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Altered Reactivity of Rat Pulmonary Arterial Smooth Muscle to Vasoactive Agents in Hypoxia[1] (39788)

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Several humoral agents either locally released or activated in the lungs have been proposed as mediators of hypoxic pulmonary vasoconstriction. These include prostaglandin $F_2\alpha$ ($PGF_2\alpha$), angiotensin II (A-II), epinephrine (EPI), norepinephrine (NE), 5-hydroxytryptamine (5-HT), and histamine. The subject has recently been reviewed by Bergofsky (1). The present studies were undertaken to determine whether acute bath hypoxia or chronic hypobaria alter the response of pulmonary artery smooth muscle to vasoactive substances proposed as mediators of hypoxic pulmonary vasoconstriction.

Methods. In this study, two groups of male Sprague–Dawley rats were employed. Group I consisted of normal rats with an average weight of 300 g (normal rats). Group II consisted of rats maintained in a hypobaric chamber at one-half atmosphere for 2 weeks (hypobaric rats). Published studies by ourselves and others indicate that hypobaria of this extent and duration results in pulmonary artery hypertension, pulmonary artery hypertrophy, and right ventricular hypertrophy in the rat (2, 3). At the end of this period, their average weight was 240 g (range 200–285). All rats were fed Purina lab chow and water *ad libitum*. Animals were sacrificed by decapitation. The main pulmonary artery (PA) was removed and immediately placed in a preoxygenated Krebs solution. The solution contained the following in millimoles per liter: NaCl, 115.3; KCl, 4.7; $CaCl_2$, 1.8; $MgSO_4$, 0.57; KH_2PO_4, 1.8; Na_2 (EDTA), 0.03; $NaHCO_3$, 22.1; and D-glucose, 7.9. The main pulmonary artery was carefully dissected and cut in helical strips approximately 7–10 mm long and 0.8–1.2 mm wide. These strips were suspended under 0.5*g* tension in a series of 10-ml isolated tissue baths (Metro Scientific, Farmingdale, N. Y.) containing the above solution at 37° and allowed to equilibrate for 1–2 hr. Two strips of pulmonary artery were obtained from each animal. One strip was aerated with 95% O_2–5% CO_2 and the other strip with 95% N_2–5% CO_2. The pH, pCO_2, and pO_2 of the bath were determined with a blood gas analyzer (IL 113). The high oxygen bath had an average pO_2 of 376 mm Hg (range 350–392), pCO_2 of 36 mm Hg, and pH of 7.46 while the low oxygen bath had an average pO_2 of 22 mm Hg (range 18–24), pCO_2 of 36 mm Hg, and pH of 7.45. The response of arterial strips to the various agonists was determined in four different experimental conditions: (i) normal animals in high oxygen baths, (ii) normal animals in low oxygen baths, (iii) hypobaric animals in high oxygen baths, and (iv) hypobaric animals in low oxygen baths. Cumulative dose response curves were obtained to $PGF_2\alpha$, A-II, 5-HT, EPI, NE, histamine, and KCl under the four different experimental conditions. Not more than two agonists were tested on each strip, and the order and the combination of the two agonists tested were altered from strip to strip. Before a new agonist was added, the tissues were washed in fresh Krebs solution and allowed sufficient time to return to baseline tension. Isometric contraction was recorded by means of a Beckman Type R Dynograph recorder and Statham G7B mechanoelectrical transducers. Similar experiments were performed on thoracic aorta (Ao) with A-II

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and $PGF_2\alpha$ which were the only two agents that demonstrated altered reactivity of pulmonary artery. Contractile response was expressed as percentage of maximal contraction to a given agonist. The dose response curve so obtained is a measure of the "reactivity" of the vessel. The maximal developed tension (MDT) to each agonist was also measured. Concentration of the drug that produced 50% of maximal contraction was determined graphically from each experiment and the group mean and standard error were determined (ED50). One way analysis of variance (ANOVA) was performed for the variables $PGF_2\alpha$, A-II, 5-HT, EPI, NE, and KCl. For each variable, exact location of significant differences between means of the four groups were investigated by the use of Duncan's multiple-range test (4, 5). The level of significance was taken as $P < 0.05$. The drugs used in this experiment were obtained from the following sources: $PGF_2\alpha$ from the Upjohn Company, Kalamazoo, Mich.; A-II (Hypertensin-CIBA) from CIBA-Geigy, Summit, N. J.; EPI, NE, 5-HT, and histamine from Sigma, St. Louis, Mo., and KCl from Mallincroft, St. Louis, Mo.

Results. Effect of lowering bath oxygen tension. (A) Reactivity. In the normal and hypobaric rats, the lowering of bath oxygen tension did not change the reactivity of PA and Ao strips to any of the agonists tested (Table I).

