K_{ATP} channel blocker does not abolish the protective effect of NHE1 inhibition against ischaemia/reperfusion in aged myocardium

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Abstract

Background and objective—Aging is associated with an increase in myocardial susceptibility to ischaemia/reperfusion (I/R) injury. Na^+/H^+ exchange (NHE) inhibition and anaesthetic preconditioning (APC) are shown to protect myocardium from I/R injury. We set out to investigate if NHE inhibition can induce protection against I/R injury and whether K_{ATP} channel inhibition can enhance this effect in aged rat myocardium.

Methods—Hearts from 24-month old rats were assigned to 4 groups: (1) Control group; (2) APC group perfused with 2.5% sevoflurane before ischemia; (3) HOE group perfused with (3-methylsulfonyl-4-piperidinobenzoyl) guanidine methanesulfonate (HOE-694) prior to ischaemia; (4) HOE+5HD group perfused with both HOE and 5-hydroxydecanoic acid before ischaemia. We measured intracellular Na^+ and Ca^{++} to quantitate the severity of myocardial injury.

Results—Both intracellular Na^+ and Ca^{++} were significantly increased at the end of ischaemia and both were attenuated by NHE inhibition. Intracellular Na^+ was 134±12 mEq/kg/dry weight in control group and 55±7 in HOE group (p<0.05). Intracellular Ca^{++} was 1764±142 nM in control group and 694±213 in HOE group (p<0.05). Infarct size was measured at 28±4% in control group vs. 17±2% in HOE group (p<0.05). High-energy phosphates and myocardial function were better preserved in HOE group compared to control (p<0.05). The beneficial effects of HOE on myocardial preservation was not blocked by 5HD nor were there any differences between APC and control groups.

Conclusions—NHE inhibition was effective in protecting myocardium from I/R injury in aged rats whereas APC was not. 5HD failed to block the protective effect of NHE inhibition.

Keywords

aged; anaesthetic preconditioning; NHE inhibition; myocardium; ischaemic/reperfusion injury

Introduction

As the population ages, more and more elderly patients will present for surgical procedures. Aging is associated with an increase in myocardial susceptibility to ischaemia and a decrease in post-ischemic recovery of function. Many investigators have observed a decreased capacity of the aged myocardium to tolerate an ischaemic/hypoxic stress in both animal models and in humans [1,2]. Due to the important implications for managing patient risk from cardiac complications and for the development of specific intraoperative cardioprotective therapies

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that minimize the risk of cardiac injury, studies have focused on both inhibition of Na^+/H^+ exchange 1 (NHE1) and volatile anaesthetic preconditioning (APC) to protect myocardium from ischaemia/reperfusion (I/R) injury [3-5]. While ischaemic preconditioning (IPC) has been shown to have a strong cardioprotective effect against I/R injury, its protective effect may be significantly attenuated in aged hearts [6,7]. Anaesthetic preconditioning shares similar benefits to IPC in protecting the heart from I/R injury including the observation that APC is also less effective against I/R injury in aged hearts [8,9]. Several studies have focused on the affect of aging on the ability of NHE inhibition and APC to protect the myocardium against I/R injury and found that NHE inhibition and APC are both effective in protecting myocardium from I/R injury in young animals [8-11]. The APC has failed to protect aged myocardium from I/R injury [8]. In the present study we set out to investigate the effect of NHE1 inhibition on myocardial protection against I/R injury in aged rat myocardium and to determine if mitochondrial K_{ATP} channel inhibition can modulate this protection.

Methods

The study protocol was approved by the Animal Care Committee of the University of California, Davis (Davis, California, USA) and all experiments were conducted in accordance with "The Guide for Care and Use of Laboratory Animals" (NIH publication vol.25 no.28, revised 1996) and policies of the University of California, Davis.

Preparation of Isolated Hearts

Hearts were obtained from male Fischer 344 rats of age 22-24 months (NIA, Maryland, USA). A total of 72 rats were used in this study. Anaesthesia was first obtained with an intra-peritoneal injection of sodium thiopental (50-75mg/kg) along with 1,000 units of heparin. Thiopentone was chosen for initial anaesthesia since this drug has been shown not to influence preconditioning [8]. Once no response to tail-clamp was obtained, the heart was excised via thoracotomy and placed in an iced solution of Krebs-Henselet buffer (KHB). The aorta was quickly cannulated and aerobically perfused with KHB on a non-recirculating Langendorff apparatus at a perfusion pressure of 90 ± 10 mmHg. The concentration of KHB reagents were (in mmoles/L): NaCl 127, KCl 4.7, $MgCl_2$ 1.25, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 10. The perfusate was continuously oxygenated with a 95% O_2 / 5% CO_2 at $37\pm0.5°$ C.

