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Ischemic Preconditioning Reduces the Severity of Ventricular Arrhythmias Induced by Coronary Artery Occlusion in Rats

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Abstract: The effects of brief ischemia (preconditioning) on early phase arrhythmias induced by coronary artery occlusion were studied in two sets of rat experiments. In the first protocol, the duration of the preconditioning coronary occlusion was varied. We used three different periods, 1 min (group 2, n=8), 3 min (group 3, n=10), and 5 min (group 4, n=10). A 10 min reperfusion period was maintained before the coronary artery was reoccluded for 30 min. The severity of arrhythmia during this 30 min reocclusion period was assessed and was compared with that in control rats (group 1, n=12) in which the coronary artery was also occluded for 30 min, without preconditioning. Total numbers of ventricular ectopics in each group were 1236 ± 262 beats (M ± SE), 1230 ± 370 beats (NS), 200 ± 60 beats ($p<0.01$) and 394 ± 152 beats ($p<0.01$), respectively. The incidence of VT in groups 1–4 was 100%, 100%, 70%, and 60% ($p<0.05$), respectively. The 3 min preconditioning occlusion had the most potent protective effects against ventricular arrhythmias induced by coronary artery occlusion. In the second protocol, the duration of reperfusion after the 3 min preconditioning occlusion was varied, the periods being 10 min, 20 min, and 30 min. The longer reperfusion period attenuated the protective effect of the 3 min occlusion preconditioning. In conclusion, preconditioning periods of more than 3 min of myocardial ischemia protected against the ventricular arrhythmias induced by prolonged coronary occlusion. The protective effect of 3 min occlusion preconditioning lasted for 30 min.

Key words: Ischemic preconditioning, Coronary occlusion induced arrhythmia, Antiarrhythmic effect, Rats

INTRODUCTION

The occurrence of arrhythmias in acute myocardial infarction is highly variable[1)–5)]. Although infarct size[4)] and heart rate[6)] have been reported to influence the occurrence of ventricular arrhythmias, some patients with acute myocardial infarction have ventricular arrhythmias, while others with acute myocardial infarction of the same size and the same location have no ventricular arrhythmia. Many studies have recently been carried out on endogenous antiarrhythmic agents, prostacyclin[7)–9)] and adenosine[10)–12)] have been reported to have antiarrhythmic effects. These substances are released in acute myocardial ischemia[8),10)]. Thus, coronary artery occlusion preconditioning may have a protective effect on the ventricular arrhythmias induced by coronary artery occlusion itself. We studied the antiarrhythmic effects of brief ischemia on early phase ventricular arrhythmias induced

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by coronary artery occlusion.

Methods

Male Sprague-Dawley rats (190–300 g) were anesthetized with sodium pentobarbitone (60 mg/kg) administered intraperitoneally. The trachea was cannulated for artificial ventilation. Systemic blood pressure was continuously monitored via a cannula inserted in the carotid artery. A standard limb lead 1 electrocardiogram was continuously recorded, together with systemic blood pressure on a Grass Model 7D recorder (Grass Instrument Co, U.S.A.). The chest was opened using a left thoracotomy, followed by sectioning of ribs 4 and 5, approximately 2 mm to the left of the sternum. Artificial ventilation with room air was begun immediately, using a volume of 1.5 m*l*/100 mg and a rate of 54 strokes/min to maintain normal pCO_2, pO_2 and pH levels[13]. After the pericardium was incised, the heart was exteriorized out of the chest, using gentle pressure on the rib cage. A 6/0 braided silk suture attached to a 10 mm micropoint reverse cutting needle was placed under the left coronary artery, as described by H. Selye *et al*[14]. The heart was replaced in the chest and the rat was left to recover for 15 min. Rats that had arrhythmias during the recovery period and/or less than 70 mmHg of mean blood pressure were discarded. After this procedure, both ends of the ligature were passed through a small plastic tube; regional myocardial ischemia could be induced at any time by pulling the suture while pressing the tube against the surface of the myocardium. Ischemia could be maintained for any desired period by clamping the tubing and the suture. Reperfusion could be initiated by unclamping and removing the plastic tube. After the reperfusion period, the coronary artery could be reoccluded by tying the suture firmly for 30 min.

