Title

Pharmacological preconditioning in type 2 diabetic rat hearts: the roles of mitochondrial ATP-sensitive potassium channels and the phosphatidylinositol 3-kinase-Akt pathway

Authors and Affiliation

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Abstract

Purpose: The authors examined whether olprinone, a phosphodiesterase type 3 inhibitor, or isoflurane, a volatile anesthetic, could protect the heart against myocardial infarction in type 2 diabetic rats and whether the underlying mechanisms involve protein kinase C (PKC), mitochondrial ATP-sensitive potassium (m-K_{ATP}) channels, or the phosphatidylinositol 3-kinase (PI3K)-Akt pathway.

Methods: All rats underwent 30 min of coronary artery occlusion followed by 2 h of reperfusion. Wistar rats received isoflurane or olprinone before ischemia with or without the PKC inhibitor chelerythrine (CHE), the m-K_{ATP} channel blocker 5-hydroxydecanoic acid (5HD), or the PI3K-Akt inhibitor LY294002 (LY). Goto-Kakizaki (GK) rats were randomly assigned to receive isoflurane or olprinone. In another group, GK rats received LY before the olprinone.

Results: In the Wistar rats, both isoflurane ($38 \pm 11\%$) and olprinone ($40 \pm 11\%$) reduced infarct size as compared to the control ($59 \pm 8\%$). In the GK rats, olprinone ($41 \pm 9\%$) but not isoflurane ($53 \pm 11\%$) reduced infarct size as compared to the GK control ($58 \pm 14\%$). The beneficial effects of olprinone were blocked by LY ($58 \pm 14\%$). In the Wistar rats, CHE, 5HD, and LY prevented isoflurane-induced reductions of infarct size. On the other hand, LY but not CHE or 5HD prevented olprinone-induced reductions of infarct size.

Conclusions: Olprinone but not isoflurane protects the heart against myocardial infarction in type 2 diabetic rats. The olprinone-induced cardioprotective effect is mediated by the PI3K-Akt pathway but not PKC or m-K_{ATP} channels. (245 words)

Key Words

Olprinone, isoflurane, diabetes, mitochondrial ATP-sensitive potassium channels, phosphatidylinositol 3-kinase-Akt

Introduction

       Large-scale clinical trials have shown that diabetic individuals are prone to developing ischemic heart disease [1]. In addition, a number of studies have shown that the cardioprotective effects of ischemic preconditioning (IPC) and anesthetic preconditioning (APC) are impaired in alloxan and/or streptozotocin-induced type 1 diabetic models [2-5]. Dysfunctional mitochondrial ATP-sensitive potassium (m-K_{ATP}) channels have been shown to occur in diabetic hearts and cause impairment of myocardial preconditioning [4, 6-9]. It is generally accepted that the m-K_{ATP} channel is an integral player in the signal transduction pathway of IPC [10, 11] and APC [12, 13], and the m-K_{ATP} channel is also known as a mediator that is closely related to protein kinase C (PKC) in cardioprotective mechanisms. Recent studies have demonstrated PKC to be upstream and downstream of the m-K_{ATP} channel opening [14, 15].

       Phosphodiesterase type 3 inhibitor (PDE3-I) increases the intracellular cyclic adenosine monophosphate (cAMP) level [16] and exerts a positive inotropic effect, which is not changed by diabetes [17]. Sanada et al. showed that preischemic administration of PDE3-Is reduces myocardial infarction via activation of the cAMP/PKA pathway independent of PKC [18]. However, whether such PDE3-Is-induced reductions in myocardial infarct size are attenuated by diabetes and whether the mechanisms of such reductions involve m-K_{ATP} channels are still unknown.

       We used Goto-Kakizaki (GK) rats, which are a selectively inbred model of type 2 diabetes developed from the Wistar rat. Type 2 diabetes in this rat has many similarities to the human form of the disease [19, 20], and these rats have been used extensively as a type 2 diabetic research model [21]. They appear to be a more appropriate diabetic research model because worldwide the most common form of diabetes is type 2 diabetes, the prevalence of which is steadily increasing [22]. Recent studies showed that IPC is impaired in type 2 diabetic models [9, 23, and 24]. However, whether the reductions in myocardial infarct size produced by APC are similarly attenuated is unknown.