Experimental Design and Groups

Six rats from each group were randomly selected. Control group was perfused for a 45-60 minute baseline interval before initiating 25 minutes of global ischemia followed by 60 minutes of reperfusion. The HOE group was perfused with NHE1 inhibitor, HOE 694 [(3-methylsulfonyl-4-piperidinobenzoyl) guanidine methanesulfonate](10 uM), for 10 minutes prior to 25 minutes of ischemia and followed by 60 minute of reperfusion. The APC group was perfused with sevoflurane (2.5%) for 10 minutes and followed by a 10-minute washout period prior to 25 minutes of global ischaemia, and finally 60 minutes of reperfusion. The HOE+5HD group was perfused with both HOE and 100 uM 5-hydroxydecanoic acid (Sigma, St. Louis, MO) for 10 minutes prior to 25 minutes of ischaemia and followed by 60 minute of reperfusion. The sevoflurane was delivered at 2.5% to the gas mixture via a standard Sevotec5 variable bypass vaporizer (Datex-Ohmeda, Milwaukee, WI, USA) at a final concentration of 0.4 ± 0.02 mM. Global ischaemia was induced by shutting off all flow of the KHB perfusate to the heart [8].

NMR spectroscopy

^{23}Na, ^{19}F, and ^{31}P nuclear magnetic resonance (NMR) were used to measure intracellular Na^+ (Na^+_i), intracellular Ca^{++} ($[Ca^{++}]_i$,), and intracellular pH (pH_i) and high energy phosphates, respectively [8]. In order to measure Na^+_i, 7.5 mM dysprosium

triethylenetetraminehexaacetic acid (DyTTHA) was substituted iso-osmotically for NaCl in the perfusate and Ca^{++} was added to reach a perfusate concentration of 2.5 mM as measured by Ca^{++} electrode. In order to measure $[Ca^{++}]_i$, hearts were perfused for 30-40 minutes prior to the control interval with perfusate containing the acetoxymethyl ester of 5F-1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (FBAPTA) at 2.5 μM. FBAPTA was then washed out of the extracellular space with control solution for 15 min before measurement of $[Ca^{++}]_i$. ^{23}Na, ^{19}F and ^{31}P experiments were conducted using a Bruker AMX400 spectrometer (Bruker, Rheinstetten, Germany). ^{23}Na, ^{19}F, and ^{31}P spectra were generated from the summed free induction decays of 1000, 1500, and 148 excitation pulses (90°, 45° and 60°) using 2K, 2K, and 4K word data files and ±4000, ±5000, and ±4000 Hz sweep widths, respectively. For all nuclei, data files were collected over 5 minute intervals. Because the NMR signal intensity reflects the time average for the interval over which data are collected, data are represented in time as corresponding to the midpoint of the appropriate 5 acquisition interval. All three nuclei were measured in separate hearts.

Na^+_i in mEq/kg dry weight was calculated from the calibrated area under the unshifted peak of the ^{23}Na spectra after subtracting out the extracellular peak. At the end of the experiment, hearts were weighed wet and dried to constant weight (at least 48 hr) at 65°C to determine dry weight. $[Ca^{++}]_i$ in nmoles/liter cell water was calculated as the product of the ratio of the areas of the Ca^{++}-bound and Ca^{++}-free peaks in the FBAPTA spectrum and the 500 nM Ca-FBAPTA dissociation constant. Intracellular pH was determined from the chemical shift of the inorganic phosphate (Pi) resonance [with reference to control phosphocreatine (PCr)] calibrated at 37° C. High-energy phosphates are reported as percent of control peak height.

Haemodynamic measurements

To measure left ventricular pressures, a latex balloon was filled with water and connected to a pressure transducer (Medex, Dublin, CA, USA) via a piece of PE40 tubing. The balloon was inserted into the left ventricle via the left atrial appendage through the mitral valve. The balloon volume was adjusted during the equilibration period to yield a left ventricular end diastolic pressure (LVEDP) of 3-5 mmHg. Pressures were recorded onto a computer using Powerlab (ADInstruments, Colorado Springs, CO, USA). The haemodynamic measurements included LVEDP and left ventricular developed pressure (LVDP) was defined as left ventricular end systolic pressure (LVESP) – LVEDP [8].

Creatine Kinase Analysis

The run-off from the coronary sinus was collected for the first 10 minutes of reperfusion. This was placed in aliquots and stored at -80° C until analysis could take place. The amount of CK was determined using CK kit from Sigma Diagnostics (Sigma Diagnostics, St. Louis, MO, USA) and a Shimadzu UV-VIS recording photospectrometer (Shimadzu, Columbia, MD, USA). Units are expressed as IU/ gram of dry weight [8].

Determination of Infarct Size

At the end of reperfusion, the hearts were quickly taken down from the Langendorff apparatus and sliced into 2 mm sections. The sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTC) staining solution and placed in a 37°C incubator for 20 minutes. Non-infarcted myocardium stains a bright red that is caused by reduction of TTC by dehydrogenases present in viable tissue. After 20 minutes, the sections were rinsed off with water and placed on transparent petri dishes. They were then scanned into a computer with Adobe Photoshop software (Adobe, San Jose, CA, USA). Standard computer planometric analysis, using NIH image 1.62 (National Institutes of Health, MD, USA), was used to determine infarct area. Infarct size was expressed by dividing the necrotic area by the total slice area of myocardium to obtain the percent necrosis [8].

Statistical Analysis

The animals were randomly assigned to each group. Data presented are mean ± SEM. Differences in data between groups were analyzed using Analysis of Variance. Two-tailed Student-Neuman-Keuls post-test was used if the Analysis of Variance was significant. A value of *P* < 0.05 was considered statistically significant.

