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Comparison of the efficacy of glibenclamide and glimepiride in reperfusion-induced arrhythmias in rats

Nasruddin E. El-Reyani, Ömer Bozdogan, István Baczkó, István Leprán *, Julius Gy. Papp

Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical University, H-6701 Szeged, P.O. Box 115, Hungary

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Abstract

The effect of glibenclamide and glimepiride, two orally active antidiabetic sulphonylurea derivatives, was investigated on the development of reperfusion-induced arrhythmias and it was compared to their blood glucose lowering action. Arrhythmias were produced by reperfusion following 6 min coronary artery ligation in anaesthetised rats. 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 significantly the oral glucose loading-induced hyperglycaemia were similar (1 and 0.1 mg/kg, respectively) after glibenclamide and glimepiride. It is concluded 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.

Keywords: Reperfusion arrhythmia; Glibenclamide; Glimepiride; (Rat, anaesthetized)

1. Introduction

ATP-dependent K^+ channels (K_{ATP}) were first described by Noma (1983) and have been found to be widely distributed particularly in pancreatic β-cells, myocardium, skeletal muscle, vascular smooth muscle cells and in neurones. In cardiac cells they may play a special role because of their high density at the cell surface, their large ion conductance and their dependence upon cell metabolism. Under normoxic conditions when ATP is available, these channels are in a closed state. However, during ischaemia, the decrease in cytosolic ATP concentration and the accumulation of ischaemic metabolites together results in opening of K_{ATP} channels. Several investigations have suggested that the activation of these channels during myocardial ischaemia provides a 'natural' protective effect (Escande and Cavero, 1992; Grover, 1994) via increasing the rate of repolarisation, thereby decreasing voltage dependent calcium influx to the myocardium and preserving ATP during ischaemia. On the other hand, opening of K_{ATP} channels during the acute phase of myocardial infarction may contribute to the development of re-entrant type arrhythmias and sudden cardiac death.

In isolated perfused hearts during regional ischaemia and/or reperfusion glibenclamide, a K_{ATP}-channel blocker, was found to be antiarrhythmic (Wolleben et al., 1989; Kantor et al., 1990; Tosaki et al., 1993; D'Alonzo et al., 1994), and increased the chance for spontaneous recovery from ventricular fibrillation (Bril et al., 1992; Rees and Curtis, 1995). There are also some in vivo investigations showing that glibenclamide may possess antiarrhythmic activity (Ballagi-Pordany et al., 1990; Bekheit et al., 1990; Billman et al., 1993; Kondo et al., 1996). However, contrary results have also been presented. For example, glibenclamide had no effect on the incidence of ventricular fibrillation developing in response to a secondary insult in anaesthetized dogs with recent myocardial infarction (Chi et al., 1989), or in vitro against reperfusion-induced arrhythmias (Cole et al., 1991; Bernauer, 1997). 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., 1997a).

* Corresponding author. Tel.: +36-62-455-676; Fax: +36-62-454-565; E-mail: lepran@phcol.szote.u-szeged.hu

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Structurally different sulphonylureas may have a different action against arrhythmias during myocardial ischaemia. It has been demonstrated by Ballagi-Pordany et al. (1990) that first-generation antidiabetic sulphonylureas may exacerbate arrhythmias, while second-generation compounds produce antiarrhythmic action both in experimental animals and in diabetic patients. The discovery of the heterogeneity of sulphonylurea receptors, as part of K_{ATP} channels (Aguilar-Bryan et al., 1998), further emphasizes the need to compare the pancreatic and extrapancreatic effects of different agents.

Glimepiride is an orally active sulphonylurea derivative, which is considered to be a more potent antidiabetic when compared to glibenclamide (Geisen, 1988; Langtry and Balfour, 1998), while producing less adverse effects in the cardiovascular system (Geisen et al., 1996). The aim of the present investigations was to compare the efficacy of glibenclamide and glimepiride in coronary artery occlusion–reperfusion-induced arrhythmias in rats and to examine whether there is a correlation between this effect and the hypoglycaemic action of these two K_{ATP} channel inhibitory drugs.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 300–350 g were used. Animals were fed a standard laboratory rat food pellet (Altromin, Gödöllö, Hungary) and allowed to drink tap water ad libitum. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the Albert Szent-Györgyi Medical University (Szeged, Hungary).

2.2. Blood glucose determination

In conscious rats, a single drop of blood was taken by cutting the tip 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 min after intraperitoneal drug treatment and 30 min after oral administration of 1 g/kg glucose in 5 ml/kg tap water.

2.3. Coronary artery ligation and reperfusion

Coronary artery ligation and reperfusion was performed as described earlier (Baczkó et al., 1997b). Animals were anaesthetised with pentobarbitone (60 mg/kg i.p. in a volume of 2 ml/kg). The left carotid artery was cannulated for measuring the blood pressure using a pressure transducer (Gould-Statham P23ID, Hugo Sachs Elektronik, March-Hugstetten, Germany) and was recorded on an oscillographic recorder (Watanabe, WTR 331, Hugo Sachs Elektronik). The catheter was filled with saline that contained heparin (500 IU/ml), but the animal was not heparinized. The trachea was cannulated for artificial respiration. The chest was opened in the fourth intercostal space, the heart was exposed and a loose loop of atraumatic silk (Ethibond 5/0, Ethicon, UK) was placed around the left main coronary artery, approximately 2 mm from its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tubing. The heart was set back in its place and artificial respiration was started using 60 strokes/min (Harvard rodent ventilator, Model 683, Harvard Apparatus, South Natick, MA, USA). The standard electrocardiogram (lead II, ECG) was recorded using subcutaneous needle electrodes. After finishing the preparation, the animals were allowed to stabilize for 10 min, then the loose loop of the coronary artery ligature was tightened and fixed by clamping on the silk and thus regional myocardial ischaemia was produced for 6 min and then followed by reperfusion for 5 min.

Incidence of arrhythmias was analysed in accordance with the Lambeth Conventions (Walker et al., 1988), i.e., as ventricular fibrillation, ventricular tachycardia and other types of arrhythmias, including single extrasystoles, bigeminy and salvos. In case of ventricular fibrillation development, no attempt was made to defibrillate the animals.

At the termination of the experiment, heparin (500 IU/kg) was given intravenously and the heart was excised. The left coronary artery was re-tightened and the heart was first perfused retrogradely with 10 ml isotonic NaCl solution then with 2 ml ethanol, for the demarcation of the occluded and the non-occluded myocardium (Leprán et al., 1983). The non-perfusable area, that remained red coloured, was cut along the epicardially visible border zone and its weight was measured and expressed in percentage of the wet weight of the ventricles. Hearts showing no arrhythmias during coronary artery ligation or reperfusion and exhibiting $< 10\%$ non-perfusable area were excluded from the final evaluation. Using these criteria altogether eight animals were excluded from the experiments.

2.4. Drug administration protocol

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

2.5. Statistical evaluation

The percentage incidence of arrhythmias was calculated and compared using the χ^2-method. All other parameters

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

Group	Dose (mg/kg)	Basal	Glucose loading
Control		3.2 ± 0.16	5.4 ± 0.26
Glibenclamide	0.01	3.2 ± 0.11	5.7 ± 0.17
	0.1	3.0 ± 0.15	4.4 ± 0.21[a]
	1.0	3.3 ± 0.08	3.4 ± 0.18[a]
Glimepiride	0.01	3.1 ± 0.16	5.9 ± 0.18
	0.1	3.2 ± 0.04	4.0 ± 0.11[a]
	1.0	3.5 ± 0.06	3.8 ± 0.19[a]

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

were expressed as mean ± standard error of the mean (S.E.) and, after analysis of variance (ANOVA with repeated measures for the blood pressure and heart rate values), were compared by the modified t-statistic (Wallenstein et al., 1980).

3. Results

3.1. Blood glucose level

During basal conditions, i.e., before any drug treatment, the blood glucose level in conscious rats was not significantly different among different groups (Table 1). Thirty minutes after the intraperitoneal administration, both glibenclamide and glimepiride significantly reduced the blood glucose concentration when applied in a dose of 1 mg/kg (2.6 ± 0.12 and 2.7 ± 0.11 mmol/l in the glibenclamide and glimepiride treated animals, vs. 3.3 ± 0.08 and 3.5 ± 0.06 mmol/l before treatment, respectively, $P < 0.05$). Both compounds already in a dose of 0.1 mg/kg inhibited the elevation of plasma glucose concentration after oral glucose loading, while the smallest dose applied (0.01 mg/kg) was without effect (Table 1).

3.2. Haemodynamic parameters

At the doses that did not influence the blood glucose concentration in conscious rats, neither glibenclamide nor glimepiride pretreatment influenced significantly the heart rate or blood pressure as measured before coronary artery ligation in anaesthetised rats (Table 2). These haemodynamic parameters did not differ from the control during coronary artery ligation. Large doses of either glibenclamide or glimepiride (i.e., 5 mg/kg i.p.), however, significantly increased the heart rate before coronary artery ligation and this remained high also during the experiment after glimepiride pretreatment (Table 2). It was difficult to measure the heart rate or blood pressure during reperfusion, due to frequent arrhythmias in this period. When it was possible to perform statistical analysis, no significant differences were observed after glibenclamide or glimepiride pretreatment as compared to the control group.

3.3. Arrhythmias during coronary artery ligation

In the present experiments, coronary artery ligation lasting for 6 min was not 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.

3.4. Arrhythmias during reperfusion

Arrhythmias induced by reperfusion after 6 min myocardial ischaemia started within 10–30 s following the release of the coronary artery ligature. Irreversible ventric-

Table 2
Effect of glibenclamide and glimepiride on the heart rate (HR) and 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
	5.0	20	432 ± 7.5[a]	104 ± 5.5	16	398 ± 15.3	49 ± 4.8[a]	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
	5.0	22	428 ± 7.7[a]	108 ± 4.6	18	415 ± 9.8[a]	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) and mean arterial blood pressure 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.
[a] $P < 0.05$ compared to the corresponding control value.

Table 3
Effect of glibenclamide and glimepiride on 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[a]
	0.1	15	53	0	33	47	87	100
	5.0	16	81[a]	0	75[a]	19[a]	100	94
Glimepiride	0.0001	8	38	0	37	63	100	100
	0.001	22	64[a]	5	32	36[a]	91	96
	0.01	18	61[a]	0	11	39[a]	100	89
	0.1	15	60[a]	0	13	40[a]	93	100
	5.0	18	67[a]	0	50[a]	33[a]	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 salvo.
[a] $P < 0.05$ compared to the corresponding control value.

ular fibrillation occurred in 73% of the control animals and there was no animal surviving without developing arrhythmias during reperfusion (Table 3). Only 2 of 18 animals recovered spontaneously from ventricular fibrillation in the control group. Both glibenclamide and glimepiride increased the survival rate during reperfusion after 6 min myocardial ischaemia in anaesthetised rats and this protective effect of glimepiride was 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. However, the dose required to produce this effect was much smaller with glimepiride (i.e., 0.001–0.1 mg/kg) than with glibenclamide (5 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, neither glibenclamide nor glimepiride treatment decreased the mean length of arrhythmic attacks or the total period that was characterized by arrhythmias during reperfusion.

