

# IRL2500, A Selective ETB Antagonist for Endothelin-Induced Vasodilatation in the Pulmonary Vascular Bed in Rats

Q Hao, A Hyman, H Lippton, F Zavisca, R Cork

## Citation

Q Hao, A Hyman, H Lippton, F Zavisca, R Cork. *IRL2500, A Selective ETB Antagonist for Endothelin-Induced Vasodilatation in the Pulmonary Vascular Bed in Rats*. The Internet Journal of Anesthesiology. 2002 Volume 7 Number 1.

## Abstract

**Introduction:** Endothelins (ET-1 and ET-2) have vasoactive effects mediated by receptors (ETA & ETB). The objective of the present study was to determine the effect of endothelins and their antagonists on the pulmonary vascular bed in vivo. To accomplish this, we employed the unique rat model and studied the pulmonary vascular beds of intact-chest animal under conditions of constant pulmonary blood flow.

**Methods:** Twenty-five rats were anesthetized with pentobarbital, and a specially designed balloon catheter was placed into rat's the pulmonary artery via the right jugular vein. The right lower lobe of the lung was perfused at constant flow ( $14 \pm 0.6$  ml/min) with blood removed from a cannulated carotid artery. The baseline pulmonary arterial pressure was  $12.8 \pm 1.1$  mmHg. In order to demonstrate vasodilator responses to testing agents, the pulmonary arterial pressure was elevated to  $34.8 \pm 1.4$  mmHg by continuous infusion of U46619, a thromboxane-like substance. The effect of various endothelin agonists and antagonists were then studied.

**Results:** Intrapulmonary arterial injections of ET-1, IRL1620 (ETB agonist) and ET-3 produced dose-dependent vasodilator responses in the pulmonary vascular bed. At a dose of 1 µg, ET-1, IRL1620 and ET-3 caused decreases in pulmonary arterial pressure of  $8.1 \pm 0.4$  mmHg,  $8.2 \pm 0.3$  mmHg and  $7.9 \pm 0.3$  mmHg respectively (all significantly different from baseline,  $n=5$ ,  $p<0.05$ ). After pretreatment with IRL2500 (ETB antagonist, 10mg/kg, i.v.), the vasodilator responses to ET-1, IRL1620 and ET-3 at the same dose were  $0.9 \pm 0.1$  mmHg,  $2.1 \pm 0.3$  mmHg and  $1.8 \pm 0.3$  mmHg respectively ( $n=5$ , not significantly differ from the baseline). This data suggests that IRL2500 can block the vasodilator responses to ET-1, IRL1620 and ET-3 in the lung. Data from a different group of animal demonstrate that the vasodilator responses to ET-1, IRL1620 and ET-3 are not altered significantly by pretreatment with BQ123 (an ETA antagonist) ( $n=5$ )

**Conclusions:** Using a new rat model, these results indicate that ET-1, IRL1620 and ET-3 dilate the pulmonary vascular bed by activation of the ETB receptor. The present data also suggest that IRL2500, as a specific ETB antagonist can be a useful pharmacological tool to study the pulmonary vascular regulation in rats. This new intact rat preparation appears to be valuable in carrying out cardiopulmonary studies in vivo.

## INTRODUCTION

Endothelin (ET) was first isolated, sequenced and cloned by Yanagisawa<sub>1</sub> in 1988. The ET isolated from supernatant of porcine aortic endothelial cells was a peptide of 21 amino acid residues with two disulfide bridges linking CYS3-CYS11. Studies by Inoue and others<sub>2,3</sub> have revealed the existence of three distinct endothelin (ET) peptides named ET-1, ET-2 and ET-3. ET receptors have been characterized and divided into three subtypes: ET<sub>A</sub> (selective for ET-1),<sub>4</sub>