Results

Our results in Figure 1 demonstrated that I/R caused significant myocardial injury. The infarct size was 28±4% in control group, and 31±3% in APC group which showed no statistical difference between the two groups (p>0.05). After treating the hearts with NHE inhibitor, the infarct size decreased to 17±2%. The mitochondrial K_{ATP} channel inhibitor, 5HD, did not change the myocardial protective effect of the NHE inhibition, and the infarct size in this group was 17±1% (p>0.05 compared to the HOE group). But, the infarct sizes in both groups were significantly smaller than that of the control and APC groups (p<0.05). In Figure 2, myocardial CK release (IU/gram dry weight) was measured during reperfusion. I/R caused significant CK release during reperfusion (325±66 in first 10 minutes in control group and 321±20 in APC group). There were no statistical differences between the two groups (p>0.05). After treated with HOE, the CK release was significantly decreased (86±50) compare to control (p<0.05) and 5HD did not alter the effect of NHE1 inhibition. The CK release in HOE+5HD group was 92±38 and there were no statistical differences compare to the HOE group (p>0.05).

Average haemodynamic variables during the pre-ischemic period are presented in Table 1. There were no significant differences in pre-ischemic haemodynamic variables among all the 4 groups. Haemodynamic measurements following 60 minutes of reperfusion are also presented in Table 1. Figure 3 demonstrated that there were no significant differences in LVDP (mmHg) between control and APC hearts (20±6.6 vs. 18±6.7, p>0.05) during reperfusion. There was a significant difference between the HOE treated hearts (54±10) and the control hearts (p<0.05), and 5HD did not block the effect of NHE1 inhibition on myocardial protection (58±12, p>0.05) at the end of reperfusion.

The Na_i^+ (mEq/kg dry weight) increased from baseline 24±4 prior to ischaemia to 134±12 (p<0.05) at the end of ischaemia and recovered to 27±2 at the end of reperfusion (Fig.4). APC had no effect on the Na_i^+ during I/R compared to control group (P>0.05). The Na_i^+ rose from 25±1 to 139±14 at the end of ischaemia and returned to 28±2 at the end of reperfusion (Fig. 4). The ischaemia induced increase of Na_i^+ was significantly blocked by adding NHE1 inhibitor prior to ischaemia (55±7 in HOE group vs. 134±12 in control group, p<0.05) at the end of ischaemia. Although Mitochondrial K_{ATP} channel inhibitor 5HD partially blocked Na effect of HOE during ischemia (72±2), it did not block the protective effects of HOE in aged hearts during reperfusion which was significantly lower than control heart (p<0.05).

Figure 5 in this study demonstrated that ischaemia caused increases in $[Ca^{++}]_i$ during I/R in aged myocardium and APC did not attenuate the increases in $[Ca^{++}]_i$. Compare to the control hearts, $[Ca^{++}]_i$ levels were significantly lower in the HOE group at the end of ischaemia (1764 ±142 vs. 694±213 nM, p<0.05) and reperfusion (1905±178 vs. 595±118 nM, p<0.05). After adding 5-HD to the HOE treated hearts, the $[Ca^{++}]_i$ levels were not different between the two groups (449±117 at the end of ischaemia and 490±67 at the end of reperfusion, p>0.05).

In Table 1, we demonstrated that there was a significant decrease of ATP during ischaemia from baseline of 103±5% to 14±3% at the end of ischaemia (p<0.05) and there were no significant difference between control and APC hearts (17±7%, p>0.05). Pi, an ATP breakdown product, is significantly increased at the end of ischaemia (1082±143%, p<0.05) in control group and was 832±91 in APC group, but did not reach statistical difference (p>0.05).

NHE1 inhibition significantly decreased the consumption of ATP to $41\pm5\%$ at the end of ischaemia ($p<0.05$). In the HOE+5HD group, the ATP was $33\pm3\%$ at the end of ischaemia which is lower than HOE group, but, it was still statistically higher than control group ($p<0.05$). Pi in HOE group was $744\pm21\%$ ($p<0.05$) and was $630\pm37\%$ after treated with mitochondrial K_{ATP} channel inhibitor which were significantly lower than control heart ($p<0.05$), but there were no statistical differences between HOE and HOE+5HD groups ($p>0.05$).

Ischaemia caused anaerobic metabolism resulting in a significant decrease in pH_i during ischaemia period (5.89 ± 0.02) (Table 1). Acute APC did not prevent the decrease in pH_i during ischaemia (5.86 ± 0.02, $p>0.05$ compared to control). After treating with NHE1 inhibitor prior to ischaemia, the decrease in pH_i was significantly less (6.13 ± 0.05, $p<0.05$, Table 1). As also shown in Figure 4, the mitochondrial K_{ATP} channel inhibitor 5HD did alter the pH_i in NHE1 inhibitor treated hearts (5.91 ± 0.04, $p<0.05$ compared to HOE group). At the end of the 60 minute reperfusion, the pH_i all 4 groups were recovered to the same level.

