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Ömer BOZDOĞAN[1],
István LEPRÁN[2],
Julius Gy. PAPP[2]

Effect of the Combination of Glibenclamide, an ATP-dependent Potassium Channel Blocker, and Metoprolol, a Cardioselective β-adrenoceptor Blocker, During Myocardial Infarction in Conscious Rats

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[1]Department of Biology, Abant Izzet Baysal University, Bolu - TURKEY
[2]Department of Pharmacology, Albert Szent-György Medical University, Szeged - HUNGARY

Abstract: We investigated the possible interaction of glibenclamide and metoprolol on the occurrence of life threatening arrhythmias during the acute phase of experimental myocardial infarction. Coronary artery ligation was performed in conscious rats and ECG was recorded for 15 min following ligation. Neither metoprolol (2 mg/kg i.p., 20 min before coronary artery ligation), nor glibenclamide (5 mg/kg i.p., 30 min before ligation) pretreatment increased significantly the survival rate during the acute phase of myocardial infarction (10 % and 22 %, respectively vs. 9 % in controls). Combination of glibenclamide with metoprolol, however, significantly improved the survival rate (62 %, $P < 0.05$). These results suggest that the combination of a cardioselective β-blocking agent with an ATP-dependent potassium channel inhibitor may result in an enhanced antiarrhythmic effect during the acute phase of myocardial infarction.

Key Words: Glibenclamide, Metoprolol, Arrhythmias, Sudden cardiac death, Conscious rats

Introduction

Orally acting sulfonylureas are widely accepted for the treatment of non-insulin dependent diabetes mellitus (NIDDM). The incidence of cardiovascular complications and myocardial infarction among patients is very high and it is still unclear how these drugs influence the outcome of myocardial infarction. It has been suggested that second generation sulfonylureas may decrease the incidence of fatal myocardial infarction in NIDDM [1, 2].

Glibenclamide is a second generation antidiabetic agent that blocks ATP-dependent potassium channels (KATP) and inhibits the shortening of the action potential duration of myocardial cells during ischemia [3]. Such an effect may inhibit the development of electric inhomogeneity, leading to severe arrhythmias during acute myocardial infarction. This has been demonstrated by many investigators using isolated perfused heart [4-7]. Previously, we observed that this compound increased the survival rate following ischemia-reperfusion injury of the heart [8, 9], as well as during the acute phase of experimental myocardial infarction in conscious rats [10]. Glibenclamide pretreatment did not decrease the incidence of different arrhythmias and ventricular fibrillation, but rather it increased the chances of spontaneous recovery from fibrillation [10-12]. However, glibenclamide may inhibit the development of a natural protective mechanism during myocardial ischaemia via the inhibition of the opening of KATP channels during hypoxia [13]. As a consequence, glibenclamide may increase calcium influx to myocytes through voltage-dependent calcium channels by prolonging the action potential duration. This increased calcium entry during myocardial ischaemia may result in calcium overload and may further precipitate myocardial ischaemia. Such an effect is intensified by the activation of the sympathetic autonomic nervous system due to the blood-glucose-lowering action of glibenclamide, which

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may also contribute to calcium overload and may lead to the progression of myocardial ischaemia and arrhythmias during the acute phase of myocardial infarction.

The present experiments were designed to investigate whether inhibition of the activation of the sympathetic autonomic nervous system by metoprolol, a cardioselective β-adrenoceptor blocking agent, after the use of glibenclamide could improve the protective action during the acute phase of experimental myocardial infarction in conscious rats.

Material and Method

Male Sprague-Dawley rats weighing 370-480 g were used. The animals were fed commercial rat food pellet, and given tap water ad libitum. In a preliminary operation the animals were anaesthetised with ether and the thorax was opened in the fourth intercostal space. A loose loop of atraumatic silk (5/0, K 890 H, Ethicon, England) was placed around the left main coronary artery about 2mm from its origin. The silk was led through a polyethylene tubing, and the chest was closed with the tubing remaining inside, but both ends of the silk were carried outside the thoracic cavity [14]. The thorax was slightly compressed to prevent pneumothorax and was closed in layers with the ends of the atraumatic silk remaining under the skin. The animals recovered quickly after this operation and the mortality was very low (< 5 %). The animals were left in their cage for 7 days for complete healing after the operation. Then the silk under the skin was freed through a small incision on the skin and electrode pairs were stitched under the skin under light ether anaesthesia. The animals were put into individual cages to recover from the ether anaesthesia and to adapt to the new conditions for 2 hours.

Acute ligation of the coronary artery was performed by tightening the loose silk ligature in the conscious rats. After coronary ligation, bipolar ECG was continuously recorded from freely moving animals for 15 minutes. In the animals that survived for 16 hours the mass of the infarcted myocardium was measured. The animals were anaesthetised by pentobarbitone (60 mg/kg i.p.) and the heart was excised and washed in isotonic saline solution. The heart was sliced into 2 mm thick slices from the apex to the base and stained in 0.1% nitroblue-tetrazolium dye [15]. The wet weight of the infarcted, unstained myocardium was expressed as the percentage of the total weight of the ventricles.

