Original Research

Effect of ATP-dependent channel modulators on ischemia-induced arrhythmia change depending on age and gender

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Abstract

The number of ATP-dependent potassium channels in myocardial cells has been previously shown to change depending on gender and age. Different effects of the ATP-dependent potassium channel blocker, glybenclamide and ATP-dependent potassium channel opener, pinacidil on ischemia or reperfusion-induced arrhythmia observed in various research might depend on different ages and genders of the animals used. The aim of this study is to research the effect of ATP-dependent potassium channel modulators on ischemia-induced arrhythmia in animals of different ages and genders. Sprague-Dawley rats of different ages and genders were used in this study. Ischemia was produced by the ligation of the left coronary artery for 30 min. Electrocardiogram (ECG), blood pressure, infarct area and blood glucose were determined during the 30 min of ischemia. An arrhythmia score from an ECG recorded during 30 min of ischemia was determined by examining the duration and type of arrhythmia. Different effects of glybenclamide and pinacidil on the arrhythmias were observed in male and female young and middle-age rats. Pinacidil decreased the infarct zone in younger female rats, but differences in the type and length of ischemia-induced arrhythmias between females and males disappeared in older age. The results of this study showed that the effect of ATP-dependent potassium channel modulators on ischemia-induced arrhythmia changed due to the age and gender of rats.

Keywords: cardiac arrhythmias, myocardial ischemia, coronary ligations, glybenclamide, pinacidil, rats

***Experimental Biology and Medicine* 2013; 238: 1170–1179. DOI: 10.1177/1535370213498980**

Introduction

There are structural differences in male and female myocardial cells and the number of ATP-dependent potassium channels decreases in females, but not in males with increasing age.[1] These types of changes may affect the different responses to ATP-dependent potassium channel modulating drugs observed in experimental research in both sexes.[2,3] There are several studies relating the effect of ATP-dependent potassium channels with the arrhythmias induced by ischemia and reperfusion.[4–7] Most of these studies were performed in male animals. Although adult male animals have commonly been used in experimental studies, their weight and exact age have not usually been evaluated in the results. However, the effect of drugs may be different in animals of different age and gender used in research. The aim of this study is to research the effect of the ATP-dependent potassium channel opener, pinacidil and blocker ATP-dependent potassium channel glybenclamide on the arrhythmia caused by coronary artery ligation, and how this varies with the increasing age of rats from both genders.

Materials and methods

Animals

The animals utilized in this study were cared for and used in accordance with the approval of the Abant İzzet Baysal University Animal Research Ethical Committee, Turkey. Two age categories of male and female Sprague Dawley rats, four housed to a cage were studied, Young adults (age 7–8 months, weight 170–223 g for females, $n = 20$ and 250–328 g for males, $n = 25$) and middle-age adults (age 12–13 months, weight 180–280 g for females, $n = 23$ and 240–360 g for male's $n = 21$). There was no significant difference in body weight between young and middle-age male rats and between young and middle-age female rats. Nevertheless, it was lower in the young (7–8 months old) and older adult (12–13 months old) females than in the

corresponding males. All rats were fed a standard diet and water *ad libitum*.

Surgical procedures and determination of infarct size

Animals were anaesthetized with thiopental sodium (100 mg/kg) intraperitoneally and a tracheotomy was performed for artificial respiration. The left carotid artery was cannulated by a catheter in order to measure the blood pressure with a blood pressure transducer (Biopac System Inc. Goleta, CA, USA, under the authorized distribution of representative Commat-Ankara, Turkey). After the chest was opened in the fourth intercostal space, the heart was exposed and a loose loop of atraumatic silk was placed around the left main coronary artery, approximately 2 mm from its origin. The heart was returned to its place and artificial respiration was performed using a ventilator (0.9 mL/100 g body weight at a rate of 60 strokes/min). Following 5 min stabilization, the left coronary artery was ligated by tightening the silk for 30 min. At the end of 30 min of ligation, live animals were heparinized (500 IU/kg) and the heart was excised out. The coronaries of the heart were perfused with 10 mL of isotonic NaCI solution and then 2 mL of 96% ethanol given from cannulated aorta for demarcation of the occluded and non-occluded myocardium. The occluded myocardium coloured white and the non-occluded myocardium of a pinkish colour were separated from each other by dissection and both areas were then weighed individually. The percentage of occluded myocardium to the wet weight of whole ventricles was recorded as the risk of the infarct zone. The occluded myocardium was then sliced with a razor and was stained with 0.1% nitro-blue-tetrazolium, dissolved in Sorensen-phosphate buffer (pH 7.4) for 10 min. The infarcted myocardium was not stained and remained pale and was easily separated from the stained region. The wet weight of unstained infarcted myocardium (infarct area) was expressed as a percentage of the wet weight of whole ventricles. The method for the measurements of the risk of infarcted region and determination of infarcted area used in the current study was adapted from the methods described previously by Lepran *et al*.[8]

