (5):1011-7.
- Isomoto S, Kurachi Y. Function, regulation, pharmacology, and molecular structure of ATP-sensitive K^+ channels in the cardiovascular system. *J Cardiovasc Electrophysiol* 1997; **8** (12):1431-46.
- Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, Matsuzawa Y, Kurachi Y. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J Biol Chem* 1996, **271**; :24321-4.
- Jackson WF. Arteriolar tone is determined by activity of ATP-sensitive potassium channels. *Am J physiol* 1993; **265**: H1797-H1803.
- Johns TNP, Olson BJ. Experimental myocardial infarction: I. A method of coronary occlusion in small animals. *Ann Surg* 1954; **140**: 675-682.
- Johnson BA, Weil MH, Tang W, Noc M, McKee D, McCandless D. Mechanisms of myocardial hypercarbic acidosis during cardiac arrest. *J Appl Physiol* 1995; **78** (4): 1579-84.
- Josephson ME, Horowitz LN, Farshidi A. Recurrent sustained ventricular tachycardia: 1. Mechanisms. *Circulation* 1978; **57**: 431-439.
- Kakei M, Noma A, Shibasaki T. Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. *J Physiol Lond* 1985; **363**: 441-462.
- Kannel WB, Dannenberg AL, Levy D. Population implications of electrocardiographic left ventricular hypertrophy. *Am J Cardiol* 1987; **60**: 85I-93I.
- Kantor PF, Coetzee WA, Carmeliet EE, Dennis SC, Opie LH. Reduction of ischemic K^+ loss and arrhythmias in rat hearts: effect of glibenclamide, a sulfonylurea. *Circ Res* 1990; **66**: 478-85.
- Kantor PF, Coetzee WA, Dennis SC, Opie LH.. Effects of glibenclamide on ishaemic arrhythmias. *Circulation* 1987; **76** (Suppl. 4): 17.
- Katz AM. Effects of ischemia on the contractile processes of heart muscle. *Am J Cardiol* 1973; **32**: 456-60.
- Kleber AG. Resting membrane potential, extracellular potassium and intracellular sodium activity during global ischemia in isolated perfused guinea pig hearts. *Circ Res* 1983; **52** (4): 442-50.
- Klein RC, Beckman J, Peake G. Absence of differential inhibition of thromboxane A2 and prostacyclin by low and high dose aspirin in coronary artery disease. *Can J Cardiol* 1987; **3** (6):288-92.
- Kondo T, Kubota I, Tachibana H, Yamaki M, Tomoike H. Glibenclamide attenuates peaked T wave in early phase of myocardial ischemia. *Cardiovasc Res* 1996; **31**: 683-687.
- Krumholz HM, Goldberger AL. Reperfusion arrhythmias after thrombolysis: electrophysilogic tempest, or much ado about nothing. *Chect* 1991; **99** (Suppl S): 135-140.
- Kuck KH, Sghofer J, Schluter M, Bleifeld W, Mathey D. Reperfusion arrhythmias in man: influence of intrvenous lidocaine. *Eur Heart J* 1985; **6** (Suppl E): 163-167.