4. Discussion

Transient coronary artery ligation followed by reperfusion in the anaesthetised rat is a widely used and accepted method to investigate the efficacy of antiarrhythmic treatments. In the present investigations, we applied 6 min occlusion of the left main coronary artery that was not long enough for a significant amount of ischaemia-induced arrhythmias to develop, however, it was purposely chosen to prime the heart to develop severe arrhythmias consistently 10–30 s following the start of reperfusion.

The present results demonstrate that pretreatment with two sulphonylureas, glibenclamide or glimepiride, significantly decreased the incidence of irreversible ventricular fibrillation during reperfusion following a brief period of myocardial ischaemia in anaesthetised rats. Moreover, this cardioprotective action of glimepiride occurred in smaller doses than that producing a blood glucose lowering effect.

It has been suggested that sulphonylureas may inhibit the opening of K_{ATP} channels during myocardial ischaemia, thereby preventing the loss of intracellular K^+ from jeopardised cells and the non-uniform shortening of the action potential 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 re-entrant pathways, resulting in antiarrhythmic, antifibrillatory action. Such an idea is supported by many 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 (Bekheit et al., 1990; Billman et al., 1993; Kondo 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.

The reason for the observed difference between glibenclamide and glimepiride, and the divergence of the blood glucose lowering potency and the 'antiarrhythmic' activity is not known. However, there are some data that also 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 phos-

pholipase A_2 were not influenced. On the other hand, glibenclamide inhibited both the cyclooxygenase and 12-lipoxygenase enzymes and also the phospholipase A_2. Muller et al. (1994) described that glimepiride, in spite of its higher blood glucose lowering potency in diabetic patients than that of glibenclamide, had a 2.5–3-fold lower affinity to membranes isolated from rat pancreatic β-cells. 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 effect. Perhaps, 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. The observed difference in the blood glucose lowering and the 'antiarrhythmic' activity of sulphonylurea compounds may also correlate with the multiplicity of sulphonylurea receptors that regulate the opening of ATP-dependent K^+ channels. The contribution of these possibilities to the difference between glibenclamide and glimepiride revealed in the present study needs further investigations.

The observed differences between the two sulphonylurea compounds may suggest the possibility to develop 'cardioselective' compounds that inhibit K_{ATP}-channels without decreasing blood glucose level. This conclusion is supported by recent findings that a novel 'cardioselective' K_{ATP} 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 possible candidates for the drug treatment of cardiac arrhythmias with a selective action during myocardial ischaemia.

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EFFECT OF GLIBENCLAMIDE AND GLIMEPIRIDE TREATMENT ON THE DEVELOPMENT OF MYOCARDIAL INFARCTION IN RATS

Nasruddin E. El-Reyani, István Baczkó, István Leprán, Julius Gy. Papp

Department of Pharmacology and Pharmacotherapy, University of Szeged and Research Unit for Cardiovascular Pharmacology, Hungarian Academy of Sciences, H-6721 Szeged, Dóm tér 12., Hungary

Summary

The effect of glibenclamide and glimepiride, two orally active antidiabetic sulphonylurea derivatives, on the development of myocardial infarction has been compared. Permanent coronary artery ligation was induced 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.

Key Words: Myocardial infarct size, Glibenclamide, Glimepiride, Rat

Introduction

Orally active sulphonylurea drugs that stimulate the secretion of insulin from pancreatic β-cells by closing ATP-dependent potassium (K_{ATP}) channels are widely used in the treatment of non-insulin dependent diabetes mellitus (NIDDM). K_{ATP} channels are found not only in the pancreas, but also in the myocardium (26). In the cardiac cells they may play a special role because of their high density at the cell surface, their significant ion conductance and their dependence upon cell metabolism.

It is suggested that the activation of K_{ATP} channels during myocardial ischaemia provides a 'natural' protective effect (11, 15) via increasing the rate of repolarisation, thereby decreasing voltage dependent calcium influx to the myocardium and preserving ATP during ischaemia. On the other hand, opening of K_{ATP} channels during the acute phase of myocardial infarction may contribute to the development of re-entry type arrhythmias and sudden cardiac death.

Clinical studies found that first-generation antidiabetic sulfonylureas decreased the survival time of diabetic patients as compared to second-generation compounds (28). This finding was confirmed also by experimental studies (4). In isolated perfused heart during regional ischaemia and/or reperfusion glibenclamide, a K_{ATP}-channel inhibitor, was found to be antiarrhythmic (8, 17, 38, 41), and increased the chance for spontaneous recovery from ventricular fibrillation (6, 32). Previously we also demonstrated that in *in vivo* conditions glibenclamide pretreatment increased the survival rate and decreased the incidence of life-threatening arrhythmias during acute myocardial infarction in conscious rats (20) or during ischaemia/reperfusion in anaesthetised rats (3, 10).

There are, however, very few data showing the effect of K_{ATP} inhibitors on the development of irreversible tissue damage during myocardial infarction. It has been demonstrated that acute treatment may increase infarct size after transient coronary artery occlusion and reperfusion (24, 37). Many investigations showed that K_{ATP} inhibitors abolish the protective action of preconditioning on the development of myocardial infarct size after coronary artery occlusion and reperfusion (16, 23, 34, 35). However, no information is available on the influence of long-term treatment with K_{ATP} inhibitors on the development of myocardial infarction after permanent coronary artery occlusion.

Oral hypoglycaemic sulfonylurea drugs are frequently prescribed in NIDDM and the occurrence of ischaemic heart disease is common among these patients. The aim of our experiments was therefore to investigate the effects of two different sulphonylurea drugs, glibenclamide and glimepiride, on the development of myocardial infarct size after permanent coronary artery occlusion in rats.

Materials and Methods

Animals

Male Sprague-Dawley rats, weighing 300-350 g were used. Animals were fed a standard laboratory rat food pellet (Altromin, Gödöllő, Hungary) and allowed to drink tap water ad libitum. No glucose supplementation was used during the treatment period. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the Albert Szent-Györgyi Medical University (Szeged, Hungary).

Coronary artery occlusion

Coronary artery ligation was performed as described by Selye et al. (36). During ether anaesthesia, after opening the chest the left main coronary artery was ligated, approximately 2 mm from its origin, using atraumatic silk (Ethibond 5/0, Ethicon Ltd., United Kingdom). The chest was closed in layers while the thorax was slightly compressed to stop pneumothorax and to regain spontaneous ventilation. Sham operated animals were treated in the same manner except that the silk ligature around the coronary artery was left loose. In the case of the development of 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.

Determination of the infarct size

One or 7 days after coronary artery ligation the animals were anaesthetised with pentobarbitone (60 mg/kg i.p.) and their heart was excised and washed in isotonic NaCl solution. The aorta, the atria and the right ventricular free wall were carefully removed and the hearts were cut with a series of razor blades transversally into 1.6 mm thick slices. The heart slices were then incubated in 0.1 % nitroblue-tetrazolium (Fluka AG, Switzerland) to allow the demarcation of the non-infarcted (stained dark blue) and infarcted (remaining pale) myocardium (25).

After staining, slices were kept in buffered 1 % formaldehyde until further evaluation within 1 day. Slices were ranked in order from the apex to the base and digitalized using a desktop scanner (ScanJet IIc, Hewlett-Packard) with 400 dpi resolution, set to 'millions of colours'. Stored images were pre-processed using Photo Finish (Zsoft Corporation) by enhancing the colour differences to increase the signal to noise ratio, then the colour depth was decreased to 256 colours. Further differentiation

between the non-infarcted, infarcted and 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. Using the cursor pointer a pixel was selected, representing the totally infarcted tissue (not staining with nitroblue-tetrazolium), or partially infarcted tissue (showing some staining), or normal myocardium (well stained), as well as the left ventricular cavity. The program automatically redefined the colours of other pixels, having similar colour in a prefixed range to the representative one. 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 scanned together with the heart slices and the thickness of the slices (1.6 mm), the computer can automatically calculate the volume of the above mentioned tissues.

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

All images were processed by the same person (N.E.E.) in a blinded manner and the same colour settings were used for differentiating the infarcted and non-infarcted myocardium.

Animals showing <10 % myocardial infarction were excluded as representing unsuccessful coronary artery occlusion. Based on this criterion 3 animals were excluded from the final evaluation.

Drug treatment

Glibenclamide (Sigma; 0.1 or 5 mg/kg), glimepiride (Hoechst; 0.001 or 5 mg/kg) or vehicle (DMSO : EtOH 1:1) was applied i.p. twice a day with a Hamilton syringe in a volume of 100 µl/kg. The first treatment made 30 min prior to coronary artery ligation. These doses were chosen on the basis of previous experiments (10) 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 did not influence plasma glucose concentration in metabolically healthy rats and produced only marginal effect on the incidence of arrhythmias.

Statistical evaluation

The survival rate was compared using the χ^2-method. All other parameters were expressed as mean ± standard error of the mean (S.E.) and after analysis of variance were compared by means of the modified 't'-statistical method of Wallenstein et al. (40).

Results

Survival after coronary artery ligation

The sham operation, i.e. anaesthesia, rapid opening and closing of the chest wall without coronary artery ligation, did not cause death of the animals. Out of the 146 rats with myocardial infarction 16 (11 %) died during the first 4 hours after coronary artery ligation in spite of using mechanical taping on the chest wall to revert the animals from ventricular fibrillation. Further 32 (22 %) rats died after the 4th hour but during 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 among different groups concerning the survival rate during the first 4 hours or later.

Infarct size

After 7 days coronary artery ligation the volume of the heart used for the determination of the infarct size (i.e. the heart without the large vessels, the atria and the right ventricular free wall) was significantly decreased in the control group and in the glibenclamide treated animals, as compared to the corresponding 1 day old infarction (Table I). The volume of the heart was smaller after 5 mg/kg glibenclamide treatment both at 1 day and 7 days compared to the corresponding control values. Glimepiride treatment, however, did not influence the volume of the heart.

Coronary artery ligation resulted in extensive loss of nitroblue-tetrazolium staining that involved the anterior and lateral free wall of the heart and extended to 45.3±2.5 % of the left ventricular myocardium after 1 day infarction in control animals (Table I). After 7 days the volume of the infarcted myocardium became somewhat 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 was significantly enlarged from 12.1±0.7 % to 19.9±1.2 % at 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 during 1 day coronary artery occlusion. This treatment, however, considerably decreased the volume of the infarcted myocardium 7 days after coronary artery ligation, while the enlargement of the ventricular cavity did not differ from the control group (Table I). The smaller dose of glibenclamide did not influence the development of myocardial infarction.

The larger dose of glimepiride (5 mg/kg b.i.d.) significantly decreased the enlargement of the left ventricular cavity during the evolvement of myocardial infarction, while not influencing the size of the infarcted myocardium (Table I). The smaller dose of the compound (0.001 mg/kg b.i.d.) did not influence the development of infarct size, although the enlargement of the ventricular cavity was somewhat smaller (Table I).

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

Thickness of myocardium

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

Glibenclamide treatment in the larger dose resulted in more intense thinning of the infarcted myocardium as compared to the control. Both doses of glibenclamide

inhibited the development of hypertrophy of the non-infarcted myocardium, measured as the thickness of the septum 7 days after myocardial infarction (Table II).