In the first protocol (Fig. 1) the coronary occlusion preconditioning period was varied: 1 min in group 2 (n=8), 3 min in group 3 (n=10), and 5 min in group 4 (n=10). The reperfusion period was fixed at 10 min. Reocclusion (second occlusion) was then carried out for 30 min. The severity of arrhythmias during this 30 min reocclusion was assessed and was compared with that in the control group (group 1, n=12), in which the coronary artery was also occluded for 30 min, without coronary occlusion preconditioning.

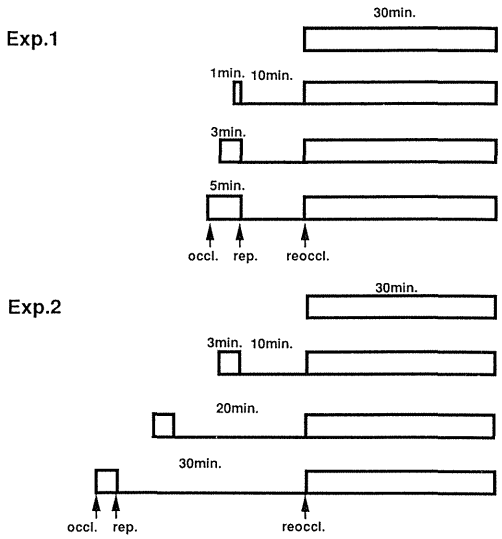

Fig. 1. Experimental protocol.
occ: occlusion, rep: reperfusion, reocc: reocclusion.

In the second protocol (Fig. 1), coronary occlusion preconditioning period was maintained at 3 min, and the reperfusion period was varied, being 10 min in group 2 (n=10), 20 min in group 3 (n=9), and 30 min in group 4 (n=10). Following reperfusion, reocclusion (second occlusion) was carried out for 30 minutes. The severity of arrhythmias during the second occlusion period was assessed and was compared with that in control rats (group 1, n=12), in which the coronary artery was occluded for 30 min, without any preconditioning occlusion.

Ventricular tachycardia (VT) was defined as a run of 4 or more consecutive ventricular ectopics. The total number of ventricular premature complex (VPC), and the hemody-

namic data were assessed in rats which survived for the duration of the experiment. The incidence of events was assessed in all rats subjected to the experiment. All values are expressed as means ± S.E.M. Changes within each group were compared using a paired Student's t-test, and differences between group were assessed using an independent Student's t-test. Changes in the incidence of events were analyzed by Fischers exact probability test. Differences were regarded as significant if the p values were less than 0.05.

The experiment was performed in accordance with the Guidelines for Animal Experiments, Yamanashi Medical College.

Results

A) Arrhythmias during the reperfusion period

As shown in Table 1, there were a few premature ventricular beats during the reperfusion period after 1 min and 3 min occlusion preconditioning. However, 5 min occlusion preconditioning induced severe reperfusion arrhythmias. Seventy-one percent of

Table 1. Reperfusion arrhythmias following 1 min, 3 min, and 5 min coronary artery occlusion. There were a few ventricular ectopic beats during reperfusion following 1 min and 3 min occlusion. However, 5 min occlusion induced severe reperfusion arrhythmias. Fifty-eight percent of the rats died as a result of reperfusion arrhythmia. 1 min: 1 min occlusion, 3 min: 3 min occlusion, 5 min: 5 min occlusion

	No. of VPC	Incidence of VT	Incidence of VF	Mortality Rate
1 min. (N=8)	1±1	0%	0%	0%
3 min. (N=17)	10±7	0%	0%	0%
5 min. (N=24)	306±145	83%	71%	58%

