

Comparison of thiopental and ketamine+xylazine anesthesia in ischemia/reperfusion-induced arrhythmias in rats

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Background/aim: To investigate the influence of thiopental (85 mg/kg, intraperitoneally (ip)), and ketamine+xylazine (ketamine 75 mg/kg and xylazine 8 mg/kg, ip) anesthesia on the incidence and duration of ischemia/reperfusion-induced arrhythmias.

Materials and methods: Myocardial ischemia was induced by a 6-min ligation of the left anterior descending coronary artery, followed by a 6-min reperfusion. Measurements were taken of the incidence and duration of ventricular arrhythmias, the mean arterial blood pressure and heart rate, and the pressure rate-product (as an index of myocardial oxygen consumption).

Results: The arrhythmia score and the incidence of ventricular fibrillation and tachycardia were significantly decreased in the ketamine+xylazine-anesthetized rats compared with the thiopental-anesthetized group (arrhythmia score: 2.0 ± 2.1 versus 3.7 ± 1.2 , $P < 0.05$). The heart rate was significantly lower in the ketamine+xylazine group during the entire experiment, whilst the pressure-rate product was also significantly lower in the ketamine+xylazine group at different time points of the ischemia and reperfusion periods.

Conclusion: Ketamine+xylazine anesthesia has a strong antiarrhythmic effect and an apparent depressive action on the heart rate and the myocardial oxygen consumption index. Therefore, ketamine+xylazine anesthesia is not appropriate for the evaluation of possible antiarrhythmic agents. Thiopental anesthesia is preferable to ketamine+xylazine anesthesia in the in vivo ischemia-reperfusion arrhythmia model.

Key words: Anesthetics, ischemia/reperfusion, ventricular arrhythmias, rat

1. Introduction

Occlusion of the coronary arteries induces myocardial ischemia. Although thrombolytic and angioplastic therapy restore the blood flow through blocked arteries to save the myocardium from eventual necrosis in a hospitalized patient, the reperfusion can lead to the occurrence of life-threatening arrhythmias (1). Thus, the reperfusion-induced arrhythmias after transient episodes of ischemia represent a clinical problem that limits the benefits of thrombolytic and angioplastic therapy (2).

For decades, researchers have sought to clarify the cellular mechanisms underlying the occurrence of ventricular arrhythmias and to test the antiarrhythmic potential of drugs and substances using the in vivo ischemia-reperfusion arrhythmia model (3–5). The main issue is an appropriate choice of the anesthetic agent used in this model (6). The use of different types of anesthesia may be the reason for certain discrepancies between the results of ischemia/reperfusion (I/R)-induced arrhythmia studies in the literature. The anesthetic agents used in this

model were reported to influence the occurrence of I/R-induced arrhythmias (7–10).

Ketamine hydrochloride is a dissociative anesthetic that has certain undesirable effects, such as muscle hypertonicity, myoclonus, and violent recovery (11,12). Therefore, ketamine is commonly used in combination with an alpha-2 agonist to counteract these undesirable effects (13). Xylazine hydrochloride is a typical alpha-2 agonist of the nonopioid group with analgesic, sedative, and muscle relaxant effects (14). Thiopental, a thiobarbiturate, is also an anesthetic substance with certain side effects, such as respiratory depression (15).

Both thiopental and ketamine+xylazine (K+X) have been used in studies involving experimental cardiology (16–24). However, there are only a few studies addressing the influence of anesthetics on I/R-induced arrhythmias (7–10), whilst there has been no comparison between these two commonly used anesthetics (thiopental and K+X) in an in vivo I/R arrhythmia model. With this in mind, the aim of the present study was to compare the

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duration and severity of ventricular arrhythmias induced by coronary artery occlusion and reperfusion, as well as the hemodynamic parameters between thiopental- and K+X-anesthetized rats.

