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Isoproterenol Reduces Ischemia-Reperfusion Lung Injury Despite β-Blockade[1]

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Background. **If lungs could be retrieved from non-heart-beating donors (NHBDs), the shortage of lungs for transplantation could be alleviated. The use of lungs from NHBDs is associated with a mandatory warm ischemic interval, which results in ischemia-reperfusion injury upon reperfusion. In an earlier study, rat lungs retrieved 2-h postmortem from NHBDs had reduced capillary leak measured by filtration coefficient (Kfc) when reperfused with isoproterenol (iso), associated with an increase in lung tissue levels of cyclic AMP (cAMP). The objective was to determine if this decrease in Kfc was because of β-stimulation, or would persist despite β-blockade.**

Materials and methods. **Donor rats were treated intraperitoneally with β-blockade (propranolol or pindolol) or carrier, sacrificed, and lungs were retrieved immediately or 2 h postmortem. The lungs were reperfused with or without iso and the β-blockers in the reperfusate. Outcome measures were Kfc, wet:dry weight ratio (W/D), lung levels of adenine nucleotides and cAMP.**

Results. **Lungs retrieved immediately after death had normal Kfc and W/D. After 2 h of ischemia, Kfc and W/D were markedly elevated in controls (no drug) and lungs reperfused with β-blockers alone. Isoproterenol-reperfusion decreased Kfc and W/D significantly ($P < 0.01$) even in the presence of β-blockade. Lung cAMP levels were increased only with iso in the absence of β-blockade.**

Conclusions. **The attenuation of ischemia-reperfusion injury because of iso occurs even in the presence of β-blockade, and may not be a result of β-stimulated increased cAMP.**

Key Words: **lung ischemia-reperfusion injury; filtration coefficient; non-heart-beating lung donor; β-blockade; β-adrenoreceptor; cyclic AMP.**

INTRODUCTION

Lung transplantation is a successful therapy that palliates many patients with a variety of types of end stage lung disease, but its widespread use is limited by a lack of suitable donors [1]. If lungs could be retrieved from non-heart-beating donors (NHBDs), the critical shortage of lungs for transplantation could be alleviated. This shortage of donor lungs has prompted our laboratory to investigate the use of NHBDs in experimental models of lung transplantation [2–7]. Evidence to support the concept of NHBDs in thoracic transplantation has recently been reviewed [8].

Using an isolated perfused rat lung model (IPRLM), we have investigated the impact of increasing postmortem ischemic time on the early phase of ischemia-reperfusion injury (IRI) independent of circulating leukocytes, assessed by measuring the filtration coefficient (Kfc) using a blood free reperfusate. We demonstrated that rat lungs retrieved 1 h post-mortem from NHBDs had substantially increased Kfc and wet:dry weight ratio (W/D), ameliorated by ventilation of the cadaver with oxygen, and that rat lungs retrieved 2 h postmortem from NHBDs had very elevated Kfc and W/D ratio irrespective of cadaver ventilation [9]. Subsequent studies demonstrated reduced Kfc and W/D after reperfusion with 10 μM isoproterenol (iso), associated

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with an increase in lung cyclic AMP (cAMP) [10]. Similar benefit was seen by reperfusion with the phosphodiesterase IV inhibitor rolipram [11], or the combination of iso and rolipram [12]. The role of cAMP in the attenuation of lung IRI has been documented by other investigators using various pharmacological agents, including a cAMP analogue [13–16]. We concluded that this effect of iso was related to an increase in lung cAMP, although we did not demonstrate in increase in lung cAMP in lungs reperfused with rolipram [11].