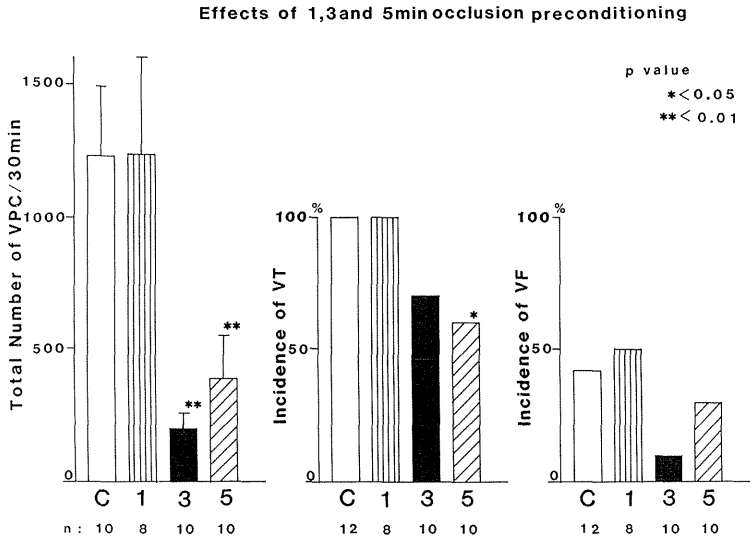

Fig. 2. Antiarrhythmic effects of 1 min, 3 min, and 5 min occlusion preconditioning. Three min and 5 min occlusion preconditioning had protective effects against the early phase ventricular arrhythmias induced by coronary artery occlusion. C: control, 1: 1 min occlusion, 3: 3 min occlusion, 5: 5 min occlusion, *: p value<0.05, **: p value<0.01 vs control.

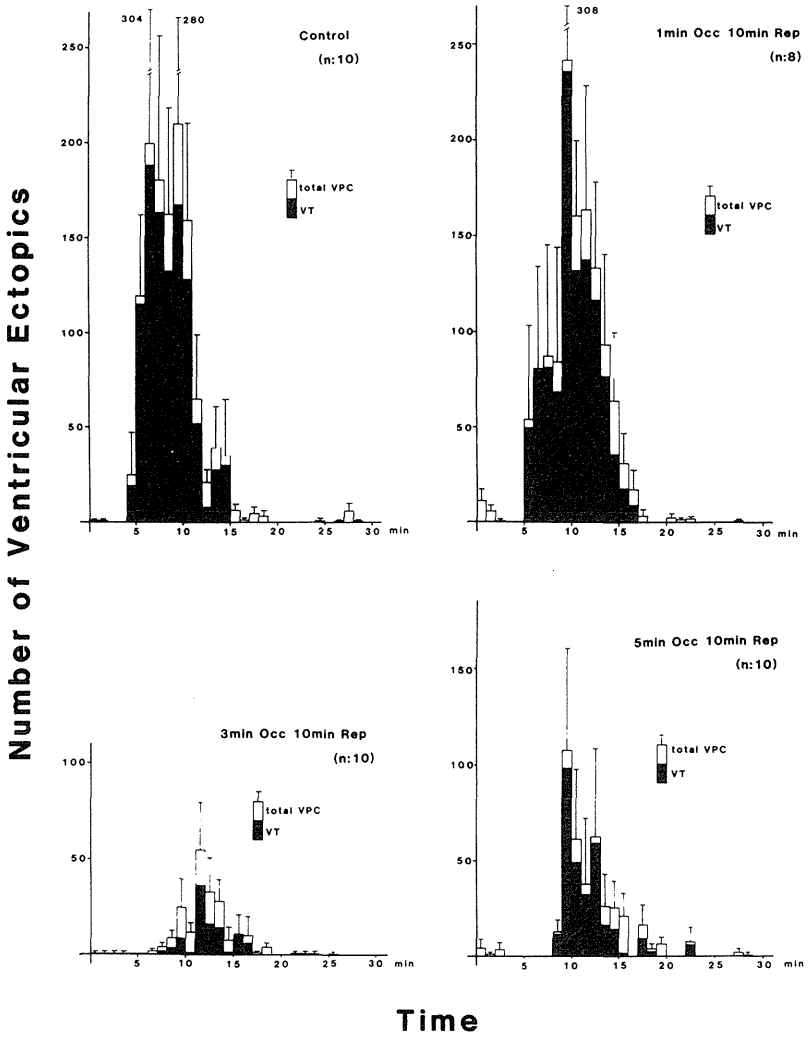

Fig. 3. Distribution of ventricular arrhythmias induced by coronary artery occlusion in control (top left) and under conditions when prolonged (30 min), occlusion was preceded by a short preconditioning occlusion of 1 min (top right), 3 min (bottom left), or 5 min (bottom right). The reperfusion period in each case was 10 min. A 3 min or 5 min occlusion preconditioning markedly reduced the ventricular arrhythmias occurring during subsequent prolonged occlusion. Black columns indicate the numbers of ventricular ectopics which appeared in ventricular tachycardia.

the rats had ventricular fibrillation and 58% of the rats died during the reperfusion period after 5 min occlusion.