2. Materials and methods

2.1. Animals

Twenty-six male Wistar albino rats (6–7 month olds weighing 340 ± 30 g) were used in the present study. They were provided by the Faculty of Medicine, Bülent Ecevit University, Zonguldak. The animals were kept in a room at a temperature of 21 ± 2 °C, with 40%–65% humidity and a 12-h light/dark cycle. The animals consumed tap water and standard rat pellet food ad libitum. The experimental procedures the animals underwent were in accordance with the guidelines and recommendations of the World Medical Association. All of the experimental procedures in this study were discussed and approved by the Animal Research Local Ethical Committee of Bülent Ecevit University, Zonguldak (protocol no: 2010-16-27/05).

2.2. Anesthetic agents

Thiopental sodium (Abbott, İstanbul, Turkey), at a dose of 85 mg/mL/kg was administrated intraperitoneally (ip). Ketamine, at a dose of 75 mg/kg (Alfamine 10%, Alfasan Int., Woerden, Netherlands), and xylazine at a dose of 8 mg/kg (Alfazyne 2%, Alfasan Int., Woerden, Netherlands), were administrated ip in a volume of 1 mL/kg. Satisfactory anesthesia was defined by lack of reflexes in response to pinch stimuli in the tail and foot, as well as the presence of deep respiration. When necessary, 10% of the initial anesthetic dose was applied ip to reach the anesthetic stage.

2.3. Surgical procedures and hemodynamic measurements

The ischemia and reperfusion protocols performed in our study were previously defined by Bozdoğan et al. (24). Briefly, the rats were anaesthetized via an injection of thiopental sodium ip (85 mg/kg) and placed on an animal rectal temperature controller (RTC 9404-A, Commat Ltd, Ankara, Turkey) to maintain their body temperature in the range of 37 ± 1 °C during the experimental period. The trachea and the left carotid artery were cannulated for artificial respiration and the measurement of arterial blood pressure, respectively (blood pressure transducer, SS 13 L, Biopac Systems, Goleta, CA, USA). A standard limb lead II electrocardiogram (ECG) and arterial blood pressure were recorded and monitored throughout the experimental period (data acquisition system MP35, Biopac Systems) (Figure 1). Thoracotomy was performed by cutting the fourth and fifth ribs on the left side of the chest. The animal respirator was started immediately, using room air to provide artificial ventilation (60 strokes/min, at a tidal volume of 1.5 mL/100 g; SAR 830, IITC Life

Science, Woodland Hills, CA, USA). The pericardium was incised and then the hearts were gently exteriorized. A 5/0 silk suture was passed around the left anterior descending coronary artery (LAD) approximately 2–3 mm from its origin. The heart was then replaced and allowed to stabilize for 10 min. During this period, rats with a sustained decrease in mean arterial blood pressure (MABP) values below 70 mmHg or ventricular arrhythmias prior to the ligation were excluded. After heart rate (HR) and blood pressure stabilization, the slip loop was made using the loose ends of the previously placed silk suture. The coronary artery occlusion was induced by ligation of the LAD with the slip loop. The slip loop was then loosened by pulling the loose end of the loop to permit reperfusion.

The heart was removed and cannulated through the aorta following the termination of the reperfusion period. In order to wash out the coronary vessels, the heart was subjected to retrograde perfusion with 10 mL of saline solution at 37 °C. The heart was then perfused with 2 mL of 96% ethanol to specify the zone at risk, followed by reocclusion of the left coronary artery. The nonischemic zone of the heart was thoroughly perfused with ethanol and appeared white. The zones that were not perfused with ethanol were defined as the zone at risk and remained red (original tissue color). Finally, the total ventricle and zone at risk were weighed; the zone at risk was measured as the percentage of total ventricle weight (25).

Various results were seen in all rats for which a successful coronary artery occlusion had been performed. This included ST segment elevation and increased QRS amplitude on ECG, a 20%–40% reduction in arterial blood pressure compared to the preischemic values, and zone at risk values greater than 40%. Reversal of ischemia-induced ST segment changes and recovery of MABP were observed in all rats that had undergone successful reperfusion. A total of 6 animals were omitted on the basis of these criteria.

