Turk J Biol
28 (2004) 39-46

The Effect of Sex on Ischemia - Reperfusion Induced Arrhythmias and the Role of ATP - Dependent Potassium Channel Blockage

Ersöz GONCA, Eylem SUVEREN TİRYAKİ, Ömer BOZDOĞAN
Department of Biology, Faculty of Art and Science, Abant İzzet Baysal University, 14280 Gölköy, Bolu - TURKEY

Received: 01.07.2003

Abstract: The presence of more ATP-dependent potassium channels in females has been thought to play an important role in protection against myocardial ischemia. In this study, we investigated whether there is a difference in the severity of ischemia and reperfusion induced arrhythmias due to sex and, if there is, what the role of ATP-dependent potassium channel in this difference is. The left anterior descending coronary artery in rats was ligated and arrhythmias were recorded in ECG during 6 min. of ischemia and reperfusion. The duration of arrhythmias was shorter in females than in corresponding males. Glibenclamide was effective in decreasing the duration of arrhythmias in males but not in females. This may be due to the presence of more ATP-dependent potassium channels in females.

Key Words: Coronary Ligation, Ischemia-Reperfusion, Arrhythmias, Sex, Glibenclamide

Cinsiyetin Miyokardiyal İskemi - Reperfüzyon ile Uyarılan Aritmiler Üzerine Etkisi ve ATP - Bağımlı Potasyum Kanal Blokajının Rolü

Özet: ATP bağımlı potasyum kanal sayısının dişilerde fazla oluşunun, miyokardiyal iskemiye karşı korumada önemli bir rol oynadığı düşünülmektedir. Bu çalışmada, dişilerde iskemi ve reperfüzyon sonrası oluşan aritmi şiddetinde cinsiyete bağlı olarak farklılık olup olmadığı ve varsa bu farklılıkta ATP bağımlı potasyum kanallarının rolü araştırıldı. Sıçanlarda koroner ligasyon sol koroner arter bağlanarak yapıldı ve altı dakikalık iskemi ve reperfüzyon periyotları boyunca EKG kaydedildi. Reperfüzyon boyunca aritmi süreleri dişi cinsiyette erkek cinsiyete göre daha az bulundu. Glibenklamid aritmi sürelerini azaltmada erkeklerde etkili ancak dişilerde değildi. Bu sonuçlara göre, dişilerde ATP bağımlı potasyum kanallarının sayıca fazla olması aritmilere karşı koruyucu olabilir.

Anahtar Sözcükler: Koroner Ligasyon, İskemi-Reperfüzyon, Aritmiler, Cinsiyet, Glibenklamid

Introduction

Glibenclamide is a sulfonylurea compound that blocks the ATP-dependent potassium channel in ischemic cells. Since extracellular potassium accumulation leads to the nonuniform shortening of the action potential duration of myocardial cells and contributes to the generation of malignant arrhythmias, glibenclamide is expected to protect against these arrhythmias. Likewise, several investigations have suggested that the blockage of these channels by glibenclamide, which selectively blocks the ATP-dependent potassium channel, decreases the incidence and duration of ventricular arrhythmias in males (1,2) in vitro and (3-6) in vivo. Although these findings indicate that glibenclamide is an antiarrhythmic agent, it should be noted that some investigators have described ATP-dependent potassium channel blockers as proarrhythmic during the acute phase of myocardial infarction (7,8).

Sex differences in susceptibility to cardiac disease have been described extensively in the literature. The rate of coronary heart disease in women before menopause is much lower than that in men of the same age (9). The major mechanism responsible for this protection is thought to be the antiatherogenic action of female sex hormones (10). Besides the chronic effect of estrogen on lipid profiles, it has been shown that 17-β estradiol reduces myocardial infarct size (11) and the incidence of ischemia and reperfusion induced arrhythmias, (12,13) and decreases myocardial damage and preserves left ventricular function (14,15). One possible mechanism of the cardioprotective effect of estrogen is suggested to be enhanced nitric oxide (NO) production.