- Langtry HD, Balfour JA. Glimepiride: a review of its use in the management of type 2 diabetes mellitus. *Drugs* 1998; **55**: 563-584.
- Leprán I, Baczkó I, Varró A, Papp JGy. ATP-sensitive potassium channel modulators: both pinacidil and glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rats. *J Pharmacol Exp Ther* 1996: **227**: 1215-1220.
- Leprán I, Koltai M, Siegmund W, Szekeres L. Coronary artery ligation, early arrhythmias, and determination of the ischemic area in conscious rats. *J Phamacol Meth* 1983; **9**: 219-230.
- Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**:1561-1566.
- Levy D, Salomon M, D'Agostino RB, Belanger AJ, Kannel WB. Prognostic implications of baseline electrocardiographic features and their serial changes in subjects with left ventricular hypertrophy. *Circulation* 1994; **90**:1786-1793.
- Li JY, Northover BJ. Antiarrhythmic and electrophysiologic effects of amiodarone, lidocaine, and penticainide in anaesthetised rats. *Cardiovasc Res* 1992, **26**: 1116-1120.
- Liu X, Tosaki A, Engelman RM, Das DK. Salicylate reduces ventricular dysfunction and arrhythmias during reperfusion in isolated rat hearts. *J Cardiovasc Pharmacol* 1992; **19**: 209-215.
- Lomuscio A, Vergani D, Marano L, Castagnone M, Fiorentini C. Effects of glibenclamide on ventricular fibrillation in non-insulin-dependent diabetics with acute myocardial infarction. *Coron Artery Dis* 1994; **9**:767-71.
- Loubatiéres A. Effects of sulfonylurea on the pancreas, in The Diabetic Pancreas (Volk BW, Welmann RE, eds). *Bailliere Tindall, London* 1977: 489-515.
- Lu HR, Remeysen P, De Clerck F. Does the antiarryhthmic effect of ischemic preconditioning in rats involves the L-arginine nitric oxide pathway?. *J Cardiovasc Pharmacol* 1995; **25**: 524-530.
- Mackenzie I, Saville VL, Waterfall JF. Differential class-III and glibenclamide effects on action potential duration in guinea-pig papillary muscle during normoxia and hypoxia/ischaemia. *Br J Pharmacol* 1993; **110**: 531-538.
- Maggioni AP. Prevalence and prognostic significance of ventricular arrhythmias after acute myocardial infarction in the fibrinolytic era: GISSI-2 results. *Circulation* 1993; **87**:312-322.
- Manning AS, Coltart DJ, Hearse DJ. Ischemia and reperfusion-induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with allopurinol. *Circ Res* 1984; **55**: 545-548.
- McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985; **312**: 159-63.
- McHowatt J, Yamada KA, Wu J, Yan GX, Corr PB. Recent insights pertaining to sarcolemmal phospholipid alterations underlying arrhythmogenesis in the ischemic heart. *J Cardiovasc Electrophysiol* 1993; **4**: 288-310.
- Miki T, Nagashima K, Seino S. The structure and function of the ATP-sensitive K^+ channel in insulin-secreting pancreatic beta-cells. *J Mol Endocrinol* 1999; **22** (2):113-23.
- Miura T, Goto M, Sakamoto J, Shimamoto K, Iimura O. Glibenclamide, a blocker of ATP-sensitive potassium channels, abolishes infarct size limitation by preconditioning in rabbits

anaesthitized with xylazine pentobarbial but not with pentobarbital alone. *J Cardiovasc Pharmacol* 1995; **25**: 531-538.

- Mourre C, Ben Ari Y, Bernardi H, Fosset M, Lazdunski M. Antidiabetic sulphonylureas: localization of binding sites in the brain and effects on the hyperpolarization induced by anoxia in hippocampal slices. *Basic Res* 1989; **486**: 159-164.
- Muller G, Hartz D, Punter J, Okonomupulos R, Kramer W. Differential interaction of glimepiride and glibenclamide with the beta-cell sulfonylurea receptor.: I. Binding characteristics. *Biochem Biophys Acta* 1994; **1191**; 267-277.
- Munch-Ellingsen J, Bugge E, Ytrehus K. Blockade of the KATP-channel by glibenclamide aggravates ischemic injury, and counteracts ischemic preconditioning. *Basic Res Cardiol* 1996; **91**: 382-8.
- Murohara Y, Yui Y, Hattori R, Kawai C. Effect of superoxide dismutase on reperfusion arrhythmias and left ventricular function in patients undergoing thrombolysis for anterior wall acute myocardial infarction. *Am J Cardiol* 1991; **67**: 765-767.
- Nelson MT. Ca2+-activated potassium channels and ATP-sensitive potassium channels as modulators of vascular tone. *Trends Cardiovasc. Med* 1993; **3**: 54-60.
- Noma A. ATP-regulated K^+ channels in cardiac muscle. *Nature* 1983; **305**: 147-148.
- O'Callaghan PA, McGovern BA. Evolving role of sotalol in the management of ventricular tachyarrhythmias. *Am J Cardiol* 1996, **78**: 54-60.
- Opie LH, Nathan D, Lubbe WF. Biochemical aspects of arrhythmogenesis. *Am J Cardiol* 1979; **43**: 131-48.
- Ozaki Y, Yatomi Y, Kume S. Effects of oral hypoglycaemic agents on platelet functions. *Biochem Pharmacol* 1992; **44**: 687-691.
- Panten U, Schwanstecher M, Schwanstecher C. Pancreatic and extrapancreatic sulfonylurea receptors. *Horm Metab Res* 1992; **24** (12): 549-54.
- Parks DA, Granger DN. Contribution of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol* 1986; **250**: G749-G753.
- Parratt JR, Kane KA. K_{ATP} channels in ischaemic preconditioning. *Cardiovasc Res*, 1994; **28**: 783-787.
- Patterson CE, Rhoades RA. Protective role of sulphydryl reagents in oxidant lung injury. *Exp Lung Res* 1988; **14**: 1005-1019.
- Pfeffer MA, Braunwald E. Ventricular remodelling after myocardial infarction. Experimental observations and clinical implication. *Circulation* 1990; **81**: 1161-72.
- Pogatsa G, Koltai MZ, Balkanyi L, Devai I, Kiss V, Koszeghy A. The effect of various hypoglycaemic sulphonylureas on the cardiotoxicity of glycosides and arrhythmogenic activity due to myocardial ischaemia. *Acta Physiol Hung* 1988; **71**: 243-250.
- Pogatsa G, Koltai MZ, Jermendy G, Simon J, Aranyi Z, Ballagi-Pordany G. The effect of sulphonylurea therapy on the outcome of coronary heart diseases indiabetic patients. *Acta Med Hung* 1992-93; **49:** 39-51.
- Pogwizd SM, Corr PB. Reentrant and nonreentrant mechanisms contribute to arrhythmogenesis during early myocardial ischemia: results using three-dimensional mapping. *Circ Res* 1987a; **61**: 352-71.

