Pulmonary vasoregulation by endothelin in conscious dogs after left lung transplantation

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Doi, Shouzaburoh, Nicholas Smedira, and Paul A. Murray. Pulmonary vasoregulation by endothelin in conscious dogs after left lung transplantation. J. Appl. Physiol. 88: 210–218, 2000.—We tested the hypothesis that regulation of the pulmonary circulation by endogenous endothelin (ET) during normoxia and hypoxia was altered in conscious dogs 1 mo after left lung autotransplantation (LLA). Sham-operated control and post-LLA dogs were chronically instrumented to measure the left pulmonary vascular pressure-flow (LP-Q˙) relationship. LP-Q˙ plots were generated on separate days during normoxia and hypoxia (arterial PO2 ~50 Torr) in the intact condition, after selective ETA-receptor inhibition (BQ-485), and after combined ETA-ETB-receptor inhibition (bosentan). Although LLA resulted in a chronic increase in pulmonary vascular resistance, the ET-receptor antagonists had no effect on the LP-Q˙ relationship during normoxia in either group. The magnitude of hypoxic pulmonary vasoconstriction (HPV) was flow dependent in both groups, and the HPV response was potentiated post-LLA compared with control. ETA-receptor inhibition attenuated the HPV response to the same extent in both groups. ETB-receptor inhibition attenuated the HPV response to a greater extent than did ETA-receptor inhibition alone, and this effect was greater post-LLA compared with control. Plasma ET-1 concentration only increased during hypoxia in the LLA group. These results indicate that ET does not regulate the baseline LP-Q˙ relationship in either group. Both ETA- and ETB-receptor activation mediate a component of HPV in conscious dogs, and the vasoconstrictor influence of ETB-receptor activation is enhanced post-LLA.

Pulmonary vascular pressure-flow relationship; endothelin-receptor antagonists; hypoxic pulmonary vasoconstriction

ENDOTHELIN (ET)-1 is a 21-amino acid peptide released from vascular endothelial cells (53). ET has a potent vasoconstrictor effect mediated by several receptor subtypes (ETA and ETB receptors) (43, 46, 49). ET is known to be increased in some forms of chronic pulmonary hypertension (2, 3, 8). ET has also been implicated as a mediator of acute hypoxic pulmonary vasoconstriction (HPV) (5, 33, 48).

Our laboratory has been systematically investigating chronic changes in pulmonary vascular regulation that occur after left lung autotransplantation (LLA). This experimental model allows an assessment of the specific effects of the surgical transplantation procedure on the pulmonary circulation while avoiding the important but confounding effects of lung preservation techniques, immunosuppressive therapy, and tissue rejection. We have observed that LLA results in a chronic increase in pulmonary vascular resistance (26) and is characterized by abnormalities in neural (29), humoral (9), and local (30) mechanisms of pulmonary vasoregulation.

Because ET is known to be elevated in some forms of pulmonary hypertension, in the present study we tested the hypothesis that ET-receptor inhibition would attenuate the increase in pulmonary vascular resistance that occurs after LLA. Because of the putative role of ET in the HPV response, we also tested the hypothesis that ET-receptor inhibition would attenuate the magnitude of HPV in normal, conscious dogs. Finally, because the effects of lung transplantation on HPV have not been systematically examined, and because LLA results in endothelial dysfunction (30), we tested the hypothesis that the magnitude of HPV would be increased post-LLA.

Our studies utilized dogs that were chronically instrumented to measure the left pulmonary vascular pressure-flow (LP-Q˙) relationship. This experimental model allows us to perform experiments with animals in the conscious state, which eliminates the effects of anesthetic techniques, immunosuppressive therapy, and tissue rejection. Our studies were performed ~4–5 wk after the surgical procedure; therefore, the effects of acute surgical trauma were minimized. Finally, the use of LP-Q˙ plots avoids the problems inherent in the interpretation of single-point calculations of pulmonary vascular resistance (14) and allows us to distinguish between active and passive changes in the pulmonary circulation in response to the various interventions.

METHODS

All surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee.