In recent years, it has been shown that the density of the sarcolemmal ATP- dependent potassium channel was higher in females than in males (16). In accordance with studies suggesting that the activation of an ATP-

dependent K^+ channel is an important part of the endogenenous cardioprotective effect that promotes cellular survival under metabolic stress conditions (17), it was reported in this in vitro study that the presence of more cardiac ATP-dependent K^+ channels contribute to higher resistance against ischemia and reperfusion.

The main objectives of our study were to establish whether there is a sex difference in ischemia and reperfusion arrhythmias and to clarify the effect of ATP-dependent K^+ channel modulation by a channel blocker, glibenclamide.

Materials and Methods

Animals

Male and female Sprague-Dawley rats weighing 220-300 g and 7-8 months old were used. The animals were fed commercial rat food pellets and allowed to drink tap water ad libitum. All guiding principles declared by Hacettepe University Ethics Committee, Turkey, for animal care and use were followed.

Ischemia and reperfusion in anesthetized rats

The animals were anesthetized with thiopenthal sodium (60 mg/kg). The trachea was cannulated for artificial respiration. The left carotid artery was cannulated for measuring the blood pressure transducer (PowerLab, AD Instruments, United Kingdom) . The chest was opened in the fourth intercostal space and the heart was exposed (a loose loop of atraumatic silk was placed around the left main coronary artery approximately 2 mm from its origin). Both ends of the ligature were led out of the thoracic cavity through a flexible tubing. The heart was replaced and artificial respiration was started using an animal respirator (Ugo Basile Rodent Ventilator, Italy) at 60 strokes/min. The animals were allowed to stabilize for 5 min; then the loose loop of the coronary artery ligature was tightened and fixed by a clamp on the silk and thus regional myocardial ischemia was produced. The 6 min of ischemia was followed by 6 min of reperfusion produced by loosening the clamping on the silk.

At the end of the experiment, in the surviving rats, the heart was excised and perfused through the aorta with solutions: first with 10 ml of NaCl and then with 2 ml of ethanol for demarcation of the occluded and nonoccluded myocardial regions. The nonperfused area that remained red was separated from the well-perfused area that seemed white . The nonperfused myocardium was cut with scissors and weighed. The percentage of this area to the total weight of the ventricle was calculated. Bipolar ECG and arterial blood pressure was recorded during the 6 min of coronary ligation and reperfusion and stored on computer for later analysis.

Drug application

Glibenclamide was first dissolved in dimethylsulfoxide and then mixed with ethanol in a 1/1 ratio. It was intraperitoneally given in 5 mg /kg /100 µl, and 25 min before coronary artery ligation. In the control groups, instead of the glibenclamide, the same value of solvent was applied.

Statistical analyses

The mean and standard errors were determined for all parameters including the duration of arrhythmias, blood pressure and heart rate. The duration of arrhythmias, blood pressure and heart rate were compared by analyses of variance (multivariate ANOVA combined with the LSD post hoc test). The survival rate and incidence of arrhythmias were compared by the chi-square test (Fisher's exact test, 2 tailed).

Results

Changes in blood pressure and heart rate during ischemia and reperfusion

Coronary ligation was not performed in rats having decreased blood pressure below 70 mmHg before ligation. In ECG, ST segment elevation or QRS changes were observed in all animals following ligation. These changes were not seen in unsuccessful ligation. Blood pressure did not vary before coronary ligation but decreased ($P < 0.05$) immediately after ligation in all groups of animals. Blood pressure in the female control group was significantly high with respect to that in the male control group during coronary ligation (Table 1).

Following reperfusion, blood pressure gradually increased. In female control group, blood pressure was nonsignificantly higher than that in the corresponding male group during reperfusion. However, blood pressure after 3 and 5 min of reperfusion was significantly high in female groups compared with that in the male group treated with glibenclamide ($P < 0.05$), (Table 2).

Table 1. Heart rate and mean arterial pressure during 6 min of coronary artery ligation in anaesthetized rats.

