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Vasoconstrictor effect to 5HT, histamine and phenylephrine in pulmonary artery and its modulation by hypoxia

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Abstract
Hypoxia in lung diseases like chronic obstructive pulmonary disease, pulmonary arterial hypertension, pulmonary oedema etc. exhibit vasoconstriction, remodelling of the pulmonary vessel wall and thrombosis which leads to increased pulmonary vascular resistance. The pulmonary artery of *Capra hircus* is taken as pulmonary vascular model to evaluate effects of several vasocontractile agents so as to understand the mechanisms involved as pharmacological basis for therapeutics in the treatment of pulmonary arterial hypertension. The secondary branch of pulmonary artery mounted in automatic organ bath was exposed to vasotonic agent like 5HT, Histamine and α-adrenergic agonist under normoxic and hypoxic conditions. Hypoxia reduced the affinity of 5HT by 1.06 log units and E_{max} by 63%. 5HT induced contractile response was reduced by 35.1% and 70% with decrease in affinity by 1.32 and increase in affinity by 0.36 log unit by Ondansetron in normoxic and hypoxic rings, respectively, when compared with respective control. Hypoxia reduced affinity of Histamine by 0.16 log units and E_{max} by 63.6%. The Histamine induced contractile response was reduced by 37.7% with decrease in affinity by 0.32 and 31.11% with decrease in affinity by 0.05 log unit by Ranitine in normoxic and hypoxic rings, respectively, when compared with respective control. Hypoxia increased affinity of PE by 0.1 log units and E_{max} by 80%. The PE induced contratile response was reduced by 77% and 88% with decrease in affinity by 0.52 and 0.73 log unit by Prazosin in normoxic and hypoxic rings, respectively as compared with respective control. The inhibition of serotonergic, histaminergic & α_1-adrenergic receptor mediated vasototonic response is more in hypoxic than normoxic tissue suggesting hypoxia significantly reduced the sensitivity of serotonergic, histaminergic & α_1-adrenergic receptor. The inhibition of serotonergic vasototonic response by Ondansetron ; histaminergic vasototonic response by Ranitine and α_1-adrenergic receptor mediated vasototonic response by Prazosin more in hypoxic than normoxic tissue suggests that Ondansetron significantly augmented the vasorelaxation effect of 5HT; Ranitine significantly augmented the vasorelaxation effect of Histamine and Prazosin significantly augmented the vasorelaxation effect of α_1-adrenergic receptor agonist contracted rings more in hypoxic than normoxic state.

Keywords: Hypoxia, 5HT, Histamine, α-adrenergic receptors, pulmonary arterial hypertension

1. Introduction
The tone of pulmonary vasculature regulates pulmonary circulation that maintains concentration of oxygen in the blood and the function of the heart. Changes in the pulmonary vascular resistance could bring about changes in the function of the lungs and eventually the right ventricle [1]. A low regional pressure of oxygen (Po_2) in the lungs causes reflex contraction of vascular smooth muscles which is well known as hypoxic pulmonary vasoconstriction (HPV). Occurrence of HPV may be low (20-30mins), moderate (2hrs) and chronic [2]. A long term HPV could cause pulmonary arterial hypertension (PAH) and this may lead to right ventricular hypertrophy and eventually right heart failure. Chronic hypoxia-induced pulmonary vascular remodelling arising from heterogeneous group of diseases may lead to hypoxic pulmonary hypertension (PH) and this significantly worsens morbidity and mortality [3]. Animal model studies showed that pulmonary vessels contain adrenergic (α_1&β_2), serotonergic (5HT), histaminergic (H_1&H_2) and other receptors which mediate vasoconstriction of pulmonary artery. The vasoconstriction in normoxic pulmonary arteries has been reported to be mediated by 5HT receptor in control and hypertensive rats [4], histamine receptor in cat, dog, rabbit, man [5] and α_1-adrenergic receptor in pulmonary vasculature, rat [6, 7]. Effect of hypoxia on pulmonary vasoconstriction to PE in isolated pulmonary arteries of mice [8], PA-SMC growth promotion to 5-HT [9], 5-HT gene expression in PA[10] have been demonstrated.