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

Discussion

Coronary artery ligation in the rat is widely used for the evaluation of different phases of myocardial infarction in pharmacological investigations when large numbers of animals are used in the experiments. In this model the acute phase (i.e. the first hours) of myocardial infarction is characterised by high mortality due to severe arrhythmias (21, 39), whereas the later phase is characterised by scar formation and anatomical remodelling of the ventricles (1, 29).

Detailed morphologic evaluation of myocardial infarction with classical histologic methods (i.e. serial sectioning, histologic staining, microscopic evaluation, etc.) is a laborious and expensive procedure. There are several techniques that make these large-scale measurements easier and simpler. Among them computer-assisted planimetry is very helpful to shorten the time of evaluation, but previous preparation is still a problem.

Recently Porzio et al. (31) described a method for simultaneous measurement of the infarct size and left ventricular geometry in the rat. Accordingly, after cryostat sectioning, nitroblue-tetrazolium staining and mounting on slides, video images were analysed by a sophisticated computer technique. Our method is a simpler one using

macroscopic sectioning and staining, no mounting, but a direct digitalisation of stained slices of the heart using a flatbed scanner. Furthermore, during the evaluation of stored images the differentiation of infarcted and non-infarcted tissues was performed automatically by the software using constant settings of the colours representing these areas. In our method no manual delineation of the infarcted area is needed as in previous planimetric techniques. Thus, a subjective component of the differentiation did not influence the evaluation and myocardial infarction in small patches or the finger type border zone could also be evaluated.

Using this method it was demonstrated that in the control animals the infarct size was somewhat 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 morphologic methods for the evaluation of experimental myocardial infarction in the rat (7, 12, 30, 33).

Glibenclamide treatment, in a high dose that according to previous experiment (10) decreased basal plasma glucose concentration and inhibited its elevation upon oral glucose loading, significantly decreased the volume of the infarcted myocardium. This effect appeared to be due to the increased scar thinning 7 days after coronary artery ligation. The smaller dose of glibenclamide, exhibiting no significant effect on the plasma glucose concentration (10) did not influence scar formation, however, it still inhibited the thickening of the non-infarcted myocardium.

The reasons why glibenclamide treatment resulted in increased scar thinning and inhibition of the hypertrophy of the non-infarcted myocardium is not known. Death of the animals having larger myocardial infarction cannot be the reason for this difference since in our conditions death was rare after 1-day coronary artery ligation. K_{ATP} inhibitors may increase the energy requirement of the myocardium by inhibiting the shortening of action potential duration due to decreased intracellular ATP concentration in myocardial ischaemia (11, 15). Such an effect might result in a more intense loss of the viable myocardium within the infarcted region.

K_{ATP} inhibitors may also decrease hypoxic vasodilatation during hypoxia and ischaemia (2, 9, 19). 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 the development of myocardial hypertrophy.

Hypoglycaemia itself evoked by glibenclamide is not likely to be responsible for scar thinning and the inhibition of the development of hypertrophy since glimepiride, in a high dose which caused an even more intense decrease in plasma glucose level than that produced by glibenclamide (13), 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 that in the vehicle treated controls.

The reason for the difference between the effects of the two compounds on the development of myocardial infarction is not known. It might bear importance that glimepiride inhibits vascular K_{ATP} channels to a lesser extent than glibenclamide (5, 14). It was also found that glimepiride inhibited the cyclooxygenase pathway of isolated human platelets, while not influencing 12-lipoxygenase and phospholipase A_2 (27). On the other hand, glibenclamide inhibited both the cyclooxygenase and the lipoxygenase

pathways, as well as phospholipase A_2. This difference might be relevant to our present findings, since phospholipase A_2 inhibition by steroid anti-inflammatory drugs also adversely influences the healing process and results in increased scar thinning and mummification of the infarcted myocardium (18, 22).

In conclusion, our results suggest that glibenclamide may increase scar thinning and inhibit the development of hypertrophy in the non-infarcted myocardium after permanent coronary artery ligation in metabolically healthy rats. Such an effect may deteriorate the heart function and promote the shift from a compensated state to decompensation after myocardial infarction. Glimepiride, on the other hand, did not promote scar thinning and inhibited the dilatation of the left ventricular cavity. Whether these dissimilarities in the effects of the two K_{ATP} inhibitors also result in differences in the long-term survival and in the mechanical function of the infarcted heart in diabetic animals requires further investigation.

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Table I. 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 II. 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 I.

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Antiarrhythmic and electrophysiological effects of GYKI-16638, a novel *N*-(phenoxyalkyl)-*N*-phenylalkylamine, in rabbits

István Baczkó [a], Nasruddin E. El-Reyani [a], András Farkas [a], László Virág [a], Norbert Iost [a], István Leprán [a,*], Péter Mátyus [b], András Varró [a], Julius Gy. Papp [a]

[a] *Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged and Research Unit of the Hungarian Academy of Sciences, Dóm tér 12, P.O. Box 427, H-6701 Szeged, Hungary*

[b] *Institute of Organic Chemistry, Semmelweis University of Medicine, Hőgyes u. 7., H-1092 Budapest, Hungary*

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Abstract

The effect of *N*-[4-[2-*N*-methyl-*N*-[1-methyl-2-(2,6-dimethylphenoxy)ethylamino]-ethyl]-phenyl]-methanesulfonamide.hydrochloride (GYKI-16638; 0.03 and 0.1 mg/kg, i.v.), a novel antiarrhythmic compound, was assessed and compared to that of D-sotalol (1 and 3 mg/kg, i.v.) on arrhythmias induced by 10 min of coronary artery occlusion and 10 min of reperfusion in anaesthetized rabbits. Also, its cellular electrophysiological effects were studied in rabbit right ventricular papillary muscle preparations and in rabbit single isolated ventricular myocytes. In anaesthetized rabbits, intravenous administration of 0.03 and 0.1 mg/kg GYKI-16638 and 1 and 3 mg/kg D-sotalol significantly increased survival during reperfusion (GYKI-16638: 82% and 77%, D-sotalol: 75% and 83% vs. 18% in controls, $P < 0.05$, respectively). GYKI-16638 (0.1 mg/kg) significantly increased the number of animals that did not develop arrhythmias during reperfusion (46% vs. 0% in controls, $P < 0.05$). In isolated rabbit right ventricular papillary muscle, 2 μM GYKI-16638, at 1 Hz stimulation frequency, lengthened the action potential duration at 50% and 90% repolarization (APD_{50-90}) without influencing the resting membrane potential and action potential amplitude (APA). It decreased the maximal rate of depolarization (V_{max}) in a use-dependent manner. This effect was statistically significant only at stimulation cycle lengths shorter than 700 ms. The offset kinetics of this V_{max} block were relatively rapid, the corresponding time constant for recovery of V_{max} was 328.2 ± 65.0 ms. In patch-clamp experiments, performed in rabbit ventricular myocytes, 2 μM GYKI-16638 markedly depressed the rapid component of the delayed rectifier outward and moderately decreased the inward rectifier K^+ current without significantly altering the slow component of the delayed rectifier and transient outward K^+ currents. These results suggest that in rabbits, GYKI-16638 has an in vivo antiarrhythmic effect, comparable to that of D-sotalol, which can be best explained by its combined Class I/B and Class III actions.

Keywords: Antiarrhythmic drug; Reperfusion arrhythmia; Action potential duration; V_{max}

1. Introduction

The analysis of the Cardiac Arrhythmia Suppression Trials (CAST-I and CAST-II) prompted the reconsideration of prophylactic antiarrhythmic treatment after myocardial infarction. The results shed light on the controversy that Class I/C type Na^+ channel blockers, i.e. flecainide and encainide, increased mortality in survivors of myocardial infarction despite their ability to reduce the number of premature ventricular beats (The Cardiac Arrhythmia Suppression Trial (CAST) Investigators, 1989; The Cardiac Arrhythmia Suppression Trial II Investigators, 1992). The results of these trials and those of the ESVEM and CASCADE trials shifted the attention to cardiac K^+ channel blockers (Mason, 1993; The CASCADE Investigators, 1993).

As a disappointment, in the SWORD trial D-sotalol, a so-called 'pure' Class III antiarrhythmic drug, which is known to block cardiac K^+ channels selectively, was shown to increase mortality in subsets of patients with myocardial infarction and lowered ejection fraction (Waldo et al., 1996).

* Corresponding author. Tel.: +36-62-545-676; fax: +36-62-544-565.
E-mail address: lepran@phcol.szote.u-szeged.hu (I. Leprán).

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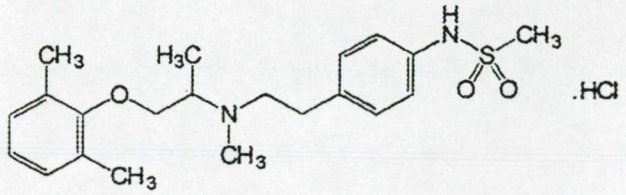

Fig. 1. Chemical structure of GYKI-16638.

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 K^+ channel blocker and β-adrenoceptor antagonist) and amiodarone (a K^+ channel blocker possessing Na^+ and Ca^{2+} channel blocking properties and antiadrenergic activity). Amiodarone has been shown to exert a strong antiarrhythmic effect in a number of studies and is currently considered to be one of the most efficacous antiarrhythmic drugs available in clinical practice. 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.

N-[4-[2-*N*-methyl-*N*-[1-methyl-2-(2,6-dimethylphenoxy) ethylamino]-ethyl]-phenyl]-methanesulfonamide. hydrochloride (GYKI-16638; Fig. 1) 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 is expected to show amiodarone-like electrophysiological effects, i.e. both Class I/B and Class III properties.

In the present study, we investigated the effect of GYKI-16638 and D-sotalol on the incidence of coronary artery occlusion and reperfusion-induced arrhythmias in anaesthetized rabbits. We also studied the cellular electrophysiological effects of GYKI-16638 in rabbit right ventricular papillary muscle and in rabbit single isolated ventricular myocytes.

2. Materials and methods

2.1. Animals

Male rabbits weighing 2–3 kg were used for the experiments. The animals were allowed to have tap water and laboratory rabbit chow (Altromin, Gödöllő, Hungary) ad libitum until the experiment. The animal handling protocol was reviewed and approved by the Ethics Committee for the Protection of Animals in Research of the Faculty of Medicine, University of Szeged, Szeged, Hungary.

2.2. Coronary artery ligation and reperfusion

The animals were anaesthetized with 30 mg/kg pentobarbitone-Na given intravenously in a volume of 1 ml/kg into the marginal vein of the right ear. Acute coronary artery occlusion and reperfusion were performed as described by Coker (1989). To measure blood pressure, a catheter filled with isotonic saline containing 500 IU/ml heparin (the animals were not heparinized) was introduced into the right carotid artery. The catheter was connected to a pressure transducer (Gould-Statham, P23ID, Hugo Sachs Electronik, March-Hugstetten, Germany) and blood pressure was recorded on an oscillographic recorder (Watanabe, WTR 331, Hugo Sachs Electronik). For the infusion of drugs, another catheter was introduced into the marginal vein of the left ear.