Isoproterenol is a potent β-adrenoreceptor agonist that activates adenylate cyclase to increase intracellular cAMP. Propranolol (pro) is a non-selective β-blocker that acts as a competitive inhibitor of iso [17]. However, Tromba *et al*. have demonstrated the inactivation of potassium conductance induced by iso was not blocked by the β-blocker pro [18]. This action was mimicked by a cAMP analogue. Accordingly, it is possible that the attenuation of IRI because of iso is independent of β-adrenoreceptors. Therefore, the present study was designed to determine if the beneficial effect of iso-reperfusion on rat lungs from NHBDs is dependent on β-stimulation. We chose to employ the β-blocker pro, a competitive inhibitor of iso, and later confirmed our findings by performing additional experiments with the β-blocker pindolol.

MATERIALS AND METHODS

Challenge Test

Separate experiments established the appropriate dose of pro (Sigma Chemical Co., St. Louis, MO) and pin (ICN Biomedical Inc., Aurora, OH) to achieve β-blockade in the rat. Male Sprague-Dawley rats were anesthetized by i.p. injection of pentobarbital sodium (60 mg/kg) (Abbott Laboratories, Chicago, IL), and the trachea was cannulated. Then, β-blocked rats were treated with different doses of pro (2 mg/ml saline) or pin (0.4 mg/ml saline) i.p. The control rat (no β-blocker or iso-only group) received a similar volume of saline. After waiting for 15 min to allow time for absorption and attainment of therapeutic serum levels, the degree of β-blockade was demonstrated by i.v. administration of 0.1 mg/kg iso (Sigma Chemical Co.) administered via the penile vein in a concentration of 0.1 mg/ml saline [19]. The control rat (no drug) received a similar volume of saline. Heart rate was continuously monitored by placing electrodes through the skin of the anesthetized rats, attached to a Hewlett-Packard echocardiogram (ECG) monitor with continuous display of heart rate.

Isolated Perfused Rat Lung Model

The isolated perfused rat lung model (IPRLM) was first used to measure Kfc in dogs [20, 21] and later modified for use in rats [22, 23]. This model provides a sensitive and reproducible method with which to assess alterations in permeability of pulmonary microcirculation, the critical initial phase of lung IRI. The specific details of this preparation have been outlined previously [9, 10].

Briefly, 60 donor rats weighing 250 to 400 g were anesthetized by i.p. injection of pentobarbital sodium (60 mg/kg) (Abbott Laboratories), and the trachea was cannulated. Then, rats were treated with the β-blockers (4 mg/kg pro or 0.8 mg/kg pin) or a similar volume of saline i.p. After 15 min, a small laparotomy incision was made and 600U of heparin (Elkins-Sinn, Cherry Hill, NJ) was injected i.t. under direct vision. The rats were sacrificed with an i.t. injection of pentobarbital sodium (100 mg/kg). Cardiac arrest was documented by observation of cardiac motion transmitted through the diaphragm and by palpation. The heart-lung block was left *in situ* in an effort to simulate the NHBD's clinical situation as closely as possible.

The heart, lungs and mediastinal structures were retrieved en bloc via median sternotomy immediately after death or 2 h postmortem from non-ventilated NHBDs. Subsequently, the main pulmonary artery and the left atrium were cannulated for ventilation and perfusion in the IPRLM as described in detail in an earlier publication [10]. Perfusate was a modified Earle's balanced salt solution [containing (in mmol) 2.4 $CaCl2 \cdot 2H_2O$, 0.4 $MgSO_4$ (anhydrous), 5.4 KCl, 116 NaCl, 0.88 NaH_2PO_4 (anhydrous), 5.5 D-glucose, and 0.3 phenol red] (ICN Biomedical Inc., Aurora, OH) containing 0.21% $NaHCO_3$ and 4% bovine serum albumin (Fisher Scientific, Pittsburgh, PA) with or without 10 μM iso, 10 μM pro and 2 μM pin depending on the study conditions.

Pressure transducers (Cope Laboratories, Lakewood, CO) were positioned at the hila of the lungs, zeroed to atmospheric pressure, and calibrated with a mercury manometer. Pulmonary arterial (Ppa) and venous (Ppv) pressures were measured continuously. In addition, pulmonary airway pressure (Paw) was continuously monitored by positioning a T-tube on the inspiratory limb of the respiration plumbing. All pressure measurements and changes in weight gain were collected by Lab View SCXI Pressure Weight Acquisition System (National Instruments, Austin TX) on a Gateway personal computer.