ET<sub>B</sub> (equally selective for ET-1, ET-2 and ET-3)<sub>5</sub> and ET<sub>C</sub> (selective to ET-3)<sub>6</sub>. Activation of the ET<sub>A</sub> receptor is associated with pronounced vasoconstriction, whereas activation of the ET<sub>B</sub> receptor occupation is associated with vasodilatation. ET<sub>C</sub> has been identified although its physiological significance is uncertain.<sub>7</sub>

Endothelin receptors are widely distributed in many tissues and involved in numerous physiologic and pathophysiologic responses.<sub>8</sub> Discoveries of the specific agonists and

antagonists for ET receptors are essential for characterization of the responses to endothelins. BQ123 has been proven as the most specific ET<sub>A</sub> receptor antagonist.<sup>9</sup> IRL1620, a specific ET<sub>B</sub> agonist, is vasoactive in some vascular beds.<sup>10,11</sup> IRL2500, a small molecular weight compound, is highly selective at attenuating decreases in arterial pressure by ET-1 by blocking the ET<sub>B</sub> receptor.<sup>12</sup> Whether or not the pulmonary vasodilator responses to endothelins are mediated by ET<sub>B</sub> receptor are unknown. Therefore, the present study was undertaken to determine the influence of IRL2500, a specific ET<sub>B</sub> antagonist on the pulmonary vasodilator responses to ET isopeptides. We studied this problem using intact chest rats under conditions of constant pulmonary blood flow.

## MATERIALS AND METHODS

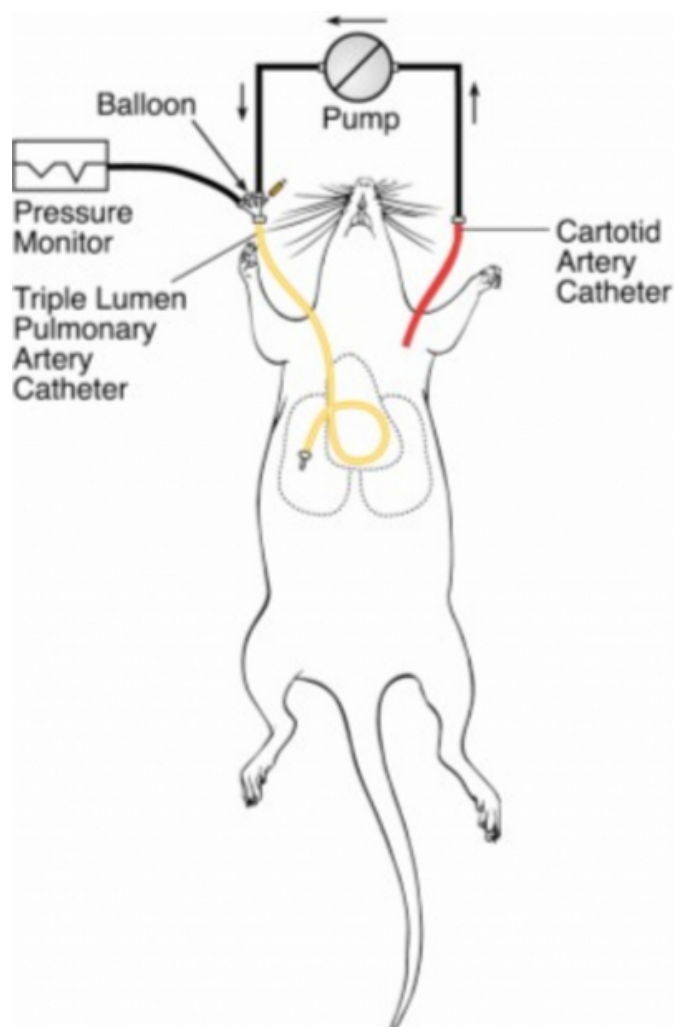
The protocol of the present study was approved by the Animal Research Committee of the Tulane University Medical Center. Male Charles River rats (260-340 gm) were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg), and allowed to breathe room air enriched with oxygen through a tracheotomy. The anesthetized animals were strapped in a supine position on a fluoroscopic table, and catheters were inserted in the femoral artery for systemic arterial pressure measurement and the femoral vein for drug administration. A specially designed triple lumen balloon catheter was used (Nu-Med, Hopkinton, N.Y.). This catheter is 145 mm in length and 1.1 mm in O.D. It had a specially curved tip to facilitate passage through the right heart and main pulmonary artery, and then into the artery supplying the right lower lobe. At the distal tip of the catheter is a pressure port through which a 0.25 mm soft tip coronary artery angioplasty guide wire is inserted. Two mm proximal to this port is a perfusion port, which permits passage of a 0.34 mm soft-tipped coronary guide wire. A plastic non-dispensable balloon is affixed to a third port just proximal to the perfusion port. When fully distended with contrast material, the balloon is 4.0 mm in diameter and 3.5 mm in length. Before introduction, this catheter curve was initially straightened with 0.45 mm straight wire in the pressure port to facilitate passage from the right jugular vein into the right atrium at the tricuspid valve. As the straight wire was removed, the natural curve permitted easy entry into the right ventricle. The catheter was then passed over a 0.25 mm soft-tipped guiding catheter to the main pulmonary artery and then into the right lower lobe artery.