       Tsang et al. showed that repeated IPC stimulus is necessary to achieve the threshold for cardioprotection and the critical level of Akt phosphorylation that protects the diabetic myocardium [23]. PKC, m-K_{ATP} channels, and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway mediate isoflurane-induced preconditioning in the non-diabetic rat heart [25, 26]. We have previously demonstrated that cardioprotection induced by olprinone, a PDE3-I, involves PI3K-Akt in non-diabetic rat hearts [27]. Thus, the present study was carried out to clarify whether olprinone or isoflurane could protect the heart against myocardial infarction in GK rats and whether the underlying mechanisms involve PKC, m-K_{ATP} channels, or the PI3K-Akt pathway.

Materials and Methods

All experimental procedures and protocols described in this study were approved by the Institutional Animal Care and Use Committee of the Nagasaki University School of Medicine.

Drugs

Olprinone was purchased from Eisai (Tokyo, Japan). Isoflurane was purchased from Abbott Japan (Tokyo, Japan). Chelerythrine (CHE), sodium 5-hydroxydecanoic acid (5-HD), LY294002 (LY), patent blue dye, and 2,3,5-triphenyltetrazolium chloride (TTC) were purchased from Sigma (St. Louis, MO, USA).

General Preparation

Male Wistar rats weighing between 270 and 440 g and male GK rats weighing between 260 and 400 g were anesthetized with sodium pentobarbital (a 50 mg/kg intraperitoneal bolus followed by an intravenous infusion of 10-20 mg/kg/h). The rats were adequately sedated to ensure that pedal and palpebral reflexes were absent throughout the experimental protocol. Catheters were inserted into the right jugular vein and the right carotid artery for fluid or drug administration and measurement of arterial blood pressure, respectively. After a tracheotomy had been performed, the trachea was intubated with a cannula connected to a small animal ventilator (model SAR-830 CWE, PA, USA), and the lungs were ventilated with pure oxygen. Arterial blood gas pH was maintained within a physiological range by adjusting the respiratory rate and tidal volume throughout the experiment. A left thoracotomy was performed in the fifth intercostal space, and the pericardium was opened. A 7-0 prolene ligature was placed around the proximal left anterior descending coronary artery (LAD) and vein in the area immediately below the left atrial appendage. The ends of the suture were threaded through a small plastic tube to form a snare for reversible LAD occlusion. Coronary artery occlusion was produced by clamping the snare onto the epicardial surface of the heart and was confirmed by the appearance of epicardial cyanosis. Reperfusion was achieved by loosening the snare and was verified by observing an epicardial hyperemic response. Hemodynamics were continuously monitored with a transducer (blood pressure monitor link sck-9082; Becton Dickinson, Tokyo, Japan) and an AP-641G blood pressure amplifier (Nihon-Kohden, Tokyo, Japan) and shown on a polygraph system (Nihon-Kohden).

Experimental Protocol

The experimental design used in the current investigation is illustrated in Figure 1. All rats underwent 30 min of coronary artery occlusion followed by 2 h of reperfusion.

The Wistar rats were randomly assigned to one of 12 groups. In 3 of these groups, the rats received saline (control), a 1.0% end-tidal concentration of isoflurane for 30min starting at 45 min before LAD occlusion, or an olprinone (10 μg/kg) i.v. bolus at 45 min before the LAD occlusion. In other the nine groups, the rats received a PKC inhibitor, CHE (5 mg/kg); an m-K_{ATP} channel blocker, 5-HD (10 mg/kg); or a PI3K-Akt inhibitor, LY (0.3 mg/kg) i.v. bolus at 50 min before the LAD occlusion followed by saline, isoflurane, or olprinone.

The GK rats were randomly assigned to one of 4 groups. In 3 of these groups, the rats received saline (control), isoflurane, or olprinone at the same time as the Wistar rat groups. In another group, the GK rats received an LY (0.3 mg/kg) i.v. bolus at 50 min before the LAD occlusion followed by olprinone.

The doses of CHE [25], 5-HD [25], and LY [28] were set on the basis of a previous study. Isoflurane was administered via a vaporizer (ISOTEC3, Ohmeda, Steeton, UK). The end-tidal concentrations of isoflurane were measured using an infrared gas analyzer that was calibrated with known standards before and during experimentation. Following discontinuation of the volatile anesthetic, the end-tidal concentrations of isoflurane decreased to zero before the LAD occlusion.