Pulmonary capillary pressure (Ppc) was estimated by the double-occlusion technique described by Townsley [24]. Simultaneous occlusion of arterial and venous catheters results in equilibration of Ppa and Ppv to the same pressure. This equilibrated pressure equals the Ppc and also reflects the capillary pressure when the lung is not isogravimetric. Pulmonary arterial (Ra), and venous (Rv) vascular resistances and Kfc were calculated as previously described [10]. Kfc was normalized using baseline wet lung weight and expressed as ml/min/cmH_2O/100 g lung weight.

Wet-to-Dry Weight Ratios

At the end of the experiment, the right upper lobe was excised and immediately weighed, then re-weighed after being dried for 48 h in an oven kept at 60°C for wet-to-dry ratios (W/D). Pieces of the remaining right lung were snap frozen in liquid nitrogen, and stored at −80°C for later analysis.

Adenosine Monophosphate (AMP), Adenosine Diphosphate (ADP), Adenosine Triphosphate (ATP), and Cyclic AMP (cAMP) Analysis by HPLC

Frozen lung tissue samples were homogenized with ice-cold 0.6 N perchloric acid (6 ml/g tissue) using a tissue tearor (Biospec Products, Bartlesville, OK) at 30,000 rpm for 30 s. After centrifugation for 10 min at 10,000 rpm, the supernatant was removed and neutralized with cold 1M potassium phosphate dibasic (pH 12) to achieve a pH of 6.8. The supernatant was separated from precipitated salt by centrifugation for 2 min at 10,000 rpm and washed four times with five volumes of water saturated diethyl ether. After ether wash, samples were filtered through a 0.45-mm acrodisc filter (Pall Gelman, Ann Arbor, MI) and analyzed by Beckman System Gold HPLC apparatus (Beckman Instruments, Fullerton, CA). ATP and cAMP concentrations were determined by Partisil 10 Sax column and ADP, and AMP concentrations were determined by LC-18-T column (Supelco, Bellefonte, PA). There was 100 μl of each sample injected into the HPLC apparatus. A flow rate of 1.0 ml/min was used, and detection was performed by ultraviolet (UV, 254 nm) light absorbance. Chromatograms were analyzed on an IBM 486 DX 33-MHz computer with Peak Simple software (Beckman Instruments). Standard curves were made by performing serial dilutions for ATP, ADP, AMP, and cAMP (Sigma Chemical Co.) and data were corrected for lung water

content by expressing results as μmoles/g dry weight. Total adenine nucleotide levels (TAN) were defined as TAN = AMP + ADP + ATP, and are expressed as μmoles/g dry weight.

Specific Protocol

Sixty pairs of lungs were divided into 10 groups: (1) control (no drug), retrieval immediately after death (0 h control), (2) retrieval immediately after death and pro (0 h pro), (3) retrieval immediately after death and iso (0 h iso), (4) retrieval immediately after death and iso + pro (0 h iso + pro), (5) control, retrieval 2 h postmortem (2 h control), (6) retrieval 2 h postmortem and pro (2 h pro), (7) retrieval 2 h postmortem and iso alone (2 h iso), (8) retrieval 2 h postmortem and iso + pro (2 h iso + pro), (9) retrieval 2 h postmortem and pin (2 h pin), (10) retrieval 2 h postmortem and iso + pin (2 h iso + pin). In previous studies, 2 h of non-ventilated ischemia has resulted in a reproducible severe degree of IRI with reperfusion in the IPRLM manifested by significant increases in Kfc and W/D. All lungs were allowed to equilibrate, after initial reperfusion, for 15 to 20 min to achieve an isogravimetric state. Lungs that did not achieve an isogravimetric state were discarded.