Experimental groups and drug treatments

The experiment was designed in four main groups: (1) young males, (2) middle-age males, (3) young females and (4) middle-age females. These groups were divided into three subgroups as control, glybenclamide and pinacidil treated groups. Glybenclamide was prepared in dimethyl sulphoxide/ethanol and pinacidil in dimethyl sulphoxide/saline, 1/1 daily. In the control group, there was no drug treatment but the solvent of drug (1 mL/kg) including dimethyl sulphoxide/ethanol, 1/1 was given intraperitoneally (i.p.) 20 min before ligation and coronary ligation was performed. Glybenclamide (5 mg/kg) i.p. 20 min before ligation and pinacidil (0.1 mg/kg) intravenously (i.v.) was applied into the tail vein 10 min before coronary ligation. The doses of drugs chosen were used commonly as an effective dose in previous experimental research. Glybenclamide and pinacidil were purchased from Sigma Chemical Co (St Louis, MO, USA).

Recording

Bipolar lead II electrocardiograms (ECGs) were recorded using needle electrodes, and blood pressure was measured from the carotid artery using a blood pressure transducer and stored on a computer using the Biopac System (representative in Turkey; Commat-Ankara, Turkey) Pro 3.7 software. The duration of the arrhythmia and heart rate were determined from ECG using BSL Pro 3.7 software of Biopac. The incidence of arrhythmias was analysed in accordance with the Lambeth Conventions,[9] as ventricular fibrillations (VFs), ventricular tachycardia (VT) and other types of arrhythmias including extrasystoles (ventricular premature contraction [VPC]), ventricular bigeminy and salvos. An arrhythmia score was used to indicate the incidence and duration of arrhythmias, where 0 indicates no arrhythmia, 1 indicates an arrhythmia duration of less than 10 s, 2 indicates an arrhythmia duration of 11–30 s, 3 indicates an arrhythmia duration of 31–90 s, 4 indicates an arrhythmia duration of 91–180 s or reversible VF for less than 10 s, 5 indicates arrhythmia duration longer than 180 s or reversible VF for more than 10 s and 6 indicates irreversible VF or death of the animal.

Biochemical analyses

Blood glucose just before ligation was measured using a glucometer (Glucotrend2, Roche Group, UK) in all groups. The aim of measuring blood glucose was to research whether there is a different effect of the drug on blood glucose in rats of different ages and to evaluate the relation of blood glucose and the arrhythmia.

Statistical analyses

All data are expressed as the mean ± SE. Differences between groups were calculated using one way analyses of variance (LSD *post hoc* test). The survival rate and the incidence of arrhythmia were compared by χ^2 method (Fisher exact test).

Results

ST segment elevation and an increase in QRS amplitude and heart rate were observed following coronary artery ligation in all animals (Figure 1). The animals having low blood pressure below 70 mmHg before ligation (five animals) in control group were excluded from the experiment. Fourteen animals, two of them from old male groups, four from old female groups, four from young male groups and four from young female groups, died during surgery or ligation due to severe bradycardia and cardiac arrest associated with a low blood pressure below 20 mmHg. Four animals, one of them from young female adult groups and three from old male groups, were excluded from the study due to incorrect ligation that was observed after perfusion of coronary artery.