2.4. Recording and arrhythmia analysis

By using a data acquisition system, ECG and blood pressure recordings were analyzed to determine the HR and MABP, and systolic and diastolic blood pressure (SABP, DABP) parameters at regular intervals throughout the ischemia and reperfusion periods (MP 35, Biopac Systems). The pressure-rate product (PRP) is the product of mean blood pressure and heart rate ($PRP = SABP \times HR/1000$), which corresponds to the oxygen and energy demand of the myocardium. PRP was calculated as an index of myocardial oxygen consumption according to Baller et al. (26).

In accordance with the Lambeth Conventions (27), arrhythmias were identified during the ischemia and reperfusion periods as ventricular fibrillation (VF), ventricular tachycardia (VT), and other types of

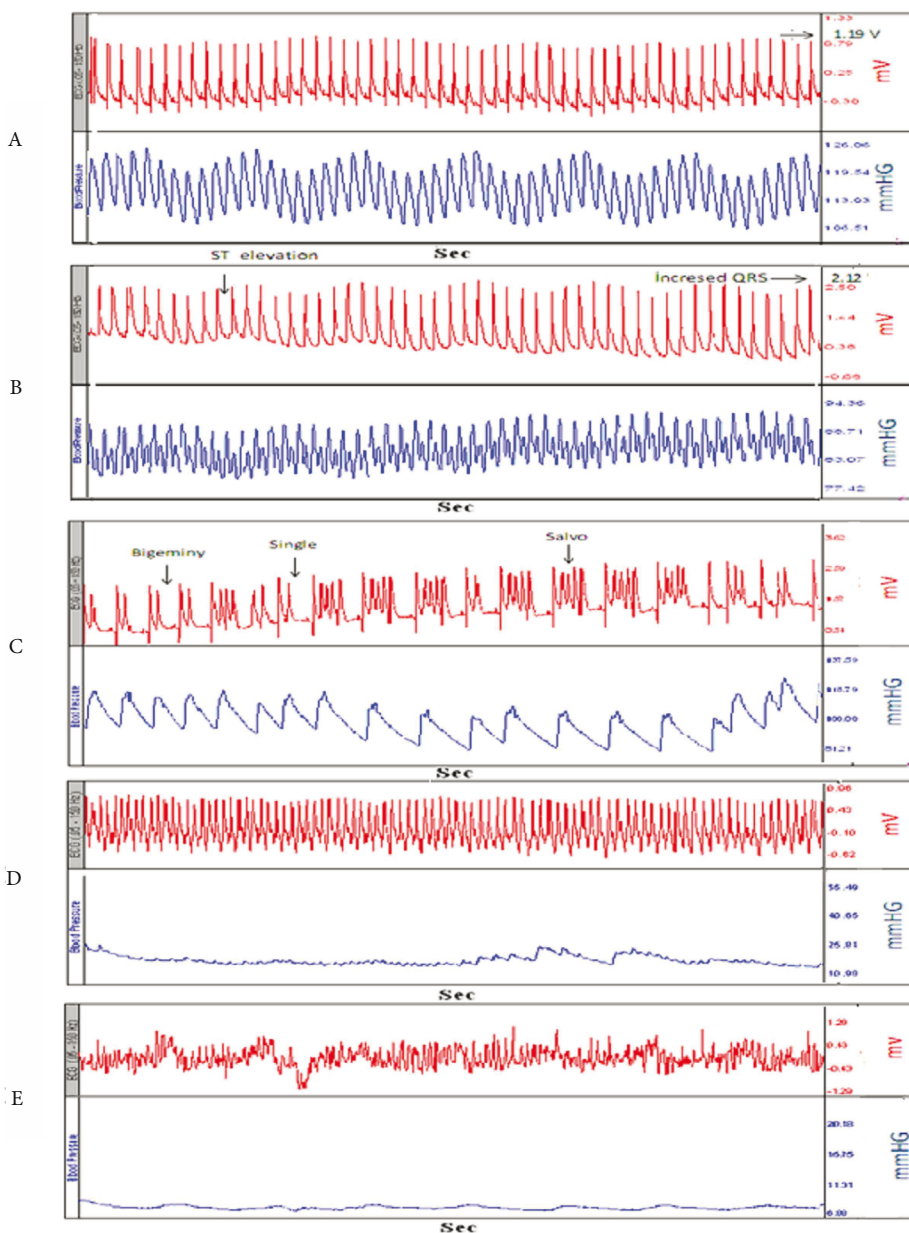


Figure 1. Original electrocardiogram tracings (recorded at speed 80 mm/s) and arterial blood pressure recordings: (A) Sinus rhythm just before the coronary ligation; (B) ST-segment elevation and the increase in QRS amplitude; (C) Other types of arrhythmias (VPC) including bigeminy, single extrasystole, and salvo; (D) ventricular tachycardia (VT); (E) ventricular fibrillation (VF).

arrhythmias (ventricular premature contraction (VPC)) including bigeminy, salvos and single extrasystoles (Figure 1). A grade was given to each animal as an index of the severity of arrhythmias (arrhythmia score), according to the following scale: 0- no arrhythmia; 1- in the absence of VF, the duration of VT and/or VPC is shorter than 10 s or equal to 10 s; 2- in the absence of VF, the duration of VT and/or VPC is between 11 and 30 s; 3- in the absence of VF, the duration of VT and/or VPC is between 31 and 90 s; 4-