- Pogwizd SM, Corr PB. Electrophysiologic mechanisms underlying arrythmias due to reperfusion of ischemic myocardium. *Circulation* 1987b; **76**: 404-26.
- Pogwizd SM, Corr B. The contribution of nonreentrant mechanisms to malignant ventricular arrhythmias. *Basic Res Cardiol* 1992; **87** (Suppl 2):115-29.
- Ponce Zumino A, Risler NR, Schanne OF, Ruiz Petrich E, Carrion A. Magnesium: effects on reperfusion arrhythmias and membrane potential in isolated rat hearts. *Mol Cell Biochem* 1997; **171**: 85-93.
- Priori SG, Mantica M, Napolitano C, Schwartz PJ. Early afterdepolarizations induced in vivo by reperfusion of ischemic myocardium. A possible mechanism for reperfusion arrhythmias. *Circulation* 1990; **81**: 1911-1920.
- Quast U, Cook NS. Potent inhibitors of the effect of the K^+ channel opener BRL 34915 in vascular smooth muscle. *Br J Phamacol* 1988; **93**: 204P.
- Quayle JM, Standen NB. KATP channels in vascular smooth muscle. *Cardiovasc Res* 1994; **28**: 797-804.
- Rees SA, Curtis MJ. Pharmacological analysis in rat of the role of the ATP-sensitive potassium channel as a potential target for antifibrillatory intervention in acute myocardial ischaemia. *J Cardiovasc Pharmacol* 1995; **26**: 280-288.
- Rorsman P, Trub G. Evidence that glucose-induced depolarization in the pancreatic β-cells involves the closure of an ATP-dependent K^+ channel. *Acta Physiol Scand* 1985; **124**: 147.
- Russell DC, Oliver MF. Ventricular refractoriness during acute myocardial ischemia and its relationship to ventricualr fibrillation. *Cardiovasc Res* 1978; **12** (4): 221-7.
- Saffitz JE, Corr PB, Sobel BE. Arrhythmogenesis and ventricular dysfunction after myocardial infarction. Is anomalous cellular coupling the elusive link? *Circulation* 1993; **87**: 1742-1745.
- Schotborg CE, Wilde AAM. Sulfonylurea derivatives in cardiovascular research and in cardiovascular pateints. *Cardiovasc Res* 1997; **34**: 73-80.
- Schultz JE, Qian YZ, Gross GJ, Kukreja RC. The ischemia-selective K-ATP channel antagonist, 5-hydroyxdecanoate, blocks ischemic preconditioning in the rat heart. *J Mol Cell Cardiol* 1997; **29**: 1055-1060.
- Selye H, Bajus E, Grasso S, Mendell P. Simple techniques for the surgical occlusion of coronary vessels in the rat. *Angiology* 1960; **11**: 398-407.
- Sewell WH, Koth DR, Huggins CE. Ventricular fibrillation in dogs after sudden return of flow to the coronary artery. *Surgery* 1955; **38**: 1050-1053.
- Simon RJ. Maxawell, Gregory YH. Lip. Reperfusion injury: a reviw of the pathophysiology, clinical manifistation and therapeutic options. *Int J Cardiol* 1997; **58**: 95-117.
- Singh BN. Current antiarrhythmic drugs: an overview of mechanisms of action and potential clinical utility. *J Cardiovasc Electrophysiol* 1999, **10**: 283-301.
- Smith RC, Leung JM, Keith FM, Merrick S, Mangano DT. Ventricular dysrhythmias in patients undergoing coronary artery bypass graft surgery: incidence, characteristics, and prognostic importance. *Am Heart J* 1992; **123**: 73-81.