Mean pressures in the right lower lobe artery and the aorta

were continuously recorded. After intravenous injection of Heparin (1000 units/kg), the balloon was then distended with radiopaque material until the lobar arterial pressure fell to pulmonary capillary wedge pressure. The distal portion of the right lower lung lobe was then perfused with blood removed from a carotid artery with an extra corporeal pump (Masterflex Quick-Load Rotary Pump Model #7021-24). The volume of extra corporeal tubing was 1.5 ml. At a perfusion rate of  $14.0 \pm 0.62$  ml/min, pressure in the perfused lobar artery approximated that in the main pulmonary artery. Since this catheter perfused approximately one-sixth of the lung by weight, this perfusion rate approximated physiologic flow for that lung area. Fig 1.

**Figure 1**

Fig 1: Experimental Preparation



In order to demonstrate the vasodilator responses the pulmonary vascular bed, the pulmonary vasomotor tone was raised by a continuous intralobar arterial infusion of U46619, a prostaglandin-like substance, at 1.5-2.5 g/min.

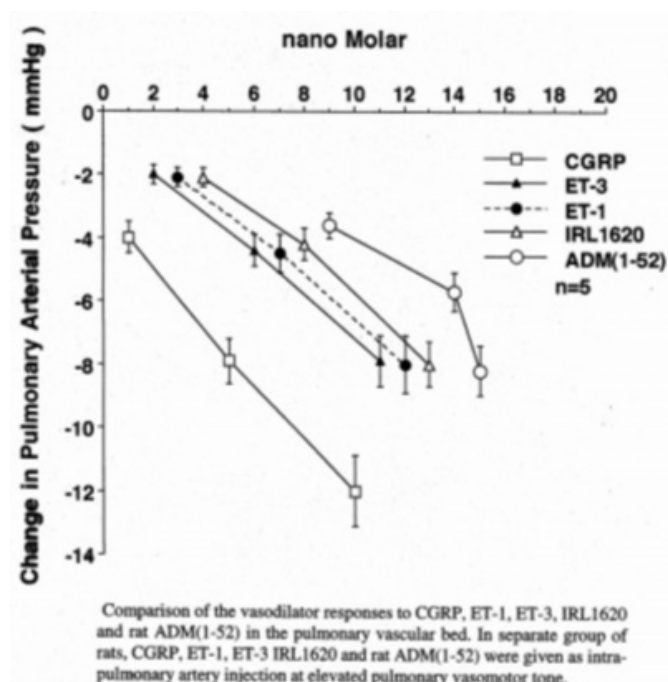
After pressures were stabilized, intralobar arterial bolus injections of testing agents were given. These agents included endothelin-1 (ET-1), IRL1620 (an ET<sub>B</sub> agonist), endothelin-3 (ET-3), as well as several other potent pulmonary vasodilators such as calcitonin-gene-related peptide (CGRP), adrenomedullin (1-52)(ADM-52), a peptide isolated from pheochromocytoma, nitroglycerin (GTN) and isoproterenol (ISO). IRL2500 (ET<sub>B</sub> antagonist, 10mg/kg) and BQ123 (ET<sub>A</sub> antagonist, 1mg/kg) were given as intravenous injections. ET-1, ET-3, IRL1620, IRL2500, ADM (1-52) and CGRP were purchased from Phoenix Pharmaceutical Inc. BQ123 was bought from RBI, GTN and ISO from Sigma Chemical Company. Data was presented as Mean  $\pm$  S.E.M. Statistical analysis was done by Student's t-test.