Thus, PH is generally considered as a disease resulted from alveolar hypoxia and prevalent in human beings residing in high altitude areas and small ruminants like goats climbing to high altitude areas for browsing. We hypothesize that hypoxia could be affecting functions or regulations of serotonergic, histaminergic and adrenergic receptors in mediating altered vasoconstriction in goat pulmonary artery in the line of observations made in other animal model. Hence, the present study has been undertaken to identify the sensitivity of 5HT, histaminergic and adrenergic receptor mediating pulmonary vasoconstriction of *Capra hircus* under normoxic and hypoxic state.

2. Materials and methods

The apical lobe of lungs containing branches of pulmonary artery obtained after sacrifice of goat for food purpose in the local abattoir. The tissues were transferred in ice cold MKHS to the laboratory. The arteries were cleared of connective tissues, fascia then cut into segments of circular rings measuring 1.5-2 mm in length and employed for isometric contraction studies. Freshly prepared arterial rings were mounted with the isometric force transducer (MLT 0201) positioned on a micro-positioner (Panlab S.I., Spain). Then the arterial rings were equilibrated in MKHS under a resting tension of 1.0 g for a period of 60 min with washing at 15 min interval with MKHS maintained at pH of 7.2-7.4. Following the equilibration period the pulmonary artery rings were mounted in automatic organ bath. 5HT(10 ηM - 100μM), Histamine(ηM 100μM) and α-adrenergic (10 ηM - 100μM) was added cumulatively to bath in order to elicit a concentration related contractile response while maintaining the tissues under normoxic (95% O_2 +5% CO_2) and hypoxic (1% O_2 + 4% CO_2 + 95% N_2) conditions. The isometric contraction was recorded by PC with the help of Lab chart 7 pro software (AD Instrument software, Australia). All the experiments were carried after approval from IAEC, C.V.Sc &AH (Regd No.433CPCSEA /CVS/ 2007)

2.1 Statistical Analysis

All values will be expressed as mean ± standard error of mean (SEM) of measurements in 'n' experiments. The net contraction was expressed as mean gm. The data will be compared using unpaired student's 't' test using GraphPad Software Quick Calcs. The mean–logEC_{50} and maximal contraction (E_{max}) was calculated using Graph-Pad Prism 5 software (GraphPad Prism5, GraphPad Software Inc., San Diego, CA, U.S.A). A 'p' value < 0.05 and p < 0.001 will be considered statistically significant.

3. Results

3.1 5HT (1ηM -100μM) induced concentration related contractile (CRC) response in absence (E_{max}) or presence (E_{Bmax}) of Ondansetron (10 μM), in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*

The CRC response curve of 5HT (EC_{50} 5.77±0.01, E_{max} 1.34±0.16 g, n=6) elicited in normoxic rings was shifted to right with significant (p<0.001) decrease in (EC_{50} 4.71±0.01μM, and E_{max} 0.50 ±0.004 g, n=6) in hypoxic rings. 5HT induced CRC response curve elicited in presence of Ondansetron (10μM) was shifted to right with significant (p < 0.001) decrease in EC_{50} and E_{Bmax}(4.45±0.02 μM, 0.87± 0.03g, n=6) in normoxic condition as compared to 5HT control. Similarly, 5HT induced CRC response curve elicited in presence of Ondansetron was shifted to right with significant (p<0.001) increase in EC_{50} (5.07 ±0.001 μM) and significant (p< 0.001) decrease in E_{max} (0.15±0.02 g, n=6) in hypoxic rings in comparison with that of EC_{50} and $E_{Bmax}$of normoxic treated one (table 1, Fig 1).

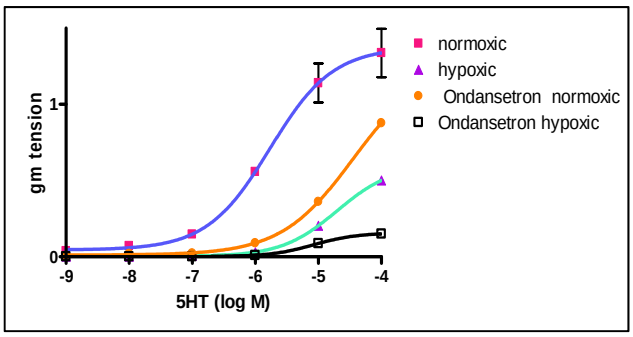

Fig 1: 5HT (1ηM -100 μM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of Ondansetron in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*.