Discussion

In the present study, NHE1 inhibition limited severity of myocardial injury and myocardium functional recovery following ischaemia and reperfusion by limiting the increase in Na^+_i and $[Ca^{++}]_i$, preserving high-energy phosphates and decreasing CK release and infarct size. However, inhibition of mitochondrial K_{ATP} channel did not modify the myocardial protective effect induced by NHE1 inhibition in aged rats. Over the past 30 years, several experimental interventions have been shown to protect the myocardium from I/R injury. The two areas that have gained most attention are NHE1 inhibition and myocardial preconditioning. NHE is the pivotal membrane protein in the maintenance of intracellular pH and ion homeostasis. The key step in NHE1 inhibition is by inhibition of Na^+ entry into the cell thereby preventing subsequent cellular Ca^{++} overload that in turn is mediated through inhibition of Na^+-coupled Ca^{++} influx. Intracellular Ca^{++} overload is identified as a major factor associated with irreversible cell damage. In contrast, mitochondrial K_{ATP} channel activation is proposed as the primary mediator for the protective mechanism of myocardial preconditioning [5,10,12-18]. Nonetheless, there is considerable interest in the possible interactions between NHE1 inhibition and K_{ATP} activation that may possibly enhance myocardial protection when both mechanisms are activated at the same time [19,20].

The selective and potent NHE1 inhibitor, HOE 694, a compound similar to cariporide (HOE 642) used in clinical trials [5], provides cardioprotective in various experimental models. It was shown to reduce infarct size, improve myocardial function, decrease the incidence of arrhythmias, and attenuate of apoptosis following ischaemia and reperfusion [15,21].

As the aging process continues, metabolic and structural differences begin to develop in senescent myocardium [11]. Several investigators have reported that the hearts of senescent animals are less tolerant to I/R injury when compared to young adult animals. These reports provided evidence of diminished anti-oxidative capability, decreased ATP content, and increased intracellular calcium in senescent hearts following I/R injury when compared to young adult hearts [8,11,22,23]. We asked the question whether NHE1 inhibition could provide the same or similar protection against I/R injury in senescent myocardium that was observed in previous studies in young adult hearts. Our results demonstrated that even in senescent myocardium, NHE1 inhibition confers cardioprotection by limiting the ischaemia induced intracellular H^+ production, Na^+ influx, and Ca^{++} overload and further leads to significant recovery of both systolic (LVDP) and diastolic (LVEDP) functions during reperfusion as demonstrated in newborn and young animal models by inhibition of NHE1 [12,15,21,24]. There is also other evidence that Na^+_i overload can also independently cause mitochondrial damage [25] as increased Na^+_i during ischaemia induced depolarization of the mitochondrial

membrane potential and depression of a mitochondrial state 3 respiration, which directly impaired the cells abilities to generate ATP. Our findings are also consistent with this observation as our results showed a limited increase in Na^+_i and an improvement in ATP levels at the end of ischaemia in the HOE group.

The myocardial protective effect of APC in I/R injury is well supported in several studies [4, 8,26]. The proposed mechanism for this effect was activation of the K_{ATP} channel particularly in the mitochondrial membrane by the anaesthetic agent [18,27]. As a result volatile anaesthetics have been investigated as pharmacological agents for myocardial preconditioning against I/R injury in both newborn and young adults in animal studies with proven benefit [8,16]. In contrast, clinical studies conducted in patients undergoing coronary artery bypass graft (CABG) surgery yielded both positive and negative results [28-30]. As the majority of patients undergoing CABG are older, this raises the question of the efficacy of preconditioning in aged heart. Other studies, together with our current and previous studies in isolated perfused rat heart model, have shown that both APC and IPC failed to induce cardio-protection in the senescent myocardium [6-8,31]. The observed metabolic and structural differences in the aged myocardium could be one of the explanations for these results. For example, in one recent published study, the authors demonstrated that the aged myocardium had an elevated (ROS) baseline level compared to young adult hearts; the ROS level failed to further increase in response to I/R stimulation [31].

As both myocardial preconditioning and NHE1 inhibition exert potent myocardial protective effect against I/R injury [12,13,32-34], we asked the question whether there is a relationship between the NHE inhibition and the K_{ATP} channel activation that could confer additive protection to myocardium following I/R. NHE is known to exist not only in plasmatic cell membrane, but also in the inner mitochondrial membrane [35]. This may be important as during ischaemia the normal mechanism of H^+ extrusion associated with electron transport stops, subsequently the mitochondrial matrix becomes overloaded with H^+, which results in collapse of the H^+ gradient across the mitochondrial membrane leading to the cessation of ATP synthesis [36,37]. In a recent study, the authors suggested that NHE inhibitors could exert their anti-ischaemic effect at the mitochondrial level by first favouring the opening of mitochondrial K_{ATP} channel and mitochondrial K_{ATP} channel blocker abolished the protective effect of NHE inhibition [38], and second, delaying mitochondrial matrix acidification to inhibit ATP depletion during ischaemia [37]. In a related study, the authors found that pH-mediated arteriolar dilation caused by NHE inhibition was attenuated by K_{ATP} channel blockade [39]. Our results were consistent with the previous observation as the mitochondrial K_{ATP} channel blocker, 5HD, blocked the effect of HOE 694 on intracellular pH during ischaemia when used in conjunction with the NHE1 inhibitor. However, although the intracellular acidosis was greater with 5HD blockade, we did not observe any changes in the other intracellular anion concentrations and myocardial function or infarct size changes as observed by others [38].