- Spruce AE, Standen NB, Stanfield PR. Studies of the unitary properties of adenosine-5`-triphosphate-regulated potassium channels of frog skeletal muscle. *J Physiol* 1987; **382**: 213-236.
- Sturgess NC, Ashford ML, Cook DL, Hales CN. The sulfonylurea receptor may be an ATP-sensitive potasium channel. *Lancet* 1985; **31**: 471-475.
- Sullivan JM, Vander Zwaag RV, el-Zeky F, Ramanathan KB, Mirvis DM. Left ventricular hypertrophy: effect on survival. *J Am Coll Cardiol* 993; **22**: 508-513.
- Tachibana H, Kubota I, Yamaki M, Watanabe T, Tomoike H. Discordant S-T alternans contributes to formation of reentry: a possible mechanism of reperfusion arrhythmia. *Am J Physiol* 1998; **275**: H116-21.
- The Cardiac Arrhythmia Suppression Trial Investigators: Preliminary report: Effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. *N Engl J Med* 1989; **32**: 406-412.
- The cardiac Arrhythmia Suppression Trial II Investigators: Effect of the antiarrhythmic agent moricizine on survival after myocardial infarction. *N Engl J Med* 1992; **327**: 227-233.
- 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 Pharmacolo Exp Ther* 1993; **267**: 1355-1362.
- Tsuchiya K, Wang W, Giebisch G, Welling P. ATP is a coupling-modulator of parallel Na/KATPase-K channel activity in the renal proximal tubule. *Proc Natl Acad Sci* 1992; **89**: 6418- 6422.
- Tweedie D, Henderson C, Kane K. Glibenclamide, but not class III drugs, prevents ischaemic shortening of the refractory period in guinea-pig hearts. *Eur J Pharmacol* 1993; **240**: 251-257.
- Undrovinas AI, Burnashev N, Eroshenko D, Fleidervish I, Starmer CF, Makielski JC, Rosenshtraukh LV. Quinidine blocks 5`-triphosphate-sensitive potassium channels in heart. *Am J Physiol* 1990; H1609-1612.
- Vanheel B, Hemptinne A. Influence of KATP channel on modulation on net potassium efflux from ischaemic mammalian cardiac tissue. *Cardiovasc Res* 1992; **26**: 1030-9.
- Varro A, Rabloczky G. Antiarrhythmic effect of acute and chronic amiodarone treatment in conscious rats. *J Pharm Pharmacol* 1986, **38**: 772-773.
- Venkatesh N, Lamp ST, Weiss JN. Sulfonylureas, ATP-sensitive K^+ channels and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ Res* 1991; **69**: 623-37.
- Vesterqvist O. Measurements of the in vivo synthesis of thromboxane and prostacyclin in humans. *Scand J Clin Lab Invest* 1988; **48** (5): 401-7.
- Vos MA, Verduyn SC, Gorgles AP, Lipcsei GC, Wellens HJ. Reproducible induction of early afterdepolarizations and torsade de points arrhythmias by d-sotalol and pacing in dogs with chronic atriventricular block. *Circulation* 1995, **91**: 864-872.
- Walker MJA, Curtis MJ, Hearse DJ., *et al.* The Lambeth Conventions: Guidlines for the study of arrhythmias in ischemia, infarction, and rperfusion. *Cardiovasc Res* 1988; **22**: 447-455.
- Waldo AL, Camm AJ, De Ruyter H. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. *Lancet* 1996, **348**: 7-12.