3.2 Histamine (1ηM -100μM) induced concentration related contractile response curve either in presence or absence of Ranitidine (10μM) in normoxic and hypoxic rings.

The CRC response curve of Histamine elicited in normoxic rings (EC_{50} 5.01±0.01 μM, E_{max} 1. 22±0.06 g, n=6) was shifted to right with significant (p<0.001) decrease in EC_{50} (4.85±0.01μM) and E_{Bmax} (0.45 ±0.05 g) in hypoxic rings. Histamine induced CRC response curve elicited in presence of ranitidine (10μM) was shifted to right with significant (p < 0.001) decrease in EC_{50} (4.69 ± 0.02μM) and E_{Bmax} (0.76±0.03g) in normoxic condition as compared to Histamine control. Similarly, Histamine induced CRC response curve elicited in presence of Ranitidine was shifted to right with significant (p<0.05) increase inEC_{50} (4.80±0.01 μM), and with significant (p<0.001) decrease in E_{max} (0.31±0.01 g) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax}normoxic one (Table 1, Fig 2).

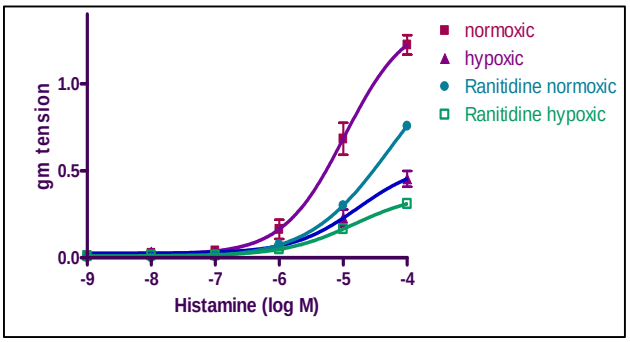

Fig 2: Histamine (1ηM -100 μM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of Ranitine in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*

3.3 PE (1ηM -100μM) induced concentration related contractile response curve either in presence or absence of Prazosin (10μM) in normoxic and hypoxic rings.

The CRC response curve of PE (EC_{50} 5.18±0.09 μM, E_{max} 0.60±0.01 g, n=6) elicited in normoxic rings was shifted to right with non-significant increase in EC_{50} (5.28±0.03μM), and significant (p< 0.001) decrease in E_{max} (0.12 ±0.01 g) in hypoxic rings. PE induced CRC response curve elicited in

presence of prazosin (10μM) was shifted to right with significant ($p < 0.05$) decrease in EC_{50} (4.66±0.2 μM) and was shifted to right with significant ($p < 0.001$) decrease in, E_{Bmax} (0.14± 0.004g) in normoxic condition as compared to non-treated normoxic control (Figure 6). Similarly, PE induced CRC response curve elicited in presence of prazosin was shifted to right with significant ($p<0.001$) decrease in (EC_{50} 4.55 ±0.02 μM) and significant ($p< 0.001$) decrease in (E_{max} 0.02 ±0.002 g) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax} normoxic treated one (table 1, Fig 3).

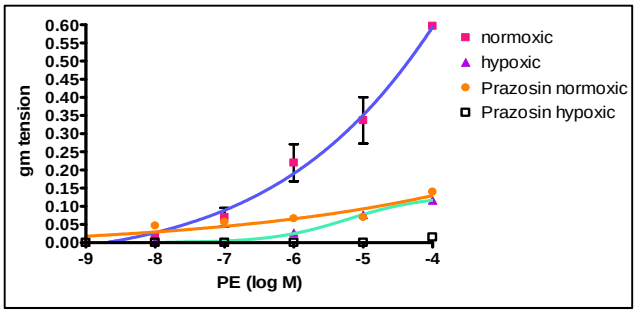

Fig 3: PE (1ηM - 100 μM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of Prazosin in normoxic and hypoxic pulmonary arterial rings of *Capra hircus.*

Table 1: 5HT (1ηM -100μM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of ondansetron(10 μM), in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*