Although evidence indicating that the protection produced by preconditioning and NHE1 inhibition occurs via different mechanisms, controversy remains concerning the relationship between those two modes of protective interventions. Principally, controversy persists in relation to the attenuation of intracellular acidosis that occurs during ischaemia that is a hallmark of preconditioning [8,13,40-42] as the use of 5HD to block preconditioning causes intracellular pH to decrease.

There are several limitations in the present study. First, the purpose of the study was to investigate whether the mitochondrial K_{ATP} channel inhibition can affect NHE1 inhibition induced protection against I/R injury in aged rat myocardium. We only used aged animals and we made no comparisons between young and old. Second, although we are fully aware that there are other pathways involved in myocardial protection, we only investigated the pathways

we outlined in this paper. Third, the NMR spectroscopy we used here can only measure one nucleus at a time; for this reason the pH_i, Na^+_i, and $[Ca^{++}]_i$ were measured in separated hearts.

Conclusion

In conclusion, the NHE1 inhibition reduced myocardial infarct size and CK release, and improved myocardial function recovery during reperfusion in aged rats. It prevented the myocardial overload of Na^+ and Ca^{++} during I/R. APC with sevoflurane did not provide myocardial protection against I/R injury in this age group. Mitochondrial K_{ATP} channel antagonist, 5-HD, did not block the beneficial effects provided by NHE1 inhibition against I/R injury in aged myocardium. These findings will assist us in future research and development against myocardial I/R injury aged population.