Arrhythmias were recorded according to the Lambeth Conventions [16] as ventricular fibrillation (VF) ventricular tachycardia (VT), and other arrhythmias, including ventricular extrasystole (PVC), bigeminy and salvos. The incidence and duration of each type of arrhythmia were determined during the 15 min period after the coronary artery ligation. An arrhythmia score was given according to the incidence and duration of arrhythmias for each animal as follows: 0 = no arrhythmia; 1 = <10 s VT or other arrhythmias, no VF; 2 = 11-30 s VT or other arrhythmias, no VF; 3 = 31-90 s VT or other arrhythmias, no VF; 4 = 91-180 s VT or other arrhythmias and/or <10 s reversible VF; 5 = >180 s VT or other arrhythmias and/or >10 s reversible VF; 6 = irreversible VF.

Glibenclamide (5 mg/kg) and/or metoprolol (2 mg/kg) were applied i.p. 30 min before coronary ligation. The control animals were given 100 μl/kg DMSO:ethanol 1:1 solution as a solvent for the drugs.

The survival rate and the incidence of arrhythmias were statistically compared by the χ^2- test. The other parameters were expressed as mean ± SE and after the analysis of variance (one-way ANOVA) were compared by means of the modified "t" statistic of Wallenstein et al. [17].

Results

Arrhythmias occurred within 3-5 min following coronary ligation in all of the animals. Only 1 of 11 control animals survived the first 15 min following ligation. Glibenclamide or metoprolol pretreatment alone did not significantly influence the occurrence of arrhythmias or the survival rate (Table 1). Although the combination of glibenclamide and metoprolol did not influence the incidence of arrhythmias during the first 15 min after coronary artery ligation, the survival rate significantly improved (Table 1). This effect was due to the improved chances of spontaneous recovery from ventricular fibrillation, which occurred in 5 out of 10 fibrillating animals after using the combination, while there was only 1 case out of the 11 fibrillating controls. The arrhythmia score was also significantly decreased by the combination of glibenclamide with metoprolol when compared to the controls.

Ventricular tachycardia or other arrhythmias were short-lived in the control animals and rapidly converted to irreversible ventricular fibrillation (Table 2). Neither glibenclamide nor metoprolol pretreatment alone could influence the length of different arrhythmic attacks. The combination of these drugs, however, significantly shortened the period of ventricular fibrillation. On the

Table 1. Combined effects of glibenclamide and metoprolol on the survival rate and the incidence of arrhythmias during the acute phase of myocardial infarction in conscious rats.

Group	Dose	N	Surviving 15 min	Surviving 16 h	Incidence of arrhythmias (n/%)			Arrhythmia
	(mg/kg)		(n/%)	(n/%)	VF[a]	VT[b]	Other	Score
Control		11	1 / 9	1 / 9	11 / 100	11 / 100	8 / 73	5.73±0.27
Glibenclamide	5	9	2 / 22	2 / 22	9 / 100	7 / 78	7 / 78	5.56±0.34
Metoprolol	2	10	1 / 10	0 / 0	10 / 100	9 / 100	8 / 80	5.90±0.10
Glibenclamide								
+ Metoprolol	5	13	8 / 62[d]	3 / 23	10 / 77	11 / 85	12 / 92	4.77±0.32[d]
	2							

N = total number of animals in a group; n = number of animals with the given response;

[a] ventricular fibrillation;

[b] ventricular tachycardia;

[c] extrasystoles, bigeminia and salvo.

Drugs were applied i.p. 30 min before coronary artery ligation.

[d] Significantly different ($P < 0.05$) from the control group.

Table 2. Effect of glibenclamide and metoprolol on the length of arrhythmic attacks during the first 15 min of myocardial infarction in conscious rats.

Group	Dose	N	Length of arrhythmic attacks (s)			
	(mg/kg)		VF	VT	Other	Total
Control		11	610 ± 63.1	16 ± 3.1	21 ± 7.9	647 ± 62
Glibenclamide	5	9	556 ± 97.1	34± 19.1	30 ± 15.5	620 ± 80.7
Metoprolol	2	10	559 ± 50.5	41 ± 31	9 ± 1.8	608 ± 26.2
Glibenclamide + Metoprolol	5	13	281 ± 98.4[abc]	44 ± 11.4	65 ± 17.6[ac]	389 ± 80.3[abc]
	2					

Results are mean ± SE of N animals.

Significantly different ($P < 0.05$) from the [a] control, [b] glibenclamide, or [c] metoprolol-treated animals, respectively.

For other details see Table 1.

other hand, the period characterized by other, less severe arrhythmias was increased.

The first minutes after coronary artery ligation in conscious rats were characterized by tachycardia (Table 3). The heart rate could not be determined after the 5th min because of frequent arrhythmias in all of the animals. Metoprolol treatment significantly decreased the heart rate measured before coronary artery ligation. The combination of glibenclamide and metoprolol produced an even more marked decrease in heart rate and this remained significantly lower after coronary artery ligation, as compared to the control or glibenclamide-treated animals, until the time when the heart rate could be measured.