	Normoxic (N)		Hypoxic(H)	
Treatments	EC_{50}	E_{max}/E_{Bmax} (gm)	EC_{50}	E_{max}/E_{Bmax} (gm)
5HT	5.77±0.01	1.34±0.16	4.71±0.01[c]	0.50±0.004[c]
5HT+ Ondan	4.45± 0.02[a]	0.87±0.03[a]	5.07±0.001[ac]	0.15±0.02[ac]
Hist	5.01±0.01	1.22±0.06	4.85±0.01[c]	0.45±0.05[c]
Hist+ Ranitidine	4.69±0.02[a]	0.76±0.03[a]	4.8±0.01[ad]	0.31±0.01[ac]
PE	5.18±0.09	0.60±0.01	5.28±0.03	0.12±0.01[c]
PE+ Prazosin	4.66±0.20[b]	0.14±0.004[a]	4.55±0.02[a]	0.02±0.002[ac]

a ($p<0.001$),b ($p<0.05$) represents level of significance between the rows within each column. Data of each row (hypoxic) is compared with the data of normoxic (control) within corresponding column. c ($p<0.001$),d ($p<0.05$) represents level of significance between the sub-columns (N and H) within each row. Data of each 'H' column in a particular row is compared with the corresponding data of 'N' column.

4. Discussion

The major findings are (i) the goat pulmonary artery is differentially sensitive to 5HT, histamine, PE and the vasoconstriction effect to these agents are in the order of potency 5HT>histamine>>phenylephrine in both normoxic and hypoxic rings indicating that the vascular smooth muscles contains serotonergic, histaminergic and adrenergic receptor, (ii) 5HT, histamine and PE- induced maximal contractile response was reduced by 63%, 64% and 77% in hypoxic rings as compared to normoxic rings suggesting that functions and regulations of these receptors are reduced in low P_{O2} (iii) ondansetron, ranitidine and prazosin inhibited 5HT, histamine and PE-induced contractile response by 35%, 37%, 77% in normoxic and by 70%, 31%, 83% in hypoxic rings indicating that there is increased function or regulation of 5-HT_3 receptor in hypoxia.

5HT has been implicated in aetiology of both clinical [11, 12] and experimental [13, 14] pulmonary hypertension. Out of several 5HTsubtypes of this receptor $5HT_{1B/1D\ /2A}$ has been reported as major contributor of vasoconstriction [15, 16]. There are regional differences in 5-HT sensitivity between larger to smaller branches of PA. The resistance arteries and capacitance arteries are more sensitive to 5-HT in normoxic hypoxic rats, respectively [4]. It has also been shown that the 5-HT_{1B} receptor plays a major role in mediating vasoconstriction in the human isolated small muscular pulmonary arteries [17]. Pulmonary arteries express 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 receptors and their activation in vascular smooth muscle (VSM) is important for pulmonary circulation [18]. 5-HT_{2A} and 5-HT_{2B} receptors in particular, and the 5-HT transporter (SERT/5HTT) cause constriction and proliferation of pulmonary VSM cells [19, 20]. In the present study we observed that the threshold concentration and maximal contractile response to 5HT were about 1ηM and 1.34 gm, respectively. The EC_{50} for 5HT was about 1μM. In human pulmonary vasculature the maximum response to 5-HT obtained in control rings at the end of the CRC was 1.63±0.23 g in artery and 1.76±0.21 g in vein preparations [21]. The vasoconstrictor effect of 5-HT in pulmonary vascular preparations isolated from different animal species including human has been ascribed to activation of 5-HT_{2A} receptors [22, 23, 24, 25].It has been observed that vasoconstriction response to 5HT in bovine and human pulmonary artery is mediated by primarily 5-$HT_{1B/1D}$ and secondarily $5HT_{2A}$ receptor [25].The maximum response to 5HT obtained at the end of CRC in GPA (goat pulmonary artery) is about 18% less than that found in human PA. Hence, vasoconstriction to 5HT is less sensitive to 5HT receptor as compared to HPA and could be mediated by 5-$HT_{1B/1D}$ and $5HT_{2A}$ receptors. Long term hypoxic pulmonary hypertension leads to proliferation of pulmonary tissues which increases the pulmonary vasoconstriction to 5HT in human [17, 26], rat [27, 28] and mice [29, 30] that is mainly due to increased expression of 5-HT_{1B} [17, 29, 30]. This demonstrate that the sensitivity to 5HT is increased in PH. But we observed that two hrs exposure of PA to hypoxia reduced the affinity of 5HT by 1.06 log units and E_{max} by 63% as compared to that of normoxic indicating a decreased sensitivity to 5-HT in GPA. In chronic hypoxic rats, the increased vasoconstriction to 5-HT is mediated by both the 5-HT_{2A} and 5-HT_{1B} receptor [4] with increase in mRNA for the 5-HT_{1B} receptor is in the pulmonary arteries from these rats [31]. Under hypoxia the reduced sensitivity to 5HT in GPA appears to be due to subdued function or expression of 5-HT_{2A} and 5-HT_{1B} receptor. There is no evidence showing that the vasoconstriction to 5HT in pulmonary artery is mediated by $5HT_3$ receptor [32]. Vasocontraction in GPA was elicited in presence of Odansetron, a $5HT_3$ receptor and we observed that the maximum contractile response to 5HT was reduced by 35.1% and 70% normoxic and hypoxic rings, respectively,

