ELSEVIER

European Journal of Pharmacology 324 (1997) 77–83

ejp

# $K_{ATP}$ channel modulators increase survival rate during coronary occlusion-reperfusion in anaesthetized rats

István Baczkó, István Leprán *, Julius Gy. Papp

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

Received 10 October 1996; revised 15 January 1997; accepted 17 January 1997

**Abstract**

We investigated the effect of ATP-sensitive $K^+$ channel ($K_{ATP}$) openers (pinacidil and cromakalim), and a $K_{ATP}$ blocker (glibenclamide) on reperfusion-induced arrhythmias in pentobarbitone-anaesthetized rats. Arrhythmias were induced by reperfusion following a 6 min ligation of the left main coronary artery. Rats were pretreated with pinacidil (0.1 or 0.3 mg/kg), or cromakalim (28 or 56 μg/kg), or glibenclamide (5 mg/kg), or vehicle. Pinacidil and cromakalim produced dose-related reductions in blood pressure. Pinacidil (0.1 mg/kg) and cromakalim (56 μg/kg) significantly decreased the incidence of reperfusion-induced ventricular fibrillation and increased survival. Glibenclamide did not decrease ventricular fibrillation incidence, yet improved survival by increasing the possibility of recovery from ventricular fibrillation. The present study suggests that both opening and blocking $K_{ATP}$ channels may increase survival during coronary occlusion and reperfusion in anaesthetized rats. 

*Keywords:* Reperfusion arrhythmia; Pinacidil; Cromakalim; Glibenclamide; Anesthetized rat

## 1. Introduction

Since the identification of the ATP-dependent $K^+$ channels ($K_{ATP}$) by Noma (1983), their role in cardiac myocytes under physiologic and pathologic conditions has been extensively investigated. Data on the effect on cardiac arrhythmias of $K_{ATP}$ modulators, however, have been inconsistent in the setting of myocardial ischaemia and reperfusion.

In the normally oxygenated heart these channels are found mainly in a closed state; however, during myocardial ischaemia, as the intracellular ATP concentration falls and ischaemic metabolites (ADP, lactate, $H^+$) accumulate, the open probability of $K_{ATP}$ channels increases. Thereby they enhance the repolarizing $K^+$ current that, at least partly, may be responsible for the shortening of the action potential duration in ischaemic myocytes (Gasser and Vaughan-Jones, 1990). This effect could also result in a decrease in voltage-dependent calcium current and in a decrease in myocardial contractility. Such a mechanism may have a natural protective role during myocardial ischaemia that preserves the function of the myocardium for a longer time (Escande and Cavero, 1992). Non-uniform shortening of the action potential duration may result in increased electrical inhomogeneity and may be responsible for development of arrhythmias during acute myocardial ischaemia. Accordingly, $K_{ATP}$ openers have been found by many authors to be arrhythmogenic during acute ischaemia in vitro in isolated, perfused heart preparations (Wolleben et al., 1989; Di Diego and Antzelevitch, 1993; Fagbemi et al., 1993; Tosaki et al., 1993). However, we have demonstrated an antiarrhythmic effect of pinacidil during the acute phase of myocardial infarction in conscious rats (Lepran et al., 1996), while Rees and Curtis (1995) found that the $K_{ATP}$ opener RP 49356 did not have any action on the incidence of ischaemia-induced ventricular fibrillation in isolated rat heart. Smallwood et al. (1993) found that intra-coronarily administered pinacidil increased the incidence of ventricular fibrillation in a 90 min occlusion/5 h reperfusion in vivo canine model.

Inhibition of the opening of $K_{ATP}$ channels during myocardial ischaemia could prevent the shortening of the action potential duration and may offer an antiarrhythmic effect. This has been demonstrated in in vitro experiments using isolated perfused hearts (Wolleben et al., 1989; Kantor et al., 1990; Zhang et al., 1991; Tosaki et al., 1993; D'Alonzo et al., 1994). Similar data have been published

* Corresponding author. Tel.: (36-62) 455-676; Fax: (36-62) 321-107; e-mail: lepran@phcol.szote.u-szeged.hu

0014-2999/97/$17.00 
*PII* S0014-2999(97)00064-2

by Linz et al. (1994) who found glibenclamide and 5-hydroxydecanoate to be protective against reperfusion-induced arrhythmias in isolated working rat hearts. More recently, Rees and Curtis (1995) found glibenclamide not to be antifibrillatory in acute myocardial ischaemia in isolated rat hearts; however, the compound did reduce the incidence of sustained ventricular fibrillation.