Blood Glucose Measurement

Blood samples were collected to measure blood glucose in the Wistar rat control group and each GK rat group. The blood glucose level was determined by the glucose oxidase method using a Glutest sensor and Glutest Ace (Sanwa Kagaku Kenkyusho, Nagoya, Japan). Samples were taken before ischemia, just after starting reperfusion, and 2 h after starting reperfusion.

Determination of Infarct Size

Myocardial infarct size was measured as previously described [25]. Briefly, at the end of each experiment, the LAD was reoccluded, and patent blue dye was administered intravenously to stain the normal region of the left ventricle (LV), and the heart was rapidly excised. LV tissue was isolated and cut into approximately 10 cross-sectional pieces of equal thickness. The nonstained LV area at risk (AAR) was separated from surrounding blue-stained LV normal zone, and both regions were separately incubated at 37°C for 15 min in 1% TTC in 0.1 M phosphate buffer adjusted to pH 7.4. The tissues were fixed overnight in 10% formaldehyde. AAR and blue-stained LV normal zone region were weighed for determination AAR / LV. TTC stains living tissue a deep red color, but necrotic tissue is TTC-negative and appears white within the AAR slices. Each slice was scanned at 1200 dpi with a commercial scanner (Canoscan LiDE 60; Canon, Japan), and infarcted and noninfarcted areas were measured using an image analysis program. Myocardial infarct size was

expressed as a percentage of the AAR.

Statistical Analysis

        Statistical analysis of hemodynamic data and blood glucose concentrations within and between groups was performed with analysis of variance for repeated measures followed by Dunnett's test. Inter-group differences in body weight, age, LV weight, AAR weight, the ratio of AAR to LV, and the ratio of infarct size to AAR were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test. Statistical significance was defined as $P < 0.05$. All values were expressed as mean ± SD. Statistical analysis was performed using SPSS 15.0 software (SPSS Japan, Tokyo, Japan) or GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

Results

There were no significant differences in body weight or age among the groups. One hundred and seventy-five rats were instrumented to obtain 157 successful myocardial infarct size experiments. Nine rats were excluded as a result of technical difficulties with the experimental preparation. Malignant ventricular arrhythmias developed in nine other rats before completion of the experiment, and these rats were excluded from further analysis.

Hemodynamics

Mean arterial pressure (MAP) decreased in the CHE-treated Wistar rat group and GK control groups during reperfusion as compared to baseline, but no significant differences in MAP were observed under baseline conditions, before or during LAD occlusion or during reperfusion as compared with Wistar control group (Table 1).

Infarct Size

LV weight, AAR weight, and the ratio of AAR to total LV mass were similar among the groups (Table 2). In the Wistar rats, both isoflurane (38 ± 11%) and olprinone (40 ± 11%) reduced infarct size as compared to the control (59 ± 8%) (Fig. 2). In the GK rats, olprinone (41 ± 9%) but not isoflurane (53 ± 11%) reduced infarct size compared to the GK control (58 ± 14%). The beneficial effect of olprinone was blocked by LY (58 ± 14%) (Fig. 2). In the Wistar rats, CHE (56 ± 10%), 5-HD (60 ± 10%), or LY (52 ± 12%) alone did not affect infarct size, but each of these inhibitors prevented the isoflurane-induced reduction of infarct size (51 ± 10%, 58 ± 9%, 54 ± 16%, respectively) (fig. 3). On the other hand, LY (54 ± 17%) but not CHE (42 ± 8%) or 5-HD (40 ± 11%) prevented the olprinone-induced reduction of infarct size (fig. 3).

Blood Glucose Measurement

The Wistar rats had normal concentrations of blood glucose at the baseline and had increased concentrations during coronary occlusion and reperfusion (table 3). The GK rats had abnormally high concentrations of blood glucose at the baseline and further increased concentrations during coronary occlusion and reperfusion. There were no significant differences in blood glucose concentration among the GK rat groups at any measured point.

Discussion

　　The major results of the current study are that olprinone protects both diabetic and non-diabetic hearts against ischemia-reperfusion injury, however, a 1.0% end-tidal concentration of isoflurane exerts a cardioprotective effect in non-diabetic hearts but not in type 2 diabetic hearts. Isoflurane-induced preconditioning in non-diabetic hearts is dependent on PKC, m-K_{ATP} channels, and PI3K-Akt, whereas olprinone-induced cardioprotection is mediated by PI3K-Akt independently of PKC and m-K_{ATP} channels.