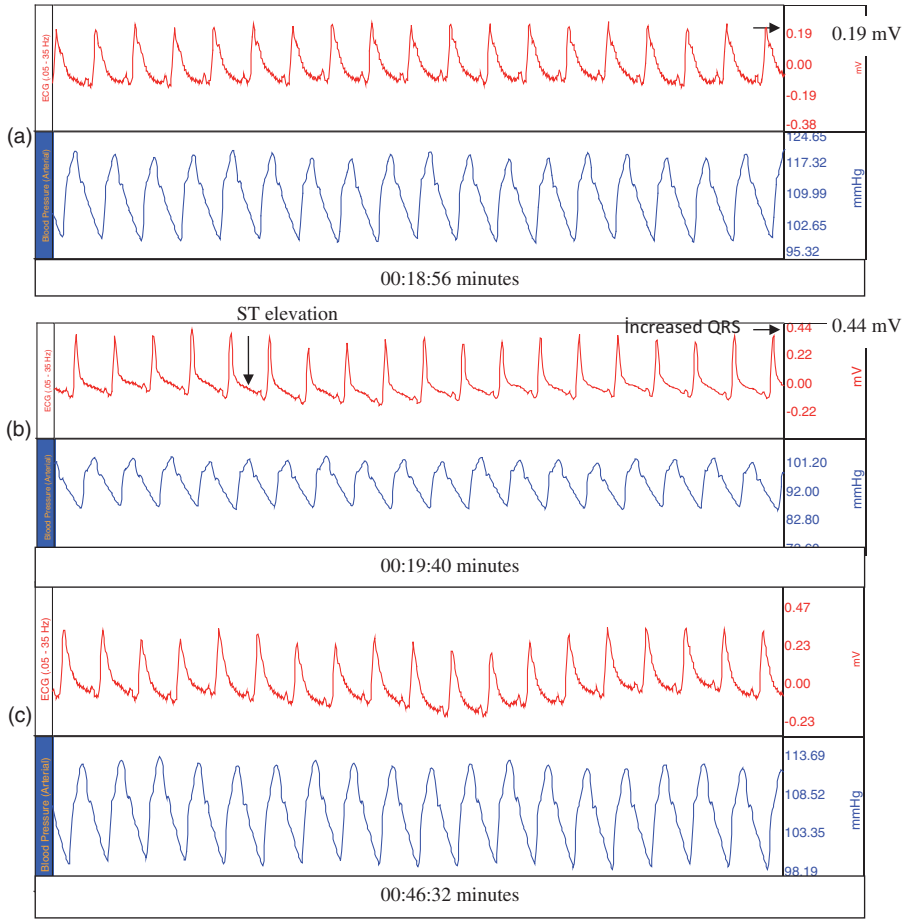

Figure 1 ECG and blood pressure recorded in male control rats. (a) Before ligation, (b) 30 s after ligation and (c) at 30 min of ligation. (A color version of this figure is available in the online journal)

Blood pressure and heart rate

Blood pressure decreased immediately after ligation but returned to control values following 1 min of ligation (Table 1). There were no differences in blood pressure between control groups of male and female rats. Blood pressure following coronary ligation did not show much difference among any of the groups; however, it decreased significantly in pinacidil-treated young adult male rats in respect to its control after 15 min of coronary ligation. Similarly, no significant change was found between heart rates, which were measured during 30 min of coronary ligation (Table 2). Heart rates in young male rats in the control groups before coronary ligation were higher than in the corresponding middle-aged rats; but the same difference was not found between control groups of young and older female rats.

Arrhythmias in male and female

The survival rate at the end of 30 min of coronary ligation was no different between the female and male rats, or between drug treated groups and their corresponding controls (Table 3). The arrhythmic period, which is the time between the beginning and the end of arrhythmia, appeared within 30 min of coronary ligation and was higher in male rats than female rats in both age groups (Figure 2). Incidence of VT and scores of arrhythmia and the arrhythmic period were significantly lower in the young adult female rats rather than the older females. On the other hand, no difference was found in the incidence of VT and score of arrhythmia and arrhythmic period between young and old male control groups. The arrhythmia score was significantly higher in young adult males rather than the corresponding females (4.3 ± 0.4 in male; 2.7 ± 0.5 in female) although there was no difference between older adults of both genders (Figure 3). Ventricular arrhythmias used in calculation of arrhythmia score were shown in Figure 4.

Effects of glybenclamide and pinacidil on arrhythmia

Different effects of glybenclamide and pinacidil on the ischemia-induced arrhythmias were observed in male and female rats of both age groups. Glybenclamide decreased the incidence of VT in old adult male rats in respect to corresponding controls, but was ineffective in old adult females (Table 3). Conversely, pinacidil increased the incidence of VT and fibrillation in young adult females but not in old adult females in respect to their controls. Pinacidil did not change the incidence of VT and fibrillation in male rats in either age in respect to their corresponding control groups. The incidence of VPC and VF was similar in all