when compared with respective control. Hence blocking effect of ondansetron was almost doubled in hypoxic when compared with nomoxic one. This finding clearly demonstrate that vasoconstriction to 5HT in GPA is partially mediated by $5HT_3$ receptorin addition to $5\text{-}HT_{1B}$ receptor as reported in human and bovine PA. The explanation to the decreased maximum contractile response to 5HT could be arising from reduced function or expression of $5\text{-}HT_{1B}$ receptor and increased sensitivity to ondansetron could be due to increased function or expression of $5\text{-}HT_3$ receptor in hypoxia as compared to normoxia.

It has been well established that in guinea-pig pulmonary artery low concentration and higher concentration of histamine cause vasorelaxation and vasoconstriction which has been proposed to be mediated by H_2 and H_1-receptor [33, 34, 35]. The responses to histamine in isolated vascular preparations vary considerably depending upon the species [36], the type of vessels [37] and the region within the vascular bed from which the tissue has been derived [38, 39]. The vasoconstriction effect to histamine in GPA was examined in the present study to identify the role of H_1 & H_2 receptor in normoxic and hypoxic conditions. The EC_{50} and maximal contractile response of histamine obtained from CRC were 5.01±0.01M and 1.22±0.06g, respectively in normoxic GPA. So the EC_{50} of histamine observed in GPA is less than the pD_2 of 2-methylhistamine (5.31 ± 0.28M) reported in guinea-pigs PA [35]. This finding showed that vasoconstriction to histamine could be mediated by H_1 & H_2 receptor in this tissue. Vasoconstriction to histamine in hypoxic GPA was reduced with decrease in its EC_{50} and E_{max} by 0.16 M and 63.6%. Ranitidine, a H_2 receptor antagonist inhibited the contractile response to histamine with decrease in EC_{50} and E_{max} by 0.32M and 37.7 % in normoxic and by 0.05M and 31.11 % hypoxic GPA. An almost identical H_2 receptor blocking effect in both normoxic and hypoxic PA clearly indicates that the reduced vasoconstriction to histamine in hypoxic GPA could be due to reduced function or regulation of H_1 receptor. The reduced vasoconstriction to histamine observed in hypoxic GPA may be attributed to the stimulation of the endothelial H1 receptor liberates vasodilator substance and possibly activates smooth muscle granulate cyclise to accumulate cellular cyclic guano sine monophosphate as reported in human coronary arteries [40].