After tracheal cannulation, thoracotomy was performed in the fourth intercostal space and artificial ventilation was started with room air (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA), with respiratory volume and rate subsequently adjusted to keep blood gases and pH within the normal range (7 ml/kg/stroke, 40 strokes/min, respectively). Following pericardiotomy, a loose loop of 4–0 atraumatic silk (Ethicon, Edinburgh, UK) was placed around the first branch of the left circumflex coronary artery just under its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tube.

After stabilization of blood pressure and heart rate (approximately 10 min), saline or 0.03 or 0.1 mg/kg GYKI-16638 or 1 or 3 mg/kg D-sotalol 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 110 4 CH, Multiline, 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 (QT_c) was calculated using the following equation of Carlsson et al. (1993a): $QT_c = QT - 0.175 \times (RR - 300)$.

Arrhythmias were detected and diagnosed in accordance with the Lambeth conventions as ventricular tachycardia, ventricular fibrillation and other types of arrhythmias, including single extrasystoles, bigeminy, salvos and bradycardia (Walker et al., 1988).

At the end of the experiment, heparin-Na (500 IU/kg, i.v.) was administered and the animals were killed. The hearts were cut out from the chest in order to determine the size of the occluded zone. After the ligation was

tightened, the hearts were retrogradely 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 is expressed as a 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.

2.3. Drug administration protocol

D-Sotalol (1 or 3 mg/kg) was dissolved in saline, and 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.

2.4. Measurement of action potential parameters in rabbit right ventricular papillary muscle

Following cervical dislocation, the heart of each animal was rapidly removed through a right lateral thoracotomy. The hearts were immediately rinsed in oxygenated Tyrode's solution containing (in mM): NaCl, 115; KCl, 4; $CaCl_2$, 1.2; $MgCl_2$, 1; $NaHCO_3$, 21.4; and glucose, 11. The pH of this solution was 7.35–7.45 when gassed with 95% O_2 and 5% CO_2 at 37°C. The papillary muscles from the right ventricle were individually mounted in a tissue chamber (volume ≈ 50 ml). Each preparation was initially stimulated (HSE [Hugo Sachs Electronik] stimulator type 215/II, March-Hugstetten, Germany) at a basic cycle length of 500 ms (frequency = 2 Hz), using 2-ms long rectangular constant voltage pulses isolated from ground and delivered across bipolar platinum electrodes in contact with the preparation. We applied the following types of stimulation in the course of the experiments: stimulation with a constant cycle length of 500 ms (2 Hz); stimulation with different constant cycle lengths ranging from 300 to 5000 ms taking the measurement after the 25th beat.

To determine the recovery of V_{max}, extra test action potentials were elicited using single test pulses (S_2) in a preparation driven at a basic cycle length of 500 ms. The S_1–S_2 coupling interval was increased progressively from the end of the effective refractory period up to 10 s. The time constant for recovery of V_{max} was fitted to a single exponential function, starting at the 40 ms diastolic interval and ending at 5 s.

Before the control measurement, at least 1 h was allowed for each preparation to equilibrate while being continuously superfused with Tyrode's solution. The temperature of the superfusate was kept constant at 37°C. Transmembrane potentials were recorded using a conventional microelectrode technique. Microelectrodes filled with 3 M KCl and having tip resistances of 5–20 mΩ were connected to the input of a high impedance electrometer (HSE microelectrode amplifier type 309), which was referenced to the ground. The first derivative of transmembrane potentials (V_{max}) was electronically derived by an HSE differentiator (type 309). The voltage outputs from all amplifiers were displayed on a dual-beam memory oscilloscope (Tektronix 2230 100 MHz digital storage oscilloscope, Beaverton, OR).

The maximum diastolic potential (MDP), action potential amplitude (APA), and action potential duration measured at 50% and 90% repolarization (APD_{50-90}) were obtained using software developed in our department (HSE-APES). GYKI-16638 was dissolved in dimethyl sulfoxide (DMSO) as a 1-mM stock solution. After the control measurements, GYKI-16638 was added to the tissue bath to obtain a final concentration of 2 μM and the measurements were repeated after a 30-min incubation time.

To select the single in vitro concentration, we were guided by pharmacokinetic studies with GYKI-16638. In these measurements, the obtained plasma concentration after GYKI-16638 administration correlated well with the concentration used in our in vitro studies with GYKI-16638.

2.5. Whole-cell configuration of the patch-clamp technique

Single ventricular myocytes were obtained by enzymatic dissociation from New Zealand rabbits (1–2 kg) by a technique described earlier in detail (Varró et al., 1996).

One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope (TMS; Nikon, Tokyo, Japan), and at least 5 min were allowed for individual myocytes to settle and adhere to the bottom of the chamber before superfusion was started. Myocytes that were used were rod-shaped with clear striations. HEPES-buffered Tyrode solution served as the normal superfusate in all experiments. This solution contained (mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES 5.0 at pH 7.4.

Patch-clamp micropipettes were made from borosilicate glass capillaries (Clark, Reading, UK) using a P-97 Flaming/Brown micropipette puller (Sutter Instrument Co, Novato, CA, USA). These electrodes had resistances between 1.5 and 2.5 MΩ when filled with pipette solution containing (in mM): K-aspartate 100, KCl 45, K_2ATP 3, $MgCl_2$ 1, EGTA 10, and HEPES 5. The pH of this solution was adjusted to 7.2 by addition of KOH. Nisoldipine (1 μM; Bayer, Leverkusen, Germany) in the external solution eliminated the inward Ca^{2+} current (I_{Ca}). An Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA) was used to record the membrane current in the whole-cell

Table 1
Effect of intravenous administration of D-sotalol and GYKI-16638 on mean arterial blood pressure, heart rate, QT and QT_c intervals in anaesthetized rabbits

Group	Dose (mg/kg)		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
		QT_c		162±3.4	162±3.8
D-sotalol	1.0	MBP	13	97±3.2	97±2.7
		HR		272±9.4	252±8.9[a]
		QT		142±4.7	162±5.2[a]
		QT_c		156±3.9	172±4.0[a]
	3.0	MBP	13	95±3.5	100±3.3
		HR		265±9.7	247±7.9[a]
		QT		150±6.8	167±6.7[a]
		QT_c		163±5.9	176±5.8[a]
GYKI-16638	0.03	MBP	14	93±3.4	93±2.8
		HR		273±5.2	259±6.3[a]
		QT		150±4.6	159±7.3
		QT_c		164±4.1	171±6.5
	0.1	MBP	17	94±2.7	93±2.5
		HR		270±8.7	253±7.3[a]
		QT		140±4.4	166±6.4[a,b]
		QT_c		153±3.4	173±4.7[a]

n = Number of animals, MBP = mean blood pressure (mm Hg), HR = heart rate (1/min), QT = QT interval (ms), QT_c = QT_c interval.
[a] $P < 0.05$, compared to the preinfusion value of the same group.
[b] $P < 0.05$, compared to the control group.

configuration of the patch-clamp technique. After a high (1–10 GΩ) resistance seal was established by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by application of 1.5 V electrical pulses applied for 1–5 ms. Series resistance was typically 4–8 MΩ prior to compensation (50–80%, depending on the voltage protocol utilized). Experiments, where the series resistance was high, or where it increased substantially during measurement, were terminated and the data were discarded. Membrane currents were digitized using a 333-kHz analog-to-digital converter (Digidata 1200, Axon Instruments) under software control (pClamp 6.0, Axon Instruments). Analyses were performed using Axon (pClamp 6.0) software after low-pass filtering at 1 kHz. All patch-clamp data were collected at 37°C.

GYKI-16638 was diluted at the time of use from a 10-mM stock solution containing 100% DMSO. DMSO at the resulting concentrations (0.2%) produced no discernible effect on APD or the membrane currents assessed. All stock solutions were prepared using HEPES-buffered Tyrode solution as the solvent.

2.6. Statistical evaluation

For the evaluation of data obtained from the cellular electrophysiology experiments, Student's t-test for paired data was used. All data are expressed as means ± standard error of the mean (S.E.M.).

The incidence of arrhythmias was calculated and compared by using the χ^2 method. All other variables are expressed as means ± S.E.M. and, after analysis of variance, were compared by means of the modified t statistic of Wallenstein et al. (1980). Differences were considered significant when P values were less than 0.05.

3. Results

3.1. Effect of GYKI-16638 on haemodynamic variables in anaesthetized rabbits

There were no significant differences between the mean arterial blood pressures of control and D-sotalol- or GYKI-16638-treated animals. Mean arterial blood pressure fell significantly in all groups due to coronary artery occlusion as compared to preocclusion values (74 ± 3.9 vs. 101 ± 2.8 mm Hg, 78 ± 4.5 vs. 97 ± 3.2 mm Hg, 84 ± 2.6 vs. 95 ± 3.5 mm Hg, 69 ± 3.8 vs. 93 ± 3.4 mm Hg and 74 ± 3.9 vs. 94 ± 2.7 mm Hg in controls, 1 and 3 mg/kg D-sotalol-, 0.03 and 0.1 mg/kg GYKI-16638-treated animals, respectively, all $P < 0.05$).

The infusion of 1 and 3 mg/kg D-sotalol, as well as 0.03 and 0.1 mg/kg GYKI-16638, significantly decreased the heart rate of rabbits compared to the basal values

Table 2
Effect of D-sotalol and GYKI-16638 on the incidence of arrhythmias during 10 min of coronary artery occlusion in anaesthetized rabbits

Group	Dose (mg/kg)	n	Incidence of arrhythmias (N/%)			
			None	VF	VT	Other
Control		19	4/19 (21%)	8/19 (42%)	2/19 (11%)	14/19 (74%)
D-Sotalol	1.0	13	5/13 (38%)	1/13 (8%)	0/13 (0%)	8/13 (62%)
	3.0	13	7/13 (54%)	1/13 (8%)	0/13 (0%)	6/13 (46%)
GYKI-16638	0.03	14	3/14 (21%)	3/14 (21%)	2/14 (14%)	11/14 (79%)
	0.1	17	5/17 (29%)	4/17 (24%)	0/17 (0%)	12/17 (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.

Table 3
Effect of D-sotalol and GYKI-16638 on the incidence of arrhythmias during 10 min of reperfusion following 10 min of coronary occlusion in anaesthetized rabbits

Group	Dose (mg/kg)	n	Incidence of arrhythmias (N/%)			
			None	VF	VT	Other
Control		11	0/11 (0%)	9/11 (82%)	7/11 (64%)	5/11 (46%)
D-Sotalol	1.0	12	4/12 (33%)	3/12 (25%)[a]	4/12 (33%)	8/12 (67%)
	3.0	12	4/12 (33%)	2/12 (17%)[a]	4/12 (33%)	9/12 (75%)
GYKI-16638	0.03	11	3/11 (27%)	2/11 (18%)[a]	4/11 (36%)	9/11 (82%)
	0.1	13	6/13 (46%)[a]	3/13 (23%)[a]	6/13 (46%)	8/13 (62%)

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.
[a] $P < 0.05$.

(Table 1). Coronary occlusion did not change heart rate significantly compared to preocclusion values. No significant changes occurred in the heart rate of animals during reperfusion.

3.2. Effect of GYKI-16638 on QT and QT_c intervals in anaesthetized rabbits

D-Sotalol infusion, in the dose of 1 and 3 mg/kg, significantly lengthened QT and QT_c intervals (Table 1). GYKI-16638, in the dose of 0.03 mg/kg, had no effect on QT and QT_c intervals, but caused a significant increase of both variables in the dose of 0.1 mg/kg. No significant changes occurred in the QT or QT_c intervals during reperfusion.

