原 著

Isoflurane Preserves ATP in Stunned Canine Heart without Depending on the Reduction in Cardiac Work

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Abstract

Sustained dysfunction of myocardial contractility following short periods of coronary artery occlusion and reperfusion is termed "stunned myocardium". Isoflurane may improve the recovery of regional myocardial contractility in stunned myocardium. The purpose of the present study was to determine whether: 1) isoflurane improves the regional myocardial contractility, 2) isoflurane prevents depletion of high energy phosphates (HEP); and 3) the relief in cardiac work during isoflurane anesthesia contributes to the preservation of the HEP metabolism in stunned myocardium in an acute canine model. The left anterior descending coronary artery (LAD) was occluded for 15 min and then reperfused for 60 min under isoflurane (1.5 % end-tidal concentration) anesthesia. Regional myocardial contraction was evaluated by ultrasonic segment shortening (SS), and postsystolic shortening (PSS) measurements. In another group, mongrel dogs were allocated into three groups: Controls, anesthetized with urethane and chloralose; the ISO group, given isoflurane before ischemia; and the ISOc group, whose heart rate and mean blood pressure were controlled to approximately match baseline values. Full thickness samples of myocardium were obtained from the reperfused area (supplied by the LAD) and the non-ischemic area (supplied by the left circumflex coronary artery). The levels of adenosine monophosphate, adenosine diphosphate, adenosine triphosphate (ATP), creatine phosphate, and lactate in the endocardial portion of the myocardium were measured. After 60 min of reperfusion, percent SS was 67.7±6.6 % vs 23.9±9.4 %, isoflurane and control, respectively; PSS was 10.5±4.6 % isoflurane vs 40.5±8.8 % control. Although ATP of the reperfused area in the control group showed significantly lower levels 60 min after reperfusion, the ISO and ISOc groups preserved significantly higher levels of ATP than the control group. In conclusion, after a brief period (15 min) of myocardial ischemia followed by 60 min reperfusion, isoflurane improved the regional myocardial contractility, and resulted in higher myocardial ATP than the control groups even when the cardiac work was restored to near baseline values. The relief in cardiac work plays only a minor role in the ATP-sparing effect of isoflurane.

Key words : Stunned Myocardium, Isoflurane, Contractility, ATP

Introduction

A brief period of ischemia that do not produce myocardial necrosis can result in persistent contractile dysfunction; termed "stunned myocardium"[1]. Depletion of myocardial high energy phosphate (ATP) levels has been postulated as one of the causes of this sustained myocardial dysfunction[2]~

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[4]; during ischemia, ATP is degraded to metabolites such as adenosine, hypoxanthine and inosine, which readily diffuse across cell membranes and are therefore washed out of the myocardium into the coronary sinus at the time of reperfusion[5]. The loss of these precursors from the reperfused myocardium limits the resynthesis pathway and is believed to account, in part, for the prolonged ATP depletion observed after reperfusion.

Although isoflurane has been used prevalently in cardiac or noncardiac surgery with ischemic heart disease, the cardioprotective effects of isoflurane are still debated. Davis and Sadi[6] reported that isoflurane reduced the size of myocardial necrosis after coronary occlusion in dog. In contrast, isoflurane is reported to exacerbate the myocardial dysfunction in dogs with critical coronary artery stenoses[7] and may cause "coronary steal" in ischemic heart[8].

Furthermore, it is not clear whether isoflurane acts as cardioprotective on myocardial contractility involving mechanism in stunned myocardium. Halothane and isoflurane were reported to accelerate postischemic recovery in stunned myocardium when inhaled before ischemia[9,10] or depress contraction when inhaled after ischemia[11]. The purpose of the present study was to determine whether: 1) isoflurane improves the regional myocardial contractility, 2) isoflurane prevents depletion of high energy phosphates (HEP); and 3) the relief in cardiac work during isoflurane anesthesia contributes to the preservation of the HEP metabolism in stunned myocardium in an acute canine model.

Materials and Methods

Surgical preparation

This study was approved by our Institutional Committee on Animal Research.

Mongrel dogs of either sex weighing between 10 and 15 kg were anesthetized with sodium thiamylal (25 mg/kg, iv). After tracheal intubation the lungs were mechanically ventilated with room air to maintain normocarbia. Anesthesia was maintained with a combination of morphine (1.5 mg/kg, iv) and a mixture of α-chloralose and urethane (45 mg: 450 mg/mℓ, 1 mℓ/kg, iv for initial dose and 0.3 mℓ/kg/hr for continuous injection) during the experiment. A 14G catheter was inserted into the aorta *via* the left carotid artery and connected to a transducer for continuous blood pressure monitoring and blood sampling. A precalibrated electromagnetic flow probe (Nihon Kohden, Japan) was placed around the ascending aorta to measure the aortic flow (AoF). A 0.5 cm segment of the left anterior descending (LAD) coronary artery was dissected free from the surrounding tissue distal to the first diagonal branch. The ECG was monitored using limb lead II and myocardial ECG to detect ischemic signs.