## RESULTS

In the intact chest rat model, vasoactive responses to endothelins were studied in the pulmonary and systemic vascular bed under the conditions of constant pulmonary blood flow. Since the pulmonary perfusion flow was kept constant throughout the experiment, the changes in the pulmonary arterial pressure directly reflected the changes in the pulmonary vascular resistance. The mean systemic arterial pressure was  $135.0 \pm 15.0$  mmHg, and did not change significantly throughout the experiments. The baseline pulmonary arterial pressure was  $12.8 \pm 1.1$  mmHg. Following continuous intrapulmonary arterial infusion of U46619, the pulmonary arterial pressure was elevated to  $34.8 \pm 1.4$  mmHg.

Fig 2 illustrates the pulmonary vasodilator responses to ET-1, ET-3, IRL1620 (ET<sub>B</sub> agonist), CGRP and rat ADM (1-52) under condition of elevated pulmonary vasomotor tone. All these compounds tested in vivo produced a dose-dependent pulmonary vasodilator response. On the molar bases, CGRP was the most potent endogenous substance, whereas, ratADM (1-52) was the least potent. The pulmonary vasodilator potency order for endothelins was ET-3 > ET-1 > IRL1620. These responses were observed in different animal groups and a limited number of injections were given to each animal to avoid desensitization of ET isopeptides. Injections of ET-1, ET-3 and IRL1620 produced little, if any, pulmonary response at basal vasomotor tone.