Both types of $K_{ATP}$ modulatory agents are used in therapy, $K_{ATP}$ opening drugs (e.g., pinacidil, nicorandil) for the treatment of hypertension and angina pectoris (Friedel and Brogden, 1990; Licata et al., 1995; Kaski, 1995; Knight et al., 1995), while $K_{ATP}$ blocking sulphonylureas (e.g., glibenclamide) have been administered for many years to patients suffering from type II diabetes mellitus (Nattrass, 1986). The reasons for the conflicting results in the literature concerning their possible antiarrhythmic/antiischaemic effect may be due to differences in the animal models and species used and other experimental conditions. Therefore, the aim of the present experiments was to compare the effects of representative $K_{ATP}$ openers (cromakalim, pinacidil) and a $K_{ATP}$ blocker (glibenclamide) on arrhythmias induced by reperfusion following a brief period of myocardial ischaemia in anaesthetized rats.

## 2. Materials and methods

### 2.1. Animals

The experiments were performed on male Sprague-Dawley rats, weighing 300–350 g. The animals were housed six to a cage and allowed to have tap water and laboratory rat chow (Altromin, Gödöllö, Hungary) ad libitum until the experiment. 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. Coronary artery ligation and reperfusion

The animals were anaesthetized with pentobarbitone sodium (60 mg/kg i.p. in a volume of 2 ml/kg). Acute coronary ligation and reperfusion were achieved according to Kane et al. (1984). After tracheal canulation thoracotomy was performed in the fourth intercostal space and the heart was exposed. A loose loop of 5-0 atraumatic silk (K 890 H, Ethicon, Edinburgh, 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 tube. The heart was set back in its place and artificial respiration was started immediately with 60 strokes/min (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA).

Blood pressure was measured from the carotid artery using a pressure transducer (Gould-Statham, P23ID, Hugo Sachs Electronik, March-Hugstetten, Germany) and was recorded on an oscillographic recorder (Watanabe, WTR 331, Hugo Sachs Electronik). The catheter was filled with isotonic saline that contained heparin (500 IU/ml), but the animals were not heparinized. The electrocardiogram (lead II) was recorded using subcutaneous needle electrodes.

After stabilization of the blood pressure and heart rate (approximately 5 min), the loose loop of the coronary ligature was tightened and fixed by clamping on the silk and thus regional myocardial ischaemia was produced. After 6 min, the ligature was released to permit reperfusion for 5 min.

Arrhythmias were detected during ischaemia and reperfusion 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). The onset and duration of arrhythmias were also measured (Lepran et al., 1983). An arrhythmia score was used to evaluate the incidence and duration of different arrhythmias by giving a grade to each animal as follows: 0 = no arrhythmias; 1 = < 10 s ventricular tachycardia or other types of arrhythmias, no ventricular fibrillation; 2 = 11–30 s ventricular tachycardia or other types of arrhythmias, no ventricular fibrillation; 3 = 31–90 s ventricular tachycardia or other types of arrhythmias, no ventricular fibrillation; 4 = 91–180 s ventricular tachycardia or other types of arrhythmias, and/or < 10 s reversible ventricular fibrillation; 5 = > 180 s ventricular tachycardia or other types of arrhythmias, and/or > 10 s reversible ventricular fibrillation; 6 = irreversible ventricular fibrillation.

No attempt was made to revert ventricular fibrillation to sinus rhythm during ischaemia or reperfusion. At the end of the experiments the hearts were excised and after re-tightening the ligation they were perfused retrogradely with 10 ml saline and 2 ml of 96% ethanol through the aorta for determining the extent of the perfusable and non-perfusable areas (Lepran et al., 1983). The bright white area represented the perfusable area of the heart while the colour of the non-perfusable myocardium did not change. The hearts were cut along the epicardial border line and the wet weight of the non-perfusable myocardium was expressed as the percentage of the total weight of the ventricles.

If the perfusion proved that the ligature was at an inadequate place (the whole heart could be perfused) and no change in the electrocardiogram (QRS distorsion) and no decrease in blood pressure occurred after tightening of the ligature, the given animal was excluded from the final evaluation. On the basis of these criteria altogether five animals were excluded.

### 2.3. Drug administration protocol

All drugs were dissolved in dimethyl sulfoxide/saline, 1:1 mixture and were applied in a volume of 100 μl/kg. Each dose was prepared on the day of the experiment.

Cromakalim (Sigma, St. Louis, MO, USA) was administered at doses of 28 and 56 μg/kg i.v. 5 min prior to coronary artery ligation. Pinacidil (Research Biochemicals International, Natick, MA, USA) was given 5 min before coronary occlusion in 0.1 and 0.3 mg/kg doses i.v., and glibenclamide (Sigma) was administered i.p. at 5 mg/kg 20 min before the occlusion.

### 2.4. Statistical evaluation

The percentage incidence of arrhythmias was calculated and compared by using the $\chi^2$ method. All other variables were expressed as mean ± standard error of the mean (S.E.M.). For the comparison of the arrhythmia scores Gehan's generalized Wilcoxon test was applied. The other parameters, after analysis of variance, were compared by means of the modified '*t*' statistic of Wallenstein et al. (1980).