(B) Maximal developed tension. In normal rats, the lowering of bath oxygen tension did not change the MDT developed by PA with any of the agonists tested but there was a significant decrease of MDT of Ao strips to A-II. In previously hypoxic rats, the bath hypoxia decreased MDT developed by PA with $PGF_2\alpha$, 5-HT, and KCl but not with A-II, NE, or EPI. In the aorta, MDT was decreased with A-II and $PGF_2\alpha$ (Table II).

Effect of hypobaric environment. (A) Reactivity. The reactivity of PA strips from hypobaric animals to $PGF_2\alpha$ was increased but that of PA to A-II was decreased ($P < 0.05$; Table I). On the contrary, the reactivity of Ao strips from hypobaric animals to $PGF_2\alpha$ and A-II did not change.

(B) Maximal developed tension. Chronic hypobaria resulted in an increased MDT of the PA to $PGF_2\alpha$, 5-HT, and KCl but not to A-II, EPI, and NE. In the Ao strips, chronic hypobaria decreased the MDT to A-II (Table II). Noteworthy is that the expected increase in MDT of hypertrophic PA was not observed with A-II, EPI, or NE.

The rat PA smooth muscle did not constrict *in vitro* to histamine in either high or low oxygen; for this reason, the effects of bath hypoxia and hypobaria on histamine response of the PA could not be ascertained in this experiment. Unresponsiveness of PA's to histamine *in vitro* has been found by Smith and Cox (6). It is believed that hista-

TABLE I. ED50 VALUES FOR VARIOUS AGENTS ON PA AND AO.[a]

Agent	Molarity (M)	Normal rats		Hypobaric rats	
		High O_2 bath	Low O_2 bath	High O_2 bath	Low O_2 bath
Pulmonary artery					
$PGF_{2\alpha}$	10^{-6}	6.86 ± 0.65^a (10)	6.34 ± 0.99^b (11)	2.39 ± 0.47^a (9)	2.02 ± 0.22^b (9)
A-II	10^{-8}	1.33 ± 0.19^c (9)	1.20 ± 0.17^d (9)	2.33 ± 0.35^c (10)	2.36 ± 0.43^d (8)
EPI	10^{-8}	3.94 ± 1.00 (7)	5.34 ± 1.31 (7)	5.53 ± 1.28 (6)	6.90 ± 1.16 (6)
NE	10^{-8}	2.58 ± 0.93 (8)	2.31 ± 0.59 (8)	4.74 ± 1.59 (7)	3.27 ± 0.57 (6)
5-HT	10^{-6}	2.82 ± 1.13 (8)	3.73 ± 1.93 (6)	1.74 ± 0.45 (12)	1.79 ± 0.41 (11)
KCl	10^{-2}	2.12 ± 0.35 (6)	1.83 ± 0.16 (6)	4.28 ± 1.22 (6)	3.32 ± 1.06 (6)
Aorta					
$PGF_{2\alpha}$	10^{-6}	1.45 ± 0.45 (8)	3.26 ± 0.72 (8)	3.49 ± 0.82 (8)	3.80 ± 0.49 (7)
A-II	10^{-8}	1.14 ± 0.25 (10)	0.84 ± 0.14 (6)	1.39 ± 0.13 (9)	1.48 ± 0.10 (7)

[a] All values for ED50 are mean ± SEM multiplied by the molarity indicated in the second column. The values in parentheses represent the number of experiments. Two values designated with the same letter superscript are significantly different ($P < 0.05$) from each other.

TABLE II. MDT VALUES FOR VARIOUS AGENTS ON PA AND AO.[a]

Agent	Normal rats		Hypobaric rats	
	High O_2 bath	Low O_2 bath	High O_2 bath	Low O_2 bath
Pulmonary artery				
$PGF_{2\alpha}$	231.0 ± 41.1[a] (10)	216.9 ± 37.2[b] (11)	658.2 ± 60.2[a,c] (9)	432.9 ± 49.0[b,c] (9)
A-II	215.0 ± 38.5 (9)	164.2 ± 13.8 (9)	235.2 ± 43.2 (10)	123.5 ± 29.4 (8)
EPI	416.3 ± 37.0 (7)	289.4 ± 18.5 (7)	439.0 ± 63.1 (6)	314.3 ± 40.9 (6)
NE	318.5 ± 53.1 (8)	270.0 ± 25.7 (8)	412.6 ± 81.7 (7)	286.3 ± 62.2 (6)
5-HT	136.3 ± 42.9[d] (8)	221.3 ± 46.5 (6)	415.9 ± 71.5[d,e] (12)	238.0 ± 45.2[e] (11)
KCl	173.7 ± 17.6[f] (6)	236.0 ± 44.8 (6)	502.3 ± 53.4[f,g] (6)	339.3 ± 32.5[g] (6)
Aorta				
$PGF_{2\alpha}$	538.5 ± 61.2 (8)	447.8 ± 52.7 (8)	728.5 ± 51.2[h] (6)	464.0 ± 53.0[h] (7)
A-II	380.6 ± 41.5[i,l] (10)	179.0 ± 23.3[j,l] (6)	200.9 ± 33.6[i,k] (9)	69.7 ± 12.7[j,k] (7)

[a] All values for MDT are mean ± SEM in milligrams. The values in parentheses represent the number of experiments. Two values designated with the same letter superscript are significantly different ($P < 0.05$) from each other.

mine acts primarily on the pulmonary vein.