the duration of reversible VF is shorter than 10 s or equal to 10 s and/or the duration of VT and/or VPC is between 91 and 180 s; 5- the duration of VF is longer than 10 s and/or the duration of VT and/or VPC is longer than 180 s; and 6- irreversible VF (28). For both groups measurements were taken of the durations of arrhythmic attacks and arrhythmic periods, which are the time intervals between the onset and the end of the arrhythmias, the incidence of arrhythmia types and mortality and the arrhythmia scores.

A HR below 200 beats/min was defined as bradycardia, and the incidence of bradycardia was determined for both ischemia and reperfusion periods.

2.5. Statistical analysis

The GraphPad statistical software package was used for statistical analysis (GraphPad Prism 5, San Diego, CA, USA). Fisher's exact test was used for the statistical analysis of the survival rate and the incidence of arrhythmias. The other parameters were analyzed using Student's two-tailed t test. Preocclusion and postocclusion MABP/HR values were also compared using Student's two-tailed t test. If the data did not follow normal distribution, the nonparameter Mann-Whitney U test was applied. Data were expressed as mean \pm standard deviation (SD).

3. Results

3.1. Hemodynamic parameters

Characteristically, MABP fell immediately following the ligation in both groups upon occlusion of the LAD ($P < 0.001$). Following reperfusion, the MABP recovered and approached the preischemic values in thiopental-anesthetized rats. Because the MABP did not recover in the K+X-anesthetized rats during the late phase of reperfusion, the MABP of this group was significantly lower compared with that of the thiopental-anesthetized rats at 3 and 5 min of reperfusion ($P < 0.05$) (Figure 2). The DABP values of the K+X-anesthetized rats were also significantly lower than those of the thiopental-anesthetized group at the same time points ($P < 0.05$) (data not shown).