## 3. Results

### 3.1. Haemodynamic variables

No significant differences were detected in the heart rate values measured before coronary artery ligation between the control animals and those given $K^+$ channel activators or blockers. The respective values 5 min after pretreatment were: for control animals 412 ± 7.4/min ($n$ = 17), for cromakalim 413 ± 8.9/min ($n$ = 15) and 402 ± 8.2/min ($n$ = 18) after doses of 28 and 56 μg/kg, respectively, for pinacidil 404 ± 9.6/min ($n$ = 22) and 397 ± 11.3/min ($n$ = 18) after doses of 0.1 and 0.3 mg/kg, respectively, and for glibenclamide 20 min after the administration 431 ± 7.5/min ($n$ = 20). The heart rate (during sinus rhythm) did not change significantly in the course of coronary artery occlusion or during reperfusion between groups.

In the cromakalim- and pinacidil-pretreated groups the mean arterial blood pressure was significantly decreased in a dose-related manner immediately after drug administration (Fig. 1). This decrease was maintained for 5 min and was present at the time of coronary occlusion with 56 μg/kg cromakalim and 0.3 mg/kg pinacidil. However, blood pressure with 28 μg/kg cromakalim or 0.1 mg/kg pinacidil had recovered by the time of tightening the coronary ligation. After coronary artery ligation blood pressure fell in all groups. This fall was significantly attenuated by 28 μg/kg cromakalim, with all other groups exhibiting similar falls in pressure. In surviving animals, blood pressure recovered to levels approaching baseline in all groups (Fig. 1). The mean blood pressure of the glibenclamide-treated group showed no significant difference compared to the control throughout the experiment (104 ± 5.5 vs. 102 ± 4.6 mmHg 1 min before and 58 ± 7.4 vs. 56 ± 6.5 mmHg 1 min after occlusion).

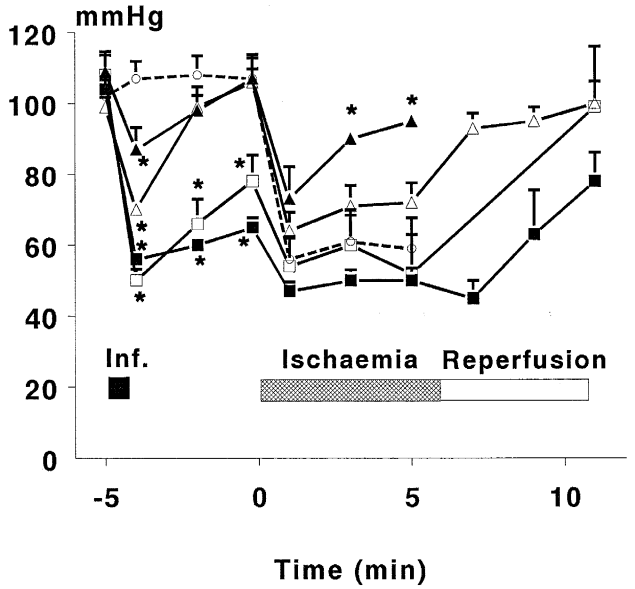


Fig. 1. Effect of pinacidil and cromakalim on mean blood pressure during coronary artery occlusion and reperfusion in anaesthetized rats. The broken line with open circles represents the control group. Open triangles, 0.1 mg/kg pinacidil; open squares, 0.3 mg/kg pinacidil; filled triangles, 28 μg/kg cromakalim; filled squares, 56 μg/kg cromakalim. Pinacidil and cromakalim were given during a 1 min infusion (Inf.) 5 min before the start of coronary artery ligation. Asterisks denote statistically significant difference from the control group ($P < 0.05$).

The underperfused region of the heart, measured at the termination of the experiment by the in vitro perfusion method, was well demarcated and extended to the proximal and lateral free wall of the left ventricle. The values in the control, 0.1 and 0.3 mg/kg pinacidil, 28 and 56 μg/kg cromakalim and glibenclamide groups amounted to 37.7 ± 2.0% ($n$ = 10), 38.1 ± 0.9% ($n$ = 18), 39.0 ± 1.1% ($n$ = 15), 38.5 ± 1.5% ($n$ = 12), 38.8 ± 1.2% ($n$ = 16) and 38.6 ± 1.5% ($n$ = 20) of the wet weight of the ventricles, respectively.

### 3.2. Arrhythmias during ischaemia

The main focus of this study was to investigate reperfusion-induced arrhythmias, therefore we applied 6 min of ischaemia which is not enough for the development of severe ischaemia-induced arrhythmias. In all groups ischaemia-induced arrhythmias started at 5 min of the occlusion period. In case of ventricular fibrillation at the time of the onset of reperfusion the experiment was terminated and the animal was considered dead during ischaemia.

Significantly more animals survived this period in the high-dose cromakalim-treated group compared to the control animals (94% vs. 65%, $P < 0.05$).