Surgical Procedures

Fourteen conditioned male mongrel dogs weighing 28 ± 1 kg were premedicated with 10 mg intramuscular morphine sulfate and anesthetized with 20 mg/kg intravenous pentobarbital sodium and 15 µg/kg fentanyl citrate. An endotracheal tube was inserted, and the lungs were mechanically ventilated. Anesthesia was maintained with ~1.2% end-tidal

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Experimental Protocols

All experiments were performed with each healthy unseeded conscious dog lying on its right side in a quiet laboratory environment. The seven LLA dogs were studied 30 ± 3 days after surgery. The seven sham-operated controls were studied 37 ± 3 days after surgery. Continuous LP-Q˙ plots were used to assess the pulmonary vascular effects of hypoxia and the ET antagonists. LP-Q˙ plots were constructed by continuously measuring the pulmonary vascular pressure gradient [pulmonary arterial pressure (PAP) – left atrial pressure (LAP)] and LQ˙ during gradual (~1 min) inflation of the hydraulic occluder implanted around the right pulmonary artery. LP-Q˙ plots are highly reproducible and have no effect on systemic hemodynamics, blood gases, or the zonal condition of the lung (28). Each dog was studied on 3 separate days in the intact condition, after selective ETA-receptor block, and after combined ETA1-B-receptor block. The experiments were performed in random order with regard to group and ET antagonists. Because of technical failure (flow probes defective), LP-Q˙ plots were obtained in six dogs in each group.

Protocol 1: Effect of LLA on the magnitude of hypoxic pulmonary vasoconstriction. We tested the hypothesis that LLA would result in an increase in the magnitude of HPV compared with normal conscious dogs. A baseline LP-Q˙ plot was obtained during normoxia in the conscious state in six control dogs and six LLA dogs. A conical face mask was then placed over each dog’s snout. Room air was administered via the mask by using a semiclosed circulation system. After 15 min, a normoxia LP-Q˙ plot with face mask was obtained. The delivered room air was then blended with gases from sources consisting of 100% nitrogen, oxygen, or carbon dioxide. The gas flows were titrated to the fraction of inspired oxygen tension (~11.2) that resulted in a gradual decrease in systemic arterial P02 to ~50 Torr. After a new steady state was reached (~30 min), a hypoxic LP-Q˙ plot was generated.

Protocol 2: Effect of selective ETA-receptor block on the LP-Q˙ relationship during normoxia and hypoxia. On a separate day, the procedures utilized in protocol 1 were repeated in the same dogs after pretreatment with the selective ETA-receptor antagonist BQ-485 (perhydroazeptin-1-yl-L-leucyl-D-tryptophanyl-D-tryptophan: 10 µg·kg−1·min−1 intravenous; a gift from Banyu Pharmaceuticals, Tsukuba, Japan). The efficacy of ETA-receptor block was demonstrated by the complete inhibition of the systemic pressor response to intravenous ET-1 (400 ng/kg). LP-Q˙ plots were generated during normoxia, during normoxia after BQ-485, and during hypoxia. We tested the hypotheses that ETA-receptor inhibition would cause a downward shift in the baseline LP-Q˙ relationship in LLA dogs and that ETA-receptor block would attenuate the magnitude of HPV in both control and LLA dogs.

Protocol 3: Effect of combined ETA1-B-receptor block on the LP-Q˙ relationship during normoxia and hypoxia. On a separate day, the procedures utilized in protocol 1 were repeated in the same dogs after pretreatment with the combined ETA1-B-receptor antagonist bosentan (Ro 47–0203; 4-tert-butyl-3-ethyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2’-bipyrimidin-4-yl]-benzene sulfonamide: 10 mg·kg−1·min−1 intravenous; a gift from Hoffmann-LaRoche, Basel, Switzerland). The efficacy of ETA1-B-receptor block was demonstrated by the complete inhibition of the systemic pressor response to intravenous ET-1 (400 ng/kg). LP-Q˙ plots were generated during normoxia, during normoxia after bosentan, and during hypoxia. We tested the hypotheses that combined ETA1-B-receptor inhibition would inhibit the magnitude of HPV to a greater extent than would...
ET$_A$-receptor inhibition alone and that this effect would be more pronounced in LLA dogs.