B) Change in severity of arrhythmias related to duration of preconditioning occlusion

Figures 2 and 3 show the effects of 1 min, 3 min, and 5 min preconditioning occlusion compared with control data. In control rats, the total number of VPC was 1236 ± 262 beats/30 min, the incidence of VT and ventricular fibrillation (VF) was 100% and 42%, respectively, and the duration of VT was 95.6

± 28.7 sec. One min preconditioning occlusion had no significant effect. The total number of VPC was 1230 ± 370 beats/30 min, the incidence of VT and VF was 100% and 50%, respectively, and the duration of VT was 109.8 ± 30.9 sec. These values were almost the same as these of the controls. Three min preconditioning occlusion had a significant protective effect against ventricular arrhythmias. The total number of VPC was 200 ± 60 beats/30 min ($p<0.01$), the incidence of VT and VF was 70% and 10%, respectively, and the duration of VT was 12.6 ± 3.8 sec ($p<0.05$). Very few rats survived after the 5 min occlusion period because of the high incidence of VF in the reperfusion period after this occlusion time. However, in those rats that survived, 5 min preconditioning occlusion had the same protective effect on ventricular arrhythmias as 3 min preconditioning occlusion. The total number of VPC was 394 ± 152 beats/30 min ($p<0.01$), the incidence of VT and VF was 60% ($p<0.05$) and 30%, respectively, and although the reduction was not significant, the duration of VT was reduced to 55.1 ± 20.3 sec.

C) Change in the protective effect of 3 min preconditioning occlusion related to the duration of the reperfusion period

Three min preconditioning occlusion seemed to have the most potent protective effect on the ventricular arrhythmias induced by coronary occlusion. Thus, the duration of reperfusion after 3 min occlusion was varied from 10 min to 30 min, to estimate the duration of the protective effect afforded by this occlusion preconditioning.

As shown in Figs. 4 and 5, 3 min preconditioning occlusion followed by a 10 min reperfusion period reduced the total number of VPC from 1380 ± 228 beats/30 min to 264 ± 94 beats/30 min ($p<0.01$), the incidence of VT and VF was reduced from 100% to 60% ($p<0.05$) and from 42% to 20% (not significant), respectively and the duration of VT was reduced from 106.4 ± 26.1 sec to 26.5 ± 6.8 sec ($p<0.05$).

After the 20 min reperfusion period, the total number of VPC was 457 ± 147 beats/30

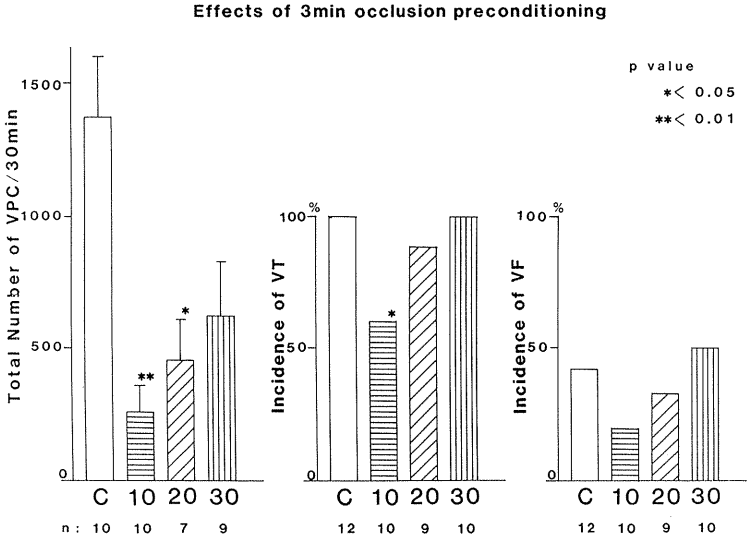

Fig. 4. Changes in protective effects of 3 min occlusion preconditioning related to duration of reperfusion. Longer reperfusion periods attenuated the protective effects of 3 min occlusion preconditioning. C: control, 10: 10 min reperfusion, 20: 20 min reperfusion, 30: 30 min reperfusion, *: p value<0.05 **: p value<0.01 vs control.