Group	n	Heart Rate (beats/min)				Blood Pressure (mmHg)			
		Basal	1 min	3 min	5 min	Basal	1 min	3 min	5 min
Male Control	9	336 ± 11	326 ± 15	314 ± 21	317 ± 16	179 ± 7	110 ± 13	113 ± 15	125 ± 11
Male Glibenclamide	10	338 ± 20	333 ± 22	333 ± 33	331 ± 19	185 ± 10	123 ± 15	125 ± 17	131 ± 14
Female Control	9	336 ± 10	372 ± 12	365 ± 13	360 ± 14	187 ±13	149 ± 8*	150 ± 10*	151 ± 12*
Female Glibenclamide	8	352 ± 27	363 ± 25	362 ± 13	352 ± 30	180 ± 10	141 ± 13	148 ± 16	130 ± 15

Data are mean ± SE. * $P < 0.05$, compared to corresponding male control value.
n: number of animals. Basal: Before coronary artery ligation and 1,3 and 5 min after coronary ligation.

Table 2. Heart rate and mean arterial pressure during 6 min of reperfusion.

Group	n	Heart Rate (beats/min)			Blood Pressure (mmHg)		
		1 min	3 min	5 min	1 min	3 min	5 min
Male Control	8	307 ± 21	321 ± 21	288 ± 23	121 ± 13	127 ± 11	142 ± 17
Male Glibenclamide	10	302 ± 10	323 ± 22	310 ± 21	108 ± 9	118±15	115 ± 15
Female Control	9	373 ± 28	348 ± 17	367 ± 20*b	144 ± 20	169 ± 10*c	161 ± 10*c
Female Glibenclamide	8	389 ± 36*a	325 ± 26	340 ± 24	93 ± 10	146 ± 16*c	158 ±12*c

Data are mean ± SE.
* $P < 0.05$; a: compared to male glibenclamide, b: compared to corresponding male control, c: compared to male glibenclamide
n: number of animals. 1, 3 and 5 min after reperfusion.

Heart rate was significantly higher in the female glibenclamide group than that in the male glibenclamide group after 1 min and also higher in the female control group than in the male control group after 5 min of reperfusion ($P < 0.05$), (Table 2).

Arrhythmias during ischemia and reperfusion in anesthetized rats

The amount of myocardium supplied by the occluded coronary artery did not differ among the groups; the percentage of ischemic area to total ventricular weight was 47 (n = 36). During the first 6 min after coronary artery ligation, arrhythmias rarely occurred, and only one of the male control animals died from ventricular fibrillation (VF) before reperfusion (Table 4).

Arrhythmias usually appeared after 5-15 sec following reperfusion. However they occured later in male glibenclamide group compared to the control values and the female glibenclamide group ($P < 0.05$). The length of arrhythmic periods during reperfusion was nonsignificantly lower in the female control group than that in the corresponding male control group (Table 3).

The incidence of arrhythmias differed among the groups. The incidence of VF was significantly lower in glibenclamide treated males than that in the controls ($P < 0.05$). The arrhythmia score was significantly lower in glibenclamide treated males compared with the male control group. Furthermore, the arrhythmia score in the female control group was significantly lower than that in the corresponding male control group ($P < 0.05$), (Table 4).

Table 3. Duration of arrhythmias during reperfusion after 6 min of coronary ligation in surviving rats.

Group	n	Appearance of arrhythmias (min)	Duration of arrhythmias (min)	Duration of Arrhythmia Attack (s)				
				VF	VT	Other	Total	Bradycardia
Male Control	8	0.12 ± 0.02	4.40 ± 0.66	50 ± 24	53 ± 19	52 ± 16	155 ± 38	0
Male Glibenclamide	10	0.67 ± 0.25*a	4.67 ± 0.5	1 ± 1*a	10 ± 5	47±10	57 ± 8*a	0
Female Control	9	0.13 ± 0.05	2.77 ± 0.55 Ψc	3 ± 2*a	44 ± 23	26 ± 6	72 ± 25*a	29 ± 29
Female Glibenclamide	8	0.16 ± 0.07*b	4.16 ± 0.60	3 ± 2*a	63 ± 28	35 ± 6	101 ± 32	0

Data are mean ± SE.
*P < 0.05; a: compared to male control, b: compared to male glibenclamide. ΨP= 0.058; c: compared to male control.
Total= ventricular fibrillation (VF) + ventricular tachycardia (VT) + other types of arrhythmias including ventricular premature contraction (VPC), ventricular gemini, AV nodal arrhythmia and salvo.
n: number of animals at the beginning of reperfusion.