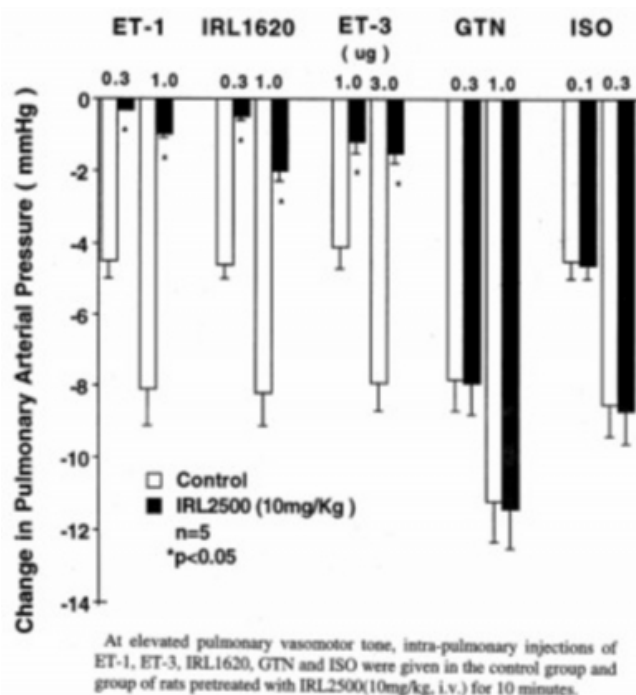
**Figure 2**



Similar to the responses to ET-1, IRL1620, ET-3, the bolus intrapulmonary arterial injections of nitroglycerin (GTN) and isopretanalol (ISO) also produced dose-dependent vasodilator responses in the lung at elevated vasomotor tone. (Fig.3). The pulmonary vasodilator responses to ET-1, IRL1620 and ET-3 were significantly reduced in the animal group pretreated with IRL2500 (ET<sub>B</sub> antagonist, 10 mg/kg, i.v.). At a dose of 1ug, ET-1, IRL1620 and ET-3 produced decreases in pulmonary arterial pressure of  $8.1 \pm 0.4$  mmHg,  $8.2 \pm 0.3$  mmHg and  $7.9 \pm 0.3$  mmHg, respectively (n=5, p<0.05 from constricted baseline). In animals pretreated with IRL2500 (ET<sub>B</sub> antagonist), the vasodilator responses to ET-1, IRL1620 and ET-3 at the same dose were  $0.9 \pm 0.1$ mmHg,  $2.1 \pm 0.3$  mmHg and  $1.8 \pm 0.3$  mmHg respectively, whereas the vasodilator responses to nitroglycerin and isopretanalol (through different mechanisms) were not changed by the pretreatment with IRL2500 (Fig. 3). Injection of IRL2500 alone did not change the control pressure in pulmonary and systemic vascular beds.

**Figure 3**

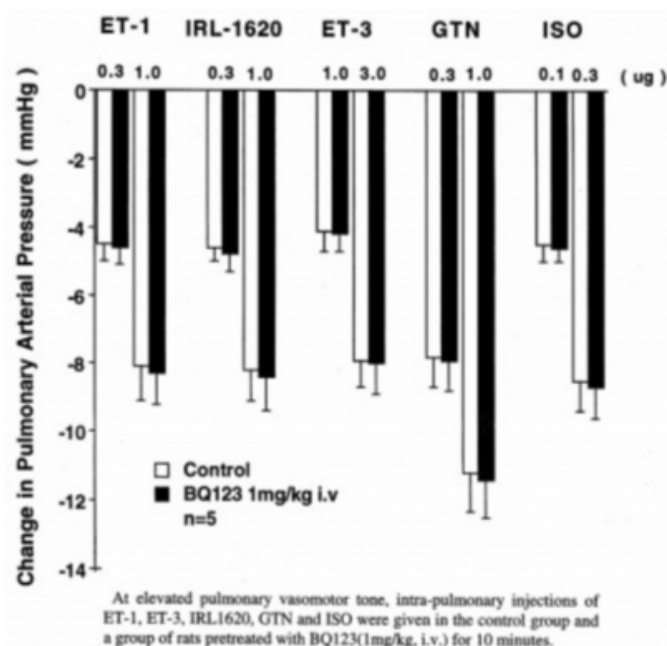
Fig 3: At elevated pulmonary vasomotor tone, intra-pulmonary injections of ET-1, ET-3, IRL1620 (ET<sub>B</sub> agonist), nitroglycerin (GTN) and isopretanolol (ISO) were given in the control group and group of rats pretreated with IRL2500 (ET<sub>B</sub> antagonist, 10mg/kg, i. v.) for 10 minutes. The vasodilator responses to ET-1, ET-3 and IRL1620, not GTN or ISO were significantly attenuated by the pretreatment of IRL2500.



In another group of rats, pretreatment with BQ123 (an ET<sub>A</sub> antagonist, 1mg/kg, i. v.) did not alter the pulmonary vasodilator responses to ET-1, ET-3, IRL1620, nitroglycerin and isopretanolol at elevated vasomotor tone significantly (Fig 4).

Fig 4. At elevated pulmonary vasomotor tone, intra-pulmonary injections of ET-1, ET-3, IRL1620 (ET<sub>B</sub> agonist), nitroglycerin (GTN) and isopretanolol (ISO) were given in the control group and a group of rats pretreated with BQ123 (ET<sub>A</sub> antagonist, 1mg/kg, i. v.) for 10 minutes. BQ123 did not alter the vasodilator responses to these substances in the lung.