During ischaemia the number of animals without any arrhythmia was significantly higher in the 56 μg/kg cromakalim group and the 0.1 mg/kg pinacidil group (50% and 64% vs. 12%, $P < 0.05$), and the incidence of ventricular tachycardia (6% and 18% vs. 47%, $P < 0.05$)

Table 1
Effect of pinacidil, cromakalim and glibenclamide on the incidence of arrhythmias during reperfusion following 6 min of coronary occlusion in anaesthetized rats

| Group | Dose (mg/kg) | *n* | Died (No./%) | Incidence of arrhythmias (No./%) | | | | Arrhythmia score |
|---|---|---|---|---|---|---|---|---|
| | | | | None | VF | VT | Other | |
| Control | | 11 | 10/91 | 0/0 | 11/100 | 7/64 | 6/55 | 5.91 ± 0.09 |
| Pinacidil | 0.10 | 19 | 6/32 [a] | 4/21 | 7/37 [a] | 14/74 | 13/68 | 3.42 ± 0.54 [a] |
| | 0.30 | 15 | 9/60 | 1/7 | 12/80 | 14/93 [a] | 12/80 | 5.13 ± 0.41 |
| Cromakalim | 0.028 | 12 | 11/92 | 0/0 | 11/92 | 10/83 | 9/75 | 5.75 ± 0.25 |
| | 0.056 | 17 | 5/29 [a] | 0/0 | 6/35 [a] | 15/88 | 13/77 | 4.47 ± 0.30 [a] |
| Glibenclamide | 5.00 | 16 | 3/19 [a] | 0/0 | 13/81 | 16/100 [a] | 15/94 [a] | 4.69 ± 0.24 [a] |

*n* = total number of animals; No. = 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] $P < 0.05$ compared with the control group.

and ventricular fibrillation (6% and 14% vs. 41%, $P < 0.05$) was significantly lower than in the control group.

### 3.3. Reperfusion arrhythmias

Arrhythmias induced by reperfusion started within 10–30 s following the release of the coronary artery ligature. Significantly more animals survived the reperfusion period in the high-dose cromakalim, 0.1 mg/kg pinacidil and in the glibenclamide groups, compared to the control group (Table 1). The incidence of ventricular fibrillation was significantly decreased by 56 μg/kg cromakalim and 0.1 mg/kg pinacidil. In spite of the significant protective effect of glibenclamide on the survival rate, the incidence of ventricular fibrillation did not change (Table 1). However, the possibility to revert fibrillation spontaneously was significantly improved (10/13 vs. 1/11 in controls, $P < 0.01$).

The arrhythmia scores, representing the incidence and duration of arrhythmias and the survival rate as a single number, were significantly reduced by 0.1 mg/kg pinacidil, 56 μg/kg cromakalim and 5 mg/kg glibenclamide (Table 1).

The appearance of arrhythmias in animals having no dysrhythmias was defined at 5 min of reperfusion. No significant differences were noted concerning the appearance of the first arrhythmia and the duration of the period that was characterized by arrhythmias (time between first and the end of the last arrhythmic attack) during reperfusion, except in the animals pretreated by 0.1 mg/kg pinacidil, where – due to many animals showing no dysrhythmias at all – the calculated appearance of arrhythmias occurred significantly later than in the control group (1.2 ± 0.46 vs. 0.1 ± 0.04 min, $P < 0.05$). The lengths of arrhythmic attacks were calculated taking only the surviving animals into consideration. As only one animal survived the reperfusion period in the control group, statistical comparison concerning the duration of different arrhythmic attacks was not possible (i.e., ventricular fibrillation and tachycardia, other arrhythmias).

## 4. Discussion

Inducing reperfusion arrhythmias, appearing after the restoration of blood flow following a brief myocardial ischaemia, is a widely used method for the investigation of potentially antiarrhythmic drugs. These arrhythmias are easily reproducible under precise conditions both in vitro and in vivo. The duration of preceding ischaemia is one determinant of the severity of reperfusion arrhythmias (Manning and Hearse, 1984). In the present experiments we intended to produce severe and reproducible arrhythmias during reperfusion. To avoid sustained ischaemia-induced arrhythmias that would confound the analysis of reperfusion arrhythmias we applied 6 min of coronary artery ligation. This duration of coronary occlusion is sufficient to prime the heart to develop severe arrhythmias consistently 10–30 s following the onset of reperfusion (Lepran and Szekeres, 1992). The model may relate to reperfusion following cardiac surgery, thrombolysis or spontaneous release of a coronary artery vasospasm in variant angina in clinical settings (Manning and Hearse, 1984).

The present results demonstrate that pretreatments either with $K_{ATP}$ channel opener drugs (pinacidil or cromakalim) or a $K_{ATP}$ blocker (glibenclamide) may offer significant beneficial effects against reperfusion-induced arrhythmias in anaesthetized rats albeit by different mechanisms. Both types of pretreatments significantly decreased the incidence of sustained ventricular fibrillation and markedly increased the number of the surviving animals during reperfusion following a brief period of ischaemia.