Acknowledgments

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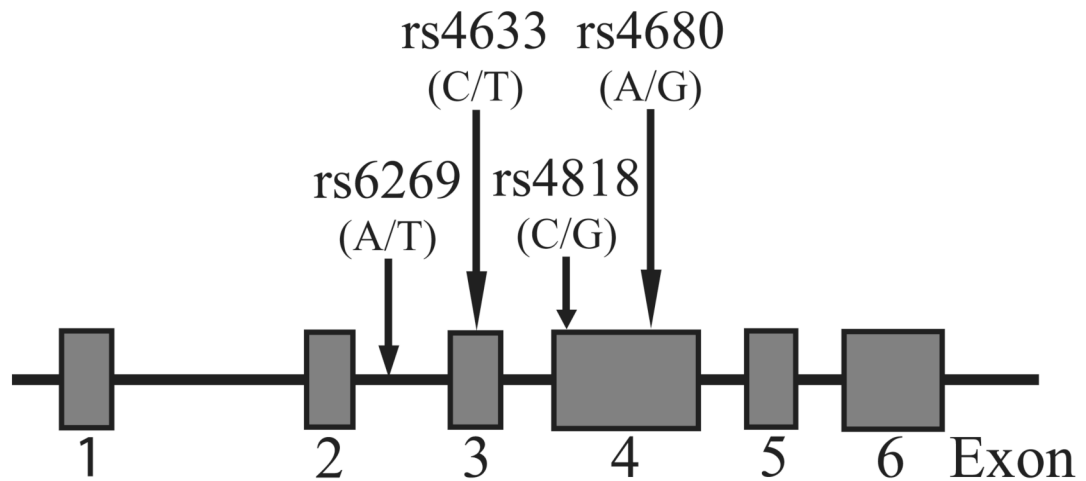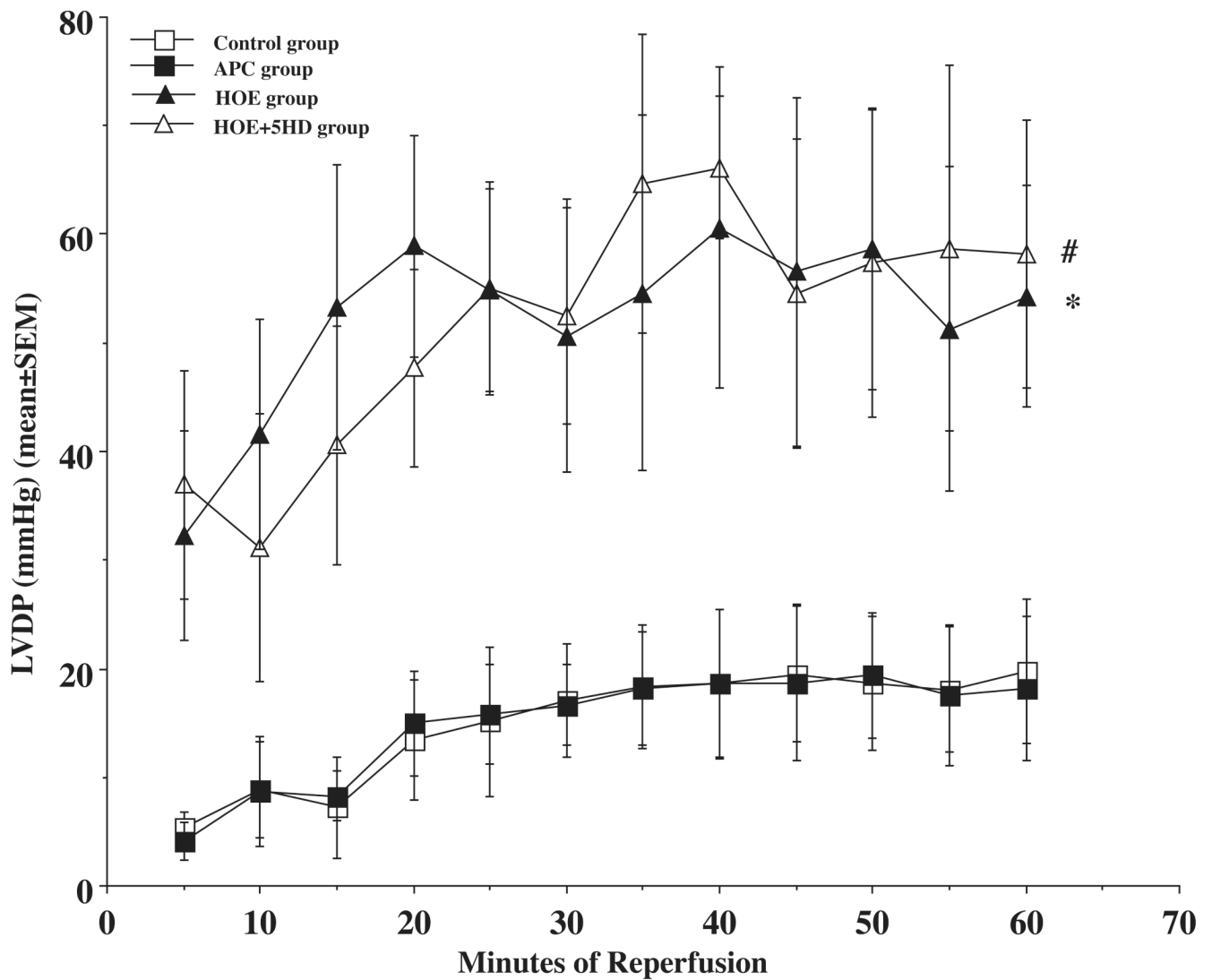
Figure 1.
Ischaemia caused myocardial infarction (control group) and volatile anaesthetic preconditioning (APC) with sevoflurane did not decrease infarct size (APC group). Na^+/H^+ exchanger inhibitor, HOE 694 (HOE group), decreased the infarct size. Mitochondrial K_{ATP} channel blocker, 5-HD, did not affect the HOE effects during reperfusion in aged rat hearts. HOE+5HD: both HOE 694 and 5-HD were added to the perfusate 10 minutes prior to ischaemia. * HOE group vs. control group, $P<0.05$; # HOE+5HD group vs. control group, $P<0.05$. N = 6 in each group. Unit: % area change.
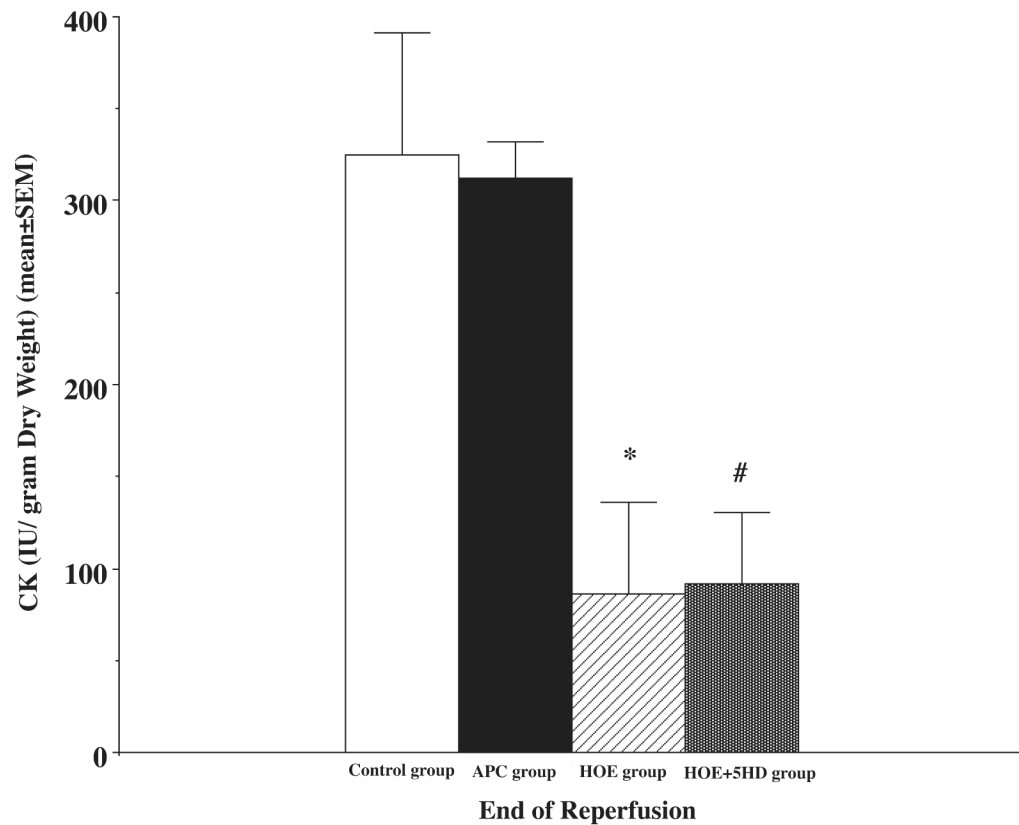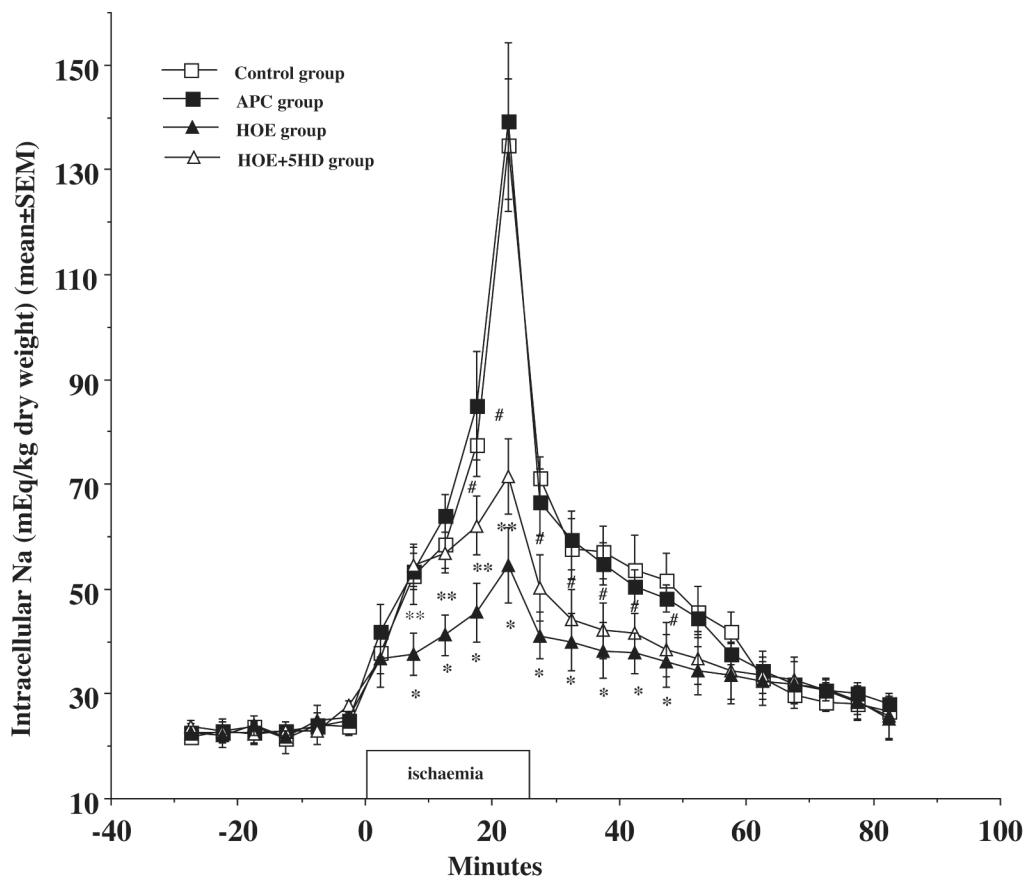
Figure 2.
Ischaemia caused a significant increase in release of myocardial creatine kinase (CK) (control group). Volatile anaesthetic preconditioning (APC) with sevoflurane did not decrease CK release (APC group). Na^+/H^+ exchanger inhibitor, HOE 