Myocardial segment function was measured in the regions perfused by the LAD coronary artery with a pair of piezoelectric ultrasonic crystals (Sonotek Corp., CA) inserted into the subendocardium. The tracings were recorded on an eight-channel Polygraph Recorder (Nihon Kohden). Values for regional dimensions were measured using hard copies printed at 2.5 cm/sec paper speed. End-diastolic segment length (EDL) was determined from the onset of positive dP/dt, and end-systolic segment length (ESL) was determined at maximal negative dP/dt. Diastolic minimal length (LminD) was defined as minimum segment length during diastole, and maximal segment length (Lmax) was defined as maximum segment length in an overall contraction. We used two indices to evaluate the regional myocardial contractility as follows:

Percent segment shortening (% SS) = [(EDL − ESL)/EDL] × 100

Percent postsystolic shortening (% PSS) = [(ESL − LminD)/(Lmax − LminD)] × 100

Experimental protocol

Experiment 1. Effects of isoflurane on regional myocardial contractility

Anesthesia was maintained with either 1MAC isoflurane (end-tidal concentration of 1.5 %) in the isoflurane group or continuous infusion of a combination of α-chloralose and urethane (0.3 mℓ/kg

/hr) in the control group. End-tidal concentrations of isoflurane were measured by a calibrated anesthetic agent monitor (Capnomac, Datex, Finland). After surgery was completed, the preparation was allowed to stabilize for at least 30 min. Control hemodynamics and regional contractile function were then recorded in both groups. The LAD was occluded for 15 min with hemodynamic measurements at 5, 10, and 15 min. The LAD then was reperfused, and data were collected at 5, 10, 15, 30, and 60 min of reperfusion.

Experiment 2. Effects of isoflurane on HEP

After surgery was completed, the preparation was allowed to stabilize for at least 30 min. Dogs were allocated into three groups. The control group received only continuous infusion of a combination of α-chloralose and urethane (0.3 mℓ/kg/hr). The ISO group was given 1MAC isoflurane before ischemia. The ISOc group was administered the same dose of isoflurane as the ISO group, but also, atrial pacing was used in combination with constriction of the descending aorta to maintain the heart rate and mean blood pressure at the same values as that observed in each individual before isoflurane.

Baseline values of heart rate (HR), systolic, diastolic and mean blood pressure (SBP, DBP and MBP, respectively) and aortic flow (AoF) were recorded on an eight-channel pen recorder (Polygraph, Nihon Kohden). Rate pressure products (RPP) were calculated from the above hemodynamic variables as an index of cardiac work. Thereafter, the LAD was occluded for 15 min and reperfused for 60 min. Neither intraventricular catheterization nor implantation of ultrasonic crystals was employed to minimize potential myocardial injury. Also, we did not administer any antifibrillatory agents during the experiment.

Full thickness samples of the myocardium were rapidly removed with scissors from the center of the LAD area (the myocardium supplied by the LAD coronary artery), and the LCx area (the myocardium supplied by the left circumflex coronary artery). Samplings of the myocardium were performed at 60 min after reperfusion in each group. Myocardial ischemic and reperfusion area was confirmed by the regional cyanosis and color change (cyanosis to hyperemia) of the myocardial surface. The removed pieces of myocardium were immediately pressed and frozen in clamps chilled in liquid nitrogen, so that the endocardial portion of the myocardium could be taken separately for analysis.

The endocardial samples were pulverized in mortar with a pestle precooled with liquid nitrogen and extracted with perchloric acid. The levels of adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), creatine phosphate (CP), and lactate in neutralized perchloric acid extracts were determined according to standard enzymatic procedures[12].

Statistical Analysis

All values in graphs, figures, and text are mean ± SEM. Differences between groups and within each group in hemodynamics were compared by two-way ANOVA with repeated measures and Fisher's least significant difference. Metabolic data were compared by one-way ANOVA and Fisher's least significant difference. Where appropriate, the chi-squared test was performed. A value of $p<0.05$ was considered statistically significant.

Results

Experiment 1.