It has been shown in a number of studies that drugs opening $K_{ATP}$ channels offer protection against ischaemia and reperfusion-induced myocardial injury and arrhythmias (Yao and Gross, 1994; Grover, 1994; Yao et al., 1993; Cole et al., 1991). Others have found that these agents may aggravate reperfusion injury and result in proarrhythmic activity (Kaiser et al., 1994; Black and Lucchesi, 1994; Delacoussaye et al., 1993; Chi et al., 1990; Tosaki and Hellegourach, 1994). The action potential duration shortening effect of $K_{ATP}$ channel openers can

indeed be proarrhythmic (Haverkamp et al., 1995), yet it may result in inhibition of arrhythmias caused by non-re-entrant mechanisms, especially spontaneous and triggered arrhythmias (Wilde and Janse, 1994).

The sensitivity of myocytes to $K_{ATP}$ openers during ischaemic conditions may increase, whereby the hitherto subthreshold doses can produce a pharmacologic effect. For this reason the action potential duration shortening effect (i.e., the arrhythmogenic potential) of $K_{ATP}$ channel openers in the normally oxygenated myocardium may have been overemphasized (Wilde, 1994). According to Fish et al. (1990), $K_{ATP}$ channel openers can diminish triggered arrhythmias at concentrations that do not affect the action potential duration in non-ischaemic tissue. Grover et al. (1995) have also concluded that the action potential duration shortening effect of cromakalim does not correlate with its cardioprotective effect in anaesthetized dogs.

The present in vivo experiments confirm that $K_{ATP}$ channel openers indeed protect against reperfusion-induced arrhythmias in anaesthetized rats. The 'antiarrhythmic' action offered by these agents, however, did not correlate with their blood pressure lowering effect. In higher doses, when both pinacidil and cromakalim significantly decreased the mean arterial blood pressure of the animals, only cromakalim was effective against ventricular fibrillation, the antiarrhythmic effect of pinacidil diminished at high dosage. Furthermore, the changes in blood pressure as a response to the lower doses of $K_{ATP}$ channel openers were transient and returned to the baseline values by the time of occlusion. This return was not due to increased heart rate at the time of the coronary ligation. Therefore, a peripheral vasodilating effect and a decrease in afterload may not be a contributing factor in the protective effect; moreover, the sustained fall in blood pressure may aggravate the effect of ischaemia/reperfusion because of the decrease in coronary perfusion pressure. The question, whether the loss of protection by pinacidil in a higher dose was due to direct cardiac or to some vascular effects, may be resolved by consideration of the actions of cardioselective $K_{ATP}$ channel openers. Indeed, Grover et al. (1995) found that a cardioselective $K_{ATP}$ channel opener, BMS-180448, possessed a cardioprotective effect similar to that of pinacidil and cromakalim, while having virtually no vasodilating effect in peripheral arterial smooth muscle.

The possible mechanisms of the observed beneficial effects of $K_{ATP}$ channel opener drugs against ischaemia and reperfusion injury and arrhythmias are still the subject of speculation. An increase in coronary collateral blood flow may limit the extent of developing ischaemia (Nienaber et al., 1983) and thereby reperfusion-induced myocardial damage. However, although pinacidil and cromakalim could improve the collateral flow due to their vasodilatory effect, this mechanism is unlikely to play a key role in the prevention of arrhythmias in the rat because of the very limited collateral flow present in this species (Maxwell et al., 1987).

The decrease in calcium influx caused by the shortening of the action potential duration selectively in ischaemic myocardial fibres may be an important factor in the prevention of reperfusion arrhythmias (Brooks et al., 1995). Since the voltage-dependent calcium influx decreases following the activation of $K_{ATP}$ channels, $K_{ATP}$ opener drugs may exert their beneficial effects against ventricular fibrillation through the inhibition of calcium accumulation in myocytes both during myocardial ischaemia and reperfusion. Such an effect may inhibit the development of re-entry arrhythmias and also results in decreased contractility and energy sparing, thereby slowing the development of ischaemic changes.

One of the electrophysiological consequences of regional myocardial ischaemia is the shortening of the action potential duration in hypoxic myocytes that leads to a marked electrical inhomogeneity between normoxic and ischaemic tissues, increasing the possibility of the development of severe arrhythmias (Janse and Wit, 1989). This inhomogeneity exists also within different areas of the hypoxic tissues, between severely and less seriously injured myocardial fibres. $K_{ATP}$ opener compounds could further shorten the action potential duration of the slightly injured fibres in the heart during ischaemia and reperfusion, making the injured myocardium more homogenous and diminishing the development of life threatening ventricular arrhythmias.