　　The present results show that isoflurane-induced preconditioning is attenuated in type 2 diabetic rat hearts, confirming previous reports. Tanaka et al. showed that isoflurane-induced preconditioning is impaired in alloxan and streptozotocin-induced type 1 diabetic dogs and infarct size is directly related to blood glucose concentration [5]. Hyperglycemia alone, independent of diabetes, has also been shown to prevent isoflurane-induced preconditioning [29]. The present results demonstrate that in contrast to isoflurane, olprinone protects hearts against ischemia-reperfusion injury in type 2 diabetic rats to the same extent as in non-diabetic rats and that its protective effect is reversed by PI3K-Akt inhibition. PDEI induces cardioprotection through the elevation of the level of cAMP and PKA activation independent of PKC [30]. This transient pre-ischemic activation of PKA inhibits Rho-kinase [18]. The Rho-kinase inhibition activates the phosphorylation of Akt [31]. Our previous study showed that the activation of the PI3K-Akt pathway plays an important role in olprinone-induced cardioprotection [27]. It is likely that olprinone-induced Akt phosphorylation is preserved even in the diabetic myocardium. Tsang et al. reported that three cycles of IPC but not less reduced infarction in GK rats and that the effect was commensurate with significant Akt phosphorylation after three cycles of IPC [23]. They suggested that repeated IPC stimuli are needed to achieve the threshold for cardioprotection and a critical level of Akt phosphorylation is necessary to protect the diabetic myocardium. Thus, the effect of olprinone is comparable to the effects of repeated IPC stimuli in terms of Akt phosphorylation.

　　PKC, m-K_{ATP} channels, and the phosphatidylinositol 3-kinase (PI3K)-Akt pathway mediate IPC and isoflurane-induced preconditioning [25, 26, and 32]. The present results also show that the PKC inhibitor CHE, the m-K_{ATP} channel blocker 5-HD, and the PI3K-Akt inhibitor LY, prevent isoflurane-induced reductions of infarct size in non-diabetic hearts. Furthermore, our results demonstrate for the first time that olprinone-induced cardioprotection is mediated by PI3K-Akt independent of PKC and m-K_{ATP} channels. Kersten et al. showed that the preconditioning evoked by the m-K_{ATP} channel opener, diazoxide, is blocked by diabetes and hyperglycemia, and the authors assumed that the blockage of m-K_{ATP} channels is the underlying mechanism of the lack of protection induced by volatile anesthetics in type 1 diabetics and acutely hyperglycemic animals [6]. Thus, it is

likely that olprinone-induced cardioprotection is mediated by a pathway independent of m-K_{ATP} channels and that this pathway is not attenuated by diabetes. Based on these results, new cardioprotective strategies that are independent of the m-K_{ATP} pathway would be effective for diabetic patients.

The dose of olprinone was set on the basis of the clinical loading dose and our preliminary study, which showed that pretreatment with 10 μg/kg but not 3 μg/kg olprinone reduces infarct size (data not shown). Previous reports have demonstrated that lower but not higher concentrations of isoflurane-induced preconditioning are attenuated by diabetes and hyperglycemia and suggested that isoflurane stimulates but glucose inhibits m-K_{ATP} channel activity [5, 29]. In the present study, a 1.0% end-tidal concentration of isoflurane exerted a cardioprotective effect in non-diabetic but not in type 2 diabetic hearts. The preischemic inhalation of a 1.0% end-tidal concentration of isoflurane and pretreatment with 10 μg/kg olprinone produced similar reductions in infarct size, suggesting that this concentration of isoflurane was appropriate for our experimental model.

There was no difference in infarct size between the Wistar rat and GK rat control groups. It is unclear as to whether hearts from diabetic animal models are more sensitive or less sensitive to ischemia reperfusion injury. It was reported that hearts from type-2 diabetic rats suffer from larger infarct sizes after ischemia reperfusion injury than those from non-diabetic rats [9], whereas Tsang et al. [23] and Kristiansen et al. [24] reported smaller infarct sizes in diabetic hearts compared with non-diabetic hearts after ischemia-reperfusion. In the majority of human studies, both type 1 and type 2 diabetes increased the susceptibility of the heart to ischemia-reperfusion injury as shown by the worse outcome of diabetic patients after acute coronary syndromes [33]. In animal studies, differences in protocol or species might have caused the discrepancies in the results.