The infarct size was measured in the surviving animals 16 hours after coronary artery ligation. The infarcted area as determined by nitroblue-tetrazolium staining, extended to the anterior and lateral region of the left ventricle. Since very few animals survived the acute phase of myocardial infarction and developed myocardial necrosis, this measurement was used only to verify the proper coronary artery ligation. There were no differences in the infarct size among different groups.

Table 3. Effect of glibenclamide and metoprolol on the alteration of heart rate during myocardial infarction in conscious rats

Group	Dose (mg/kg)	N	Heart rate (beats/min) Basal	1st min	3rd min
Control		11	339 ± 8.7	430 ± 26.4	395 ± 26.8
Glibenclamide	5	9	316 ± 6.3	405 ± 9.6	411 ± 16.5
Metoprolol	2	10	312 ± 10.6	390 ± 7.2	381 ± 14.5
Glibenclamide + Metoprolol	5	13	289 ± 7.3ab	339 ± 14.3 ab	335 ± 15.7 ab
	2				

Results are mean ± SE of N animals.

Significantly different ($P < 0.05$) from the [a] control, or [b] glibenclamide treated animals, respectively.

For other details see Table 1.

Discussion

The present results demonstrate that the combination of a K_{ATP} inhibitory antidiabetic sulfonylurea, glibenclamide, with a cardioselective β-blocker, metoprolol, offers protection against the development of life-threatening arrhythmias during the acute phase of experimental myocardial infarction in conscious rats. The protective effect is manifested by improved recovery from ventricular fibrillation.

Metoprolol was used in a dose that, according to previous investigations, shifted the dose-response curve to isoproterenol-induced tachycardia 3 times to the right [18]. This threshold dose of metoprolol, as in the previous findings using the same model [19], did not offer significant protection against the development of arrhythmias in the acute phase of myocardial infarction.

Glibenclamide alone in the present experiments did not prevent the development of fatal ventricular fibrillation after acute myocardial infarction. This finding conflicts with the findings of our previous investigations with the same model [10], and with those of a study of myocardial ischaemia/reperfusion induced arrhythmias in anaesthetized rats [8]. The possible reason for this discrepancy may be the use of older animals in the present experiments. Older rats may respond to the same coronary artery ligation with a higher incidence of ventricular fibrillation and lower survival rate [20]. On the other hand, younger animals may better tolerate the blood-glucose-lowering effect of orally acting sulfonylureas. Such differences between younger and older animals may mask the 'antifibrillatory' effect of glibenclamide in the present experiments using older animals. These findings suggest that using older animals may result in false conclusions when the possible antiischaemic-antiarrhythmic effects of KATP-inhibitors are investigated.

The combination of glibenclamide and metoprolol resulted in a significant improvement in recovery from ventricular fibrillation and in a higher survival rate during the acute phase of myocardial infarction. This beneficial drug interaction might be the consequence of the synergistic effect in inhibiting the development of electrophysiologic inhomogeneity during myocardial ischaemia. Dispersion of the refractory period due to non-uniform shortening of the action potential may have a significant role in the development of fatal ventricular arrhythmias during acute myocardial infarction [21]. Glibenclamide, as an inhibitor of K_{ATP} channels, may prevent the shortening of the action potential duration. This effect could be specific for the ischaemic cells, where these channels open due to the loss of intracellular ATP. Metoprolol, an β-adrenoceptor blocker, inhibits sympathetic excitation during the acute phase of myocardial infarction, which would otherwise also result in shortening of the action potential duration in myocardial ischaemia [22]. Thereby both agents, glibenclamide and metoprolol, act against the development of regional differences in the refractory period and their combination decreases the incidence of fatal arrhythmias. Furthermore, treatment with glibenclamide produces a fall in blood glucose concentration, which may result in a reflex activation of the sympathetic autonomic nervous system [23]. Such an effect on the heart is not desirable during acute myocardial infarction and its inhibition by metoprolol could also contribute to the significant protection afforded by the combination treatment in the present experiments.

The combination of orally acting sulfonylureas and β-adrenoceptor blockers in diabetic patients is generally not recommended, owing to the decrease in metabolic control and a concomitant increase in the risk of hypoglycaemia [24]. Such a combination increases the risk of hypoglycaemia because β-blockers inhibit the effects of catecholamines on gluconeogenesis and glucogenolysis. These drugs may also mask the alarming sympathetically mediated symptoms associated with the decrease in blood glucose concentration. The obvious untoward interaction in the metabolic control observed in diabetic patients may not have been so expressed in the present experiments when metabolically healthy animals were used.

The present results may suggest that the combination of new, 'cardioselective' K_{ATP} inhibitors, which have less influence over the metabolic control, and a cardioselective β-blocker could provide more significant antiischaemic-antiarrhythmic effects during myocardial infarction.

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