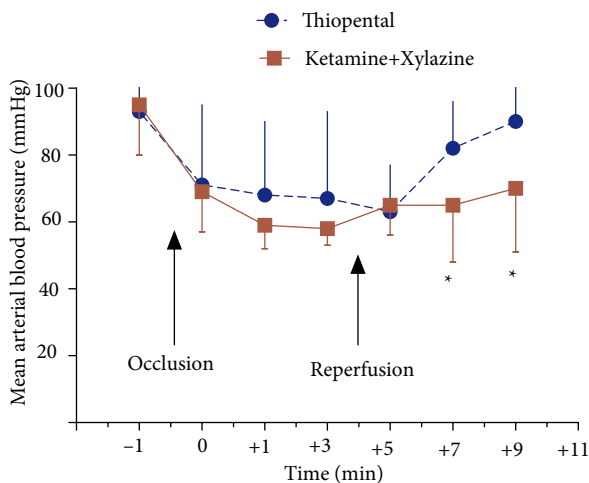


Figure 2. Mean arterial blood pressure (mmHg) values plotted against time in anesthetized rats with thiopental (85 mg/kg, ip, broken lines) and ketamine-xylazine (75 mg/kg and 8 mg/kg, respectively, ip, solid lines) during ischemia and reperfusion periods. Arrows indicate coronary artery occlusion and reperfusion. Values represent means \pm standard deviation. * $P < 0.05$: significantly different from thiopental. $n = 9-9$.

The HR values of the K+X-anesthetized rats were significantly lower than those of the thiopental-anesthetized group before coronary artery occlusion and during the entire occlusion and reperfusion period ($P < 0.0001$) (Figure 3).

In the K+X-anesthetized group, PRP values were lower than in the thiopental-anesthetized group. These differences reached significance during the ligation period ($P < 0.05$) and at 3 and 5 min of the reperfusion period ($P < 0.0001$) (Table 1).

3.2. Arrhythmias during ischemia and reperfusion

The myocardial zone at risk was equal between the two groups (Table 2). The ligation of the LAD resulted in the generation of arrhythmias, occurring as VPC. However, the incidence and the duration of VPC were not significantly different between the groups. Indeed, neither group experienced any VF or VT. Bradycardia was only observed in 3 animals anesthetized with K+X during ischemia. However, the incidence of bradycardia was not different between the two groups (data not shown).

The reperfusion-induced arrhythmias were more severe than the arrhythmias that developed during the 6 min of ischemia, as expected, and appeared within 10–30 s immediately following the reperfusion. In the K+X-anesthetized group, the incidence of VF and VT during reperfusion was significantly lower than in the thiopental-anesthetized animals ($P < 0.05$ and $P < 0.0001$, respectively), whilst the incidence of bradycardia was nonsignificantly higher than in the thiopental-anesthetized animals ($P = 0.867$) (Table 3).

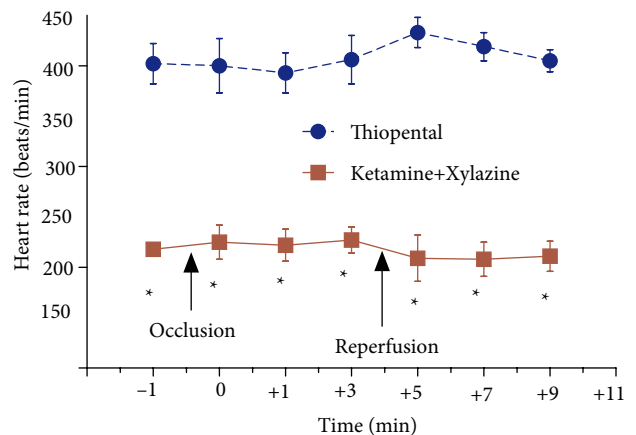


Figure 3. Heart rate (beats/min) values plotted against time in anesthetized rats with thiopental (85 mg/kg, ip, broken lines) and ketamine-xylazine (75 mg/kg and 8 mg/kg, respectively, ip, solid lines) during ischemia and reperfusion periods. Arrows indicate coronary artery occlusion and reperfusion. Values represent means \pm standard deviation. * $P < 0.0001$: significantly different from thiopental. $n = 9-9$.

Table 1. The effects of thiopental and ketamine+xylazine anesthesia on pressure rate product ($\text{mmHg min}^{-1} \times 1000$).