It was shown that among diabetic patients, receiving first-generation oral antidiabetic drug treatment (tolbutamide, phenformin), the mortality from cardiovascular causes was significantly higher than in patients treated with insulin or placebo (Meinert et al., 1970; Gilbert et al., 1975). Oral sulphonylurea antidiabetics, by blocking $K_{ATP}$ channels also in the myocardium, seem to impair the natural metabolic protection offered by the opening of these channels during myocardial ischaemia. In a more recent study, however, investigating 232 patients with non-insulin-dependent diabetes mellitus, the second-generation antidiabetic agent glibenclamide protected against arrhythmias in the acutely ischaemic myocardium (Cacciapuoti et al., 1991). Similar results were published by Lomuscio et al. (1994). Thus it seems reasonable to distinguish between first- and second-generation sulphonylurea compounds concerning their cardiac effects. Ballagi-Pordany et al. (1990) demonstrated an antiarrhythmic effect after using second-generation sulphonylurea antidiabetic drugs, and a proarrhythmic effect by using first-generation compounds in the ischaemic rat heart in vivo. Pogatsa et al. (1988) found that in digitalized, non-insulin-dependent diabetic patients glibenclamide protected, while tolbutamide offered no protection against multifocal ectopic beats. In our in vivo experiments, glibenclamide also protected against the development of reperfusion-induced irreversible ventricular fibrillation in anaesthetized rats. This significant protection occurred in spite of the well known hypoglycaemic effect of the drug

that can be harmful during myocardial infarction. We assume that the blockade of $K_{ATP}$ channels by glibenclamide was beneficial because it probably prevented the shortening of action potential duration and that of the refractory period of ischaemic myocardial fibres (Tweedie et al., 1993). Thus it seems conceivable that the decrease in electrical inhomogeneity of the heart may play a role in the prevention of fatal ventricular arrhythmias during the early phase of reperfusion.

In conclusion, both the opening and inhibition of ATP-sensitive $K^+$ channels may result in protection against reperfusion-induced arrhythmias in anaesthetized rats. This acute effect against lethal ventricular arrhythmias is probably due to the decreased electrical inhomogeneity between the ischaemic and non-ischaemic myocardium or within the ischaemic zone between more and less severely injured tissue islets during ischaemia and reperfusion. Such an effect could be achieved differently by the two types of the studied compounds, i.e., openers and blockers of the $K_{ATP}$ channels. The $K_{ATP}$ channel openers probably shorten the action potential duration of less severely damaged myofilaments, thereby making them similar to the more seriously injured tissues. $K_{ATP}$ channel blockers, on the other hand, possibly inhibit the shortening of the action potential duration in less severely ischaemic tissues, making their action potential duration similar to that in normoxic myocytes. Both mechanisms may tend to decrease the electrical inhomogeneity among islets of myocytes that were created by the developing ischaemia and were further intensified by rapid reperfusion.

## Acknowledgements

We thank Dr. M.J. Curtis for his invaluable remarks during the discussion of our results. We thank Mrs. Zsuzsa Abraham-Kovacs and Mrs. Maria Gyorffy-Kosztka for their skillful technical assistance. This work was supported by the Hungarian National Research Fund (OTKA Grant No. T 5270) and the Ministry of Welfare (ETT Grant No. T06521).

## References

Ballagi-Pordany, G., Koszeghy, A., Koltai, M.Z., Aranyi, Z., Pogatsa, G., 1990. Divergent cardiac effects of the first and second generation hypoglycemic sulfonylurea compounds. Diabetes Res. Clin. Pract. 8, 109–114.

Black, S.C., Lucchesi, B.R., 1994. Potassium channel openers are likely to be proarrhythmic in the diseased human heart. Cardiovasc. Res. 28, 923.

Brooks, W.W., Conrad, C.H., Morgan, J.P., 1995. Reperfusion induced arrhythmias following ischaemia in intact rat heart: role of intracellular calcium. Cardiovasc. Res. 29, 536–542.

Cacciapuoti, F., Spiezia, R., Bianchi, U., Lama, D., D'Avino, M., Varricchio, M., 1991. Effectiveness of glibenclamide on myocardial ischemic ventricular arrhythmias in non-insulin-dependent diabetes mellitus. Am. J. Cardiol. 67, 843–847.

Chi, L., Uprichard, A.C.G., Lucchesi, B.R., 1990. Profibrillatory actions of pinacidil in a conscious canine model of sudden coronary death. J. Cardiovasc. Pharmacol. 15, 452–464.

Cole, W.C., McPherson, C.D., Sontag, D., 1991. ATP-regulated $K^+$ channels protect the myocardium against ischemia/reperfusion damage. Circ. Res. 69, 571–581.

D'Alonzo, A.J., Darbenzio, R.B., Hess, T.A., Sewter, J.C., Sleph, P.G., Grover, G.J., 1994. Effects of potassium on the action of the $K_{ATP}$ modulators cromakalim, pinacidil, or glibenclamide on arrhythmias in isolated perfused rat heart subjected to regional ischaemia. Cardiovasc. Res. 28, 881–887.

Delacoussaye, J.E., Eldedjam, J.J., Bruelle, P., Peray, P.A., Bassoul, B.P., Gagnol, J.P., Sassine, A., 1993. Electrophysiologic and arrhythmogenic effects of the potassium channel agonist BRL 38227 in anesthetized dogs. J. Cardiovasc. Pharmacol. 22, 722–730.

