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EXPERIMENTAL / LABORATORY STUDIES

Mechanisms of Glybenclamide-Mediated Anti-Arrhythmia and Ischemic Conditionining in a Rat Model of Myocardial Infarction: Role of yohimbine Treatment

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Abstract: Glibenclamide (Glyburide), a widely used second-generation sulfonylurea hypoglycemic agent, is a known specific blocker of adenosine triphosphate (ATP) sensitive potassium (K+) channels. Although the blockage of the ATP dependent potassium channel reduces arrhythmia, it increases enfarct size. Yohimbine is an alpha-2 blocker that reduces infarct size as suggested by several studies. We hypothesized that a combination of yohimbine and glibenclamide may be more effective in reducing the arrhythmia following ligation. The arteria descendence (LAD) branch of the left coronary artery in conscious rats was ligated and arrhythmia was recorded during the 15 min following coronary ligation. Sixteen hours after coronary ligation, infarcted myocardium was determined by histological analysis. One and five mg/kg of yohimbine and 5mg/kg of glibenclamide were given intraperitoneally 25 min before ligation. Yohimbine in a 5 mg/kg dosage reduced both the occurrence and the duration of ventricular fibrillation (VF) and ventricular tachycardia. The combination of yohimbine and glibenclamide did not exhibit any additive or synergistic effects on the antiarrhythmic effect of each drug alone following coronary ligation. Moreover, yohimbine in 5mg/kg doses and a combination of 1 mg/kg of yohimbine and glibenclamide increased the survival rate after coronary ligation compared to the non-treated group. The combination of glybenclamide with yohimbine had no clear additive effects such as reducing arrhythmia following cooronary ligation. The antiarrhythmia produced by glibenclamide may not only depend on the blockage of the ATP dependent potassium channels, but also on adrenergic preconditioning.

Key Words: ATP sensitive potassium channels, Hypoglycemic sulfonylurea compounds, Myocardial ischemia, Arrhythmia, Yohimbine

Introduction

The modulation of adenosine triphosphate (ATP) dependent potassium channels plays an important role in the suppression of arrhythmias following coronary ligation. Although there are several studies indicating that blockage of the channel reduces the propensity to arrhythmias (1-6), others maintain that it is proarrhythmic (7-9). One of the deleterious effects of glibenclamide is on infarct size. It increases infarct size as well as reducing arrhythmia. The increased infarct size induced by glibenclamide may be due to increased calcium overload and contraction. In our previous study, we used the beta adrenergic selective blocker metoprolol to reduce the ischemic effect of glibenclamide (10). In that study metoprolol in combination with glibenclamide was very effective in reducing the incidence of lethal arrhythmia. Metoprolol is a cardioselective beta adrenergic blocker and reduces heart rate and contraction. On the other hand, yohimbine is an alpha-2 adrenergic blocker and increases heart rate. Although these 2 drugs have an adverse effect on heart rate, it has suggested by several studies that yohimbine reduces infarct size and the intensity of ventricular fibrillation following coronary ligation (11-14). Since yohimbine reduces infarct size, the combination of glibenclamide with yohimbine may be effective in reducing ischemia induced arrhythmia.

In the present study, our aim was to research the effect of yohimbine, alone or in combination with glibenclamide on arrhythmia and survival rate following

acute coronary ligation. No studies investigating the effect of glibenclamide in combination with an alpha-2 adrenergic blocker on arrhythmia following coronary ligation have been found.

Materials and Methods

Fifty four male Sprague-Dawley rats weighing 232-330 g were used. The animals were fed on commercial rat food pellets and were allowed to drink tap water ad libitum. All the animals were treated in accordance with the guiding principles in the care and handling of animals as recommended by of Helsinki Declaration.