Measurement of Plasma ET-1 Concentration

Blood samples were simultaneously collected from the main pulmonary artery and the left atrium to measure plasma ET-1 concentration during normoxia and after 30 min of hypoxia in seven control and seven post-LLA dogs. The blood samples were placed in chilled tubes containing protease inhibitors. After centrifugation, the plasma was stored at $-20^\circ$C. After extraction through pretreated Sep-Pak C$_{18}$ columns (Waters Associates, Milford, MA), ET-1 concentration was measured in triplicate by radioimmunoassay (Peninsula Laboratories, Belmont, CA).

Data Analysis

Phasic and mean vascular pressures and LQ were displayed continuously on an eight-channel strip-chart recorder (model 2800, Gould, Eastlake, OH). Mean values for pressures and LQ, measured at end expiration, were obtained with passive electronic filters with a 2-s time constant. All vascular pressures were referenced to atmospheric pressure before and after each LP-Q˙ plot. The analog pressure and LQ signals were digitally converted and multiplexed (model PCM-8, Medical Systems, Greenvale, NY) and stored on videotape (videocassette recorder model AG-1260, Panasonic, Secaucus, NJ) for later playback and analysis. The LP-Q˙ relationship was measured continuously over the empirically measured range of LQ in each individual experiment. In all protocols, the LP-Q˙ relationship was linear by inspection over the empirically measured range of LQ. Thus linear regression analysis was used to calculate the slope and intercept for PAP = LAP (or PAP = 0 if LAP < 0 mmHg) as a function of LQ in each individual experiment. The correlation coefficient for the LP-Q˙ relationship for each protocol averaged $>0.98$. The composite LP-Q˙ plots in Figs. 1, 2, 4, and 6 were generated by using the regression parameters from each individual continuously measured LP-Q˙ plot to calculate PAP = LAP at 10 ml·min$^{-1}$·kg$^{-1}$ intervals of LQ over the empirically measured range of LQ. The minimum and maximum values of LQ in each composite LP-Q˙ plot represent the average minimum and maximum values of LQ for the dogs studied in that protocol. Multivariate analysis of variance in the form of Hotelling's $T^2$ was used to assess the effects of mask, hypoxia, BQ-485, bosentan, and LLA on the regression parameters obtained in each individual experiment, compared with values measured at baseline (50). One-way and two-way analyses of variance were used to assess the effects of interventions on steady-state hemodynamics and blood gases. Student's t-test for intragroup and intergroup comparisons was used to compare the effects of BQ-485 and bosentan on the magnitude of the HPV response and to assess changes in plasma ET-1 concentration between normoxia and hypoxia. All values are presented as means $\pm$ SE.

RESULTS

Effect of LLA on the Magnitude of HPV

As we have previously reported (26), LLA resulted in a marked leftward shift in the baseline LP-Q˙ relationship compared with conscious sham-operated, control dogs; i.e., LLA resulted in a chronic increase in pulmo-

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**Fig. 1.** Composite left pulmonary vascular pressure-flow (LP-Q˙) plots in 6 conscious, sham-operated control dogs and 6 conscious dogs after left lung autotransplantation (LLA). PAP, pulmonary arterial pressure; LAP, left atrium pressure. Compared with control, LLA resulted in a marked leftward shift ($^*P < 0.01$) in baseline LP-Q˙ relationship, which indicates a chronic increase in pulmonary vascular resistance. Values are means $\pm$ SE. In some cases, error bars fall within symbol.