Di Diego, J.M., Antzelevitch, C., 1993. Pinacidil-induced electrical heterogeneity and extrasystolic activity in canine ventricular tissues. Does activation of ATP-regulated potassium current promote phase 2 reentry? Circulation 88, 1177–1189.

Escande, D., Cavero, I., 1992. $K^+$ channel openers and 'natural' cardioprotection. Trends Pharmacol. Sci. 13, 269–272.

Fagbemi, O.S., Chi, L., Lucchesi, B.R., 1993. Antifibrillatory and profibrillatory actions of selected class I antiarrhythmic agents. J. Cardiovasc. Pharmacol. 21, 709–719.

Fish, F.A., Prakash, C., Roden, D.M., 1990. Suppression of repolarization-related arrhythmias in vitro and in vivo by low-dose potassium channel activators. Circulation 82, 1362–1369.

Friedel, H.A., Brogden, R.N., 1990. Pinacidil. A review of its pharmacodynamic and pharmacokinetic properties, and the therapeutic potential in the treatment of hypertension. Drugs 39, 929–967.

Gasser, R.N.A., Vaughan-Jones, R.D., 1990. Mechanism of potassium efflux and action potential shortening during ischaemia in isolated mammalian cardiac muscle. J. Physiol. (London) 431, 713–741.

Gilbert, J.P., Saracci, R., Meier, P., Zelen, M., Rumke, C.L., White, C., 1975. Report of the Committee for the Assessment of Biometric Aspects of Controlled Trials of Hypoglycemic Agents. J. Am. Med. Assoc. 231, 583–608.

Grover, G.J., 1994. Protective effects of ATP-sensitive potassium-channel openers in experimental myocardial ischemia. J. Cardiovasc. Pharmacol. 24 (Suppl. 4), S18–S27.

Grover, G.J., McCullough, J.R., D'Alonzo, A.J., Sargent, C.A., Atwal, K.S., 1995. Cardioprotective profile of the cardiac-selective ATP-sensitive potassium channel opener BMS-180448. J. Cardiovasc. Pharmacol. 25, 40–50.

Haverkamp, W., Borggrefe, M., Breithardt, G., 1995. Electrophysiologic effects of potassium channel openers. Cardiovasc. Drug Ther. 9, 195–202.

Janse, M., Wit, A.L., 1989. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. Physiol. Rev. 69, 1049–1169.

Kaiser, J., Englert, H.C., Kurjat, M., Linz, W., 1994. Profibrillatory effect of pinacidil and its antagonism by low concentrations of glibenclamide in isolated guinea pig hearts. Naunyn-Schmiedeberg's Arch. Pharmacol. 349 (Suppl.), R46.

Kane, K.A., Parratt, J.R., Williams, F.M., 1984. An investigation into the characteristics of reperfusion-induced arrhythmias in the anesthetized rat and their susceptibility to antiarrhythmic agents. Br. J. Pharmacol. 82, 349–357.

Kantor, P.F., Coetzee, W.A., Carmeliet, E.E., Dennis, S.C., Opie, L.H., 1990. Reduction of ischemic $K^+$ loss and arrhythmias in rat hearts. Effect of glibenclamide, a sulphonylurea. Circ. Res. 66, 478–485.

Kaski, J.C., 1995. Management of vasospastic angina: role of nicorandil. Cardiovasc. Drug Ther. 9, 221–227.

Knight, C., Purcell, H., Fox, K., 1995. Potassium channel openers: clinical applications in ischemic heart disease – overview of clinical efficacy of nicorandil. Cardiovasc. Drug Ther. 9, 229–236.

Lepran, I., Szekeres, L., 1992. Effect of dietary sunflower seed oil on the

severity of reperfusion-induced arrhythmias in anesthetized rats. J. Cardiovasc. Pharmacol. 19, 40–44.

Lepran, I., Koltai, M., Siegmund, W., Szekeres, L., 1983. Coronary artery ligation, early arrhythmias and determination of the ischaemic area in conscious rats. J. Pharmacol. Methods 9, 219–230.

Lepran, I., Baczko, I., Varro, A., Papp, J.Gy., 1996. ATP-sensitive potassium channel modulators: both pinacidil and glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rats. J. Pharmacol. Exp. Ther. 277, 1215–1220.

Licata, G., Ganguzza, A., Galletti, F., Poeta, F., Boschetti, E., Pirrelli, A., Vulpis, V., Ravera, B., Guglielmi, M., Stornello, M., Tamburino, G., Malatino, L., Iovino, G., Rodegher, P., 1995. Multicenter, double-blind clinical trial with different doses of pinacidil in patients with mild-to-moderate essential hypertension. Curr. Ther. Res. 56, 445–456.

Linz, W., Bartsch, C., Crause, P., Englert, H.C., 1994. Characterisation of blockers of the ATP-dependent $K^+$-channel in rat models of reperfusion-induced arrhythmias. Naunyn-Schmiedeberg's Arch. Pharmacol. 349 (Suppl.), R47.