Phenylepherine, a α_1 adrenergic receptor agonist has been implicated to mediate vasoconstriction in several vascular bed including pulmonary artery of human [41], rat [42] and piglet [43]. The pEC_{50} of phenylephrine in human pulmonary artery has been demonstrated to be 5.94 [41]. The contraction to phenylephrine of the rat thoracic aorta, mesenteric artery and pulmonary artery are mediated in part via the α1D-subtype of adrenoceptor and the pA_2 for prazosin in blocking the PE –induced contractile response was 9.7 [44].The heterogeneity of α_1 adrenergic receptor subtypes has been identified in several animal species from receptor cloning studies. The EC_{50} (5.19M) and E_{max} (0.6g) for obtained in our present study clearly demonstrated that phenylephrine exhibited a low sensitivity to α_1 adrenergic receptor in pulmonary artery of goat under normoxic condition. Prazosin, α_1-adrenergic receptor blocker inhibited PE-induced contractile response with decrease in EC_{50} by 0.52 log unit and E_{Bmax} by 77% in normoxic tissues. A greater inhibition of PE-induced maximal response by prazosin indicates that the contractile response to PE could be mediated by α_{1D}-subtype of adrenoceptor as reported in rat and human PA. In hypoxic goat pulmonary arterial rings, PE mediated contractile response was decreased by 80% as compared to that of normoxic without any change in affinity. Similarly, in hypoxic rings, the prazosin inhibited PE-contraction with decrease in EC_{50} by 0.63 log unit and E_{Bmax} by 97%. This observation clearly indicated that the blockade of α_1-adrenergic receptor mediated contractile by prazosin is greater in hypoxic than normoxic pulmonary artery of goat. This blocking effect of prazosin was augmented in hypoxic state which could be due to either reduced function/expression of α_1-adrenergic receptor or augmented expression of high affinity binding sites that result in full blockade by prazosin. The high affinities of prazosin for PE contractions in the rat thoracic aorta, mesenteric artery and pulmonary artery have been reported to be mediated via α_{1D}-adrenoceptors(s) [44]. In GPA it is quite expected that hypoxia reduced sensitivity of α_{1D}-adrenoceptors to PE that may be the possible reason for reduced vasoconstriction to PE.

5. Conclusion

In summary, these results demonstrate that (i) vasopressor agents like 5HT, histamine and phenylephrine constrict the goat pulmonary arteries with a order of sensitivity 5HT>histamine>>PE in normoxic conditions, (ii) hypoxia greatly reduced (>50%) the maximal vasocontractile effect induced by these agents in the same order of sensitivity, (iii) vasoconstriction to 5HT mediated by $5HT_{1B}$ and $5HT_3$ in normoxic PA, the function/expression of $5HT_{1B}$ and $5HT_3$ could be reduced and increased under hypoxia,(iv) histamine –induced vasoconstriction is mediated by both H_1 and H_2 receptors in both normoxic and hypoxic PA, (v) PE contractile response in normoxic GPA is mediated by prazosin sensitive α_{1D}- adrenoceptor and its sensitivity is further reduced in hypoxia.

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7. Reference

1. Chester AH, Yacoub MH. The role of endothelin-1 in pulmonary arterial hypertension. Global Cardiology Science and Practice. 2014; 2(29).
2. Lumb AB, Slinger P. Hypoxic Pulmonary Vasoconstriction: Physiology and Anesthetic Implications. Anesthesiology. 2015; 122(4):932-946.
3. Pugliese SC, Poth JM, Fini MA, Olschewski A, El Kasmi KC, Stenmark KR. The role of inflammation in hypoxic pulmonary hypertension: from cellular mechanisms to clinical phenotypes. Am J Physiol Lung Cell Mol Physio. 2015; 308:L229-L252.
4. MacLean MR, Sweeney G, Baird M, McCulloch KM, Houslay M, Morecroft I. 5-Hydroxytryptamine receptors mediating vasoconstriction in pulmonary arteries from control and pulmonary hypertensive rats. British Journal of Pharmacology. 1996; 119(5):917-30.
5. Smith DJ, Coxe JW. Reactions of Isolated Pulmonary Blood Vessels to Anoxia, Epinephrine, Acetylcholine and Histamine. American Journal of Physiology. 1951; 167(3):732-737.
6. Salvi SS. α_1-Adrenergic Hypothesis for Pulmonary Hypertension. Chest. 1999; 115(6):1708-1719.
7. Jiao H, MuY, Wang R, Lin D, Sham J, Lin M. Disruption