Table 4. The incidence of arrhythmias during reperfusion after 6 min of coronary ligation in anesthetized rats.

Group	n	Incidence of arrhythmia					Arrhythmia score
		Survival	VF	VT	Other	Bradycardia	
Male Control	8	8 / (88 %)	6 / (66%)	8 / (88%)	9 / (100%)	0	4.3 ± 0.4
Male Glibenclamide	10	10 / (100%)	1 / (10 %)*	4 / (40%)Ψ	10 / (100%)	1 / (10%)	3 ± 0.2*
Female Control	9	9 / (100%)	2 / (22%)	9 / (100%)	9 / (100%)	0	3 ± 0.4*
Female Glibenclamide	8	8 / (100%)	2 / (25%)	7 / (87%)	8 / (100%)	0	3.5 ± 0.2

Data are mean ± SE. *P < 0.05, Ψ P = 0.057: compared with male control.
VF: venricular fibrillation, VT: ventricular tachycardia, Other: arrhythmias include ventricular premature contraction (VPC), ventricular gemini, AV nodal arrhythmia and salvo.
n: number of animals at the beginning of reperfusion.

Total arrhythmia was higher in females treated with glibenclamide during coronary artery occlusion compared to female control values (P < 0.05), (Table 5).

The duration of VF and total arrhythmia during the reperfusion period was lower in the female control group than that in the corresponding male group (P < 0.05). In addition, the duration of VF and total arrhythmia was significantly lower in the male group treated with glibenclamide compared to the male control group. Glibenclamide was effective in decreasing the duration of arrhythmia in males but not in females. In contrast, it nonsignificantly increased the duration of total arrhythmias in females (Table 3).

Discussion

Our present results clearly indicate that females have greater resistance to reperfusion induced arrhythmias. These findings are in agreement with previous reports by Siegmund et al. (18). As in this study, the incidence of VF and total ventricular arrhythmias decreased in female rats with respect to males. This protective effect appears to be mediated by estrogen. Previous studies demonstrated that exogenous estrogen significantly attenuates ischemia and reperfusion induced arrhythmias (11,13) and reduces infarct size (14,15). One potential mechanism of the cardioprotective effect of estrogen may be through enhanced NO production. Since estrogen stimulates NO

Table 5. Duration of arrhythmias during 6 min of coronary ligation in surviving rats.

Group	n	Duration of Arrhythmia Attack(s)				
		VF	VT	Other	Total	Bradycardia
Male Control	9	0	0	3 ± 1	3 ± 1	0
Male Glibenclamide	10	0	0	7 ± 2	7 ± 2	0
Female Control	9	0	0	2 ± 1	3 ± 1	0
Female Glibenclamide	8	1 ± 1	2 ± 1	7 ± 2	10 ± 2 *	0

*P < 0.05: compared with female control values.
VF: venricular fibrillation, VT: ventricular tachycardia, Other: arrhythmias include ventricular premature contraction (VPC), ventricular gemini, AV nodal arrhythmia and salvo.
n: number of animals at beginning of ligation.

production, females have a greater capacity to produce NO than males (18). In another in vivo study, it was proved that augmentation of endogenous NO release induced by 17-β estradiol contributes to the alleviation of irreversible ischemia reperfusion injury and reduces infarct size (11). The cardioprotective effect of estrogen has also been shown in males and this suggested that the protection may depend on NO release from the coronary endothelium, which increases coronary blood flow (13). All of these studies suggest a protective role of endogenous NO release in females during ischemia and reperfusion. The low arrhythmia observed in females in the present study might depend on this kind of protective mechanism.