Groups	0 (Basal)	1 (Lig 1 min)	3 (Lig 3 min)	5 (Lig 5 min)	7 (Rep 1 min)	9 (Rep 3 min)	11 (Rep 5 min)
Thiopental (n = 9)							
Mean \pm SD	39 \pm 11	31 \pm 12	29 \pm 12	29 \pm 13	25 \pm 15	34 \pm 10	35 \pm 9
95% Confidence interval	(30.94–47.86)	(21.11–40.23)	(20.23–38.14)	(19.51–39.23)	(50.02–75.98)	(70.97–94.03)	(74.82–105.2)
Ketamine+xylazine (n = 9)							
Mean \pm SD	39 \pm 11	17 \pm 4*	15 \pm 4*	15 \pm 3*	15 \pm 5	16 \pm 6**	17 \pm 6**
95% Confidence interval	(30.94–47.86)	(14.07–21.07)	(12.4–18.21)	(13.46–17.44)	(56.76–74.11)	(51.37–79.63)	(54.83–83.84)
P value		0.025	0.008	0.032		0.0002	0.0002

n: The number of animals that survived after 6 min of reperfusion.

Lig: Ligation, Rep: Reperfusion. *P < 0.05, **P < 0.0001: Compared with thiopental group.

Table 2. The effects of thiopental and ketamine+xylazine anesthesia on the duration of arrhythmias during 6 min of reperfusion.

Groups	Zone at risk (% of total)	Arrhythmic period (s)	Arrhythmia score	Length of arrhythmias (s)			
				VF	VT	VPC	Total
Thiopental (n = 9)							
Mean \pm SD	49 \pm 7	207 \pm 46	3.7 \pm 1.2	8 \pm 19	47 \pm 44	20 \pm 6	75 \pm 20
95% Confidence interval	(43.39–53.01)	(102.5313.3)	(2.81–4.56)	(6.76–22.77)	(13.1–80.17)	(6.83–32.72)	(27.69–121.4)
Ketamine+xylazine (n = 9)							
Mean \pm SD	49 \pm 5	54 \pm 22**	2.0 \pm 2.1*	2 \pm 6*	5 \pm 14**	10 \pm 4	17 \pm 8*
95% Confidence interval	(43.76–54.04)	(2.54–105.5)	(0.49–3.51)	(2.46–6.24)	(5.57–15.79)	(0.19–20.41)	(1.49–36.16)
P value		0.004	0.042	0.044	0.002		0.011

n: The number of animals that survived after 6 min of reperfusion. VF: Ventricular fibrillation; VT: Ventricular tachycardia; VPC: Ventricular premature contraction; Total: The total length of VF, VT, and VPC. *P < 0.05, **P < 0.001: Compared with thiopental group.

Table 3. The effects of thiopental and ketamine+xylazine anesthesia on the incidence of arrhythmias during 6 min of reperfusion.

Groups	N	Mortality index N/%	Incidence of arrhythmias (n*/%)			
			VF	VT	VPC	Bradycardia
Thiopental						
Mean \pm SD	10	1/10	7/70	10/100	10/100	0/0
Ketamine+xylazine						
Mean \pm SD	10	1/10	1/10*	2/20**	7/70	4/40
P value			0.012	0.0007		

N: The number of animals just before the reperfusion. N: The number of dead animals after 6 min of reperfusion.

n*: The number of animals that experienced arrhythmias.

VF: Ventricular fibrillation; VT: Ventricular tachycardia; VPC: Ventricular premature contraction.

*P < 0.05, **P < 0.0001: Compared with thiopental group.

In the K+X-anesthetized group, the arrhythmia score, the duration of VF and VT, the total length of the arrhythmias, and the length of the arrhythmic period were all significantly lower than in the thiopental-anesthetized rats (arrhythmia score: 2.0 ± 2.1 versus 3.7 ± 1.2 , $P < 0.05$) (Table 2).

4. Discussion

The present results reveal the novel finding that K+X-anesthetized rats undergoing acute coronary occlusion and reperfusion exhibit a lower incidence of VF and VT, a shorter duration of VT, and a lower total length of the arrhythmias than thiopental-anesthetized rats. To date, no comparable studies have compared the effects of these two anesthetics on I/R-induced arrhythmias. However, our finding is similar to the observations reported by Baczkó et al., who compared the effects of ketamine/diazepam (instead of K+X) with the effects of pentobarbital and urethane anesthesia on I/R-induced arrhythmias in rats. These researchers reported that ketamine/diazepam anesthesia had an antiarrhythmic effect compared with the other anesthetic substances (9).