3.3. Arrhythmias during 10 min of myocardial ischaemia

In all groups, arrhythmias did not develop either during the 1-min infusion of drugs or vehicle, or between the infusion of drugs and coronary occlusion.

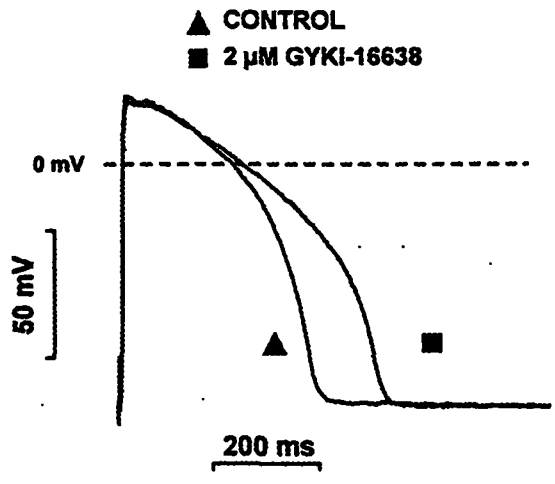

Fig. 2. Effect of 2 μM GYKI-16638 on the action potential in rabbit right ventricular papillary muscle (stimulation frequency: 2 Hz).

The incidence of arrhythmias in the control, D-sotalol- and GYKI-16638-treated groups during 10 min of coronary artery occlusion is shown in Table 2. The incidence of ventricular fibrillation was not statistically different in the D-sotalol- or GYKI-16638-treated animals compared to the control group.

There were no significant differences in the treated and control groups with respect to the incidence of other types of arrhythmias during 10 min of coronary artery ligation.

3.4. Reperfusion-induced arrhythmias

Arrhythmias induced by reperfusion appeared within 10–30 s following the release of the coronary artery ligature.

D-Sotalol (1 and 3 mg/kg) and 0.03 mg/kg GYKI-16638 pretreatment significantly reduced the incidence of reperfusion-induced ventricular fibrillation (Table 3). All drug pretreatments significantly increased the number of animals surviving reperfusion (75% and 83% with 1 and 3 mg/kg D-sotalol, 82% and 77% with 0.03 and 0.1 mg/kg GYKI-16638 vs. 18% in controls, $P < 0.05$, respectively).

The number of animals that did not develop any arrhythmia during reperfusion was significantly higher in the

Table 4
The effect of 2 μM GYKI-16638 on the action potential parameters in rabbit right ventricular papillary muscle

n = 6	Control	2 μM GYKI-16638
RP (mV)	−89 ± 1.5	−91 ± 0.8
APA (mV)	111.7 ± 2.1	112 ± 2.8
APD_{50} (ms)	158.3 ± 12.4	194.5 ± 12.7[a]
APD_{90} (ms)	205.8 ± 15.9	254.8 ± 14.9[a]
V_{max} (V/s)	208.3 ± 32.8	169.2 ± 20.8[a]

RP = resting potential; APA = action potential amplitude; APD_{50} = 50% repolarization time; APD_{90} = 90% repolarization time; V_{max} = maximum upstroke velocity; stimulation frequency: 2 Hz.
[a] $P < 0.05$.

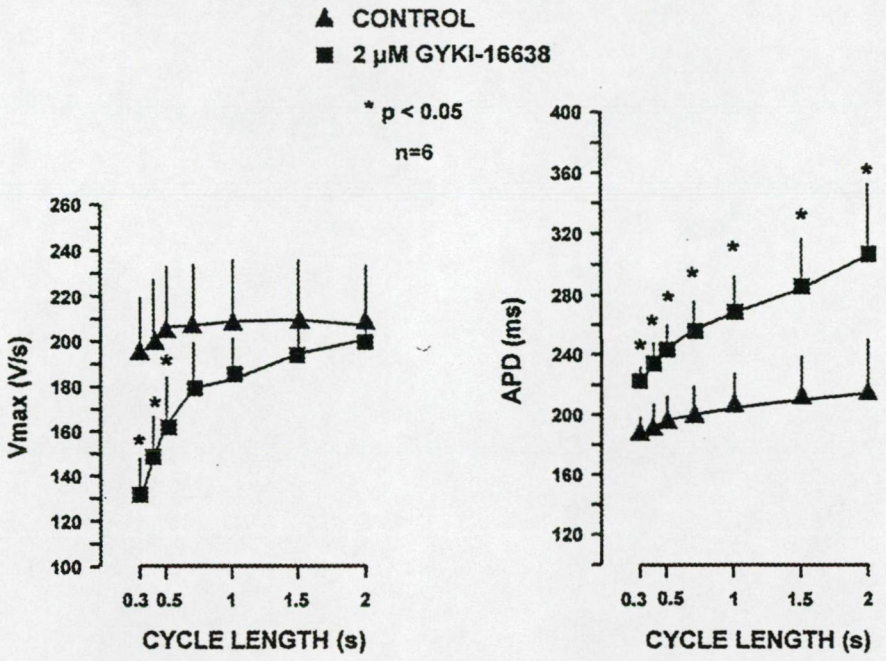

Fig. 3. Frequency-dependent effect of 2 μM GYKI-16638 on maximum upstroke velocity (V_{max}) and APD in rabbit right ventricular papillary muscle.

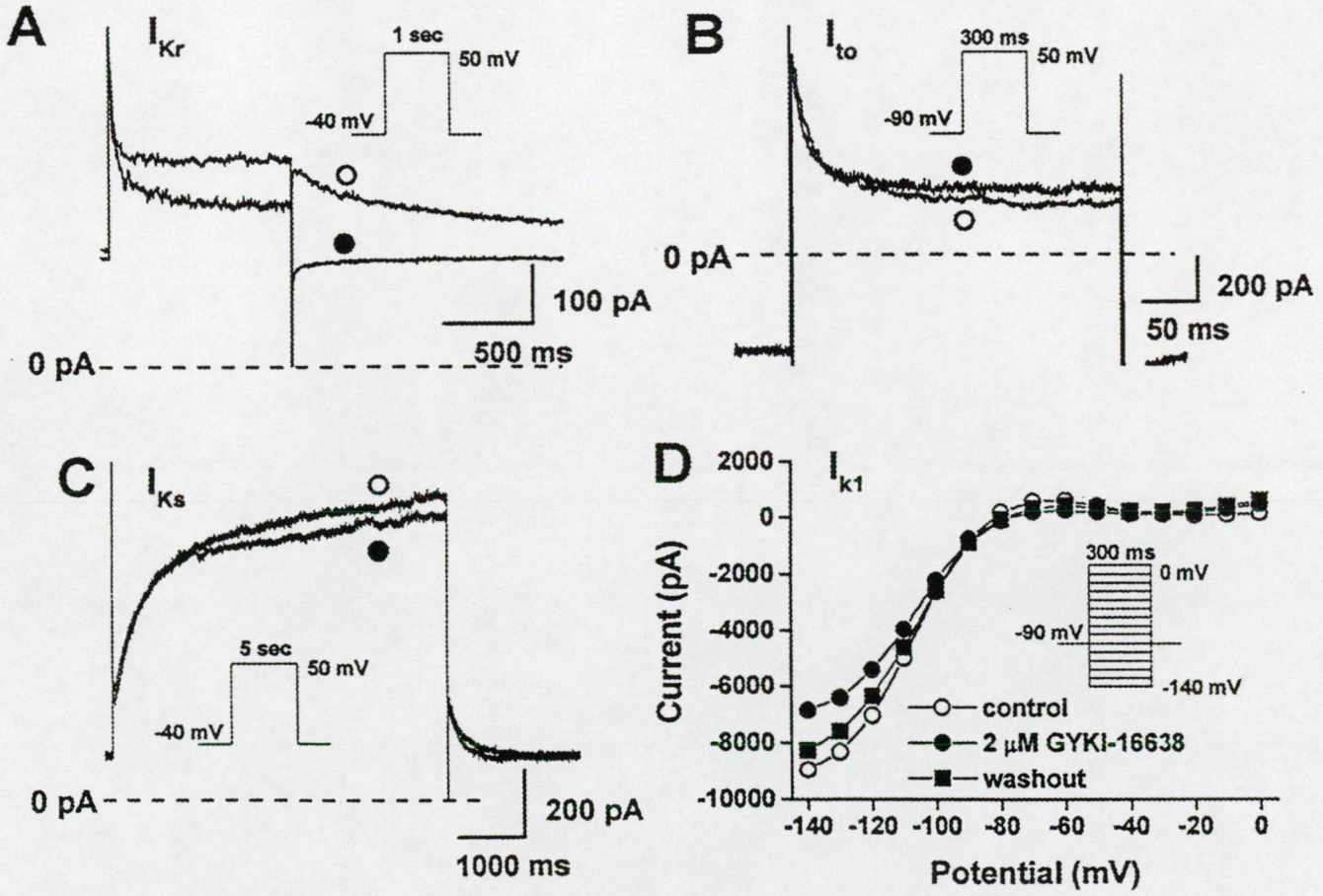

Fig. 4. Effect of 2 μM GYKI-16638 (A) on the rapid component of the delayed rectifier outward K^+ current (I_{Kr}), (B) on the transient outward current (I_{to}), (C) on the slow component of the delayed rectifier K^+ current (I_{Ks}), and (D) on the inward rectifier potassium current (I_{k1}). Pulse protocols are shown as insets.

0.1 mg/kg GYKI-16638-treated group (Table 3). There were no differences in the incidence of other types of arrhythmias between the animals receiving pretreatment and control rabbits during reperfusion (Table 3).

3.5. Effect of GYKI-16638 on the action potentials in rabbit papillary muscle

The effect of 2 μM GYKI-16638 on the action potentials at 2 Hz stimulation frequency in rabbit right ventricular papillary muscle is shown in Fig. 2 and Table 4. At 2 Hz stimulation frequency, 2 μM GYKI-16638 did not significantly influence the resting membrane potential and the APA, but it lengthened repolarization, measured as APD_{50} and APD_{90}. The maximal rate of depolarization (V_{max}) was also significantly reduced. The observed decrease of V_{max} in the presence of 2 μM GYKI-16638 was use-dependent and became significant only at stimulation cycle lengths shorter than 700 ms (Fig. 3). This was consistent with a delayed recovery of V_{max} measured in the presence of the drug ($\tau < 30$ ms in controls, and 328.2 ± 65.0 ms, ($n = 4$) with 2 μM GYKI-16638). The APD prolongation induced by 2 μM GYKI-16638 was reverse use-dependent: the slower the stimulation frequency, the more pronounced the APD prolongation (Fig. 3).