Statistics

Statistical analysis was performed using ANOVA with the Fisher's *post hoc* test for multiple comparisons. All values are reported as the mean ± SEM. Differences were considered significant if $P < 0.05$.

Animal Care

All animals received humane care in compliance with the "Guide for the Care and use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

RESULTS

Challenge Test

Figure 1 illustrates the effectiveness of β-blockade on the iso-induced heart rate response. Administration of only saline had no effect on heart rate (SS). Intravenous administration of iso resulted in a sustained increase in heart rate (SI). This increase persisted beyond 10 min from iso administration. Initial i.p. administration of 4 mg/kg pro decreased heart rate (Prl). After administration of iso to animals previously given pro, heart rate increased temporarily but quickly subsided, unlike iso alone (Prl). Pindolol (0.8 mg/kg) did not decrease heart rate like pro. However, it had the same impact as pro on the effect of administration of iso (Pil).

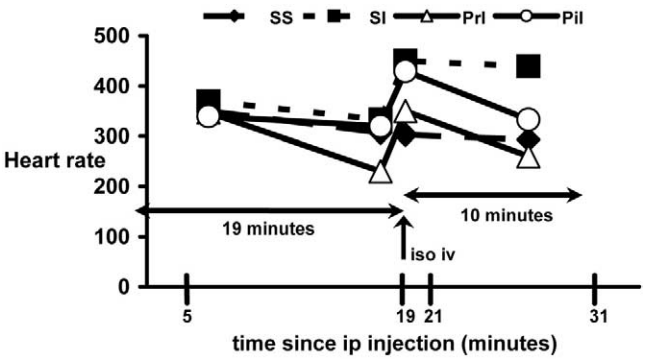

FIG. 1. The effectiveness of β-blockade on the iso-induced heart rate response (Challenge test) for each group (mean heart rate, n = 3/group). [SS; saline i.p. + saline i.v., SI; saline i.p. + iso i.v., PrI; 4 mg/kg pro i.p. + iso i.v., PiI; 0.8 mg/kg pin i.p. + iso i.v.].

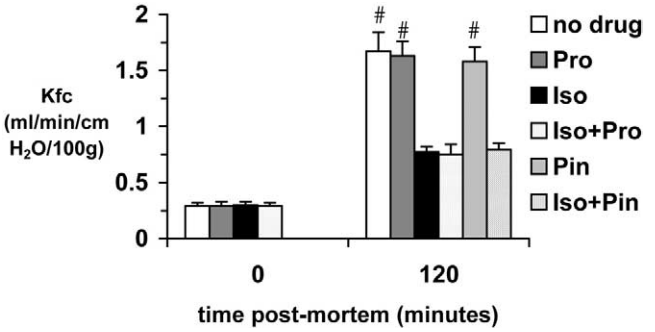

FIG. 2. Kfc for each group (n = 6/group). All values are given as the mean ± SEM. # $P < 0.01$ compared with iso-reperfusion groups at 2 h postmortem (2 h iso, 2 h iso + pro, 2 h iso + pin).

Kfc

Changes in Kfc are shown in Fig. 2. Lungs retrieved immediately had normal Kfc (at 0.3 ml/min/cmH_2O/100 g lung weight). In lungs retrieved 2 h postmortem, Kfc was markedly elevated in control and lungs reperfused with the β-blockers alone. Iso-reperfusion decreased Kfc significantly ($P < 0.01$) even in the presence of the β-blockers.