Discussion. These studies have shown that chronic hypobaria alters the reactivity of pulmonary artery smooth muscle to $PGF_{2\alpha}$ and A-II and that these changes are not seen in aortic smooth muscle. Even though intraparenchymal arteries are not used, as pointed out by Lloyd (7), it is unlikely that bidirectionality between the responses of intra- and extraparenchymal pulmonary vessels exists. In our study, acute hypoxia failed to either enhance or depress reactivity to any of the drugs tested. This disagrees with the observations of others who found that acute hypoxia enhanced the response of isolated lung preparations to $PGF_{2\alpha}$ (8, 9) and A-II (10). This may be due to the fact that in the intact lung, acute alveolar hypoxia causes release of stored or synthesized vasoactive agents (11), whereas in the isolated strip this mechanism will not be operative.

Chronic alveolar hypoxia results in species-dependent pulmonary hypertension (12) and hypertrophy of the pulmonary arteries (13). By exposing the animals to hypobaria and its resultant pulmonary artery hypertension and hypertrophy, changes in reactivity to vasoactive agonists not seen in nonexposed, nonhypertrophic pulmonary arteries were sought in our study. Chronic hypobaria indeed enhanced the reactivity of pulmonary artery to $PGF_{2\alpha}$ and decreased it to A-II. The increased reactivity to $PGF_{2\alpha}$ seen in vessels from hypobaric animals could be the result of a "receptor hypersensitivity" caused by (a) interference with prostaglandin synthesis (14) and/or (b) hypoxia-induced depletion of prostaglandin within the lungs (11). It is known that inhibition of prostaglandin synthesis potentiates the response to exogenously administered prostaglandin (15). The decreased reactivity to A-II may be viewed as a manifestation of "receptor desensitization" caused by increased endogenous A-II. The state of increased renin activity has been shown to result from chronic hypobaria in the rat (16), and A-II levels have been found to be elevated in rabbits exposed to chronic hypobaria for 2 weeks (17). A similar mechanism has been suggested for the decreased responsiveness to A-II observed in rats in sodium-depleted states (18, 19). This speculation is further supported by the observation that a significant decrease in the MDT (not seen with other agonists) to A-II was also seen in the aortic strips of hypobaric animals (Table II). Whether the changes in reactivity found in the hypertrophic, chronically hypobaric pulmonary artery were secondary to chronic hypoxia per se or to the hypertrophy resulting from chronic hypoxia is not clear from this study. Chronic alveolar hypoxia results in pulmonary artery hypertrophy and one expects to see an increased MDT to vasoactive agents in this circumstance. This was found with $PGF_{2\alpha}$, 5-HT,

and KCl but not with EPI, NE, or A-II. Bath hypoxia depressed the MDT in the hypertrophic pulmonary artery of chronically hypobaric rats and in the normally thick aorta, whereas the normally thin pulmonary artery did not show a depressed MDT. This is presumably due to the critically lowered intracellular PO_2 found in the thicker blood vessels exposed to *in vitro* hypoxia.

In conclusion, acute hypoxia did not cause a change in reactivity of pulmonary artery smooth muscle to any of the agonists tested. Acute hypoxia did decrease the MDT to $PGF_2\alpha$, A-II, and catecholamines although not significantly and this agrees with the observations of others (20, 21). A significant difference was found, however, in reactivity of chronically hypobaric pulmonary artery to $PGF_2\alpha$ (increased) and A-II (decreased). It has been suggested by others (22) that the mechanisms responsible for pulmonary vasoconstriction in chronic hypoxia may be different from those found in acute hypoxia. There is evidence that chronic *in vitro* hypoxia changed the response of aortic and pulmonary artery smooth muscle to hypoxia (20, 23) presumably through metabolic adaptation. Our evidence would suggest that these same changes may affect the reactivity of the chronically hypertrophic hypoxic pulmonary artery smooth muscle to vasoactive agents as well.

Summary. The purpose of this study was to determine whether acute hypoxia *in vitro* or chronic hypobaria alter the response of pulmonary artery smooth muscle to vasoactive substances proposed as mediators of hypoxic pulmonary vasoconstriction. Dose response curves to $PGF_2\alpha$, A-II, EPI, NE, and 5-HT were obtained on helical strips of pulmonary artery and thoracic aorta from normal and chronically hypobaric (one-half atmosphere for 2 weeks) rats, under the conditions of high and low oxygen tension in the tissue bath. Chronic hypobaria increased the reactivity of pulmonary artery to $PGF_2\alpha$ and decreased it to A-II when compared to normal rats. Acute hypoxia did not change the reactivity of pulmonary artery to any of the agents tested. Similar changes were not observed in aorta.

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