3.6. Effect of GYKI-16638 on various transmembrane K^+ currents in isolated rabbit ventricular myocytes

The effect of GYKI-16638 on various K^+ currents was studied in isolated single rabbit ventricular myocytes (Fig. 4). The rapid component of the delayed rectifier outward K^+ current (I_{Kr}) was elicited from −40 mV holding potential to various 1-s long test pulses ranging from −20 to +50 mV and then returning back to −40 mV. The amplitude of the deactivating tail current at this potential was measured as the difference between the peak tail current and the holding current level and was attributed to I_{Kr} (at +50 mV, it was 86.9 ± 16.6 pA, $n = 4$). Because of the very slow deactivation of I_{Kr}, the pulsing frequency in these experiments was 0.01 Hz. As Fig. 4A shows, 2 μM GYKI-16638 completely inhibited the I_{Kr} tail current. Similar results were obtained in three other cells.

Fig. 4B and C shows that 2 μM GYKI-16638 did not change or only minimally affected the transient outward (I_{to}) and the slow component of the delayed rectifier K^+ currents. Similar results were found in three other cells.

The effect of 2 mM GYKI-16638 on the inward rectifier K^+ current (I_{k1}) was studied at a holding potential of −80 mV and was elicited by 300-ms long voltage pulses to various potentials ranging from −140 to 0 mV. I_{k1} was determined as the steady-state current at the end of the voltage pulses. As a result of a representative experiment, Fig. 4D shows that 2 mM GYKI-16638 moderately decreased the amplitude of the steady state current–voltage relationship attributed to inhibition of I_{k1}. This effect was reversible upon 5 min of washout. The average value of the I_{k1} current ($n = 7$) at −100 mV before drug superfusion was -2648 ± 399 pA, which was significantly reduced to -2152 ± 401 pA after 5 min of superfusion with 2 mM GYKI-16638.

4. Discussion

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 D-sotalol and mexiletine.

In the present study, the antiarrhythmic effect of GYKI-16638 in anaesthetized rabbits and its electrophysiological effects in rabbit right ventricular papillary muscle preparations were investigated. We compared the antiarrhythmic effect of GYKI-16638 to that of D-sotalol, a well-known pure Class III antiarrhythmic agent.

GYKI-16638 exerted an antiarrhythmic effect in our experiments that was comparable to that of D-sotalol. Both compounds significantly decreased the number of animals that died due to lethal ventricular arrhythmias during reperfusion after 10 min of regional myocardial ischaemia. The significant improvement of survival during reperfusion occurred in spite of the fact that there were animals that had reversible ventricular fibrillation in the control group as well. This is a well-known phenomenon in experimental arrhythmia studies, i.e. relatively small hearts can recover from ventricular fibrillation, while in human and large animal hearts, this arrhythmia is irreversible (Botting et al., 1986).

The antiarrhythmic activity of GYKI-16638 was already observed after the administration of the lower dose which did not influence QT and QT_c intervals. This may suggest that GYKI-16638 has a mechanism of action that is based not solely on the prolongation of repolarization. Indeed, it was found that GYKI-16638 not only caused a significant increase in APD and, consequently, in the effective refractory period, but that it also significantly reduced the maximum upstroke velocity (V_{max}) in rabbit right ventricular papillary muscles, reflecting its fast Na^+ channel (I_{Na}) blocking ability. However, it was found that, in a higher dose, it significantly prolonged the QT and QT_c intervals in anaesthetized rabbits, as was expected from its in vitro effect on the APD. The Na^+ channel blocking effect was significant only at cycle lengths shorter than 700 ms. This was consistent with the measured time constant for recovery of V_{max}, which resembled that of Class I/B type drugs (Campbell, 1983) and amiodarone (Varró et al., 1985). Such an effect may have therapeutic importance in the inhibition of arrhythmias due to early afterdepolarizations (Papp et al., 1996).

D-Sotalol has been shown to exert an antiarrhythmic effect in a number of animal (Lynch et al., 1985; Usui et

al., 1993; Hashimoto et al., 1995) and human studies (Hohnloser et al., 1995; Koch et al., 1995) with a proposed mechanism of action of terminating re-entry (Fei and Frame, 1996). However, it was shown in the SWORD trial that D-sotalol increased mortality in patients with myocardial infarction (Waldo et al., 1996). The results shifted attention towards antiarrhythmic compounds with a combined mechanism of action. As an example, amiodarone, an antiarrhythmic agent with a complex mode of action, has attracted a great deal of interest recently. It has been shown to decrease ventricular fibrillation vulnerability in rabbit hearts following long-term pretreatment (Behrens et al., 1997), to be protective against ischaemia- and reperfusion-induced arrhythmias (Varró and Rabloczky, 1986; Coker and Chess-Williams, 1991; Li and Northover, 1992), and to be effective in the treatment of life-threatening ventricular arrhythmias in humans (Singh, 1999). Also, some multicenter clinical trials have shown that amiodarone may reduce the incidence of arrhythmia-related sudden death (Julian et al., 1997; Cairns et al., 1997). Several electrophysiological studies showed that amiodarone possessed both Class I/B and Class III antiarrhythmic properties (Singh and Vaughan Williams, 1970; Varró et al., 1985; Honjo et al., 1991; Maruyama et al., 1995), as well as Ca^{2+} channel blocking (Nattel et al., 1987) and sympatholytic effects (Polster and Broekhuysen, 1976). While effectively diminishing the development of re-entry arrhythmias, selective I_{Kr} blockers can increase the incidence of arrhythmias, by increasing the interventricular dispersion of repolarization and initiating early afterdepolarizations, leading to torsade de pointes tachycardia (Verduyn et al., 1997; Hohnloser, 1997). It was demonstrated that almokalant, a selective I_{Kr} blocker, significantly reduced the incidence of coronary artery occlusion/reperfusion-induced arrhythmias but also showed marked proarrhythmic activity (Carlsson et al., 1993a; Farkas et al., 1998). D-Sotalol has also been shown to induce torsades de pointes in animals (Buchanan et al., 1993; Vos et al., 1995) and humans (Gottlieb et al., 1997).

Amiodarone was found to have a remarkably low potential for inducing torsades de pointes tachyarrhythmias despite its ability to prolong the QT_c interval (Hohnloser et al., 1994). The decrease in the transmural dispersion of ventricular repolarization and the consequent inhibition of the development of early afterdepolarization can possibly explain this effect of amiodarone (Sicouri et al., 1997). Class I/B antiarrhythmics may reduce the occurrence of this arrhythmia. Mexiletine (Shimizu and Antzelevitch, 1997) and lidocaine in both animal (Carlsson et al., 1993b) and human studies (Assimes and Malcolm, 1998) were shown to suppress torsades de pointes induced by D-sotalol. Antiarrhythmic drugs with a Class I/B action have also been shown to be effective against coronary artery occlusion/reperfusion-induced arrhythmias (Bonaduce et al., 1986; Uematsu et al., 1986; He et al., 1992; Komori et al., 1995). Also, the combination of mexiletine and sotalol prevented ventricular tachycardia induced by programmed stimulation in dogs with chronic infarction (Chezalviel et al., 1993), and Luderitz et al. (1991) concluded in their review that in humans, the combination of mexiletine and sotalol suppressed both premature ventricular beats and complex ventricular arrhythmias more effectively than sotalol alone. These results suggest that an antiarrhythmic compound with combined Class III and Class I/B effects could reduce the incidence of re-entry arrhythmias without a high risk of inducing torsades de pointes arrhythmias.

The exact ionic mechanism of the electrophysiologic and antiarrhythmic effects of GYKI-16638 is not fully understood. As mentioned above, the use-dependent depression of V_{max} strongly argues for inhibition of the fast inward Na^+ current. The APD lengthening effect of the compound can be best explained by the marked depression of I_{Kr} and, to a lesser extent, by the decrease of the I_{k1}. Therefore, based on the cellular electrophysiological measurements, GYKI-16638 can be regarded as an antiarrhythmic compound which — like amiodarone (Varró et al., 1996; Kodama et al., 1997) — interferes with multiple transmembrane ion channels.

When administered chronically, amiodarone exhibits serious extracardiac side effects that limit 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 different chemical structure, it can be reasonably expected that this compound, unlike amiodarone, will be relatively free of extracardiac side effects. Due to its Class I/B action, it is also expected that the compound will lack the significant inhibitory effect on conduction at a normal heart rate. The compound also showed reverse frequency-dependent prolongation of APD in rabbit papillary muscle (Fig. 3), an effect which resembles that of D-sotalol or any specific I_{Kr} blocker. Therefore, further studies are needed to elucidate the possible side effects of GYKI-16638, including its capability to induce torsades de pointes or conduction disturbance-related arrhythmias.

The haemodynamic side effects of antiarrhythmic agents are of particular importance. GYKI-16638 did not change the mean arterial blood pressure, but decreased the heart rate of anaesthetized rabbits. We also found that the administration of D-sotalol significantly decreased heart rate in 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 D,L-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).

In conclusion, we demonstrated that GYKI-16638, a novel antiarrhythmic drug candidate, protected against coronary artery occlusion and reperfusion-induced arrhythmias in anaesthetized rabbits. This protection was already noticed at a lower dose, which did not lengthen the QT_c interval significantly. Based on the results of our cellular

electrophysiological investigations in rabbit right ventricular papillary muscle, it can be assumed that GYKI-16638 exerts its antiarrhythmic effect through combined Class I/B and Class III actions.

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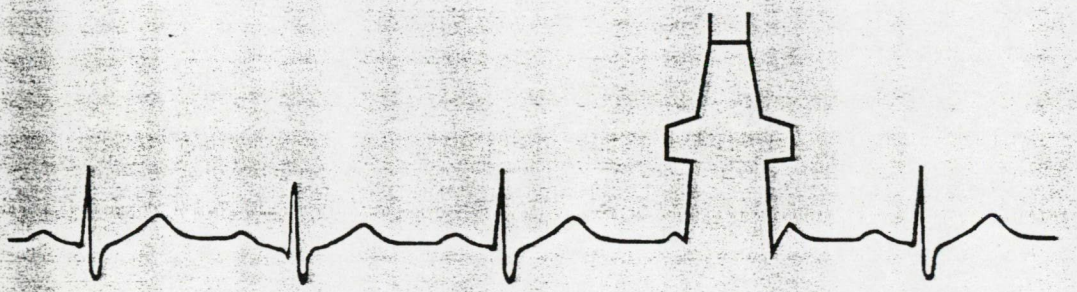

5th Alpe-Adria Cardiology Meeting in Austria at the University of Graz / Vorklinische Institute Harrachgasse 21, Graz 8.–10. May 1997

Abstracts + Posters

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A Comparison for the blood Glucose lowering and the Anti-arrhythmic effects of Glibenclamide and Glimepiride in Rats

N. E. El-Reyani, O. Bozdogan, I. Baczko, I. Lepran, J. Gy. Papp*
Department of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, Hungary

The blood glucose lowering and the antiarrhythmic effects of two sulphonylurea derivatives, glibenclamide and glimepiride, were compared in rats. Arrhythmias were induced by coronary artery ligation for 6 min followed by reperfusion in anesthetized, open-chest animals. Gibenclamide pretreatment (5 mg/kg ip. 30 min prior to coronary artery ligation) significantly increased the survival rate during reperfusion (81% vs. 27% in controls). The antiarrhythmic effect was smaller after using smaller doses of glibenclamide (survival rate was 54% and 31% after 0.1 and 0.01 mg/kg, respectively). This effect was in good correlation with the blood glucose lowering action of the drug. Glimepiride pretreatment produced similar protection against the development of fatal arrhythmias. Moreover, its effect was significant also after investigating smaller doses (eg. 0.001 mg/kg), when the survival rate was 65%. This lower dose of the compound did not produce significant lowering of the plasma glucose concentration in normoglycemic rats, nor it evoked significant effect on the oral glucose tolerance in these animals. The present results show that there is no correlation between the antidiabetic and the antiarrhythmic effects of different sulphonylurea derivatives. This finding may suggest that in various tissues the sensitivity of ATP-dependent potassium channels to drug-induced inhibition may be different.