**Fig. 2.** A: composite LP-Q˙ plots in 6 control dogs during normoxia, normoxia with face mask, and hypoxia. Compared with normoxia, there was no change in LP-Q˙ relationship during normoxia with mask. Breathing a hypoxic gas mixture caused a leftward shift ($^*P < 0.01$) in LP-Q˙ relationship. B: composite LP-Q˙ plots in 6 dogs after LLA during normoxia, normoxia with mask, and hypoxia. There was no effect of mask, whereas breathing a hypoxic gas mixture caused a leftward shift ($^*P < 0.01$) in LP-Q˙ relationship. Values are means $\pm$ SE.
In Fig. 2. Breathing room air through the face mask had no effect on the LP-Q˙ relationship in either group, whereas breathing the hypoxic gas mixture caused a leftward shift in the LP-Q˙ relationship in both control and post-LLA dogs. The HPV response (i.e., the increase in PAP – LAP from normoxia to hypoxia at each common value of left pulmonary blood flow) in control and post-LLA dogs is compared in Fig. 3. In both groups, the magnitude of HPV increased as LQ increased. Moreover, at any given value of LQ, the magnitude of HPV was increased post-LLA compared with control. Steady-state hemodynamics and blood gases are summarized in Tables 1 and 2, respectively. During normoxia, PAP was higher and LQ was lower post-LLA compared with control. Hypoxia increased PAP in both groups. Systemic arterial blood gases were similar in control and LLA dogs during normoxia. Hypoxia increased systemic arterial pH and decreased systemic arterial Pco2, Po2, and So2, as well as mixed venous Po2 and So2 to the same extent in both groups.

Effects of ET-Receptor Block in Control Dogs

The selective ETA-receptor antagonist BQ-485 had no effect on the LP-Q relationship during normoxia (Fig. 4A). After ETA-receptor block, breathing the hypoxic gas mixture still caused a leftward shift in the LP-Q relationship (Fig. 4A). However, the magnitude of the HPV response was attenuated after ETA-receptor block compared with the response measured in the intact condition (Fig. 5). Combined ETA-B-receptor block with bosentan had no effect on the LP-Q relationship during normoxia (Fig. 4B). Under these conditions, hypoxia still caused a shift in the LP-Q relationship (Fig. 4B). However, the magnitude of the HPV response was attenuated after combined ETA-B-receptor block com-

Table 1. Steady-state hemodynamics

<table>
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<th>Protocol 1</th>
<th>Protocol 2</th>
<th>Protocol 3</th>
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<td>PAP, mmHg</td>
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<td>Normoxia</td>
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<td>21 ± 1†</td>
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<tr>
<td>Hypoxia</td>
<td>104 ± 14</td>
<td>104 ± 9</td>
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Values are means ± SE. SAP, systemic arterial pressure; PAP, pulmonary arterial pressure; LAP, left atrial pressure; LQ, left pulmonary blood flow; HR, heart rate; ET, endothelin antagonist: BQ-485 in protocol 2, bosentan in protocol 3; LLA, left lung autotransplantation. Protocol 1: effect of LLA on the magnitude of hypoxic pulmonary vasoconstriction. Protocol 2: effect of selective ETA-receptor block. Protocol 3: effect of combined ETA-B-receptor block. *P < 0.05, hypoxia vs. normoxia. †P < 0.05, LLA vs. control.
pared with the intact condition (Fig. 5). The magnitude of HPV was attenuated to a greater extent during combined ET<sub>A-B</sub>-receptor block compared with selective ET<sub>A</sub>-receptor block (Fig. 5). Neither BQ-485 nor bosentan had effects on steady-state hemodynamics or blood gases (Tables 1 and 2).

### Effects of ET Receptor Block in Post-LLA Dogs

Neither selective ET<sub>A</sub>-receptor block (Fig. 6A) nor combined ET<sub>A-B</sub>-receptor block (Fig. 6B) had an effect on the LP-Q relationship during normoxia. Under these conditions, hypoxia still caused a shift in the LP-Q relationship (Fig. 6). The magnitude of HPV was attenuated by selective ET<sub>A</sub>-receptor block and was reduced to an even greater extent by combined ET<sub>A-B</sub>-receptor block (Fig. 7). BQ-485 decreased the HPV response to a similar extent in control and post-LLA dogs, whereas bosentan attenuated the HPV response to a greater extent in the LLA group (Fig. 8). Neither ET-receptor antagonist had an effect on steady-state hemodynamics or blood gases (Tables 1 and 2).

### Plasma ET-1 Concentrations During Normoxia and Hypoxia in Control and LLA Dogs

Pulmonary arterial and left atrial plasma ET-1 concentrations were similar during normoxia in control and LLA dogs (Fig. 9). Hypoxia induced a small increase in the left atrial plasma ET-1 concentration in LLA dogs but not in control dogs (Fig. 9).