W/D

W/D reflects edema accumulation in lung tissue. Changes in W/D are shown in Fig. 3. Lungs retrieved immediately had normal W/D (mean 5.8). In lungs retrieved 2 h postmortem, W/D was markedly elevated in control and lungs reperfused with the β-blockers alone. Changes in W/D mirrored changes in Kfc, that is, iso-reperfusion decreased W/D significantly ($P < 0.01$) even with the β-blockers. Fig. 4 shows the relationship between Kfc and W/D for all groups in this

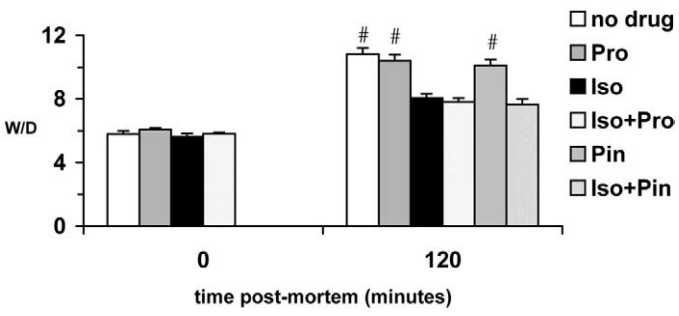

FIG. 3. W/D for each group (n = 6/group). All values are given as the mean ± SEM. # $P < 0.01$ compared with iso-reperfusion groups at 2-h postmortem (2 h iso, 2 h iso + pro, 2 h iso + pin).

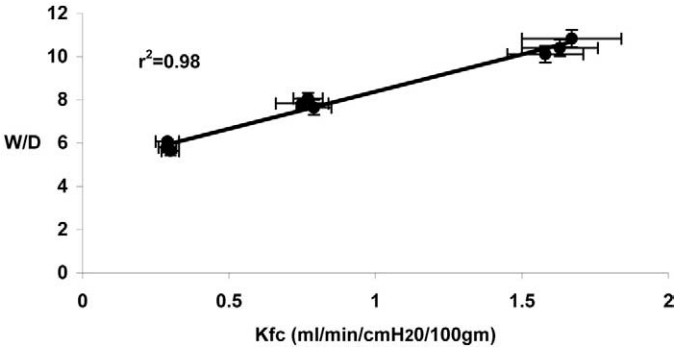

FIG. 4. Correlation between Kfc and W/D for 10 groups of rats studied (±SEM).

study, documenting the ability of changes in Kfc to reflect subsequent development of lung edema.

Hemodynamics

Hemodynamic data are shown in Table 1. In lungs retrieved 2-h postmortem, Ppa and Ra were elevated in lungs reperfused with the β-blockers alone.

cAMP and Adenine Nucleotides

cAMP levels are shown in Fig. 5. In lungs retrieved 2 h postmortem, iso-reperfusion increased cAMP significantly ($P < 0.01$) but this was prevented by β-blockade with pro. Lung levels of adenine nucleotides are shown in Table 2. TAN levels were determined by the formula: TAN = ATP + ADP + AMP. There were no significant differences between groups irrespective of β-blockade with pro or β-stimulation with iso.

DISCUSSION

If lungs could be retrieved from NHBDs at substantial intervals after circulatory arrest and death, the critical shortage of lungs for transplantation could be alleviated. We demonstrated feasibility of lung transplantation from NHBDs using a canine single lung transplant model [2]. Ventilation of the donor with oxygen appeared to be of some benefit and allowed for retrieval from the donor 4-h postmortem [3, 6]. In a rat model, we found that half of parenchymal lung cells were viable 4 h after circulatory arrest, but when dead rats were ventilated with 100% O_2, 90% of the lung cells remained viable [25] with preservation of ultrastructural integrity [26] and maintenance of adenine nucleotide levels [27]. These studies confirm that the lung is viable for a significant time interval after circulatory arrest.

Although viable, we have shown that there is considerable early capillary leak when rat lungs from NHBDs are reperfused using an IPRLM [9]. The IPRLM is a sensitive measure of capillary function in the early phase of IRI. Our method differs from other isolated perfused rat lung models [13, 14, 28] because lungs were left *in situ* after the animal was sacrificed, instead of the usual protocol of harvesting, immediate perfusing, and then making the lung ischemic. Our model more closely resembles the clinical scenario of the NHBD, which is our interest.