Lomuscio, A., Vergani, D., Marano, L., Castagnone, M., Fiorentini, C., 1994. Effects of glibenclamide on ventricular fibrillation in non-insulin-dependent diabetics with acute myocardial infarction. Coron. Artery Dis. 5, 767–771.

Manning, A.S., Hearse, D.J., 1984. Reperfusion-induced arrhythmias: mechanisms and prevention. J. Mol. Cell. Cardiol. 16, 497–518.

Maxwell, M.P., Hearse, D.J., Yellon, D.M., 1987. Species variation on the coronary collateral circulation during myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. Cardiovasc. Res. 21, 737–746.

Meinert, C.L., Knatterud, G.L., Prout, T.E., Klimt, C.R., 1970. A study of the effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. Diabetes 19 (Suppl. 2), 789–830.

Nattrass, M., 1986. Treatment of type II diabetes. Br. Med. J. Clin. Res. Ed. 292, 1033–1034.

Nienaber, C., Gottwick, M., Winkler, B., Schaper, W., 1983. The relationship between perfusion deficit, infarct size and time after experimental coronary occlusion. Basic Res. Cardiol. 78, 210–226.

Noma, A., 1983. ATP-regulated $K^+$ channels in cardiac muscle, Nature 305, 147–149.

Pogatsa, G., Koltai, M.Z., Balkanyi, I., Devai, I., Kiss, V., Koszeghy, A., 1988. The effect of various hypoglycaemic sulphonylureas on the cardiotoxicity of glycosides and arrhythmogenic activity due to myocardial ischaemia. Acta Physiol. Hung. 71, 243–250.

Rees, S.A., Curtis, M.J., 1995. Pharmacological analysis in rat of the role of the ATP-sensitive potassium channel as a potential target for antifibrillatory intervention in acute myocardial ischaemia. J. Cardiovasc. Pharmacol. 26, 280–288.

Smallwood, J.K., Schelm, J.A., Bemis, K.G., Simpson, P.J., 1993. Effect of activation of ATP-dependent potassium channels with (−)-pinacidil and (−)-3-pyridyl pinacidil on infarct size in a canine model of ischemia-reperfusion injury. J. Cardiovasc. Pharmacol. 22, 731–743.

Tosaki, A., Hellegourach, A., 1994. Adenosine triphosphate-sensitive potassium channel blocking agent ameliorates, but the opening agent aggravates, ischaemia/reperfusion-induced injury. Heart function studies in nonfibrillating isolated hearts. J. Am. Coll. Cardiol. 23, 487–496.

Tosaki, A., Szerdahelyi, P., Engelman, R.M., Das, D.K., 1993. Potassium channel openers and blockers: do they possess proarrhythmic or antiarrhythmic activity in ischemic and reperfused rat hearts? J. Pharmacol. Exp. Ther. 267, 1355–1362.

Tweedie, D., Henderson, C., Kane, K., 1993. Glibenclamide, but not class III drugs, prevents ischaemic shortening of the refractory period in guinea-pig hearts. Eur. J. Pharmacol. 240, 251–257.

Walker, M.J.A., Curtis, M.J., Hearse, D.J., Campbell, R.W.F., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W.G. et al., 1988. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. Cardiovasc. Res. 22, 447–455.

Wallenstein, S., Zucher, C.L., Fleiss, J.L., 1980. Some statistical methods useful in circulation research. Circ. Res. 47, 1–9.

Wilde, A.A.M., 1994. $K^+_{ATP}$-channel opening and arrhythmogenesis. J. Cardiovasc. Pharmacol. 24 (Suppl. 4), S35–S40.

Wilde, A.A.M., Janse, M.J., 1994. Electrophysiological effects of ATP-sensitive potassium channel modulation: implications for arrhythmogenesis. Cardiovasc. Res. 28, 16–24.

Wolleben, C.D., Sanquinetti, M.C., Siegl, P.K.S., 1989. Influence of ATP-sensitive potassium modulators on ischemia-induced fibrillation in isolated rat hearts. J. Mol. Cell. Cardiol. 21, 783–788.

Yao, Z., Gross, G.J., 1994. The ATP-dependent potassium channel: an endogenous cardioprotective mechanism. J. Cardiovasc. Pharmacol. 24 (Suppl. 4), S28–S34.

Yao, Z., Cavero, I., Gross, G.J., 1993. Activation of the cardiac $K_{ATP}$ channels: an endogenous protective mechanism during repetitive ischaemia. Am. J. Physiol. 264, H495–H504.

Zhang, H.L., Li, Y.S., Fu, S.X., Yang, X.P., 1991. Effects of glibenclamide and tolbutamide on ischaemia-induced and ouabain-induced arrhythmias and membrane potentials of ventricular myocardium from rat and guinea pig. Acta Pharmacol. Sin. 12, 398–402.