The presence of more cardiac ATP-dependent K^+ channels in females is another contribution to the higher resistance of females to ischemia. Activation of this channel is known as an endogenous cardioprotective mechanism that attenuates ischemia (17). After ischemia, the opening of the ATP-dependent K^+ channel shortens the myocardial action potential during repolarization. As a result, the voltage dependent Ca^{++} influx into the myocardial cell decreases along with ischemia. Since the density of the ATP-dependent K^+ channel is higher in females, this protective mechanism works more effectively in females than in male (16).

The data presented here support previous reports that indicate the antiarrhythmic effect of glibenclamide in males (1,3,6,9). As in these studies, the incidence of VF and total ventricular arrhythmias was significantly decreased by glibenclamide in male rats.

Several antiarrhythmic mechanisms for blockers of the ATP-dependent K^+ channel have been suggested. Blockers inhibit K^+ loss from jeopardized cells and prevent nonuniform shortening of the action potential duration during progressive myocardial ischemia (19). As in previous in vitro (1) and in vivo studies (5), glibenclamide decreases the development of electrical inhomogeneity between ischemic and nonischemic myocardium and suppreses the re-entrant pathways resulting in antiarrhythmic, antifibrillatory action. Furthermore, glibenclamide inhibits the shortening of the action potential in ischemic cells during ischemia and preserves the normal electrical stability of myocardial cells and so it may prevent arrhythmias (9). Besides studies that demonstrate the antiarrythmic effect of glibenclamide, there are several studies that indicate a proarrhythmic effect (7,8). In these studies, this effect is thought to depend on the proischemic effect of glibenclamide. In fact, glibenclamide increases Ca^{++} loading into the myocardial cell and leads to further ischemia. Furthermore, glibenclamide diminishes coronary blood flow (8) and this also potentiates ischemia. The different effect of glibenclamide may depend on 2 reason 1. Decreased electrical inhomogeneity that provides an antiarrhythmic effect; and 2. Increased ischemia that potentiates arrhythmia (3).

We did not find any research indicating an antiarrhythmic or proarrhythmic effect of glibenclamide in females. In the present study, glibenclamide in females did not cause an antiarrhythmic effect. In contrast, it nonsignificantly increased arrhythmias during reperfusion

and significantly increased the duration of total arrhythmias during ligation compared to the male control group. This may be explained by the different distribution of the ATP-dependent K^+ channel, which is higher in females than in males (16). If the structure and distribution of the ATP-dependent potassium channel were the same in males and females, a similar outcome might be expected in response to channel blockage. The ineffectiveness of glibenclamide on the arrhythmia in females may depend on its low affinity to the binding sites on the channel protein. This suggestion is consistent with the conclusion of El Reyani et al. (3) that different agents from the sulfonylurea group have different effects on arrhythmias induced by reperfusion.

In females, estrogen attenuates Ca^{++} overloading during ischemia (20,21). In addition, since females have more ATP-dependent K^+ channels, cardiomyocytes isolated from female hearts are more resistant to Ca^{++} overloading during reperfusion than male hearts (16). More NO is released due to estrogen (11,22) and endogenous NO release improves myocardial perfusion by mediating the rapid recovery of coronary flow following coronary ligation (23). In this study, the beneficial effects of these kinds of advantages against ischemia may be antagonized by pretreatment with glibenclamide, which prolongs the action potential duration and activates Ca^{++} influx; it also decreases the coronary blood flow. Because males are devoid of this protection, glibenclamide might induce more severe ischemia in females. Thus, the proarrhythmic effect instead of anthiarrhythmia was shown in the glybenclamide treated females in the present study.

Ischemia and reperfusion cause myocardial dysfunction (24). It has been demonstrated that chronic estrogen administration resulted in an enhancement of contractile function including cardiac output and aortic flow during reperfusion (15, 25). Kim et al. (15) suggested that the presevation of left ventricular function during reperfusion was dependent on the antioxidant properties of the estrogen. These studies support our findings that the rapid recovery of blood pressure in females during reperfusion may depend on alleviation of myocardial dysfunction and preservation of ventricular function by the effect of estrogen .