### DISCUSSION

The overall goal of this study was to investigate the role of endogenous ET in regulation of the pulmonary circulation after LLA. Our main findings are the following: 1) ET does not mediate the chronic increase in pulmonary vascular resistance post-LLA, 2) the magnitude of HPV is enhanced post-LLA, 3) both ET<sub>A</sub>- and ET<sub>B</sub>-receptor activation are involved in the HPV response in control and LLA dogs, and 4) the vasoconstrictor role of ET<sub>B</sub>-receptor activation during HPV is potentiated post-LLA.

As our laboratory has previously reported (26, 29–31), LLA resulted in a chronic increase in pulmonary vascular resistance. Sympathetic α<sub>1</sub>-adrenoceptor activation mediates a portion of this effect, because inhibition of α<sub>1</sub>-adrenoceptors partially reverses the shift in the LP-Q relationship post-LLA (29). Angiotensin II-receptor activation also mediates a component of the increase in pulmonary vascular resistance post-LLA (9). We postulated that endogenous ET may also be involved in the chronic increase in pulmonary vascular resistance post-LLA.
resistance post-LLA. The rationale for this hypothesis is that ET has been implicated in experimental (5, 15, 24, 47) and human (2, 3, 8) pulmonary hypertension. Moreover, LLA is characterized by profound abnormalities in endothelial function (11, 37, 54). However, neither of the ET antagonists had an effect on the baseline LP-Q˙ relationship during normoxia. Thus endogenous ET does not mediate the active increase in pulmonary vascular resistance post-LLA. However, our experimental design does not rule out the possibility that ET may exert chronic effects on the pulmonary circulation (e.g., structural changes) post-LLA.

The vascular actions of ET are mediated by at least two receptor subtypes, ET<sub>A</sub> and ET<sub>B</sub> receptors. Classically, it was thought that activation of ET<sub>A</sub> receptors on vascular smooth muscle caused vasoconstriction, whereas activation of ET<sub>B</sub> receptors on endothelial cells caused vasodilation via the release of endothelium-derived relaxing factors. However, recent evidence suggests that ET<sub>B</sub>-receptor activation can also result in vasoconstriction in human blood vessels (39), including pulmonary artery (21). At present it is uncertain whether different subtypes of ET<sub>B</sub> receptors are present on endothelial and vascular smooth muscle cells (7). One possible limitation of our study is that we did not utilize a selective ET<sub>B</sub>-receptor antagonist. We assumed that differences between the effects of BQ-485 and bosentan are due to the net effect of inhibiting ET<sub>B</sub> receptors.

Fig. 4. A: composite LP-Q plots in control dogs during normoxia, after endothelin A (ET<sub>A</sub>)-receptor block with BQ-485, and during hypoxia. BQ-485 had no effect on normoxia LP-Q relationship. Hypoxia still caused a shift (*P < 0.01) in LP-Q relationship after ET<sub>A</sub>-receptor inhibition. B: composite LP-Q plots in control dogs during normoxia, after combined ET<sub>A</sub>-B-receptor block with bosentan, and during hypoxia. Hypoxia caused a shift (*P < 0.01) in LP-Q relationship after ET<sub>A,B</sub>-receptor block. Values are means ± SE.

Fig. 5. HPV response in control dogs in intact condition, after ET<sub>A</sub>-receptor block with BQ-485, and after ET<sub>A,B</sub>-receptor block with bosentan. Magnitude of HPV was attenuated (*P < 0.01) by BQ-485 and was reduced further (†P < 0.01) by bosentan. Values are means ± SE.