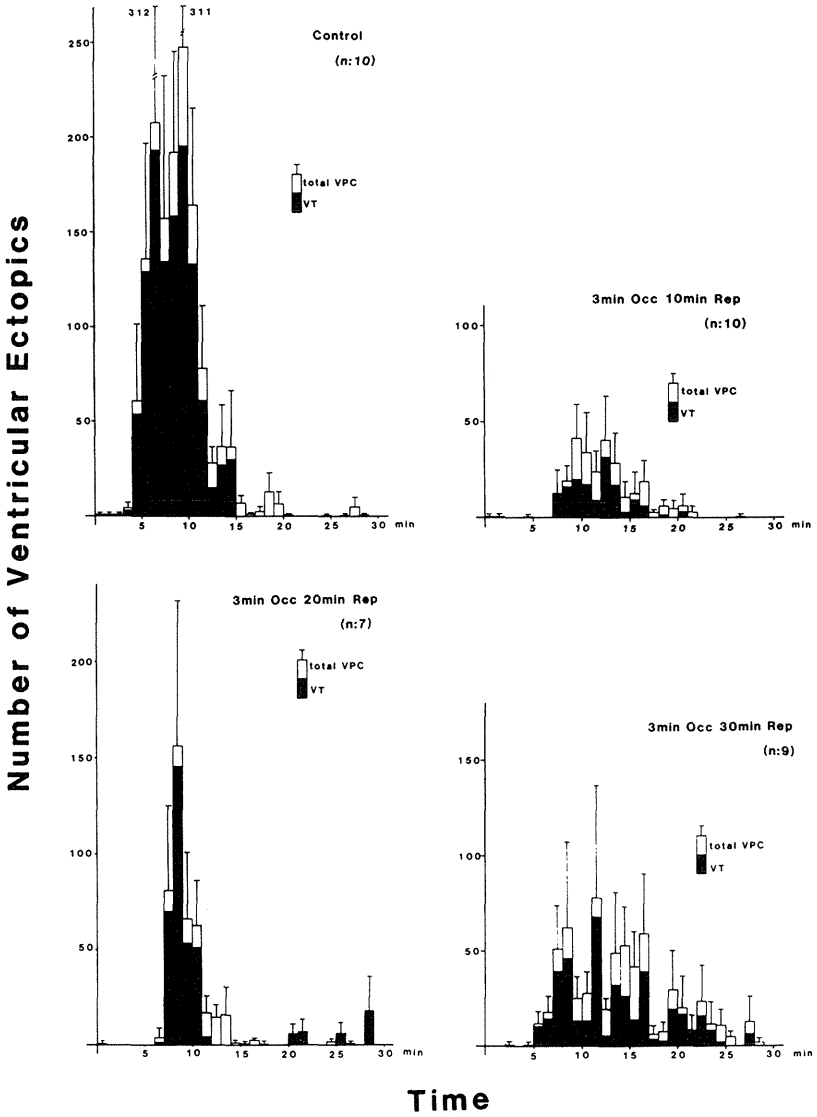

Fig. 5. Distribution of ventricular arrhythmias induced by coronary artery occlusion in control (top left) and under conditions when prolonged (30 min), reocclusion was preceded by reperfusion periods of 10 min (top right), 20 min (bottom left) and 30 min (bottom right) after 3 min occlusion preconditioning. There was a remarkable protective effect when the reperfusion period was 10 min and some protective effect when the reperfusion period was 20 min. Even after a 30 min reperfusion period, there was some evidence of protective effect, with a wider spread of arrhythmias over the occlusion period.

min ($p<0.05$), the incidence of VT and VF was 89% and 33% (a non significant reduction), respectively, and the duration of VT was 47.1 ± 17.1 sec (a non significant reduction).

After the 30 min reperfusion period, the total number of VPC was 627 ± 196 beats/30 min, the incidence of VT and VF was 100% and 50%, respectively. These values were not

Table 2. Hemodynamic data

	HR (bpm)	mBP (mmHg)
Control		
preocclusion	436±11	100±5
occlusion		
1 min	437±11	72±4**
5 min	451±13**	92±6
15 min	444±16	90±5*
30 min	429±20	90±8
1 min occlusion 10 min reperfusion		
preocclusion	415±15	89±3
occlusion		
1 min	418±15	55±4**
reperfusion		
1 min	409±13	75±6
10 min	400±16	91±7
reocclusion		
1 min	409±14	60±4**
5 min	408±12	72±7
15 min	396±14*	81±8
30 min	411±11	90±8
3 min occlusion 10 min reperfusion		
preocclusion	442±13	101±8
occlusion		
1 min	446±14	62±6**
3 min	433±15	65±8**
reperfusion		
1 min	418±12**	77±7**
10 min	443±12	107±8
reocclusion		
1 min	448±11	71±9**
5 min	442±10	79±10
15 min	458±8	102±11
30 min	458±7*	102±10
5 min occlusion 10 min reperfusion		
preocclusion	380±14	80±3
occlusion		
1 min	401±15*	61±5**
3 min	404±17	75±6
reperfusion		
1 min	386±16	79±4
10 min	387±14	90±4*
reocclusion		
1 min	399±14	72±3*
5 min	413±14	86±5
15 min	412±16	92±4
30 min	414±15	97±4**

HR: heart rate, mBP: mean blood pressure
*: p value<0.05, **: p value<0.01 vs preocclusion value

significantly reduced, compared to the control. However the duration of VT was reduced to 40.3 ± 13.9 sec ($p<0.05$). It is clear that the protective effect of 3 min preconditioning occlusion was most remarkable at the 10 min reperfusion period and varied with time. Significant protective effects were demonstrated when the reperfusion period was extended to 20 min or even to 30 min.