**Figure 4**



## DISCUSSION

The present study was the first to investigate the vasodilator responses to endothelins in the pulmonary vascular beds in intact-chest rats under conditions of constant blood flow. The animal model used in the study was developed by the authors and described in previous publications.<sup>13,14</sup> A specially designed triple-lumen balloon catheter was used to measure the pulmonary arterial pressure in rats in vivo. The right lower lobe of the lung perfused through the catheter was hemodynamically isolated in the intact chest animal by inflation of the balloon on the tip of the catheter. Since the perfusion flow was kept constant, the change in the pulmonary arterial pressure directly reflected the change in the pulmonary vascular resistance. In order to demonstrate the vasodilator responses to testing compounds in the pulmonary vascular bed, the pulmonary arterial pressure was raised and maintained to certain level by continuous infusion of U46619, a thromboxane analog.

The present data indicates that ET-1, IRL1620, and ET-3 dilate the lung through activation of ET<sub>B</sub> receptor since the depressor responses in the pulmonary vascular bed were significantly blocked by IRL2500, an ET<sub>B</sub> antagonist. The present study also extends our previous studies<sup>15</sup> by demonstrating the existence of ET<sub>B</sub> receptor in the pulmonary vascular bed in rats. Endothelins dilate the pulmonary vessels pre-constricted by U46619. However, they do not seem to play much of a role in maintaining the

basal vasomotor tone in pulmonary vascular bed, since neither intravenous injection of BQ123 (ET<sub>A</sub> antagonist) or IRL2500 (ET<sub>B</sub> antagonist) changed the baseline (not pre-constricted) pulmonary pressures. This is consistent with the finding in newborn lamb.<sup>11</sup>

Distribution of ET receptors varies between species and among tissue types, although it has been generally observed that ET<sub>A</sub> receptors predominate in arterial vessels whereas ET<sub>B</sub> receptors predominate on the lower pressure side of the circulation. Both receptors are members of the G-protein-coupled family leading to activation of multiple effector pathways. In vascular smooth muscle, an increase in intracellular Ca<sup>2+</sup> is a common feature occurring after activation of all receptor subtypes.<sup>7</sup> Gumusel reported that ET-1 and ET-3 promote calcium influx via the L-type calcium channel to promote constriction of pulmonary resistance vessels, whereas activation of potassium channels mediates the vasodilator responses to ET-1, ET-2 and ET-3 in the pulmonary vascular bed in vivo.<sup>15,16</sup>

ET receptors are widely distributed in many tissues and involved in numerous physiologic and pathophysiologic responses<sup>8</sup>. Increased plasma concentrations of ET-1 have been described in a variety of diseases, such as pulmonary hypertension, arteriosclerosis, renal failure, acute coronary syndromes, heart failure, migraine and vascular diseases. Recently an increasing number of endothelin receptor antagonists have been synthesized. These antagonists have been shown to inhibit endothelin-mediated vascular responses. Clinical studies are now ongoing to elucidate the pathophysiologic role of endothelins and the potential benefit of the blockade of the system in different disease states.<sup>17</sup>

## CONCLUSIONS

In conclusion, the present study was the first to evaluate the pulmonary vasodilator responses to endothelin isopeptides in intact chest rats under conditions of constant pulmonary blood flow. The present data suggests that ET-1, IRL 1620 and ET-3 have similar vasodilator responses in the pulmonary vascular bed by the mechanism through activation of the ET<sub>B</sub> receptor. IRL2500 can be a useful pharmacological tool as an ET<sub>B</sub> antagonist in the studies of pulmonary vascular regulation in rats in vivo. A better understanding of the hemodynamic effects of endothelins may provide an avenue to new therapeutic options for cardiovascular disorders in the future.