Fig. 6. A: composite LP-Q plots in post-LLA dogs during normoxia, after ET<sub>A</sub>-receptor block with BQ-485, and during hypoxia. BQ-485 had no effect on normoxia LP-Q relationship. Hypoxia caused a shift (*P < 0.01) in LP-Q relationship after ET<sub>A</sub>-receptor block. B: composite LP-Q plots in post-LLA dogs during normoxia, after ET<sub>A,B</sub>-receptor block with bosentan, and during hypoxia. Bosentan had no effect on normoxia LP-Q relationship. Hypoxia still caused a shift (*P < 0.05) in LP-Q relationship after ET<sub>A,B</sub> block. Values are means ± SE.
HPV is a compensatory mechanism whereby a decrease in alveolar PO2 results in constriction of adjacent pulmonary arterioles, which improves gas exchange by diverting pulmonary blood flow away from hypoxic regions to better ventilated regions of the lung. The mechanism responsible for HPV is intrinsic to the lung, because HPV has been demonstrated on numerous occasions in isolated lung preparations. There are conflicting reports in the literature concerning the role of ET in the HPV response. Evidence to support a role for ET has been reported in rats (5, 6, 15, 33), lambs (48), pigs (12, 16, 17), and dogs (51). Conversely, ET was not found to play a role in the HPV response in rats (13, 45), lambs (52), and dogs (10). Possible confounding factors that could be responsible for these conflicting results include the use of anesthetics, acute surgical trauma, artificial perfusion, and single-point calculations of pulmonary vascular resistance. Our experimental model avoids these confounding factors. Our results suggest that both ETA- and ETB-receptor activation mediate components of the HPV response. Both ET antagonists attenuated the HPV response. Moreover, the effects of combined ETA_B-receptor block were greater than those of selective ETA-receptor block.

The LLA procedure results in surgical denervation of the lung. We did not anticipate that denervation per se would alter the HPV response post-LLA, because we have previously demonstrated that the autonomic nervous system does not mediate or modulate the HPV response in conscious dogs (20). It is known in a qualitative sense that the HPV response persists in the human transplanted lung (23, 35). We observed that the HPV response was potentiated post-LLA. Although the precise mechanism that mediates HPV remains to be elucidated, it is well established that several endogenous vasodilator mechanisms (e.g., nitric oxide, prostacyclin, ATP-sensitive K channel activation) act to modulate the HPV response (18, 19, 27). Pulmonary vasodilation mediated by nitric oxide (37) and ATP-sensitive K channel activation (38) are attenuated post-LLA. Thus the potentiated HPV response post-LLA could be due to a reduction in the influence of these vasodilator pathways. In addition, in the present study we observed that the inhibitory effect of ETA-receptor block was similar in control and LLA dogs, whereas the
effect of combined ETA+β-receptor block was greater post-LLA compared with control. One possible explanation for these results is a shift in the relative contribution of endothelial and vascular smooth muscle ETβ receptors post-LLA. For example, if the vasodilator influence of endothelial ETβ-receptor activation is diminished post-LLA, this would result in a potentiated HPV response mediated by vascular smooth muscle ETβ-receptor activation.

There is a relative paucity of information concerning the role of ET in the setting of lung transplantation. Combined ETA+β-receptor block has been shown to ameliorate ischemia-reperfusion injury in canine lung allografts (40). Plasma ET concentrations are elevated after experimental (40, 41) and human (42) lung transplantation, although values return to baseline concentrations within 1 wk of the transplantation procedure. ET is also increased in the bronchoalveolar lavage fluid of patients with lung allografts (1, 36). It has been postulated that ET may be involved in chronic rejection and the development of oblitrative bronchiolitis (22, 34, 44). We did not observe an increase in plasma ET concentration during normoxia after LLA, perhaps because measurements were made 1 mo after the LLA procedure. More surprisingly, we did not observe an increase in plasma ET in response to hypoxia in the control group and only observed a small increase in the LLA group. This result appears to be inconsistent with the marked effects of the ET antagonists on the HPV response. We can only speculate that plasma concentrations of ET do not accurately reflect the local tissue concentration.

It is important to note that the magnitude of HPV was directly proportional to the level of pulmonary blood flow in both control and post-LLA dogs (Figs. 3, 5, and 7). The flow-dependent nature of HPV must be taken into account when investigating the effects of physiological or pharmacological interventions on the HPV response. This factor may account for some of the conflicting reports in the literature concerning the role of ET in the HPV response.

In summary, ET does not mediate the chronic increase in pulmonary vascular resistance post-LLA. Both ETA- and ETβ-receptor activation mediate components of the HPV response. The vasoconstrictor role of ETβ-receptor activation during HPV appears to be potentiated post-LLA.

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