Table 1 The blood pressures (mmHg) following coronary ligation in rats

	Before ligation		1 min after ligation		3 min after ligation		5 min after ligation		10 min after ligation		15 min after ligation		20 min after ligation		30 min after ligation	
	n		*n*		*n*		*n*		*n*		*n*		*n*		*n*	
Male 7–8 months old																
Control	8	70 ± 4	8	78 ± 4	8	84 ± 4	8	89 ± 5	8	92 ± 6	8	91 ± 11	8	93 ± 8	8	95 ± 7
Glybenclamide	7	70 ± 5	7	68 ± 6	7	69 ± 8	7	70 ± 10	7	69 ± 6	7	75 ± 7	7	78 ± 8	7	84 ± 7
Pinacidil	8	61 ± 7	8	60 ± 8	8	65 ± 8	8	67 ± 8	8	70 ± 7	8	54 ± 6**	8	57 ± 6**	8	54 ± 7**
Male 12–13 months old																
Control	8	78 ± 5	8	77 ± 5	8	82 ± 7	8	82 ± 8	7	78 ± 10	6	70 ± 7	6	76 ± 10	6	74 ± 12
Glybenclamide	7	77 ± 6	7	74 ± 6	7	75 ± 7	7	82 ± 5	7	88 ± 6	7	92 ± 6	7	91 ± 7	7	87 ± 9
Pinacidil	7	78 ± 4	7	77 ± 7	7	77 ± 9	7	79 ± 10	7	80 ± 9	7	91 ± 10	7	80 ± 8	7	78 ± 11
Female 7–8 months old																
Control	7	70 ± 1	7	75 ± 9	7	81 ± 9	7	86 ± 10	7	96 ± 9	7	86 ± 12	7	91 ± 10	7	83 ± 8
Glybenclamide	8	61 ± 4	8	63 ± 4	8	65 ± 6	8	69 ± 7	8	79 ± 8	8	69 ± 8	7	79 ± 10	7	77 ± 13
Pinacidil	5	86 ± 9	5	109 ± 8*	5	106 ± 6	5	109 ± 9	5	102 ± 17	5	109 ± 12	5	111 ± 6	5	87 ± 11
Female 12–13 months old																
Control	7	81 ± 7	7	79 ± 6	7	84 ± 7	7	81 ± 11	7	94 ± 11	7	92 ± 8	7	91 ± 10	7	79 ± 6
Glybenclamide	7	83 ± 6	7	83 ± 9	7	79 ± 11	7	82 ± 10	7	89 ± 6	6	87 ± 11	7	94 ± 8	7	91 ± 9
Pinacidil	7	71 ± 6	7	77 ± 9	7	78 ± 11	7	80 ± 11	7	82 ± 10	7	79 ± 14	7	81 ± 9	7	81 ± 3

n = number of animals. All data were expressed as the mean ± standard error.
*$P < 0.05$.
**$P < 0.01$ different from control of individual group.

Table 2 The heart rate (beats/min) following coronary ligation in rats

	Before ligation		1 min after ligation		3 min after ligation		5 min after ligation		10 min after ligation		15 min after ligation		20 min after ligation		30 min after ligation	
	n		*n*		*n*		*n*		*n*		*n*		*n*		*n*	
Male 7–8 months old																
Control (a)	8	369 ± 12[b]	8	363 ± 11	8	364 ± 12	8	361 ± 11	8	378 ± 15	8	355 ± 19	8	345 ± 15	8	349 ± 14
Glybenclamide	7	329 ± 13[c]	7	341 ± 13	7	333 ± 13	7	329 ± 15	7	327 ± 14	7	320 ± 13	7	320 ± 13	7	322 ± 14
Pinacidil	8	366 ± 7	8	380 ± 9	8	372 ± 8	8	371 ± 9	8	364 ± 10	8	422 ± 55	8	336 ± 21	8	346 ± 8
Male 12–13 months old																
Control (b)	8	330 ± 15	8	327 ± 18	8	326 ± 15	8	325 ± 15	7	376 ± 30	6	338 ± 15	6	342 ± 17	6	325 ± 20
Glybenclamide	7	304 ± 15	7	304 ± 17	7	303 ± 18	7	305 ± 17	7	301 ± 16[c]	7	300 ± 16	7	308 ± 15	7	289 ± 15
Pinacidil	7	358 ± 7	7	370 ± 17[c]	7	368 ± 14[c]	7	368 ± 16	7	404 ± 36	7	350 ± 17	7	364 ± 14	7	355 ± 17
Female 7–8 months old																
Control (c)	7	377 ± 7	7	380 ± 5	7	376 ± 10	7	380 ± 6	7	380 ± 6	7	384 ± 8	7	380 ± 5	7	374 ± 8
Glybenclamide	8	360 ± 19	8	365 ± 22	8	362 ± 19	8	358 ± 21	8	361 ± 23	8	366 ± 21	7	351 ± 28	7	351 ± 29
Pinacidil	5	397 ± 3	5	403 ± 25	5	410 ± 22	5	407 ± 21	5	478 ± 57[c]	5	423 ± 8	5	393 ± 22	5	387 ± 26
Female 12–13 months old																
Control (d)	7	347 ± 10	7	347 ± 10	7	345 ± 10	7	344 ± 9	7	354 ± 9	7	347 ± 14	7	351 ± 15	7	347 ± 18
Glybenclamide	7	328 ± 16	7	333 ± 17	7	313 ± 19	7	307 ± 20	7	321 ± 17	6	349 ± 22	7	316 ± 17	7	321 ± 16
Pinacidil	7	357 ± 14	7	353 ± 18	7	357 ± 18	7	358 ± 20	7	338 ± 23	7	387 ± 63	7	347 ± 25	7	354 ± 24

All data were expressed as mean ± standard error.
[b]$P < 0.05$ different from control of male 12–13 months old.
[c]$P < 0.05$ different from control of the individual group.