694 (HOE group), limited the CK release. Mitochondrial K_{ATP} channel blocker, 5-HD, did not affect the HOE effects on CK during reperfusion in aged rat hearts. HOE+5HD: both HOE 694 and 5-HD were added to the perfusate 10 minutes prior to ischaemia. * HOE group vs. control group, $P<0.05$; # HOE+5HD group vs. control group, $P<0.05$. N = 6 in each group. Unit: IU/gram dry weight.
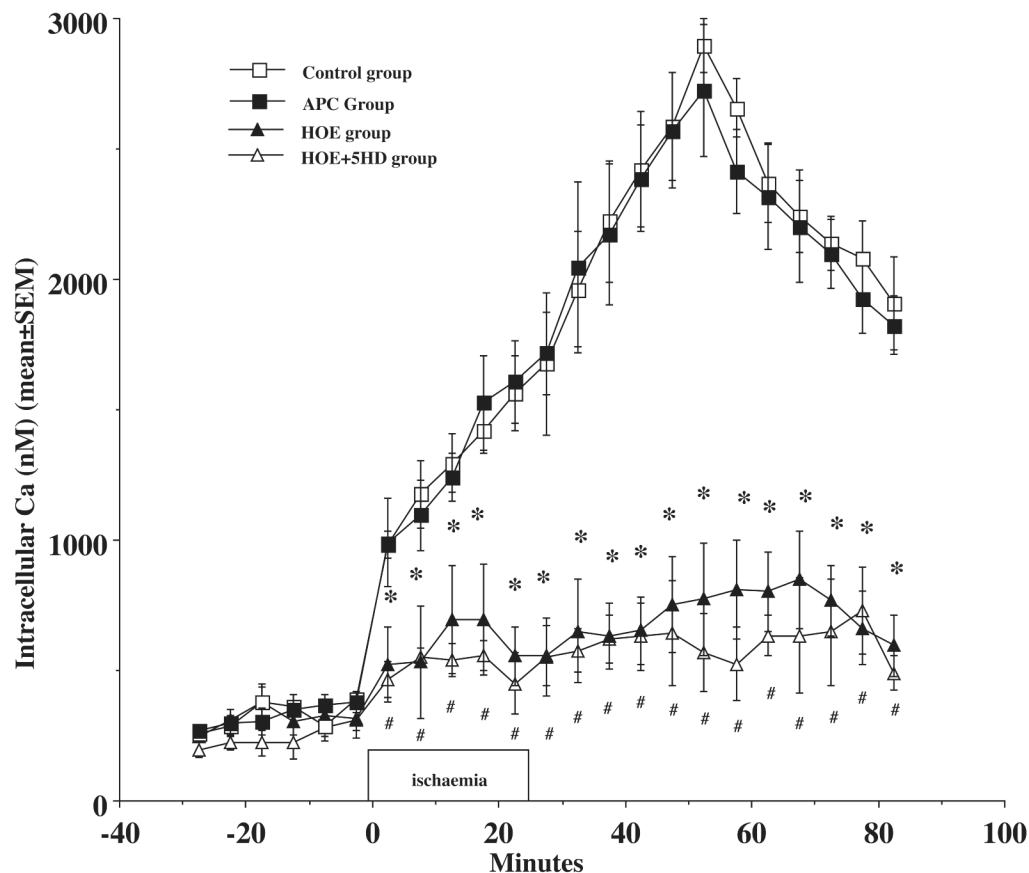
Figure 3.
During reperfusion, the left ventricular develop pressure (LVDP) only recovered to about 20% of the pre-ischaemic level in control group (open square). Volatile anaesthetic preconditioning (APC) with sevoflurane did not improve the LVDP during reperfusion (closed square). Na^+/H^+ exchanger inhibitor, HOE 694 (HOE group), improved the LVDP (closed triangle), and mitochondrial K_{ATP} channel blocker, 5-HD, did not affect the HOE effects on LVDP during reperfusion in aged rat hearts (open triangle). * HOE group vs. control group, P<0.05; # HOE +5HD group vs. control group, P<0.05. N = 6 in each group. Unit: mmHg.