Using this model, we first studied the effect of oxygen ventilation of the non-perfused NHBD lung for varying time intervals after death [9]. Lungs retrieved up to 1-h postmortem had normal Kfc if donors were ventilated with O_2. But the Kfc increased despite O_2-ventilation after 2 h of postmortem ischemia. In contrast, non-ventilated donors had significantly increased Kfc values after 30 min of postmortem ischemia. However, there was no significant difference between both groups in pulmonary parenchymal cell viability. Accordingly, if IRI can be attenuated and the integrity of the pulmonary endothelial surface is main-

TABLE 1

Hemodynamic Data

Group/Item	Ppa, mmHg	Ppv, mmHg	Paw, mmHg	Ra, mmHg · min/ml	Rv, mmHg · min/ml
0 h control (I)	6.82 ± 0.26	0.50 ± 0.12	3.65 ± 0.42	0.40 ± 0.06	0.28 ± 0.04
0 h pro (II)	7.58 ± 0.52	0.46 ± 0.09	4.66 ± 0.61	0.40 ± 0.05	0.37 ± 0.05
0 h iso (III)	6.47 ± 0.51	0.51 ± 0.06	3.90 ± 0.66	0.39 ± 0.05	0.28 ± 0.04
0 h iso + pro (IV)	7.69 ± 0.70	0.41 ± 0.07	3.96 ± 0.75	0.45 ± 0.05	0.30 ± 0.02
2 h control (V)	7.22 ± 0.58	0.45 ± 0.16	5.77 ± 0.80	0.43 ± 0.05	0.33 ± 0.05
2 h pro (VI)	9.24 ± 0.92*	0.43 ± 0.07	5.10 ± 0.83	0.52 ± 0.07†	0.45 ± 0.07
2 h iso (VII)	6.77 ± 0.30	0.36 ± 0.04	4.07 ± 0.40	0.35 ± 0.04	0.34 ± 0.04
2 h iso + pro (VIII)	7.27 ± 0.87	0.43 ± 0.08	5.45 ± 0.72	0.46 ± 0.09	0.33 ± 0.03
2 h pin (IX)	8.04 ± 0.60	0.43 ± 0.07	5.67 ± 1.33	0.51 ± 0.04†	0.39 ± 0.06
2 h iso + pin (X)	7.02 ± 0.54	0.59 ± 0.01	4.49 ± 0.68	0.43 ± 0.03	0.32 ± 0.02

Note. mean ± SEM.
* $P < 0.05$ compared to 2 h control, iso, iso + pro, iso + pin.
† $P < 0.05$ compared to 2 h NHBD without β-blocker.

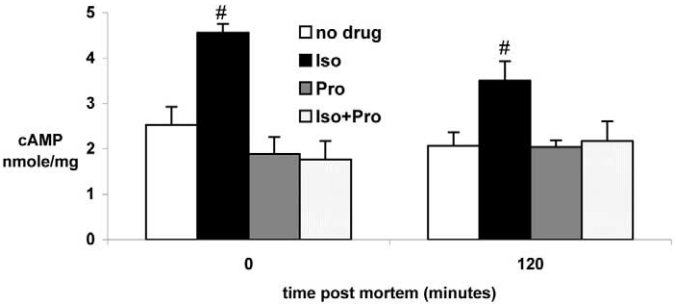

FIG. 5. Lung cAMP levels (mean ± SEM). # $P < 0.01$ compared with 2 h control, 2 h pro, and 2 h iso + pro.

tained, lungs from NHBDs might become widely available for transplant. Thus, it is important to identify strategies that will minimize capillary leak in lungs retrieved from NHBDs.