In the CHE-treated Wistar rat group and GK control groups, MAP was significantly reduced by the end of reperfusion as compared to baseline. Decrease in MAP during reperfusion period probably due to the pump function reduction, so the possibility of affecting infarct size could not be denied. However, no significant differences in MAP were observed under baseline conditions, before or during LAD occlusion or during reperfusion as compared with Wistar control group. And Sato et al. [34] reported that coronary autoregulation was observed in the pressure range 50–100 mmHg in Wistar rats. We also evaluated the relationship between MAP at the end of reperfusion and infarct size/AAR with linear regression analysis, and no correlation was found (r = 0.15, P = 0.08). Thus it is likely that decrease in MAP in the CHE-treated Wistar rat group and GK control groups has little effect on infarct size in this study.

In summary, the current investigation indicates that preischemic administration of olprinone exerts cardioprotective effects independent of m-K_{ATP} channels and PKC, and that these

protective effects are preserved in type 2 diabetic rats.

Footnotes

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Figure Legends

Fig. 1. A schematic illustration of the experimental protocols. WISTAR = Wistar rat; GK = Goto-Kakizaki rat; CON = control; ISO = isoflurane; OLP = olprinone; CHE = chelerythrine; 5HD = 5-Hydroxydecanoic acid; LY = LY294002.

Fig. 2. Myocardial infarct size expressed as a percentage of the left ventricular area at risk in Wistar or Goto-Kakizaki rats receiving saline, isoflurane, or olprinone. WISTAR = Wistar rat; GK = Goto-Kakizaki rat; CON = control; ISO = isoflurane; OLP = olprinone; LY = LY294002. Data are mean ± SD. F value obtained from these data was 7.627. *Significantly ($P < 0.05$) different from WISTAR CON. †Significantly ($P < 0.05$) different from GK CON. ‡Significantly ($P < 0.05$) different between GK OLP and GK OLP+LY.

Fig. 3. Myocardial infarct size expressed as a percentage of the left ventricular area at risk in Wistar rats receiving saline, isoflurane, or olprinone in the presence or absence of each inhibitor. WISTAR = Wistar rat; CON = control; ISO = isoflurane; OLP = olprinone; CHE = chelerythrine; 5HD = 5-Hydroxydecanoic acid; LY = LY294002. Data are mean ± SD. F value obtained from these data was 5.293. *Significantly ($P < 0.05$) different from WISTAR CON. †Significantly ($P < 0.05$) different from WISTAR ISO. ‡Significantly ($P < 0.05$) different from WISTAR OLP.
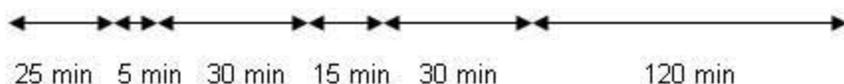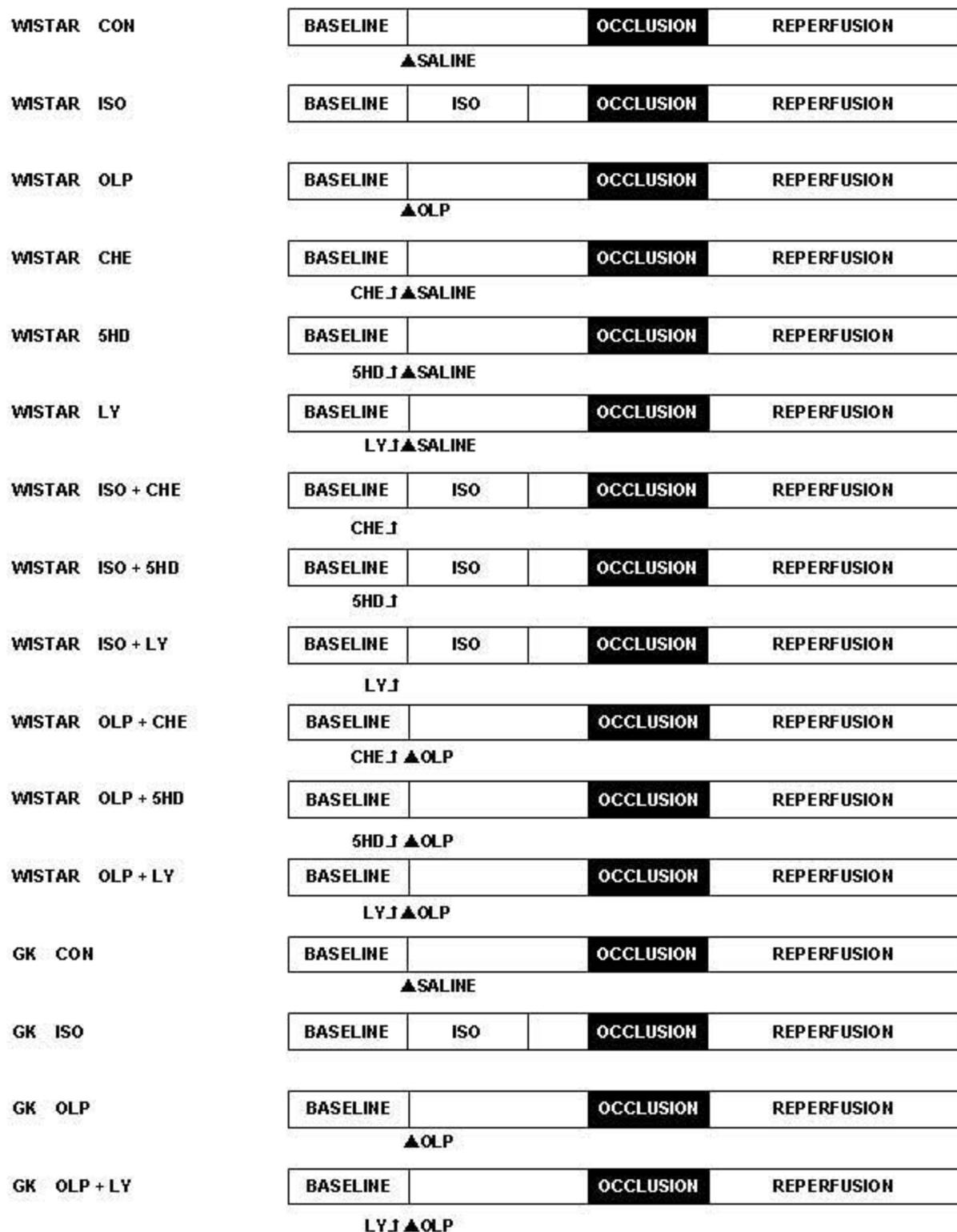
Figure 1