In preliminary surgery, a loose loop of atraumatic silk was placed around the left main coronary artery about 2 mm from its origin under ether anesthesia. The silk was passed through polyethylene tubing and the chest was closed, with the tubing remaining inside the thoracic cavity (15). The animals recovered immediately following the operation. Sometimes, however, artificial respiration (60 strokes/min, Ugo Basile Italy) with room air was performed through the mouth as animals were in deep anesthesia after the operation, and this was maintained until spontaneous respiration resumed. After 6-7 days of coronary artery ligation, the 2 ends of the loose silk loop were freed through a small incision in the skin, and electrodes were placed on both sides of the chest wall under ether anesthesia. The animals were given approximately 2 h to recover from the ether anesthesia and to adapt to their new conditions. The electrodes were connected to a recording system (Power Lab/4s, UK), and the silk ligature was tightened to produce coronary artery ligation in freely moving, conscious animals.

A bipolar ECG recording was made during the first 15 min after coronary ligation and stored on computer for later analysis. Approximately 16 h following the ligation, the animals were anesthetized with pentothal (60 mg/kg i.p.) and heparinized through the vena saphena. The heart was excised and washed in isotonic solution. The atria of the heart were removed and only the ventricles were left. The ventricles were sliced into 1 mm thick transversal sections and were stained in 0.1% nitroblue-tetrazolium dye. The wet weight of the infarcted, unstained myocardium was measured and expressed as a percentage of the total weight of the ventricles.

Heart rate was calculated from the ECG recordings for each animal before and after ligation at regular intervals. The incidence and duration of arrhythmia observed during the 15 min following coronary ligation were measured. The arrhythmias were identified as ventricular fibrillation (VF), ventricular tachycardia (VT), and other types of arrhythmia including single VEBs, bigeminia and salvos according to the Lambeth conventions (16). An arrhythmia score was given according to the incidence and duration of arhythmias for each animal as follows: 0 = no arrhythmia; 1 = < 10 s VT or other arrhythmia, no VF; 2 = 11-30 s VT or other arrhythmia, no VF; 3 = 31-90 s VT or other arrhythmia, no VF; 4 = 91-180 s VT or other arrhythmia and/or < 10 s reversible VF; 5 = > 180 s VT or other arrhythmia and/or > 10 s reversible VF; 6 = irreversible VF.

Drug treatment: glibenclamide (Sigma) was dissolved in a 1:1 mixture of dimethylsulfoxide and ethanol and was applied i.p. at a dose of 5 mg/kg and in a volume of 100 ul/kg. Yohimbine (Sigma) was dissolved in isotonic saline solution and given i.p. at a dose of 1-5 mg/kg and in a volume of 1 ml/kg. The control animals were given 1 ml/kg of isotonic saline solution and 0.1 ml/kg of the solvent for glibenclamide. All injections were performed 25 min before coronary ligation.

Determination of blood glucose concentrations: a single drop of blood was taken to determine blood glucose by cutting the tip of the tail. Blood glucose was measured by a strip test (Glucotrend 2, Roche Group, UK) before coronary ligation in 5 animals in each group to assess hypoglycemia.

Statistical analysis: the survival rate and incidence of arrhythmias were compared by chi-square test. The other parameters were expressed as mean ± SE, and after analyses of variance, one way ANOVA (post hoc test, LSD), were compared by Student's t-test (unpaired-2-tailed).

Results

ST segment elevation was observed immediately following ligation. Animals with unsuccessful ligation (23 animals), did not exhibit ST segment elevation or QRS changes and pressure decline after ligation, and no infarct was determined after 16 h. Forteen of the animals died from bleeding or respiratory failure at the end of the preliminary operation. These animals were excluded from the study.

Glibenclamide pretreatment lowered the heart rate before coronary ligation (Table 1). Heart rate increased following ligation in all groups, except in animals given 5 mg/kg of yohimbine alone and in combination with glibenclamide. The heart rates in the following minutes were not different from those of the control.