We demonstrated that lungs retrieved 2 h postmortem from NHBDs had reduced capillary leak measured by Kfc if reperfused with 10 μM iso, associated with increased lung cAMP even if donors were not ventilated with O_2 [10]. (In this earlier study, cAMP levels were erroneously reported as nmoles/g dry weight instead of μmols/g dry weight.) The role of cAMP in the attenuation of IRI has been well documented by various pharmacological agents, including cAMP analogues [13–16]. Proposed mechanisms of this effect include cAMP-mediated prevention of endothelial cell contraction, producing a reduction in the size of the inter-endothelial cell junction, or an effect on neutrophil adherence and subsequent production of capillary wall damage [14, 15, 29]. cAMP is the second messenger of β-activation [30], and several studies have demonstrated catecholamine-stimulated production of cAMP blocked by pro [29, 31, 32].

The present study was designed to determine if the effect of iso on NHBD lungs was dependent on β-stimulation. Initially, we performed a challenge test to demonstrate β-blockade and determine the maximum tolerable maximal dose of pro. Other investigators have performed similar challenge tests using iso-induced hypotension [19]. Based on this result, the investigators used an initial i.p. dose of 0.5 mg/kg pro combined with subsequent continuous infusion (0.4 mg/h) intravenously during their shock experiment. We chose to inhibit the iso-induced heart rate response because 0.5 mg/kg was inadequate to do this. Generally, almost all β-adrenoreceptors in lung vasculature and tissue are β_2-adrenoreceptors. A low dose of iso like that used in our experiment (10 μM) stimulates only β_2-adrenoreceptor, not β_1-adrenoreceptor [33]. However, it is possible that some contribution of β_1-adrenoreceptor was occurring in our experiment. To block not only β_2-adrenoreceptor but also β_1-adrenoreceptor, we used eight-fold dose of pro (4 mg/kg) compared to an earlier reported challenge test [19]. At this dose, pro itself induces hypotension like iso. This dose of pro also sometimes induced cardio-respiratory arrest. Thus, after 15 min of i.p. dwell time to allow time for absorption and attainment of therapeutic serum levels, we performed heparinization and sacrifice expeditiously. We chose to administer heparin in donors before death so that we could evaluate the impact of ischemia alone on capillary function. In the clinical scenario of lung retrieval from NHBDs, it might be possible to administer heparin to potential donors after death by intracardiac injection of heparin and a brief period of cardiopulmonary resuscitation.

We added the same mol/l (10 μM) pro as iso in the perfusion solution in the IPRLM based on prior experiments [18], and on the knowledge that pro is a competitive inhibitor of iso. We found that β-blockade with pro had no effect on Kfc in normal lungs or in lungs retrieved 2 h after death. The loading dose of pro used in the donor before sacrifice and with reperfusion in iso-reperfused lungs was sufficient to block iso-induced increases in cAMP in the lung, but did not alter the benefit of iso-reperfusion on both decreased Kfc and edema fluid accumulation (W/D). Because of the high dose of pro used before sacrifice and its concomitant administration in the IPRLM, as well as the results of our challenge test, we are confident that recipients of pro were adequately β-blocked. Because we previously demonstrated a relationship between TAN and lung

TABLE 2

Adenine Nucleotides

Group/Item	ATP	ADP	AMP	TAN
0 h control (I)	2.25 ± 0.71	2.76 ± 0.37	2.95 ± 1.19	7.96 ± 0.30
0 h pro (II)	3.36 ± 1.76	3.14 ± 0.97	3.00 ± 0.57	9.50 ± 2.34
0 h iso (III)	2.07 ± 0.59	3.13 ± 0.80	3.99 ± 0.78	9.19 ± 1.71
0 h iso + pro (IX)	2.12 ± 0.83	2.27 ± 0.49	4.30 ± 1.45	8.69 ± 1.16
2 h control (V)	3.92 ± 1.19	3.41 ± 1.01	2.81 ± 0.91	10.24 ± 2.65
2 h pro (VI)	4.40 ± 1.10	2.28 ± 0.22	2.14 ± 0.35	8.82 ± 1.46
2 h iso (VII)	3.08 ± 0.66	3.45 ± 0.16	2.95 ± 1.04	9.60 ± 0.69
2 h iso + pro (VIII)	4.17 ± 0.83	1.86 ± 0.29	1.55 ± 0.22	7.58 ± 1.09

Note. mean ± SEM (μmoles/gm dry weight).

viability [27], and between TAN and Kfc in untreated lungs retrieved from NHBDs [9], we measured TAN in lungs treated with the b-blocker pro and showed that the effect of pro was not related to levels of adenine nucleotides.