Table 3 The incidence of arrhythmia and arrhythmia score during 30 min of coronary ligation

Group	*n*	Death, *n* / %	Incidence of arrhythmia, *n* / %			Score of arrhythmia
			VPC	VT	VF	
Male 7–8 months old						
Control (a)	10	2/20	10/100	4/40	2/20	4.3 ± 0.4
Glybenclamide	7	0	7/100	2 / 28	2/28	4.0 ± 0.5
Pinacidil	8	0	8/100	4/50	1/12	4.0 ± 0.3
Male 12–13 months old						
Control (b)	7	2/28	10/100	4/57	2/28	4.6 ± 0.5
Glybenclamide	7	0	7/100	1/14[b]	1/14	3.1 ± 0.5[b]
Pinacidil	7	0	5/71	5/71	3/42	4.3 ± 0.6
Female 7–8 months old						
Control (c)	7	0	7/100	2/28	0	2.7 ± 0.5[a]
Glybenclamide	8	0	8/100	3/37	1/12	3.1 ± 0.6
Pinacidil	7	2/28	7/100	5/71[c]	2/28	3.7 ± 0.8
Female 12–13 months old						
Control (d)	9	2/28	9/100	6/67[c]	3/33	4.3 ± 0.4[c]
Glybenclamide	7	0	7/100	3/43	1/14	3.4 ± 0.5
Pinacidil	7	0	7/100	5/71	3/42	4.3 ± 0.4

[a]$P < 0.05$; different from male (7–8 months old) control.
[b]$P < 0.05$; different from male (12–13 months old) control.
[c]$P < 0.05$; different from female (7–8 months old) control.

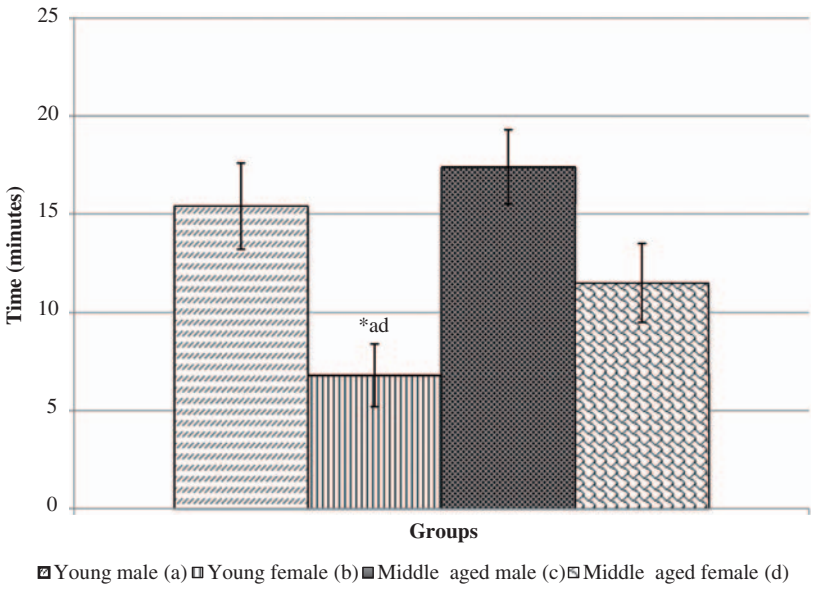

Figure 2 The arrhythmic period in 30 min of coronary ligation. [a,d]$P < 0.05$

groups of animals and there was no difference in the effect of glybenclamide and pinacidil on the incidence of VPC and VF in female and male rats of both age groups.

Blood glucose

The blood glucose levels of male and female rats of both age groups were no different from each other. There was no relation between blood glucose level and the arrhythmia score or infarct area, and glybenclamide significantly decreased the blood glucose level in all groups (Table 5). Surprisingly, pinacidil also decreased the blood glucose level in all groups, except in older adult males.