Coronary artery ligation in conscious rats resulted in severe arrhythmias that usually appeared between the 3rd and 5th mins and reached maximal severity at the 6th min of coronary ligation. If the animals survived, these arrhythmias disappeared and sinusal rhythm resumed after 12 min of coronary ligation. No differences were found in the incidence of arrhythmia among the groups. However the incidence of ventricular fibrillation was nonsignificantly lowest in the group treated with 5mg of yohimbine. Heart rate following ligation was also lowest in this group.

Although glibenclamide alone did not influence the incidence of arrhythmia, the survival rate at the end of 16 h of coronary ligation was significantly higher after combining glibenclamide with yohimbine (1 mg/kg) (Table 2). In combination with the higher dose of yohimbine, glibenclamide nonsignificantly increased the survival rate. Yohimbine alone in a 5 mg/kg dose increased the survival rate and nonsignificantly reduced total arrhythmic periods, but not in the smaller dose. The arrhythmia score was also significantly lower in this group ($P < 0.05$) (Table 3).

The duration of ventricular fibrillation during the 15 min following coronary ligation was shortened

Table 1. Heart rate before and following coronary ligation (Mean± Standard error).

Grups	Heart rate / min						
	Before ligation	1 min	3 min	5 min	8 min	12 min	15 min
Control	432 ± 16	479 ± 15	439 ± 21	434 ± 37	464 ± 27	462 ± 17	459 ± 14
Glibenclamide 5 mg/kg	354 ± 15*	435 ± 18	421 ± 19	400 ± 10	425 ± 23	388 ± 13	392 ± 15
Yohimbine 1 mg/kg	458 ± 18	504 ± 10	478 ± 13	471 ± 5	457 ± 22	403 ± 21	382 ± 18
Yohimbine 5 mg/kg	487 ± 10*	453 ± 11	416 ± 14	422 ± 16	415 ± 11	410 ± 6	394 ± 12
Glibenclamide 5 mg/kg + Yohimbine 1 mg/kg	453 ± 11	496 ± 7	490 ± 7	493 ± 10	445 ± 19	445 ± 19	454 ± 24
Glibenclamid 5 mg/kg + Yohimbine 5 mg/kg	450 ± 17	419 ± 33*	423 ± 25	467 ± 25	419 ± 15	419 ± 15	427 ± 31

*$P < 0.05$ N = Number of animals

Table 2 The survival rate and incedence of arrhythmia following coronary ligation.

	Dose, mg/kg	n	Surviving 15 min n/%	Surviving 16 h n/%	Incidence of arrhythmia (n /%)				
					None	VF	VT	Others	Bradycar.
Control		14	8/57	4/28	0	9/64	11/79	14/100	1/7
Glibenclamide 5 mg/kg	5	10	8/80	7/70	0	5/50	10/100	10/100	1/10
Yohimbine 1mg/kg	1	9	5/55	4/44	1/11	5/55	7/78	8/89	2/22
Yohimbine, 5 mg/kg	5	7	6/86	6/86*	1/14	2/29	3/42	6/86	1/13
Glibenclamide 5 mg/kg + Yohimbine 1 mg/kg	5+1	7	6/86	6/86*	0	4/57	7/100	7/100	1/14
Glibenclamide 5 mg/kg + Yohimbine 5 mg/kg	5+5	7	6/86	5/71	0	4/57	6/86	7/100	2/29

Significantly different (*$P < 0.05$) from control

Table 3. The duration of total arrhythmias observed during 15 minutes following coronary ligation and arrythmia score. Mean±Standart error