of membrane caveolae limits pulmonary vasoconstriction induced by agonists in pulmonary hypertension rats. The FASEB Journal. 2014; 28(1):1089.8
8. Patel D, Alhawaj R, Wolin MS. Exposure of mice to chronic hypoxia attenuates pulmonary arterial contractile responses to acute hypoxia by increases in extracellular hydrogen peroxide. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2014; 307(4):R426-R433
9. Eddahibi S, Raffestin B, Pham I, Launay JM, Aegerter P, Sitbon M *et al*. Treatment with 5-HT potentiates development of pulmonary hypertension in chronically hypoxic rats. Am J Physiol. 1997; 272:H1173-H1181
10. Eddahibi S, Fabre V, Boni C, Martres MP, Raffestin B, Hamon M *et al*. Induction of serotonin transporter by hypoxia in pulmonary vascular smooth muscle cell. Relationship with the mitogenic action of serotonin. Circ Res. 1999; 84:329-336.
11. Mann DA, Oakley F. Serotonin paracrine signaling in tissue fibrosis. Biochimica et Biophysica Acta. 2013; 1832(7):905-910.
12. Hervé P, Launay JM, Scrobohaci ML, Brenot F, Simonneau G, Petitpretz P *et al*. Increased plasma serotonin in primary pulmonary hypertension. Am. J. Med. 1995; 99:249-254.
13. Wanstall JC, O'Donnell SR. Endothelin and 5-hydroxytryptamine on rat pulmonary artery in pulmonary hypertension. European Journal of Pharmacology. 1990; 176(2):159-168
14. Kanai Y, Hori S, Tanaka T, Yasuoka M, Watanabe K, Aikawa N *et al*. Role of 5-hydroxytryptamine in the progression of monocrotaline induced pulmonary hypertension in rats. Cardiovascular Research. 1993; 27(9):1619-1623.
15. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ *et al*. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin).Pharmacological Reviews. 1994; 46(2):157-203
16. Templeton AGB, MCgrath JE, Whittle MJ. The role of endogenous thromboxane in contractions to U46619, oxygen, 5-HT and 5-CT in the human umbilical artery. Br. J. Pharmacol. 1991; 103:1079-1084.
17. Morecroft I, Heeley RP, Prentice HM, Kirk A, Maclean MR. 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: importance of the 5-HT1B receptor. Br J Pharmacol. 1999; 128:730-734
18. Shacham S, Orbach P, Marantz Y**,** Reddy S, Rutkowski J, Becker O *et al***.** PRX-08066: A Potent 5-HT2B Receptor Antagonist with a dual disease modifying/vasodilating mechanism for the Treatment of Pulmonary Hypertension. 2006 International PHA Conference and Scientific Sessions. Minneapolis, MN, 2006.
19. Dumitrascu R, Kulcke C, Königshoff M, Kouri F, Yang X, Morrell N *et al.* Terguride ameliorates monocrotaline induced pulmonary hypertension in rats. Eur. Respir. J. 2011; 37:1104-1118
20. Zopf DA, das Neves LAA, Nikula KJ, Huang J, Senese PB, Gralinski MR *et al.*C-122, a novel antagonist of serotonin receptor 5-HT2B, prevents monocrotaline-induced pulmonary arterial hypertension in rats. Eur. J Pharmacol. 2011; 670:195-203
21. Cortijo J, Villagrasa V, Martí-Cabrera M, Villar V, Moreau J, Advenier C *et al*. The spasmogenic effects of vanadate in human isolated bronchus. British Journal of Pharmacology. 1997; 121:1339-1349.
22. Frenken M, Kaumann AJ. Interaction of ketanserin and its metabolite ketanserinol with 5-H_{T2} receptors in pulmonary and coronary arteries of calf. Naunyn-Schmiedeberg's Arch. Pharmacol. 1984; 326:334-339.
23. Raffestin B, Cerrina J, Boullet C, Labat C, Benveniste J, Brink C. Response and sensitivity of isolated human pulmonary muscle preparations to pharmacological agents. J Pharmacol. Exp. Ther., 1985; 233:186-194.
24. Selig WM, Bloomquist, MA, Cohen ML, Fleisch JH. Serotonin-induced pulmonary responses in the perfused guinea-pig lung; evidence for 5-HT_2 receptor-mediated pulmonary vascular and airway smooth muscle constriction. Pulmon. Pharmacol. 1988; 1:93-99.
25. Maclean MR, Clayon RA, Hillis SW, Mcintyre PD, Peacock AJ, Templeton AGB. 5-HT_1-receptor-mediated vasoconstriction in bovine isolated pulmonary arteries: influences of vascular endothelium and tone. Pulm. Pharmacol. 1994; 7:65-72.
26. Cortijo J, Martí-Cabrera M, Bernabeu E, Domènech T, Bou J, Fernández *et al*. Characterization of 5-HT receptors on human pulmonary artery and vein: functional and binding studies. British Journal of Pharmacology. 1997; 122:1455-1463.
27. MacLean MR, Morecroft I. Increased contractile response to 5-hydroxytryptamin$_{e1}$-receptor stimulation in pulmonary arteries from chronic hypoxic rats: role of pharmacological synergy. British Journal of Pharmacology. 2001; 134(3):614-620.
28. MacLean MR, Herve P, Eddahibi S, Adnot S. 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. British Journal of Pharmacology. 2000; 131(2):161-168.
29. Keegan A, Morecroft I, Smillie D, Hicks MN, MacLean MR. Contribution of the 5-HT_{1B} Receptor to Hypoxia-Induced Pulmonary Hypertension. Circulation Research. 2001; 89:1231-1239
30. Morecroft I, Pang L, Baranowska M, Nilsen M, Loughlin L, Dempsie Y *et al*. *In vivo* effects of a combined 5-HT_{1B} receptor/SERT antagonist in experimental pulmonary hypertension, Cardiovascular Research, 2010; 85(3):593-603
31. Heeley RP, Prentice H, Morecroft I, Maclean MR. Evidence for increased expression of the r5-HT1B receptor in small pulmonary arteries from rats with chronic hypoxic pulmonary hypertension. Am. J Resp. Crit. Care Med. 1998; 157(Suppl.):A590.
32. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ *et al*. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacological Reviews. 1994; 46(2):157-203
33. Ash AS, Schild HO. Receptors mediating some actions of histamine. British Journal of Pharmacology and Chemotherapy. 1966; 27(2):427-439.
34. Timmerman H. Histamine agonists and antagonists. Acta Otolaryngol. 1991; 479:5-11.
35. Cardell LO, Edvinsson L. Characterization of the histamine receptors in the guinea-pig lung: evidence for relaxant histamine H3 receptors in the trachea. Br. J. Pharmacol. 1994; 111:445-454.