A previous *in vivo* study, which compared the effects of thiopental anesthesia with the effects of urethane and pentobarbital, revealed that the duration of ventricular arrhythmias in thiopental-anesthetized rats was shorter than in a pentobarbital-treated group (10). Our study may be regarded as complementary to this study, in the sense that the effect of K+X anesthesia was also compared to the effects of thiopental anesthesia on I/R-induced arrhythmias.

Thiopental has been shown to increase the duration of I/R-induced arrhythmias in previous isolated heart-model studies (29,30). These observations correspond with the results of our experiment, which revealed a significantly longer duration of ventricular arrhythmias in thiopental-anesthetized rats following reperfusion.

The present study reveals that K+X anesthesia reduces the HR of rats compared with thiopental anesthesia. In accordance with this finding, previous studies also demonstrated a sustained decrease in the HR after K+X administration in rats (31–33). The nonphysiological bradycardia caused by K+X anesthesia during the entire experiment should be reason enough to exclude this anesthetic from use in such studies. The underlying mechanism of the effect of K+X anesthesia on the HR may be explained by the effect of xylazine, which promotes a decrease in sympathetic activity and an increase in vagal activity (34). Xylazine action overrides the increased sympathetic activity and decreases vagal tone induced by ketamine (12).

In our experiments, the MABP decreased in both groups following ligation, as expected. Thiopental anesthesia had a stable influence on the MABP during the experiment,

as the MABP recovered in thiopental-anesthetized rats but not in K+X-anesthetized rats following reperfusion. This result reveals that thiopental anesthesia has a more normotensive effect during reperfusion. This finding is in agreement with the results obtained by Wixon et al., who described a cardiodepressant effect of K+X anesthesia (35).

Indeed, experimental studies have shown that the HR during acute I/R is an important determinant of susceptibility to reperfusion arrhythmias (36,37), with higher rates predisposing the rat hearts to arrhythmias. In a recent *in vivo* study, Ng et al. demonstrated that reducing the HR during ischemia reduces the incidence of reperfusion arrhythmias and suggested that the HR during ischemia is a major determinant of reperfusion arrhythmias (38). In the present study, the lower arrhythmic activity in K+X-anesthetized rats can be attributed to the anesthesia's HR-lowering effect. More specifically, the HR values were significantly lower than the physiological level during both ischemia and reperfusion periods in K+X-anesthetized rats.

The notion that HR reduction during ischemia is protective against I/R-induced arrhythmias is based on the hypothesis that lowering the HR causes a reduction in myocardial oxygen consumption and can attenuate the severity of ischemia (39,40). In our study, the myocardial oxygen consumption was also lower in the K+X-anesthetized group when we consider the PRP results. Therefore, our finding that PRP is decreased in K+X-anesthetized rats supports the suggestion that the lower arrhythmic activity observed in K+X-anesthetized rats is dependent on the anesthesia's HR-lowering effect.

It has been shown that ATP dependent potassium (K_{ATP}) channel blockage confers protection against I/R-induced arrhythmias (23,24). Ketamine inhibits the K_{ATP} channel activity in a concentration-dependent manner in the rat heart (41). Therefore, in the present study, the possible inhibition of the K_{ATP} channel by ketamine may also contribute to the antiarrhythmic effect of K+X anesthesia.

In conclusion, because K+X anesthesia does not allow for the development of severe ventricular arrhythmias, this type of anesthesia is not an appropriate choice for cardiovascular experiments designed to investigate the effect of potentially antiarrhythmic drugs. This anesthesia may be preferable for use in experiments investigating proarrhythmic agents. Thiopental anesthesia appears to be a more appropriate choice in the *in vivo* ischemia-reperfusion arrhythmia model. Future studies are needed to clarify the antiarrhythmic mechanism of ketamine+xylazine anesthesia.

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