	N	Duration of arrhythmias (seconds)				Arrythmia score
		VF	VT	Others	Total	
Control	14	267 ± 96	60 ± 40	65 ± 47	393 ± 95	4.5 ± 0.4
Glibenclamide 5 mg/kg	10	79 ± 25	48 ± 25	62 ± 16	314 ± 113	3.8 ± 0.4
Yohimbine 1 mg/kg	9	232 ± 112	84 ± 56	14 ± 4	331 ± 111	4.4 ± 0.7
Yohimbine 5 mg/kg	7	114 ± 93	26 ± 9	11 ± 3	145 ± 102f	2.5 ±0.8*
Glibenclamide 5 mg/kg + Yohimbine 1 mg/kg	7	94 ± 91	54 ± 18	42 ± 6	190 ± 79	4.1 ±0.8
Glibenclamide 5 mg/kg + Yohimbine 5 mg/kg	7	27 ± 13j	80 ± 24	84 ± 25	191 ± 47	4.4 ± 0.9

*P < 0.05
jP = 0.07,fP = 0.08 compared with the corresponding control value

nonsignificantly when a combination of 5 mg/kg of glibenclamide and 5 mg/kg of yohimbine was given (P < 0.05) (Table 3), but was not different in the animals that survived (Table 4). Glibenclamide alone and in combination with 5 mg of yohimbine nonsignificantly reduced the duration of ventricular fibrillation, but was not so effective in lowering arrhythmia when combined with a 1 mg/kg dose of yohimbine. There were no synergic effects on arrhythmia between yohimbine and glibenclamide, since there was no difference between the glibenclamide and glibenclamide plus yohimbine combinations in the arrhythmic period and the length of individual arrhythmia appeared following ligation. Yohimbine alone, in a 5 mg dose, lowered the duration of ventricular fibrillation and the arrhythmia score. This level of yohimbine was not effective in reducing the arrhythmia score in combination with glibenclamide.

Infarct size decreased only in rats treated with 5 mg of yohimbine compared to the control in surviving animals 16 h following coronary ligation (Table 3). This was associated with the less severe arrhythmia observed in this group. Glibenclamide alone or in combination with yohimbine had no effect on the infarct size.

Blood glucose concentrations were lowered by glibenclamide. Yohimbine alone did not change plasma glucose levels although it potentiated the hypoglycemic effect of glibenclamide, (Table 5). There was no correlation between increased heart rate and low blood glucose.

Discussion

The results demonstrate that yohimbine pretreatment alone significantly (P < 0.05) reduced the duration of arrhythmias (12-14). The findings that the basal heart rate decreased in the glibenclamide group in respect of the control group contradicts the results of El-Reyani et al. (6), who suggested that the basal heart rate increased in anesthetized rats treated with the same dose of glibenclamide alone. This discrepancy may be due to the animal model used in the latter study. Hypoglycemia-induced adrenergic activation may cause reflex bradycardia following tachycardia in these groups. However, although hypoglycemia was also observed in the combination group, similarly to the glibenclamide treated animals, heart rate did not decrease. This may underline another mechanism that caused the lowered heart rate in the glibenclamide group apart from hypoglycemia. The sympathetic nerve endings in the atria have been shown to contain ATP sensitive potassium channels, in which glibenclamide (a blocker of ATP sensitive potassium channels) and pinacidil (an opener of ATP sensitive potassium channels) increased the resting release of norepinephrine (17). This result may indicate that the lowering of the heart rate in the glibenclamide treated group in our study is caused by the modulation of norepinephrine release from atrial sympathetic nerve endings. Yohimbine, either alone in a higher dose or in combination with glibenclamide, may enhance the release of norepinephrine from sympathetic nerve endings before coronary ligation. This may be a reason for the higher

Table 4. The duration of arrhythmias observed at the end of 15 min and infarct size at 16 h following coronary ligation in surviving animals. Mean±Standart error

	N	Duration of arrhythmias (seconds)				N	Infarct size
		VF	VT	Others	Total		
Glibenclamide 5 mg/kg (b)	8	3 ±1	51 ± 32	62 ±17	193 ± 102	7	26 ± 7
Yohimbine 1 mg/kg (c)	5	10 ± 6	25 ± 20	21 ±6	57 ± 24	4	30 ± 4
Yohimbine 5 mg/kg (d)	6	21 ± 21	16 ± 10	8 ±6	46 ± 34	6	13 ± 8*
Glibenclamide 5 mg/kg + Yohimbine 1 mg/kg (e)	6	2 ± 0,8	63 ± 19	48 ±8	113 ± 24	6	26 ± 7
Glibenclamide 5 mg/kg + Yohimbine 5 mg/kg (f)	6	15 ± 8	65 ± 22	79 ±19	159 ± 41	5	33 ± 6

*P = 0.06

Table 5. Blood glucose levels after 25 min of drug administration.