D) Hemodynamic data

The hemodynamic data are shown in Table 2. The heart rate tended to increase just after occlusion and to decrease after reperfusion. There was no significant difference in the mean heart rate of each group at preocclusion and just before reocclusion, apart from that of 5 min preconditioning occlusion. The 5 min preconditioning occlusion group had a significantly lower heart rate at preocclusion and just before reocclusion ($p<0.05$).

The blood pressure was significantly reduced with coronary occlusion, but recovered within around 5 min. There was no significant difference in the blood pressure of each group at preocclusion and just before reocclusion.

Discussion

In the present study, we have demonstrated that brief myocardial ischemia has a protective effect against ventricular arrhythmia following a second episode of myocardial ischemia. This protective effect is acquired in a very short period and lasts up to 30 min. Gulker *et al.*[15], in their experiments with mongrel dogs, reported that when occlusions were repeated several times, the extent of the decrease in ventricular fibrillation threshold gradually became less and its duration gradually became shorter, until finally there was no significant decrease in ventricular fibrillation threshold. Podzuweit *et al.*[16], in their experiments with pigs, reported that the ventricular tachycardia induced by intramyocardial infusion or intracoronary infusion of noradrenaline or N^6, O^2-dibutyryl-cAMP was consistently stopped within 10 to 30 sec by occluding the coronary artery supplying the infusion area.

The mechanism underlying the protective effect of brief myocardial ischemia observed in the present study remains to be elucidated. The protective effect may be related to ischemia size, which might be reduced when collateral vessels are opened up during the first episode of myocardial ischemia. Rats, however, are deficient in coronary collateral vessels[14]. Therefore the opening up of collateral vessels is probably not crucial. An alternative mechanism may involve the autonomic nervous system. The sympathetic nervous system is an arrhythmogenic factor in the early ventricular arrhythmia of myocardial infarction[17]. Catecholamine depletion has been suggested to protect against myocardial ischemia induced arrhythmia[18]. Recently, it has been reported that brief ischemia has protective effects against autonomic denervation following acute myocardial infarction[19]. Thus, further studies should address the relationship between the autonomic nervous system and the protective effects of ischemic preconditioning. A third mechanism may involve metabolic factors, including extracellular potassium[20),21], cyclic AMP[22], glucose[23], lactate[24], and free fatty acid[25]. These metabolic factors are reported to play an important role in arrhythmogenesis during the acute phase of myocardial infarction. Preceding ischemia and/or reperfusion may influence these metabolic factors. Further work must be done to elicit their role in the protective effect of ischemic preconditioning. A fourth mechanism may involve the so-called endogenous antiarrhythmic substances. Adenosine and prostaglandins are released from the ischemic region during the early phase of ischemia[8),10]. Both administered endogenous antiarrhythmic substances[8),9),11),12] and enhancing endogenous antiarrhythmic substances[9] are reported to suppress early phase arrhythmia. In the present study, an antiarrhythmic effect was successfully obtained without the administration of any substance.

This could be an evidence of endogenous antiarrhythmic substances.

The severity of reperfusion arrhythmias is influenced by the duration of the preceding ischemia[26]. This finding indicates that the accumulation and/or generation of arrhythmogenic substances may play an important role in initiating reperfusion arrhythmia. In this study, we found that 3 min occlusion had the most potent protective effect against ventricular arrhythmias induced by the following coronary occlusion, without the generation of severe reperfusion arrhythmias. This finding may provide a new method for treating acute phase ventricular arrhythmia complicated with acute myocardial infarction, thus preventing sudden cardiac death.

In conclusion, a brief episode of ischemia has protective effects against early phase arrhythmias following a second episode of myocardial ischemia in rats. Although the mechanism underlying this protective effect is still unclear, this finding may help in the understanding of verntricular arrhythmia variability in acute myocardial infarction.

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