A total of 23 dogs were prepared. None of the subjects in the isoflurane group suffered lifethreatening arrhythmia, while 5 in the control group died of ventricular fibrillation during occlusion or reperfusion ($p<0.05$). Finally, hemodynamic data were obtained from 9 dogs in the both groups.

The hemodynamic data are summarized in Table 1. LAD occlusion caused an increase in heart rate (HR), and it was decreased after reperfusion in the control group. Mean arterial blood pressure (MBP) transiently decreased 5 min after LAD occlusion and turned to the baseline value after reperfusion in the control group. There were significant differ-

ences in MBP between the two groups at the baseline, 5 min after ischemia, and 5, 10, 15, 30 and 60 min after reperfusion. Left ventricular dP/dt decreased significantly from the baseline value after LAD occlusion and reperfusion in the control group. Left ventricular end-diastolic pressure (LVEDP) showed no significant changes from the baseline value thruoghout the experiment in the both groups. Aortic flow (AoF) decreased from the baseline value of 1.6 to 1.5 ℓ/min after ischemia, and was not restored after reperfusion in controls, but did not change in the isoflurane group. There were no significant differences in AoF between the two groups except the baseline values.

Calculated regional myocardial contractility are summarized in Table 1. Baseline % SS was 18.8±1.3 % in the control group and 18.5±2.2 % in the isoflurane group. (n. s.) During ischemia, 15 min after LAD occlusion, % SS dropped to −18.2±20.4 % in the control group and −22.7±24.7 % in the isoflurane group from the preischemic values. Immediately after reperfusion % SS increased, but it did not reach the baseline values in either group. After 60 min of reperfusion, % SS in the isoflurane group was 67.7±6.6 % of baseline value, in contrast with 23.9±9.4 % in the control group ($p<0.05$). With LAD occlusion % PSS increased rapidly to 71.9±7.9 % in the control group and 65.8±11.7 % in the isoflurane group. After 60 min of reperfusion, % PSS in the isoflurane group reached 10.5±4.6 %, in contrast with 40.5±8.8 % in the control group ($p<0.05$).

Experiment 2.

A total of 39 dogs were studied. Three of 13 dogs in the control group and 6 of 16 dogs in the ISOc group died of ventricular fibrillation during occlusion or reeperfusion. None of the dogs in the ISO group suffered life-threatening arrhythmia. There was a significant difference on the incidence of death of arrhythmia between the ISO group and the other two groups ($p<0.05$). Finally, metabolic data were obtained from 10 dogs in the control, ISO and ISOc groups.

There were no significant differences in hemodynamics among the three groups at the baseline

Table 1. Hemodynamic Change and Regional Myocardial Contraction during and after a 15-min LAD Occlusion in Control (C) (n=9) and Isoflurane (I) (n=9) Groups.

		baseline	Occlusion (min)			Reperfusion (min)				
			5	10	15	5	10	15	30	60
HR (bpm)	C	149±9	153±9[a]	152±9	151±10	151±10	146±10	144±10	137±10[a]	131±10[a]
	I	117±4[b]	119±4[b]	121±4[b]	121±5[b]	120±5[b]	122±6	121±6	117±6	114±6
MBP (mmHg)	C	98±7	90±6[a]	91±7	92±7	95±6	94±7	95±6	98±6	100±7
	I	72±7[b]	68±8[b]	71±8[b]	70±8[b]	72±8[b]	72±8[b]	71±8[b]	69±8[b]	71±8[b]
dp/dt MAX (mmHg/s)	C	2511±278	2000±181[a]	1887±159[a]	1944±160[a]	1911±206[a]	1887±165[a]	1833±167[a]	1827±156[a]	1738±136[a]
	I	1600±197[b]	1367±191[b]	1433±180	1458±174	1438±193	1411±216	1500±225	1431±194	1420±186
LVEDP (mmHg)	C	7±1	6±2	6±1	6±1	6±1	6±2	5±1	5±1	6±1
	I	9±1	10±1	10±1	11±1[b]	10±1[b]	10±1	10±1[b]	9±1[b]	10±1[b]
AoF (l/min)	C	1.6±0.1	1.5±0.1[a]	1.4±0.1[a]	1.4±0.1[a]	1.3±0.1[a]	1.3±0.1[a]	1.3±0.1[a]	1.3±0.1[a]	1.2±0.1[a]
	I	1.1±0.1[b]	1.1±0.2	1.1±0.2	1.1±0.2	1.2±0.2	1.2±0.2	1.1±0.2	1.1±0.2	1.1±0.2
%SS (%)	C	18.8±1.3	-4.6±2.1[a]	-4.4±1.7[a]	-3.9±3.0[a]	6.3±2.3[a]	6.0±2.1[a]	6.1±1.7[a]	5.2±2.0[a]	4.8±1.9[a]
	I	18.5±2.2	-3.1±4.3[a]	-3.6±4.6[a]	-1.9±4.7[a]	11.6±2.5[a]	10.9±3.1[a]	10.1±3.1[a]	10.2±2.7[a]	13.2±2.7[ab]
%PSS (%)	C	0±0	71.9±7.9[a]	64.9±8.7[a]	69.0±9.7[a]	36.0±8.2[a]	36.6±5.7[a]	32.0±8.2[a]	36.3±7.7[a]	40.5±8.8[a]
	I	0.6±0.6	65.8±11.7[a]	67.6±11.4[a]	58.8±11.0[a]	27.3±5.9[a]	28.2±5.9[a]	29.3±6.0[a]	29.1±7.7[a]	10.5±4.6[ab]