Group	Dose (mg/kg)	Glucose, mg/100 ml blood
Control		93 ± 5.3
Glibenclamide	5	75 ± 2.5***
Yohimbine	1	102 ± 4.2
Yohimbine	5	102 ± 2.9
Glibenclamide+Yohimbine	5 + 1	61 ± 4.6***
Glibenclamide+Yohimbine	5 + 5	48 ± 4***

Results are mean ± S.E. of 5 animals in each group
***P < 0.001 compared to the corresponding control value

heart rate observed before ligation in these groups compared to the glibenclamide treated animals. A lower dose of yohimbine was not effective against arrhythmias (18). This may be caused by less norepinephrine release.

We did not find any comparable studies in which the combined effect of glibenclamide and yohimbine on arrhythmias following coronary ligation had been investigated. Metoprolol, a cardioselective β-blocker when used in combination with glibenclamide, was protective against the development of life-threatining arrhythmias during the acute phase of experimental myocardial infarction in conscious rats in one study (10). This result indicates that metoprolol lowers myocardial contractility and increases coronary vasodilatation and so reduces the ischemic effect of glibenclamide. In contrast to metoprolol, yohimbine alone reduced the duration of arrhythmias. The opposite effect of yohimbine may be attributed, rather than to the lowered heart rate, to myocardial catecholamine release from adrenergic nerve endings (12). In combination with glibenclamide, it was not as effective on the severity of arrhythmias as glibenclamide in combination with metoprolol. However the survival rate following 16 h of infarction increased in the combination with 1 mg/kg of yohimbine, although it did not reduce the incidence or duration of ventricular fibrillation. On the other hand, the combination of a higher dose of glibenclamide reduced ventricular fibrillation nonsignificantly, but did not affect the survival rate 16 h following ligation. The underlying mechanism in the higher survival rate in the 1 mg/kg yohimbine combination cannot be identified in the present study. It can however, be said that the higher dose of yohimbine may cause greater catecholamine release and potentiate the ischemic effect of glibenclamide.

The suggestion that catecholamine release due to hypoglycemia induced by glybenclamide before coronary ligation may protect against arrhythmia was supported by the results of this study (19). Since in combination hypoglycemia and yohimbine can cause catecholamine release and may precondition the heart. This explanation is likely to account for the visible decreased survival rate and lethal arrhythmia in the combination group with 1 mg/kg of yohimbine. The greater catecholamine release may adversely influence these effects and cause more arrhythmia and a lowered survival rate.

The results of the present study can also be compared with our previous research that used the same animal model (18). In this study, the lower arrhythmia score in

the group given a higher dose of yohimbine was similar to the effect of epinephrine on the arrhythmias pretreated before coronary ligation. Low doses of yohimbine and epinephrine in both studies had no effect on arrhythmias. This means that the lower dose of yohimbine did not cause enough catecholamine release; therefore, it did not induce adrenergic preconditioning. Similar protection against arrhythmia during coronary artery occlusion was also mentioned in a recent study (20).

Conclusion

The combination of glibenclamide with yohimbine is not particularly protective against arrhythmias. Yohimbine by itself reduces the infarct size and arrhythmia score, but not with glibenclamide. Either glibenclamide or yohimbine induced synergistic catecholamine release may provide adrenergic preconditioning before coronary ligation and may increase survival rates and reduce the arrhythmic period.

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