36. Toda N. Mechanisms of histamine-induced relaxation in isolated monkey and dog coronary arteries. J Pharmacol Exp Ther. 1986; 239:529-535.
37. Van de Voorde J, Leusen I. Effect of histamine on aorta preparations of different species. Arch Int Pharmacodyn Ther. 1984; 268:95-105
38. Shirai M, Sada K, Ninomiya I. Nonuniform effects of histamine on small pulmonary vessels in cats. J Appl. Physiol. 1987; 62:451-458.
39. Tsuru H, Kohno S, Iwata M, Shigei T. Characterization of histamine receptors in isolated rabbit veins. J. Pharmacol. Exp. Ther. 1987; 243:696-702.
40. Toda N. Mechanism of histamine actions in human coronary arteries. Circulation Research. 1987; 61:280-286
41. Currigan DA, Hughes RJ, Wright CE, Angus JA, Soeding PF. Vasoconstrictor responses to vasopressor agents in human pulmonary and radial arteries: an *in vitro* study. Anesthesiology. 2014; 121(5):930-6
42. Mam V, Tanbe AF, Vitali SH, Arons E, Christou HA, Khalil RA. Impaired vasoconstriction and Nitric Oxide-Mediated Relaxation in Pulmonary Arteries of Hypoxia- and Monocrotaline-Induced Pulmonary Hypertensive Rats. The Journal of Pharmacology and Experimental Therapeutics. 2010; 332(2):455-462.
43. Meadow WL, Rudinsky BF, Strates E. Effects of phenylephrine on systemic and pulmonary artery pressure during sepsis-induced pulmonary hypertension in piglets. Dev Pharmacol Ther. 1986; 9:249-259
44. Hussain MB, Marshall I. Characterization of alpha1-adrenoceptor subtypes mediating contractions to phenylephrine in rat thoracic aorta, mesenteric artery and pulmonary artery**.** British Journal of Pharmacology, 1997; 122(5):849-58.