HR=heart rate ; MBP=mean blood pressure ; dp/dt MAX=first derivative ; LVEDP=left ventricular end-diastolic pressure ; AoF=aortic flow ; %SS=percent segment shortening ; %PSS=percent postsystolic shortening.

Values are mean ± SEM. a $P<0.05$ as compared to baseline values. b $P<0.05$ as compared to control group.

values. After inhalation of 1 MAC isoflurane, significant depletions of SBP, DBP, MBP AoF and RPP were observed in the ISO group. LAD occlusion and reperfusion did not cause significant changes of HR, SBP, DBP, MBP and RPP in the control group. Though AoF decreased slightly after the LAD occlusion, it was not affected by reperfusion in the control group.

Myocardial metabolic data are summarized in Table 2. LAD area at 60 min after LAD reperfusion showed significantly lower levels of ATP than the LCx area in all groups. ISO and ISOc showed significantly higher levels of myocardial ATP in the LAD areas than the controls at 60 min after reperfusion. Although the reperfused area showed a lower level of endocardial ATP than the non-ischemic area after reperfusion in the ISO group, it still had the highest ATP among all the groups. Myocardial ADP in the reperfused area reduced significantly in the control and ISO groups after ischemia and reperfusion. AMP in the reperfused area increased in the control and ISO groups after reperfusion; however, the ISO and ISOc group AMP levels remalned lower than that in the controls at the same time. Although CP in the reperfused area showed no significant change in the control group, it increased significantly ('overshoot') in the ISO and ISOc groups after reperfusion. Lactate levels in the reperfused area increased significantly in the control and ISO groups after reperfusion.

Discussion

The effects of anesthetics on the regional myocardial contractility of stunned myocardium have not been well defined. Warltier et al[10]. investigated the effects of halothane and isoflurane on the recoveries of regional myocardial contractility after 15 min LAD occlusion followed by 5 hr of reperfusion in chronically instrumented dogs. In their report, halothane (2% inspired) and isoflurane (2% inspired) markedly hastened the recoveries of % SS as compared with the conscious group. Fifteen minutes after reperfusion, halothane and isoflurane improved % SS to 67 % and 65 % of their respective preocclusion values, well over the 26 % recovery in conscious dogs. Two major factors may account for the apparent discrepancy of our results relative to those of Warltier. Segment shortening decreased significantly after the induction of anesthesia in both groups (from 17.7 to 10.3 % after halothane, from 17.7 to 12.4 % after isoflurane) in the Warltier study. Therefore, the relative recovery of segment shortening as compared to preocclusion values might have been overestimated in their experiment. Priebe and Foëx[7] reported that isoflurane-induced hypotension had little or no effect on regional myocardial contractility in the area supplied by the nonstenosed coronary artery,

Table 2. Effects of isoflurane on myocardial metabolism of reperfused canine myocardium after 15 min of ischemia and 60 min of reperfusion

		ATP	ADP	AMP	CP	Lactate
Control group	LAD	3.640±0.098[a]	0.874±0.040[a]	0.211±0.019[a]	3.910±0.391	5.883±0.546[ac]
(n=10)	LCx	5.000±0.096	1.224±0.088	0.136±0.010	2.252±0.387	3.162±0.229
ISO group	LAD	4.614±0.078[ab]	0.800±0.061[a]	0.141±0.004[ab]	7.090±0.794[ab]	5.761±0.579[ac]
(n=10)	LCx	5.194±0.099	1.028±0.025	0.133±0.004	3.749±0.324[b]	3.008±0.276
ISOc group	LAD	4.291±0.190[ab]	0.616±0.100	0.127±0.010[b]	6.005±0.561[ab]	3.651±0.247
(n=10)	LCx	4.753±0.081	0.970±0.174	0.155±0.019	3.151±0.437	3.774±0.297

ATP, adenosine triphosphate ; ADP, adenosine diphosphate ; AMP, adenosine monophosphate ; CP, creatine phosphate ; LAD, myocardium suplied by the LAD (ischemic and reperfused area); LCx, myocardium suplied by LCx (non-ischemic area).