## CORRESPONDENCE TO

Qingzhong Hao, MD Department of Anesthesiology, Geisinger Medical Center, 100 N. Academy Avenue, Danville, PA 17822. E-mail: qhao1@geisinger.edu

## References

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, and Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (London)*, 1988a; 332:411-415
2. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyachi T, Goto K, and Masaki T: The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. USA*. 1989a; 86:2863-2867
3. Inoue A, Yanagisawa M, Takawa Y, Mitsui Y, Kobayashi M, and Masaki T: The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J. Biol. Chem.* 1989b; 264:14954-14959
4. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S: Cloning and expression of cDNA encoding an endothelin receptor. *Nature (London)* 1990; 348: 730-732
5. Sakurai T, Yanagisawa M, Takawa Y, Miyazaki H., Kimura S, Goto K, Masaki T: Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptors. *Nature (London)* 1990; 348: 732-735
6. Masaki T, Vane J.R, Vanhoutte P.M: International Union of Pharmacology Nomenclature of Endothelin Receptors. *Pharmacol. Rev.* 1994; 46: 137-142
7. Pollock D, Keith T. L. and Highsmith R. F: Endothelin receptors and calcium signaling. *FASEB J.* 9, 1196-1204, 1995
8. Yanagisawa M. and Masaki T: Molecular biology and biochemistry of the endothelins. *Trends Pharmacol. Sci.* 1989b; 10: 374-378
9. Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K., Nishikibe, M., Yano, M: Biological profiles of highly potent novel endothelin antagonists selective for the ET<sub>A</sub> receptor. *Life Sci.* 1992; 50: 247-255
10. Pinheiro, J. M. B. And Malik A. B.: Mechanisms of endothelin-1-induced pulmonary vasodilatation in neonatal pigs. *Journal of Physiology.* 1993; 469: 739-752
11. Wong J., Vanderford P. A., Winters J., Soifer S. And Fineman J. R.: Endothelin B receptor agonists produce pulmonary vasodilation in intact newborn lambs with pulmonary hypertension. *Journal of Cardiovascular Pharmacology.* 1995; 25: 207-215
12. Macchia M, Barontini S, Ceearelli F, Galoppini C, Giusti L, Hamdan M, Lucaccini A, Martinelli A, Menchini E, Mazzoni MR, Revoltella RP, Romagnoli F and Rovero P: Toward the rational development of peptidomimetic analogs of the C-terminal endothelin hexapeptide: development of a theoretical model. *Pharmacology* 1998 Aug-Sep; 53(8-9): 545-56
13. Qingzhong Hao, Albert L. Hyman, Ella U. Choe, Lewis M. Flint and John J. Ferrara: A novel model to study the effects of burn lymph on pulmonary vascular hemodynamic variables. *The Journal of Trauma: Injury, Infection and Critical Care* Vol. 43, No. 3: 453-457; 1997
14. Albert L. Hyman, Qingzhong Hao, Allen Tower, Philip J. Kadowitz, Hunter C. Champion, Bulent Gumusel and Howard Lippton: Novel catheterization technique for the in vivo measurement of pulmonary vascular responses in rats. *Am. J. Physiol.* 274 (Heart Circ. Physiol. 43): H1218-H1229, 1998

15. Gumusel B, Hao Q, Chang J, Hyman A and Lippman H: Cardiovascular effects of endothelin, Endothelins in biology and medicine, Edited by Huggins J. P and Pelton J. T. CRC Press, 1997, pp151-170  
16. Lippman HL, Hao Q, Erdemli O, Hyman A: Role of G

Proteins in the vasodilator response to endothelin isopeptides in vivo. J. of Appl. Physiol. 78(6): 2062-9, 1995 June.  
17. Noll G, Wenzel R.R and Luscher T.F: endothelin and endothelin antagonists: Potential role in cardiovascular and renal disease. 1996; 157: 256-267

**Author Information**

**Qingzhong Hao, MD**

Department of Anesthesiology, Geisinger Medical Center

**Albert Hyman, MD**

Professor, Department of Medicine, Tulane University Medical School

**Howard Lipton, MD**

President, H. L. Labs

**Frank Zavisca, MD, PhD**

Associate Professor, Department of Anesthesiology, LSU Health Sciences Center

**Randall Cork, MD, PhD**

Professor and Chair, Department of Anesthesiology, LSU Health Sciences Center