WISTAR CON	BASELINE		OCCLUSION	REPERFUSION
	▲SALINE			

WISTAR ISO	BASELINE	ISO	OCCLUSION	REPERFUSION

WISTAR OLP	BASELINE		OCCLUSION	REPERFUSION
	▲OLP			

WISTAR CHE	BASELINE		OCCLUSION	REPERFUSION
	CHE↥ ▲SALINE			

WISTAR 5HD	BASELINE		OCCLUSION	REPERFUSION
	5HD↥ ▲SALINE			

WISTAR LY	BASELINE		OCCLUSION	REPERFUSION
	LY↥▲SALINE			

WISTAR ISO + CHE	BASELINE	ISO	OCCLUSION	REPERFUSION
	CHE↥			

WISTAR ISO + 5HD	BASELINE	ISO	OCCLUSION	REPERFUSION
	5HD↥			

WISTAR ISO + LY	BASELINE	ISO	OCCLUSION	REPERFUSION
	LY↥			

WISTAR OLP + CHE	BASELINE		OCCLUSION	REPERFUSION
	CHE↥ ▲OLP			

WISTAR OLP + 5HD	BASELINE		OCCLUSION	REPERFUSION
	5HD↥ ▲OLP			

WISTAR OLP + LY	BASELINE		OCCLUSION	REPERFUSION
	LY↥▲OLP			

GK CON	BASELINE		OCCLUSION	REPERFUSION
	▲SALINE			

GK ISO	BASELINE	ISO	OCCLUSION	REPERFUSION

GK OLP	BASELINE		OCCLUSION	REPERFUSION
	▲OLP			

GK OLP + LY	BASELINE		OCCLUSION	REPERFUSION
	LY↥▲OLP			

25 min 5 min 30 min 15 min 30 min 120 min

Figure 2

Figure 3

Table 1. Systemic Hemodynamics

	Baseline	Intervention 15 min	Preocclusion	Occlusion 30 min	Reperfusion	
					1 hr	2 hr
MBP (mmHg)						
WISTAR CON	117 ± 14	112 ± 9	103 ± 11	118 ± 16	119 ± 27	111 ± 34
WISTAR ISO	116 ± 19	87 ± 35∗	111 ± 18	119 ± 15	131 ± 11	124 ± 15
WISTAR OLP	115 ± 13	98 ± 17∗	105 ± 15	111 ± 18	113 ± 18	119 ± 19
WISTAR CHE	128 ± 18	127 ± 22	110 ± 17∗	107 ± 28∗	109 ± 15∗	101 ± 16∗
WISTAR 5HD	138± 9	136 ± 18	122 ± 32	119 ± 25	119 ± 13	100 ± 38
WISTAR LY	103 ± 26	102 ± 28	98 ± 30	93 ± 29	99 ± 21	94 ± 26
WISTAR ISO + CHE	120 ± 27	118 ± 14	119 ± 15	107 ± 26	110 ± 28	106 ± 28
WISTAR ISO + 5HD	130 ± 10	122 ± 15	116 ± 13	124 ± 25	126 ± 27	125 ± 28
WISTAR ISO + LY	117 ± 18	101 ± 25	116 ± 18	119 ± 20	128 ± 14	123± 20
WISTAR OLP + CHE	126 ± 14	125 ± 16	116 ± 17	115 ± 16	112 ± 19	117 ± 29
WISTAR OLP + 5HD	121 ± 19	125 ± 10	132 ± 19	128 ± 26	120 ± 30	119 ± 28
WISTAR OLP + LY	120 ± 17	130 ± 7	126 ± 9	120 ± 12	119 ± 24	120 ± 28
GK CON	109 ± 25	112 ± 24	117 ± 30	96 ± 11	91 ± 11	74 ± 25∗
GK ISO	114 ± 27	110 ± 24	132 ± 24	123 ± 18	119 ± 18	113 ± 16
GK OLP	99 ± 22	107 ± 16	111 ± 16	103 ± 12	104 ± 14	94 ± 24
GK OLP + LY	107 ± 11	121 ± 11	109 ± 19	109 ± 13	113 ± 21	102 ± 24

Data are mean ± SD.

MAP = mean arterial pressure; WISTAR = Wistar rat; GK = Goto-Kakizaki rat; CON = control; ISO = isoflurane; OLP = olprinone; CHE = chelerythrine; 5HD = 5-Hydroxydecanoic acid; LY = LY294002.

*Significantly ($P < 0.05$) different from baseline.

Table 2. Left-Ventricular Area at Risk

	Number	Area at Risk/Left Ventricle (%)
WISTAR CON	13	58 ± 9
WISTAR ISO	12	55 ± 5
WISTAR OLP	11	60 ± 8
WISTAR CHE	6	51 ± 6
WISTAR 5HD	9	60 ± 10
WISTAR LY	8	52 ± 12
WISTAR ISO + CHE	9	49 ± 11
WISTAR ISO + 5HD	8	58 ± 9
WISTAR ISO + LY	9	53 ± 8
WISTAR OLP + CHE	9	50 ± 8
WISTAR OLP + 5HD	9	56 ± 5
WISTAR OLP + LY	9	54 ± 6
GK CON	12	55 ± 9
GK ISO	8	52 ± 9
GK OLP	8	54 ± 5
GK OLP + LY	8	49 ± 7

Data are mean ± SD.

WISTAR = Wistar rat; GK = Goto-Kakizaki rat; CON = control; ISO = isoflurane; OLP = olprinone; CHE = chelerythrine; 5HD = 5-Hydroxydecanoic acid; LY = LY294002.

Table 3. Blood Glucose Concentrations (mg/dl)

	Baseline	Occlusion 30 min.	Reperfusion 2 hr.
WISTAR CON	114 ± 63	146 ± 69∗	142 ± 96∗
GK ISO	311 ± 145†	441± 136∗†	445 ± 115∗†
GK OLP	284 ± 83†	390 ± 147∗†	448 ± 137∗†
GK OLP + LY	271 ± 100†	414 ± 77∗†	500 ± 89∗†
GK CON	258 ± 45†	413 ± 92∗†	449 ± 135∗†

Data are mean ± SD.

WISTAR = Wistar rat; GK = Goto-Kakizaki rat; CON = control; ISO = isoflurane; OLP = olprinone; LY = LY294002. *Significantly ($P < 0.05$) different from baseline. †Significantly ($P < 0.05$) different from WISTAR CON.