Comparative Analysis of the Antiarrhythmic and Arrhythmogenic effect of Almokalant in Anaesthetized Rabbits

Farkas, I. Lepran, J. Gy. Papp*
epartment of Pharmacology, Albert

Cardiologia Hungarica

A MAGYAR KARDIOLÓGUSOK TÁRSASÁGA TUDOMÁNYOS KONGRESSZUSA

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Koltai M Zsófia, Posa Ildikó, Kocsis Erzsébet, Pogátsa Gábor, Ostadalova Ivana, Ostadal Bohuslav
Gottsegen Gyorgy Országos Kardiológiai Intézet, Budapest és Inst.Physiol., Acad. Sci., Praga

A MYOCARDIUM CONTRACTILITÁSA ÉS CALCIUM ERZÉKENYSEGE DIABETESES PATKÁNYOK UJSZULOTTJEIBEN

experimental, contractile function, metabolism

A vizsgálat célja a myocardium kontraktilitásának és calcium erzékenységének osszehasonlitása volt 26, anyagcsereegeszséges és 21, streptozotocin (230 umol/kg ip. a gestatio 8. napján) diabeteses anyától származó ujszulott patkányban. A vizsgálatokat a postnatalis élet 1, 4 és 7 napján állandó nyomással perfundált izolált szivkészitményeken (37 oC, 200 min-1) végeztuk. A myocardium kontraktilitást és annak első deriváltját isometrikus transducerrel mértuk és on line computerrel analizáltuk. Az inotrop hatást a perfusios mediumhoz adott novekvő calcium (0.06-10.0 mmol/l) adasával állapitottuk meg. Diabeteses anyák (D) ujszülöttjeinek test- (D:5.5±0.4 vs C: 5.5±0.4 g) és szivsulya (25.9±2.5 vs 26.4 ± 1.7mg) születéskor nem kulonbözött számottevöen a kontroll (C) csoportétól, azonban a késöbbi fejlödés során ezen mutatók alakulása elmaradt (testsuly: C: 14.6±1.2, D:9.6± 0.4 g, szivsuly: C:64.6 ± 3.7, D:44.2 ±1.2 mg) attól. Hasonlókeppen a kontrakciös válasz nagysága a postnatalis élet 4 és 7 napján jelentösen (p<0.05) kisebb volt a diabeteses anyák ujszülottjeiben. A novekvö calcium koncentrációra adott inotrop válasz nagysága viszont ugyanebben a csoportban a 7. napra számottevöen (p<0.05) nagyobb (a 21 2%) a kontroll csoporthoz képest és már 1.25 mmol/l calcium koncentráció esetén is megfigyelhető. A maximális kontrakció százalékában kifejezett inotrop dózis-hatas gorbe a 4. postnatalis napon a diabeteses anyák ujszülött patkányaiban jobbra tolódott, ami az extracellularis calcium iránti csökkent szenzitivitásra utal. Tisztázásra szorul, hogy ezek az eltéresek csak átmenetiek-e és visszavezethetök-e malnutritiora vagy dehydratiora.

Koltai M Zsófia, Posa Ildikó, Kocsis Erzsébet, Pogátsa Gábor, Ostadalova Ivana, Ostadal Bohuslav
Gottsegen Gyorgy Országos Kardiológiai Intézet, Budapest, Inst.Physiol., Acad. Sci., Praga, CZ

MYOCARDIAL CONTRACTILITY AND CALCIUM SENSITIVITY IN NEWBORN RATS FROM DIABETIC MOTHERS

experimental, contractile function, metabolism

The aim of the present study was to compare the early development of cardiac contractile function and inotropic response to Ca^{2+} in the offspring of 21 streptozotocin (230 umol/kg on the 8th gestation day) diabetic and 26 control mothers. The hearts isolated on the 1,4,and 7th postnatal days were perfused with Tyrode solution at constant pressure (37°C, 200 beats/min), devcloped force and its first derivative were measured by an isometric force transducer and analyzed using an on/line computer. The inotropic response was determined using an increasing concentration of Ca^{2+} (0.06-10.0 mmol/l). Absolute body (D:5.5±0.4 vs C: 5.5±0.4 g) and heart weights (25.9±2.5 vs 26.4±1.7mg) of newborn rats from diabetic (D) mothers were not different from control (C) animals,however during further development the values for the offspring of diabetic mothers were markedly lower (bodyweight: C: 14.6 ±1.2, D:9.6 0.4 g, heartweight: C:64.6±3.7, D:44.2 ±1.2 mg). Similarly, the magnitude of contraction in the diabetic group was significantly (p<0.05) lower on day 4 and 7 of postnatal life. Inotropic response to increasing concentration of Ca^{2+} was higher in 7-day-old newborns of D mothers (p<0.05, a 21±2%), starting from the Ca^{2+} concentration of 1.25 mmol/l. Inotropic response to increasing concentration of Ca^{2+} expressed as a percentage of the maximum value was different in 4-day-old experimental hearts only, the curve was shifted to the right suggesting decreased sensitivity to extracellular calcium. Whether these changes are only transient and limited to the malnutrition and/or dehydration during the suckling period remains to be clarified.

Nasruddin E. EL-Reyani, Leprán István, Papp Gyula
Szent-Györgyi Albert Orvostudományi Egyetem, Pharmacologiai Intézet, Szeged

GLIBENCLAMID HATÁSA A SZÍVINFARKTUS NAGYSÁGÁRA AZ INFARKTUS KÜLÖNBÖZÖ SZAKASZAIBAN PATKÁNYBAN

experimental, myocardial infarction

Korábbi kisérletek szerint az ATP-függö kálium csatorna blokkoló glibenclamid gátolja az életet veszélyeztetö kamrai arrhythmiák kialakulását az infarctus korai szakaszában. Jelen kisérleteinkben azt vizsgáltuk, hogy a glibenclamid miként befolyásolja az infarktus kiterjedését a szivizom infarctus különbözö időszakaiban. Koronária lekötést hoztunk létre him patkányokon, és a lekötést követö 1., 3. és 7. napon mértük az infarktusos terület kiterjedését. A szíveket szeletekre vágtuk és nitrotetrazoliumkék festékben inkubáltuk az infarktusos és neminfarktusos szövetek elkülönüléséig. A festést követöen a szeletekről digitalizált képeket készitettünk scanner segitségével. A kapott képeken az Intézetünkben kifejlesztett program segitségével a szinkülönbségeket felerösitettük és az infarktusos szövet térfogatát meghatároztuk. A kontroll csoportban a legnagyobb infaktus térfogat egy nappal a ligatúra után alakult ki (48±3.8%), amely a kötöszövet zsugorodása miatt a 3. és 7. napra csökkent (29±1.7 és 33±2.6%). Glibenclamid elökezelés (1 mg/kg i.p., b.i.d.) szignifikánsan növelte a 3. napon az infarktus térfogatát (44±3.2%). Nagyobb dózisú glibenclamid (5 mg/kg) kis mértékben csökkentette az infarktus térfogatot 1 nappal a lekötés után (39±2.9%), de növelte azt a 3. napra (40.3±2.7%). A 7. napon nem volt szignifikáns különbség a kontroll és a glibenclamid elökezelt szivek infarktus térfogatai között. Eredményeink szerint a glibenclamid növeli az infarktus térfogatát a miokardiális infarktus késöbbi szakaszában, ami nem korrelál az akut szakban az antmiákra kifejtett védöhatásával.

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Nasruddin E. EL-Reyani, István Leprán, Julius Gy. Papp.
Department of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

TIME DEPENDENT EFFECT OF GLIBENCLAMIDE ON THE INFARCT SIZE IN RATS

experimental, myocardial infarction

Previous investigations showed that glibenclamide, an ATP-dependent potassium channel blocker, inhibited the development of fatal ventricular arrhythmias during the acute phase of experimental myocardial infarction. The present experiments were devoted to study the influence of glibenclamide on the development of the infarct size during the first week after myocardial infarction. Coronary artery ligation was performed in male rats and the volume of the infarcted myocardium was measured 1, 3 and 7 days later. Hearts were excised and after slicing stained in nitroblue-tetrazolium dye for demarcation of the normal and infarcted regions. Stained slices were digitalized and stored images were processed for enhancing the colour difference. The volume of the infarcted tissue was calculated using a computer software developed in our Department. In the control animals the volume of infarction was the largest 1 day after coronary ligation (48±3.8%) and due to scar thinning it decreased by the 3rd and 7th days (29±1.7 and 33±2.6%, respectively). Glibenclamide (1 mg/kg i.p., b.i.d.) significantly enlarged the infarct size 3 days after coronary ligation (44±3.2 %). Larger dose of glibenclamide (5 mg/kg) moderately decreased the infarct volume after 1 day infarction (39±2.9%), but it also increased at the 3rd day (40.3±2.7%). No significant difference was found in infarct size at the 7th day between the control and glibenclamide treated animals. We conclude that glibenclamide may enhance the infarct size at the later stage of myocardial infarction, but this effect is not in correlation with its protection against arrhythmias during the acute phase of myocardial infarction.

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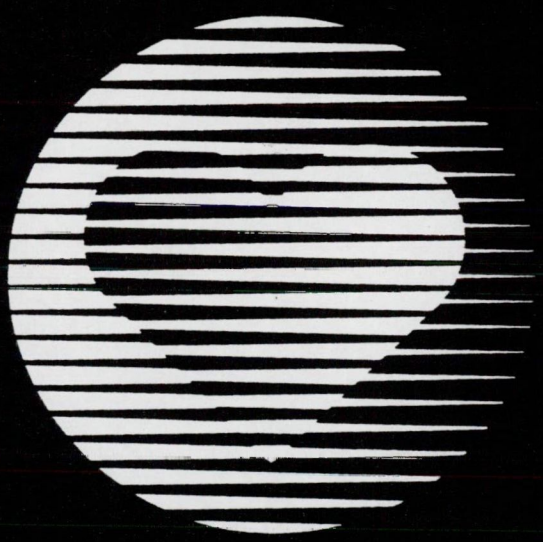

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POSITIVE SHIFT OF APPARENT ACTIVATION POTENTIAL OF TTX-SENSITIVE FAST Na^+ CURRENT IN SINOATRIAL NODE CELLS 537

Hikaru Muramatsu. Department of Cardiology, Kasugai Hospital, Yamanashi, Japan

Functional heterogeneities exist in sinoatrial node (SAN) region. Roles of TTX-sensitive Na^+ current (I_{Na}) in SAN cells are still under debate. Some pacemaker cells have I_{Na} that contributes to upstroke of spontaneous action potentials (SAP) (Am J Physiol 270: H2108-19, 1996). In this study, we examined activation potential of I_{Na} in SAP of adult rabbit SAN cells by perforated-patch clamp method. Threshold in the I-V of isolated I_{Na} of the pacemaker cells was -64±2 mV (n=12). However, take-off potential (V_T) in SAP was -43±2 mV (n=8); TTX (20 μM) further shifted V_T to -38 ±2 mV with decrease in the upstroke velocity. In ramp clamp (+0.11 V/s), I_{Na} activated at -42±4 mV (n=4). In SAP clamp, compensation current by TTX appeared at -41±3 mV (n=4). The threshold of the isolated I_{Na} became the more positive (-63 to -48 mV, n=4), as holding potential was the less negative (-88 to -68 mV). In SAN cells with more negative maximum diastolic potential (MDP, -68 to -78 mV), V_T was more negative (-45 to -58 mV, n=5). If the MDP was depolarized (-78 to -58 mV) by currents applied, V_T shifted to more positive (-58 to -38 mV, n=5). *Conclusion.* The activation potential of I_{Na} in SAP shifted in the more positive direction as MDP was the lower in the pacemaker cells. The activation potential in SAP was different from the threshold, but was determined by preconditioning potential in non-steady state.