Figure 4.

Ischaemia caused an increase in intracellular Na^+ (open square) and volatile anaesthetic preconditioning (APC) with sevoflurane did not limit the increase in Na^+ (closed square) during ischaemia and reperfusion. Na^+/H^+ exchanger inhibitor, HOE 694 (closed triangle) limited the increase at the end of ischaemia and reperfusion. Mitochondrial K_{ATP} channel blocker, 5-HD (open triangle) partially blocked the HOE effect during ischaemia, but not in reperfusion in aged rat hearts. * HOE vs. control, P<0.05, #HOE+5HD vs. control, P<0.05. ** HOE group vs. HOE+5HD group, P<0.05. N = 6 in each group. Unit: mEq/kg dry weight.

Figure 5.
Ischaemia caused an increase in intracellular Ca^{++} (open square) and volatile anaesthetic preconditioning (APC) with sevoflurane did not limit the increase in Ca^{++} (closed square) during ischaemia and reperfusion. Na$^+$/H$^+$ exchanger inhibitor, HOE 694 (closed triangle) limited the increase in of ischaemia and reperfusion. Mitochondrial K$_{ATP}$ channel blocker, 5HD (open triangle) did not block the HOE effect during ischaemia, and reperfusion in aged rat hearts. * HOE vs. control group; # HOE+5HD vs. control group, P<0.05. N = 6 in each group. Unit: no.

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Table 1

Results of LVEDP, LVDP, ATP, pH, PCr, and Pi before ischemia, at the end of ischemia, and at the end of reperfusion in four groups

	Control						APC					
	LVEDP	LVDP	ATP	pH	PCr	Pi	LVEDP	LVDP	ATP	pH	PCr	Pi
Before Ischemia	5±1.9	91±15	103±5	7.11±0.02	99±1	101±1	4.4±1.9	102±17	98±3	7.12±0.04	102±2	102±2
End of ischemia	N/A	N/A	14±3	5.89±0.02	6±2	1082±143	N/A	N/A	17±7	5.86±0.02	5±1	832±91
End of Reperfusion	26±7	20±6.6	23±5	6.99±0.06	50±7	146±20	21±4	18±6.7	19±4	7.03±0.03	32±6	158±7

	HOE						HOE+5HD					
	LVEDP	LVDP	ATP	pH	PCr	Pi	LVEDP	LVDP	ATP	pH	PCr	Pi
Before Ischemia	5.6±1.9	94±14	100±3	7.10±0.03	101±2	103±27	5±0.6	97±12	103±5	7.08±0.04	100±3	104±6
End of ischemia	N/A	N/A	41±5*	6.13±0.05*#	5±4	744±21*	N/A	N/A	33±3*	5.91±0.04	13±1	630±37*
End of Reperfusion	15±3*	54±10*	30±2*	7.08±0.03	35±4	164±24	14±2*	58±12*	31±5*	6.94±0.17	46±9	148±18

The units for LVEDP, LVDP data are mmHg. The units for ATP, PCr, and Pi expressed as % of baseline. Data are presented as mean±SEM.

*
APC, HOE, or HOE+5HD vs. Control,

#
HOE vs. HOE+5HD, $P < 0.05$ is considered significant. n=6 in each group.

LVEDP: left ventricular end diastolic pressure, LVDP: left ventricular develop pressure, PCr: phosphocreatine, Pi: inorganic phosphate.

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