Infarct area

The infarct area measured at the end of 30 min of ligation was no different in male and female control animals of both age groups (Table 5). Pinacidil only decreased the infarct zone in younger females. It had no effect on older females

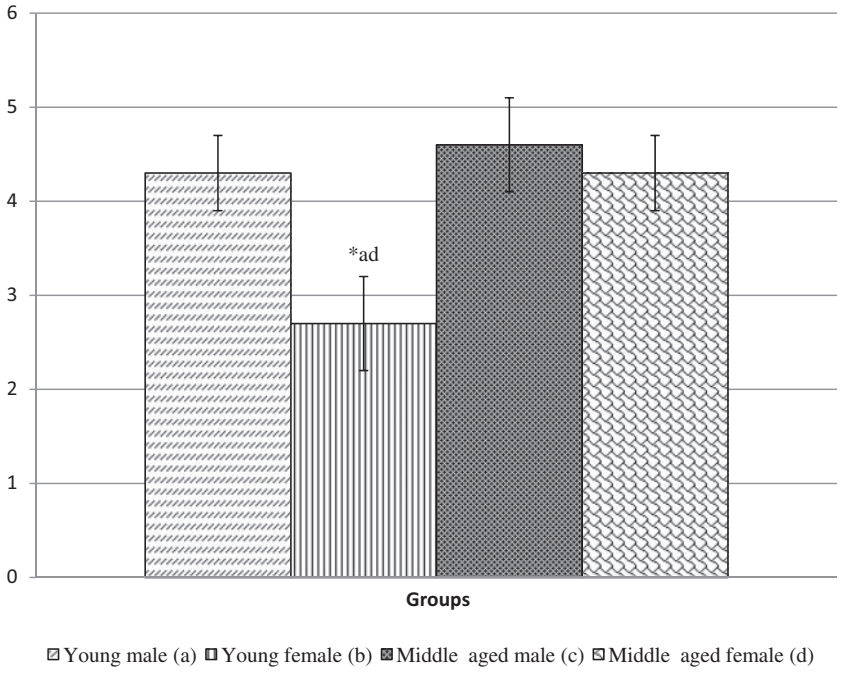

Figure 3 Score of arrhythmia. $^{a,d}P < 0.05$

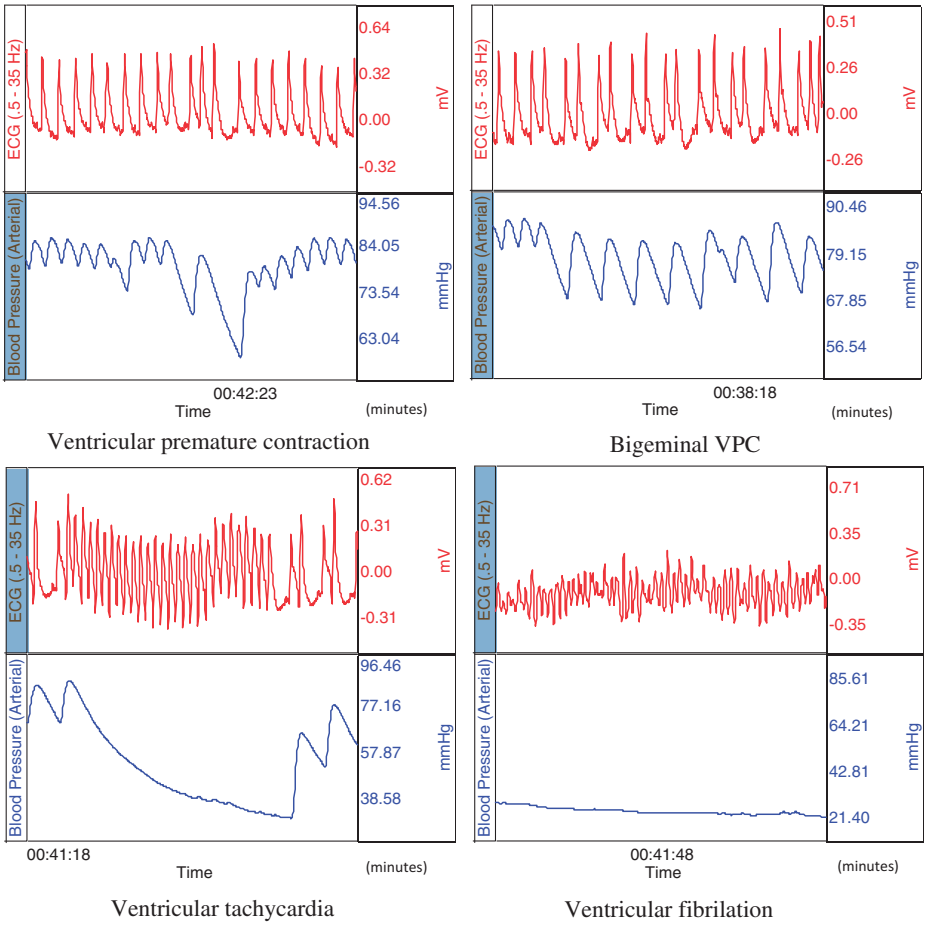

Figure 4 Ventricular arrhythmia recorded during 30 min of ligation in glybenclamide treated young male rats. (A color version of this figure is available in the online journal)