Vagal activation is more common in women than in men during coronary artery occlusion; bradycardia and decreased blood pressure are usually seen in females rather than in males (26). In rats, the heart rate lowering activity of vagal nerve stimulation is more profound in females than in males (27). Furthermore, norepinephrine (NE) release was found to be lower in females than in males during ischemia due to greater α_2 adrenoreceptor mediated presynaptic inhibition in females (28). In another study that was compatible with the findings mentioned above, heart rate increased significantly in males but decreased in females and blood pressure showed a greater recovery in males than in females during 6 min of coronary ligation (29). After the vagotomy, this difference disappeared and blood pressure and heart rate were found higher in females than in males (29). Our results contradict this report, because, in present study, blood pressure after ligation was higher in females than that in males and heart rate was similar in females and males. The reason of this discrepancy might be the anesthesia influencing the parameters studied. Thiopenthal sodium was used in our study, but pentobarbital sodium was used in the comparable study mentioned above.

Conclusion

It has been shown in this study that the incidence and duration of ischemia and reperfusion induced arrhythmias in females were lower than those in males. The different response of glibenclamide in females might be attributed to the different affinity of glibenclamide to the ATP-dependent potassium channel or the different density of the channel. Since glibenclamide was found not to affect arrhythmias in females, further research is required to clarify the opening or closing of the ATP-dependent potassium channel or other factors involved in protection against arrhythmias in females.

Acknowledgments

This study was presented as a master thesis by Ersöz Gonca.

Corresponding author:
Ömer BOZDOĞAN
Abant İzzet Baysal University,
Department of Biology, Faculty of Art and Science,
14280 Gölköy, Bolu - Turkey
e-mail: bozdogan@ibu.edu.tr