Values are mean±SEM (μmoles/g wet tissue).

a $P<0.05$ compared with LCx area.

b $P<0.05$ compared with Control group.

c $P<0.05$ compared with ISOc group.

which is in agreement with our data. Second, Warltier et al[10] . also administered lidocaine intravenously at occlusion and reperfusion. We omitted lidocaine from our study as its cardioprotective effects on ischemic myocardium have been found inconsistent and its antifibrillatory property might act to stabilize the membrane in stunned myocardium.

The timing of anesthetic inhalation might be an important factor for recovery of contractile function in stunned myocardium. Both halothane and isoflurane depressed regional myocardial contractility when they were inhaled after the establishment of stunned myocardium in an open-chest canine model[11] . It is well known that similar evidence has been found for calcium antagonist which improve the regional myocardial contractility in stunned myocardium[13] . In our present study, isoflurane demonstrated not only a significant improvement of % SS but also a significant reduction of % PSS after 60 min of reperfusion. Although postsystolic shortening, an index of early diastolic dysfunction, has been shown to be a sensitive index of myocardial ischemia[14] , there have been few reports that mentioned the effects of anesthetics on postsystolic shortening in stunned myocardium. Leone et al[15] . observed that reduction of systolic shortening, increase of postsystolic shortening and systolic bulging occurred during progressive myocardial ischemia, and the opposite phenomena occurred after reperfusion. Therefore, it is clear from this study that isoflurane improves the regional myocardial contractile function in both systolic and diastolic phase in stunned myocardium. However, a significant improvement of regional myocardial contractility immediately after reperfusion has not been demonstrated, as Warltier et al[10] . reported previously.

The mechanisms of isoflurane's cardioprotective effect in stunned myocardium are poorly understood. As described in our results, preservation of ATP is the most likely explanation for the improvement of regional myocardial contractility. Isoflurane (4.1 mM) inhibited the isolated bovine cardiac myofibrillar ATPase by 22 %[16] . This effect is potentially of favorable consequence for maintaining ATP, as it lowers ATP consumption during ischemia and reperfusion. The ATP-sparing effects of verapamil during myocardial ischemia are believed to be the reason for its cardioprotective effects[17], and it is plausible that isoflurane acts in the same way. Kashimoto[18] reported that isoflurane maintained ATP in 89 % of nonischemic hearts after 12 min following 8 min of global ischemia in isolated rat heart lung preparation. His result is in accord with our findings in this study. Although ATP utilization could be increased when the RPP was forced to match its baseline value during the ischemia and reperfusion periods, there was no significant difference on ATP content in the reperfused area between ISO and ISOc groups. This is clear evidence that the relief of cardiac work does not play an important role in the ATP-sparing effects of isoflurane in stunned myocardium. We also found a higher myocardial content of creatine phosphate in the reperfused area of the ISO and ISOc groups than in nonischemic areas or than the control group. This "overshoot", which does not occur in hearts subjected to more severe injury[19], would suggest that the phosphorylation capability of mitochondria was virtually intact, given their ability to rephosphorylate creatine. Therefore, we suggest that isoflurane also has a cardioprotective effect which does not depend on the relief of cardiac work in stunned myocardium.

The antifibrillatory effects of volatile anesthetics in acute ischemia and reperfusion have previously been shown in open-chest dog[20] and isolated guinea pig heart[21] . In the present study, none of the isoflurane-anesthetized dogs died of arrhythmians whereas 36 % of control group died of reperfusion arrhythmia. The loss of control of intracellular calcium homeostasis with calcium overload plays an important role in reperfusion injury and arrhythmia[22] . Recently, it was reported that isoflurane reduced calcium influx throgh calcium channel[23] and calcium release from the sarcoplasmic reticulum[24] in cardiac cells. These effects of

isoflurane on intracellular calcium mobilization may also act as cardioprotective to calcium overload following reperfusion in stunned myocardium.

In conclusion, after a brief period (15 min) of myocardial ischemia followed by 60 min reperfusion, isoflurane improved the regional myocardial contractility, and resulted in higher myocardial ATP than the control groups even when the cardiac work was restored to near baseline values. The relief in cardiac work plays only a minor role in the ATP-sparing effect of isoflurane.

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(Circ Cont 16 : 520～526, 1995)