Table 4 The type and duration of arrhythmias and the arrhythmic period during 30 min of coronary ligation

Group	*n*	VF	VT	Others	Total arrhythmia	Arrhythmic period
Male (7–8 months old)						
Control (a)	8	0 ± 0	1.3 ± 27	100.4 ± 17	161.7 ± 43	15.4 ± 2.2[b]
Glybenclamide	7	1.2 ± 1	9.0 ± 28	158.6 ± 41	188.8 ± 47	21.5 ± 1.6
Pinacidil	8	3.6 ± 3.6	5.2 ± 33	125.3 ± 23	161.2 ± 52	13.3 ± 1.7
Male (12–13 months old)						
Control (b)	5	0 ± 0	8.9 ± 27	117.5 ± 28	146.4 ± 38	17.4 ± 1.9
Glybenclamide	7	4.8 ± 4.8	0 ± 1.0	264.6 ± 202	270.5 ± 208	17.7 ± 3.1
Pinacidil	7	19.4 ± 11.2*	3.0 ± 23	189.8 ± 52	262.2 ± 63	16.0 ± 1.5
Female (7–8 months old)						
Control (c)	7	0 ± 0	1.3 ± 11	43.3 ± 14	54.6 ± 19	6.8 ± 1.6[a,d]
Glybenclamide	8	5.9 ± 5.9	9.9 ± 15	88.6 ± 31	114.4 ± 43	14.5 ± 4.1*
Pinacidil	5	5.0 ± 4	9.2 ± 28	60.6 ± 32	104.9 ± 61	9.7 ± 3.1
Female (12–13 months old)						
Control(d)	7	2.4 ± 1.7[c]	7.2 ± 23	83.0 ± 16	132.7 ± 36	11.5 ± 2[b]
Glybenclamide	7	0.3 ± 0.3	4.5 ± 10	94.5 ± 34	109.3 ± 33	21.1 ± 2.1*
Pinacidil	7	6.4 ± 4.0	1.7 ± 28	117.7 ± 19	175.4 ± 38	16.6 ± 2.2

*$P < 0.05$; different from control of each group.
[a]$P < 0.05$; different from male (7–8 months old) control.
[b]$P < 0.05$; different from male (12–13 months old) control.
[c]$P < 0.05$; different from female (7–8 months old) control.
[d]$P < 0.05$; different from female (12–13 months old) control. All data were expressed as mean ± standard error. VF: ventricular fibrillation; VT: ventricular tachycardia; others: ventricular premature contractions, salvo and bigemini were given in seconds. Arrhythmic period was given in minutes. All data were expressed as mean ± standard error.

Table 5 The infarct area and blood glucose level in rats

Group	*n*	Body weight	*n*	Risk of infarct zone %	*n*	Infarct zone %	*n*	Glucose
Male 7–8 months old								
Control (a)	10	283 ± 8	8	48 ± 1	8	21 ± 1	8	168 ± 4
Glybenclamide	7	275 ± 7	7	46 ± 2	7	21 ± 1	7	136 ± 6*
Pinacidil	8	275 ± 4	8	52 ± 2	8	22 ± 1	8	130 ± 9*
Male 12–13 months old								
Control (b)	7	305 ± 10	7	52 ± 2	7	23 ± 3	7	163 ± 3
Glybenclamide	7	297 ± 13	7	47 ± 3	7	22 ± 2	7	133 ± 7*
Pinacidil	7	306 ± 13	7	49 ± 1	7	21 ± 3	7	156 ± 8
Female 7–8 months old								
Control (c)	7	201 ± 6	7	49 ± 2	7	21 ± 1	7	163 ± 9
Glybenclamide	8	186 ± 6	8	48 ± 2	8	19 ± 1	8	123 ± 8*
Pinacidil	5	209 ± 6	5	38 ± 4*	5	15 ± 2*	5	137 ± 5*
Female 12–13 months old								
Control (d)	9	216 ± 6	7	48 ± 1	7	22 ± 1	9	156 ± 5
Glybenclamide	7	225 ± 8	7	43 ± 2	7	18 ± 1	7	136 ± 7*
Pinacidil	7	225 ± 14	7	52 ± 2*	7	21 ± 1	7	129 ± 8*

*$P < 0.05$; different from control of each group.

and males of both age groups. Glibenclamide did not decrease or increase the infarct zone in male and female rats of both ages.

Discussion

As shown in our previous studies, the arrhythmia score was found to be lower in young adult female rats compared to the corresponding male rats.[3,4] This difference disappeared in older age. Although the arrhythmia score increased in middle-age female rats, this increase was not seen in male animals. The arrhythmia score did not change depending on the increasing age of male rats.