To confirm the observation we made with pro, selected experiments were repeated with a different nonselective β-blocker pin. We performed the same challenge test for pin to determine an appropriate nonlethal dose. Pindolol has intrinsic sympathomimetic activity, so pin itself didn't decrease heart rate like pro. Once again, β-blockade with pin had no effect on Kfc in normal lungs or in lungs retrieved 2 h after death, but β-blockade with pin did not alter the benefit of iso-reperfusion on both decreased Kfc and edema fluid accumulation (W/D). So the attenuation of IRI because of iso occurs even in the presence of β-blockade.

In lungs retrieved 2 h postmortem, Ppa and Ra were elevated in lungs reperfused with β blockers alone. Propranolol increased Ppa and Ra more than pin, presumably because pin has intrinsic sympathomimetic activity [17].

We recognize cAMP is the second messenger of β-activation and that stimulation of β-adrenoreceptor produces cAMP [29–34]. However, our results strongly support the notion that there may be other effects of iso to ameliorate IRI in ischemic lungs. Oriowo *et al.* [35] demonstrated that β_3-adrenoreceptor agonist induced a relaxation of fragments of rat carotid artery, which is not antagonized by pro. Classical β-stimulation is not the only pathway to produce cAMP by iso. Tromba *et al.* postulated an atypical β_3-adrenoreceptor to explain their observation that β-blockade with pro failed to interfere with an iso-induced inwardly rectifying potassium conductance in Purkinje myocytes [18]. There are other reports of β_3-adrenoreceptor in bronchial [36] and vascular smooth muscle [35, 37], although Evans *et al.* failed to demonstrate β3-adrenoreceptors in rat lung [38]. However, Tamaoki *et al.* also found that the stimulation of β_3-adrenoreceptor caused bronchodilation and increased intracellular cAMP [36]. Finally, Berlan *et al.* demonstrated that iso caused a decrease in blood pressure and an increase in cutaneous blood flow even in the presence of a β-blocker. They concluded this was related to iso-stimulation of β_3-adrenoreceptor based on their other experiments using a β_3-adrenoreceptor agonist [37]. This β_3-adrenoreceptor is thought to be a reserve receptor only for extreme or stressful conditions. Accordingly, one interpretation of our data is that iso stimulated β_3-adrenoreceptors instead of classical β-adrenoreceptors that were blocked by pro or pin and attenuated IRI, but the small incremental increase of cAMP was not detectable. We surmised that this was why rolipram, a phosphodiesterase i.v. inhibitor, was effective at reducing Kfc in lungs retrieved from NHBDs although increased lung tissue levels of cAMP could not be demonstrated. Another explanation of our findings is that only a minimal amount of β-adrenoreceptor stimulation with iso is necessary to see the protective effect on IRI in this model, but this is insufficient to observe measurable increases in tissue levels of cAMP.

Vascular resistance of lungs retrieved 2 h postmortem tended to be higher in 2 h iso + pro and 2 h iso + pin than 2 h iso alone, but this difference was not significant, and was almost the same as 2 h control. But Kfc and W/D of these groups were significantly better than 2 h control. This suggests that decreased vascular resistance is not the main action for the attenuation of IRI associated with cAMP in NHBD lungs.

In conclusion, the attenuation of IRI because of reperfusion with iso occurs even in the presence of β-blockade. Thus, this effect of iso may not be because of the stimulation of classical β-adrenoreceptor. A better understanding of strategies to reduce IRI in lungs from NHBDs and the mechanism of action might lead to practical interventions that would facilitate the use of lungs from NHBDs for transplant and alleviate the critical shortage of lungs for transplant.

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