References

1. D'Alonzo AJ, Darbenzio RB, Hess TA et al. Effect of potassium on the action of the K-ATP modulators, Cromakalim, Pinacidil or Glibenclamide on arrhythmias in isolated perfused rat heart subjected to regional ischemia. Cardiovascular Research; 28: 881-887, 1994.
2. Tosaki A, Hellegouarch A. Adenosine triphosphate-sensitive potassium channel blocking agent ameliorates, but the opening agent aggravates, ischemia/reperfusion induced injury. Journal of American College of Cardiology; 23: 487-496, 1994.
3. El-Reyani N, Bozdoğan Ö, Baczko I et al. Comparison of efficacy of glibenclamide and glimepride in reperfusion–induced arrhythmias in rats. European Journal of Pharmacology; 365: 187-192, 1999.
4. Tosaki A, Szerdahelyi P, Engelman RM et al. Potassium channel openers and blockers: Do they possess proarrhythmic or antiarrhythmic activity in ischemic and reperfused rat hearts? Journal of Pharmacology and Experimental Therapeutics; 267: 1355-1362, 1993.
5. Billman GE, Avendano CE, Halliwill JR et al. The effects of ATP-dependent potassium channel antagonist, glyburide, on coronary blood flow and susceptibility to ventricular fibrillation. Journal of Cardiovascular Pharmacology; 21: 197-204, 1993.
6. Lepran I, Baczko I, Varro A et al. ATP-sensitive potassium channel modulators: both Pinacidil and Glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rats. Journal of Pharmacology and Experimental Therapeutics; 277: 1215-1220, 1996.
7. Bernauer W. Concerning the effect of the K channel blocking agent Glibenclamide on ischemia and reperfusion arrhythmias. Europan Journal of Pharmacology; 326: 147-156, 1997.
8. Valle HF, Lascano EC, Negroni JA et al. Glibenclamide effects on reperfusion-induced malignant arrhythmias and left ventricular mechanical recovery from stunning in conscious sheep. Cardiovascular Research; 50: 474-85, 2001.
9. Connor EB, Bush TL. Estrogen and coronary heart disease in women. Jama; 265: 1861-1867, 1991.
10. Bush TL, Conner EB, Cowan LD et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: Results from the Lipid Research Clinics Program Follow-up Study. Circulation; 75: 1102-1109, 1987.
11. Node K, Kitakaze M, Kosaka H et al. Amelioration of ischemia and reperfusion-induced myocardial injury by 17-β oestradiol. Circulation; 96: 1953-1963, 1997.
12. McHugh NA, Cooke SM, Schaire JL et al. Ischemia and reperfusion induced ventricular arrhythmias in dogs; Effects of estrogen. American Journal of Physiology; 268: 2569-2573, 1995.
13. Kim YD, Chen B, Bearegard J et al. 17-β estradiol prevents dysfunction of canine coronary endothelium and myocardium and reperfusion arrhythmias after brief ischemia /reperfusion. Circulation; 94: 2901-2908, 1996.
14. Delyani JA , Murahara T, Nossuli TD et al. Protection from myocardial injury by acute administration of 17-β estradiol. Journal of Molecular and Cellular Cardiology; 28: 1001-1008, 1996.
15. Zhai P, Eureel TE, Cotthaus R et al. Effect of estrogen on global myocardial ischemia–reperfusion injury in female rats. American Journal of Physiology; 279: 2766-2779, 2000.
16. Ranki HJ, Budas GR, Crawford RM et al. Gender-specific difference in cardiac ATP-sensitive K^+ channels. American Journal of Cardiology; 38: 906-915, 2001.
17. Gross JG, Fryer RM. Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circulation Research; 84: 973- 979, 1999.
18. Siegmund W, Lepran I, Szekeres L. Effect of pentobarbitone anesthesia, age and sex in the acute phase of myocardial infarction in rats, in Tardos L, Szekeres L, Papp J Gy: Pharmacologic Control of Heart and Circulation; 63-66, 1979. (Cited from Du XJ, Dart AM, Riemersma RA et al. Sex difference in presynaptic adrenergic inhibition of norepinephrine release during normoxia and ischemia in the rat heart. Circulation Research; 68: 827-835, 1991).
19. Tweedie D, Henderson C, Kane K. Glibenclamide, but not class 3 drugs, prevents ischemic shortening of the refractory period in guinea-pig hearts. Eur. J. Pharmacol; 240: 251-257, 1993.
20. Collins P, Rosana G, Jiang J et al. Cardiovascular protection by oestrogen - a calsium antagonist. Lancet; 341: 1264-65, 1993.
21. Jiang C, Wilson PAP, Philip M et al. Effect of 17-β oestradiol on contraction, Ca current and intracellular free Ca in guinea-pig isolated cardiac myocytes. British Journal of Pharmacology; 106: 739-745, 1992.
22. Kauser K, Rubanyi GM. Potential cellular signaling mechanisms mediating upregulation of endothelial nitric oxide production by estrogen. Journal of Vascular Research; 34: 229-236, 1997.
23. Pabla R, Curtis MJ. Effect of endogenous nitric oxide on cardiac systolic and diastolic function during ischemia and reperfusion in the rat isolated perfused hearts. Journal of Molecular and Cellular Cardiology; 28: 2111-2121, 1996.
24. Bolli R. Mechanism of myocardial "stunning". Circulation; 82: 723-738, 1990.
25. Kolodgie FD, Farb A, Litovsky SH. Myocardial protection of contractile function after global ischemia by physiologic estrogen replacement in the ovariectomized rat. Journal of Molecular and Cellular Cardiology; 29: 2403-2414, 1997.
26. Airaksinen K, Linnaluoto M, Tahvanainen KU et al. Gender difference in autonomic and hemodynamic reactions abrupt coronary occlusion. J Am Coll Cardiol; 31: 301-6, 1998.
27. Du XJ, Dart AM, Riemersma RA. Sex Differences in parasympathetic nerve control of rat heart. Clinical Experimental Pharmacology; 21: 485-493, 1994.

28. Du XJ, Dart AM, Riemersma RA et al. Sex difference in presynaptic adrenergic inhibition of norepinephrine release during normoxia and ischemia in the rat heart. Circulation Research; 688: 827-835, 1991.

29. Du XJ, Dart AM, Riemersma RA. Sex differences in the vagal control hearts in rats. J Mol Cell Cardiol; 25: 4, 1993.