- Wallenstein S, Zucker CL, Fleiss J L. Some statistical methodsuseful in circulation research. *Circ Res* 1980; **47**: 1-9.
- Weik R, Neumke B. ATP-sensitive potassium channels in adult mouse skeletal muscle: characterization of the ATP-binding site. *J Membr Biol* 1989; **110**: 217-226.
- Weiss JN, Venkatesh N. Metabolic regulation of ATP-sensitive K^+ channels. *Cardiovasc Drugs Ther* 1993; **7**: 499-505.
- Wibrand F, Honoré E, Lazdunski M. Opening of glibenclamide-sensitive K^+ channels in follicular cells promotes Xenopus oocyte maturation. *Proc Natl Acad Sci*, 1992; **89**: 5133-5137.
- Wild AAM, Escand D, Schumacher CA, Thuringer D, Mestre M, Fiolet JWT, Janse MJ. Potassium accumulation in the globally ischemic mammalian heart. A role for the ATP-sensitive potassium channel. *Circ Res* 1990; **67**: 835-843.
- Wild AAM, Janse MJ. Electrophysiological effects of ATP-sensitive potassium channel modulation: implications for arrhythmogenesis. *Cardiovasc Res* 1994; **28**: 16-24.
- Wild AAM. Role of ATP-sensitive K+ currnet in ischemic arrhythmias. *Cardiovasc Drugs Ther* 1993; **7**: 521-526.
- William J. Groh. The use of implantable cardioverter-defibrillators for primary prevention of sudden cardiac death. *ACC Current Journal Review* 1998; **7** (4): 48-51.
- Wit AL, Janse MJ. The Ventricular Arrhythmias of Ischemia and Infarction: Electrophysiological Mechanisms. *Futura Publishing Company, Inc Mourt Kisco, NY* 1993.
- Wolleben CD, Sanguinetti MC, Siegl PK. Influence of ATP-sensitive potassium channel modulators on ischemia-induced fibrillation in isolated rat hearts. *J Mol Cell Cardiol* 1989; **21**: 783-788.
- Woodward B, Zakaria MN. Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. *J Moll Cell Cardiol* 1985; **17**: 485-493.
- Yamamoto T, Cohen AM, Kakar NR, Yamamoto M, Johnson PE, Cho YK, Bing RJ. Production of prostanoids and nitric oxide by infarcted heart in situ and the effect of aspirin. *Biochem Biophys Res Commun* 1999; **257** (2):488-93.
- Yan GX, Yamada KA, Kléber AG, McHowat J, Corr PB. Dissociation between cellular K^+ loss, reduction in repolarization time, and tissue ATP level during myocardial hypoxia and ischemia. *Circ Res* 1993; **72**: 560-570.
- Zunkler BJ, Trube G, Panten U. How do sulfonylureas approach their receptor in the β-cell plasma membrane. *Naunyn Schmideberg's Arch Pharmacol* 1989; **340**: 328-332.

Acknowledgements

I am deeply indebted to ***Dr. István Leprán****, my supervisor, for providing me with valuable guidance and dedication of a large part of his time to my study. I would like to acknowledge the special help of* ***Prof. Dr. Julius Gy. Papp****, the Father and Head of the Department of Pharmacology and Pharmacotherapy, for going over my thesis and his comments and advice and for making the complex path to print so smooth.*

Further, I acknowledge the contribution of Prof. Dr. Seyed Saleem Ahmed for his invaluable comments and suggestions and for encouraging me throughout my scientific life.

I am grateful to Mrs. Anikó Tóth for her technical assistance, and would like to express my special thanks to my first assistant, Mrs. Zsuzsanna Ábrahám, for her experimental skill.

I am also much indebted to the staff of the Department of Pharmacology and Pharmacotherapy for their continuous help, advice, and dedication from the planning stages to the completion of the thesis.

I thank the Ministry of Education and Scientific Research, Libya, for financial support and encouragement throughout this work.

Finally, I particularly express my gratitude to my parents who have toiled endless hours in their efforts and provided me with advice and support throughout my life. The love of my brothers and sisters and the patience of my wife Amna and our children Areej, Esra and Yasin is highly appreciated.

I dedicate this work to the spirits of my recently deceased parents, my roots that never dry, and to the spirit of my sister, Kareema.....

Their memories will never fade in my mind.

Annex