A NOVEL NA^+/CA^{2+} EXCHANGE BLOCKER, KBR7943 INHIBITS CA^{2+}-ACTIVATED CL- CURRENT IN RABBIT VENTRICULAR MYOCYTES. 538

Akinori Kuruma, Masayasu Hiraoka, Seiko Kawano. Dept of Cardiovascular Disease, Med. Res. Inst. Tokyo Medical and Dental University, Tokyo, Japan

Ca^{2+}-activate chloride current ($I_{Cl(Ca)}$) is activated by Ca^{2+} transient via Ca^{2+}-induced Ca^{2+} release (CICR). Recently we have found that $I_{Cl(Ca)}$ is composed of both I_{Ca}-dependent and I_{Ca}-independent components in rabbit ventricular myocytes when cells were dialyzed with $[Na^+]_i \geq 5$ mM. To elucidate the activation mechanisms of I_{Ca}-independent $I_{Cl(Ca)}$, we examined contributions of a reverse mode of Na^+/Ca^{2+} exchange using whole cell patch clamp technique at 36 °C. I_{Ca}-independent $I_{Cl(Ca)}$ was completely blocked by 0 mM Ca^{2+}, 2 mM Ni^{2+} or internal application of 10 μM XIP. We also examined effects of KBR7943 (2-[2-[4[(4 [nitrobenzyloxy) phenyl]ethyl]isothiourea methanesulfonate), which was newly synthesized and selectively blocked the reverse mode of Na^+/Ca^{2+} exchange, on $I_{Cl(Ca)}$. The external application of KBR7943 (0.001 - 10 μM) inhibited I_{Ca}-independent $I_{Cl(Ca)}$ with IC_{50} of 2 μM and the block was complete at 10 μM. The blocking effects of KBR7934 were reversible and voltage independent. From these results, we conclude that I_{Ca}-independent $I_{Cl(Ca)}$ is activated via the reverse mode of Na^+/Ca^{2+} exchange.

EFFECT OF GLIBENCLAMIDE ON THE DEVELOPMENT OF INFARCT SIZE IN RATS 539

Nasruddin E. EL-Reyani, István Leprán, and Julius Gy. Papp. Department of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

The effect of glibenclamide, an ATP-dependent K^+-channel blocker, on the extent of the infarct volume was investigated using male Sprague-Dawley rats. Coronary artery ligation was produced during a short thoracotomy under ether anaesthesia. One day, 3 and 7 days after coronary ligation the hearts were excised and sectioned into 1.6 mm thick slices. After staining with nitrobluetetrazolium dye, slices were digitalized using a desktop scanner with 400 dpi. Stored images were processed for enhancing the colour difference. The volume of the infarcted tissue and left ventricular cavity were calculated using a computer software developed in our Department. In control animals the infarct volume was the largest after 1 day infarction (48±3.8 % of the volume of the whole left ventricle) and decreased after 3 and 7 days (29±1.7 and 33±2.6 %, respectively) due to scar thinning. The volume of the left ventricular cavity was progressively increased. Glibenclamide treatment (5 mg/kg ip., b.i.d.) offered a moderate protection against the development of infarct size 1 day after coronary artery ligation (39±2.9 %). However, after 3 days this treatment significantly increased the volume of the infarcted myocardium (40±2.9 %) and the enlargement of the left ventricular cavity was not considerably influenced. Smaller dose of the compound (0.1 mg/kg ip., b.i.d.) did not affect the infarct size during this period. These results may suggest that after an early protection against the development of infarct size 1 day after coronary artery ligation in rats, glibenclamide may exacerbate the development of myocardial infarction during the later phase.

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CAPTOPRIL INHIBITS THE PROTECTION BY CROMAKALIM DURING THE ACUTE PHASE OF MYOCARDIAL INFARCTION IN CONSCIOUS RATS 540

István Leprán, Julius Gy. Papp. Department of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, Hungary

We investigated whether the activation of renin-angiotensin system plays a role in the cardioprotective effect of cromakalim, an ATP-dependent potassium channel opener. In conscious, male Sprague-Dawley rats, acute myocardial infarction was produced by tightening a previously placed loose silk loop around the left main coronary artery. Cromakalim pretreatment (0.056 mg/kg iv. 10 min before coronary artery ligation) produced a significant increase in basal heart rate (449±9.8 vs. 349±9.1 beats/min in controls), known to be due to the blood pressure decreasing effect of the drug. This pretreatment significantly increased the survival rate during the first 15 min of myocardial infarction (56 % vs. 17 % in controls). Combined administration of cromakalim with captopril (25 mg/kg orally 1 h before coronary ligation), an inhibitor of the renin-angiotensin system by blocking angiotensin converting enzyme, did not inhibit the cromakalim-induced increase in heart rate before coronary ligation, which amounted to 494±5.1 beats/min, however, the protective action against arrhythmias did not develop and the survival rate (25%) did not differ from the control. We conclude that the reflex activation of the renin-angiotensin system due to the blood pressure lowering effect of cromakalim may contribute to the antiarrhythmic-cardioprotective action of cromakalim in conscious rats subjected to acute regional myocardial ischemia.

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ABSTRACTS

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ELSEVIER

LOBELINE INHIBITS NEURONAL Ca^{2+}-CHANNELS PS19

P.T. Tóth and E.S. Vizi
Dept. of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, P.O.Box 67, H-1450, Hungary

The effect of nicotinic receptor agonist lobeline was studied on voltage dependent Ca^{2+}-channels in cultured rat sympathetic neurons using the whole cell variant of patch clamp technique. Ca^{2+}-currents were evoked by step depolarizations from –80 mV (holding potential) to 0 mV (test potential) every 20 seconds. Lobeline (10 – 300 μM) inhibited the voltage dependent Ca^{2+}-current in a dose dependent manner. Several neurotransmitters are known to inhibit N-type of Ca^{2+}-channel in this preparatation, therefore we investigated whether lobeline is able to activate any of these receptors and inhibits the Ca^{2+}-channel via a G-protein mediated mechanism or exerts a direct effect on the Ca^{2+}-channel. The voltage dependency of the inhibitory effect of lobeline and noradrenaline were compared using a prepulse protocol. Depolarizing prepulses did not diminish the effect of lobeline, however it reduced the inhibitory effect of noradrenaline on Ca^{2+}-current. Noradrenaline was unable to inhibit the Ca^{2+}-current in the presence of GTPγS (300 μM) in the pipette solution. Activation of G-proteins by GTPγS did not influence the inhibitory effect of lobeline. These experiments suggest that lobeline does not inhibit Ca^{2+}-channels through receptor-mediated mechanisms, instead, it appears to directly modify the permeability of the voltage dependent Ca^{2+}-channels in rat sympathetic neurons.

GLIBENCLAMIDE INHIBITS LEFT VENTRICULAR HYPERTROPHY FOLLOWING PERSISTENT CORONARY ARTERY OCCLUSION IN RATS PS20

Nasruddin E. El-Reyani, István Leprán and Julius Gy. Papp
Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical University, Szeged, H-6701 Hungary.

Left ventricular hypertrophy is a known risk factor for cardiovascular mortality during heart failure. The aim of our experiments was to investigate the effect of two potent ATP-dependent potassium channel inhibitory antidiabetic drugs, glibenclamide and glimepiride, on the development of myocardial hypertrophy after myocardial infarction. Coronary artery ligation was produced in male Sprague-Dawley rats and 7 days later their heart was taken in anaesthesia and cut into 1.6 mm slices. After staining with nitroblue-tetrazolium the slices were scanned and processed for determining the infarct volume and the thickness of the left ventricular free wall (representing the infarcted myocardium) and the ventricular septum (representing the noninfarcted myocardium). In the infarcted control hearts the thickness of the septum was significantly increased 7 days after coronary artery ligation as compared to the 1-day-old infarction (2.89±0.10 mm vs. 2.45±0.08 mm, $P<0.05$). Glibenclamide treatment (0.1 or 5 mg/kg b.i.d.) significantly inhibited the thickening of the non-infarcted myocardium (2.30±0.10 mm and 2.09±0.10 mm, respectively). The same doses of glimepiride, while producing comparable blood glucose lowering effect, did not influence the thickening of the ventricular septum 7 days after myocardial infarction. These results suggest that glibenclamide treatment, while not influencing the development of myocardial infarct volume, inhibited the hypertrophy of the non-infarcted myocardium. This effect was not in correlation with the blood glucose lowering effect, since glimepiride was devoid of such action. *This work was supported by OTKA (T 22300) and ETT (06-127) grants.*

POTASSIUM IONS AND EDHF IN GUINEA-PIG CAROTID AND PORCINE CORONARY ARTERIES PS21

J-F. Quignard, M. Félétou, C. Thollon, J-P. Vilaine, J. Duhault and P.M. Vanhoutte.
Institut de Recherches Servier, 11 rue des Moulineaux, 92150, Suresnes, France.

Experiments were designed to determine in the guinea-pig carotid and the porcine coronary arteries whether or not the endothelium-derived hyperpolarizing factor (EDHF) can be identified as K^+ ions, and to determine whether or not the inwardly rectifying K^+ current and the Na^+/K^+ pump are involved in these hyperpolarizations. The membrane potential of vascular smooth muscle cells was recorded with intracellular microelectrodes in the presence of N^{ω}-L-nitro-arginine and indomethacin. In vascular smooth muscle cells of guinea-pig carotid and porcine coronary arteries, acetylcholine and bradykinin induced endothelium-dependent hyperpolarizations. The hyperpolarizations were not affected significantly by ouabain, barium chloride or the combination of ouabain plus barium. In both arteries, increasing extracellular K^+ concentration by 5 or 10 mM induced either depolarization or in a very few case small hyperpolarizations which never exceeded 2 mV. In isolated smooth muscle cells of the guinea-pig carotid artery, patch-clamp experiments shows that only 20 % of the vascular smooth muscle cells expressed inwardly rectifying K^+channels.and the current density recorded was small. These results indicate that barium sensitive-inwardly rectifying K^+ conductance and the ouabain sensitive-Na^+/K^+ pump are not involved in the EDHF-mediated hyperpolarization. Furthermore, K^+ did not mimic the effect of EDHF pointing out that K^+ and EDHF are not the same entity in those arteries.