No comparable study was found in the literature. The decrease in reperfusion-induced arrhythmias in older male rats was only observed in one of the previous studies.[10] In our study, arrhythmias recorded during ischemia were found to increase in older age, especially in females.

Although different results were observed, the result of the above and the current study suggest that the response of the heart to myocardial ischemia and reperfusion changes with increasing age. There are several studies in the literature that show the anti-arrhythmic[11–14] or proarrhythmic[15,16] effects of glibenclamide on reperfusion-induced arrhythmia. Also, there are several studies that show that pinacidil decreases reperfusion-induced arrhythmia[4,17,18] while others show that pinacidil increases these arrhythmias.[19–21] The age of animals was not usually indicated, and male animals were mostly used in these studies. Different results obtained from these studies might be due to the differences in the age and gender of the animals used. The number of ATP-dependent potassium channels was found to be greater in female rats than in male, and this difference was observed to disappear with advancing age.[1] The increasing score of arrhythmia in older aged females observed in our study might be caused by this type of decrease in ATP-dependent potassium channel in females.

The infarct area did not change with increasing age in both genders. Pinacidil decreased infarct size in young adult females, but not in older females and males of both age groups. Glybenclamide was not effective in decreasing the infarct area in animals of both ages and genders. In a previous study, it was indicated that diazoxide, the ATP-dependent potassium channel opener, reduced the infarct area in three-month-old male rats, but not in older ones.[22] Similarly, in our study the opening of ATP-dependent potassium channels using pinacidil decreased the infarct zone in young females, but not in older ones. Pinacidil was not found to be effective in decreasing arrhythmia in young males. The reason behind the different result in this study may be due to the age of the rats used; such that younger male adult rats used in the previous study were three months old, whereas the rats used in this study were eight months old. However, despite the age difference in both studies, the weight of the animals was similar. Schulman *et al*.[23] showed that the protective effect of ischaemic preconditioning on decreasing infarct area disappeared in old rats of 18–20 months old and 670–850 g in weight compared to those of three months old and 300–350 g in weight. In our study, there was no change in the infarct area depending on the increasing age of the animals. Although the above study is related to the protective effect of ischaemic preconditioning, this study suggests that the age of animals is an important determinant in the appearance of the protective effect of ischaemic preconditioning.

In current clinical applications, the dose and the type of drugs used in the treatment of cardiovascular disease are no different in both males and females of different age groups. Nevertheless, it was observed experimentally that structural and functional changes occur in the cellular activities of tissues of gradually increasing age.[1,3,24] Clinically, death following acute myocardial infarction was shown to be more likely in human beings of older age than in young people.[25,26] It was also shown that death due to acute myocardial infarction was encountered more often in males compared to corresponding females before menopause, and was the same after menopause.[27] Drugs such as antihistamines, antibiotics, antimalarials and anti arrhythmic induce the torsades de pointes type of ventricular arrhythmia much more easily in females rather than males.[28] These clinical observations show the importance of the usage of different doses of drugs in treatments. That is why animal research should be performed using animals of different ages and genders.

There was no relation between blood glucose level and arrhythmia. Pinacidil and glybenclamide were expected to have opposite effects on the level of blood glucose; however, in our study, both glybenclamide and pinacidil had the same effect on blood glucose. They produced hypoglycaemia in all groups, with the only exception being that pinacidil did not decrease blood glucose level in middle-aged male rats. We found there was no relation between ventricular arrhythmia and blood glucose level. The blood glucose lowering effect of pinacidil may be derived from an enhanced sensitivity to insulin.[29] The ineffectiveness of pinacidil on blood glucose levels in older male rats may be related to changes in the animals' sensitivity to pinacidil, with changes in its binding site or decreased number of ATP-dependent potassium channels in the myocardial cell membrane.

Conclusion

Arrhythmias occurring following coronary artery ligation in rats were seen more in male rats than in females of a younger age. This discrepancy between male and female rats disappeared in older age. The different effects of glybenclamide and pinacidil on the ischemia-induced arrhythmia were observed in rats of different ages and genders. As a result, it can be suggested that differences in the age and gender of experimental animals are important in determining the effect of ATP-dependent potassium channel modulating drugs on ischemia-induced arrhythmia.

Author contributions: ÖB: project preparation, evaluation of data and writing of the manuscript, STK and SY: operation of animals and data analysis and HSO: project preparation and reviewing the literature.

ACKNOWLEDGEMENT

We thank Abant Izzet Baysal University Research Fund for supporting this study-project number: 2010.03.01.340.

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(Received January 31, 2013, Accepted June 10, 2013)