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Hypoxic Moderation of Renal Hypertension in the Miniature Swine (41936)

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Abstract. Eleven male Yucatan miniature swine were subjected to unilateral renal artery constriction (RAC) with an intact contralateral kidney. Five pigs remained at laboratory altitude of 1524 m and served as normoxic, hypertensive controls (NH) while the other six (HH) were subjected to a simulated altitude of 4267 m for 9 weeks to determine the effect of hypoxia on the development of renal hypertension. Systemic blood pressure was increased less in HH as compared with NH. Data from three sib-pairs represented in each treatment group suggested a diverse, apparently familial response to RAC. The hypertensive process, irrespective of treatment groups, included a diminution of free circulating serum thyroxin and an increase in serum sodium. Hypoxia moderates renal hypertension in the miniature pig primarily via a decrement in stroke volume and cardiac output.

Evidence suggests that exposure to a hypoxic environment lowers systemic arterial blood pressure and protects against the development of experimental hypertension. Humans sojourning and living at high altitudes have been reported to have lower systemic blood pressures than their sea level counterparts (1–3). In experimental studies, Fregly and co-workers (4) have demonstrated that when rats with early experimental renal hypertension were exposed to hypoxia, the rise in their systemic blood pressures was less than that of normoxic controls. Similarly, hypoxia has been shown to totally block blood pressure elevation in the spontaneously hypertensive rat (5).

The mechanism by which hypoxia might protect against spontaneous or experimentally induced hypertension has not been clearly established. Local tissue hypoxia dilates systemic vessels, but it is not known whether this is the basis of the moderating effect of hypoxia on hypertension. Fregly and co-workers found in follow-up studies (6, 7) that associated with the mitigating effect of hypoxia on renal hypertension in the rat was a decreased thyroid function and a decreased aldosterone secretion. They demonstrated a thyroid-depressing factor of renal origin (8) and suggested that the thyroid gland was central to the hypoxic moderation of the renal hypertension.

If hypoxia protects against systemic hypertension, identifying the basis of this effect would increase our understanding of blood pressure regulation. Since existing experimental evidence supporting this notion has been obtained in the rat, data from an alternate animal model should prove beneficial in studying the effect of hypoxia on systemic hypertension. Accordingly, the purpose of this study was to examine the effects of hypobaric hypoxia on experimentally induced renal hypertension in Yucatan miniature swine, an animal with cardiovascular attributes similar to those of man.

Materials and Methods. Eleven 4- to 6-month-old male Yucatan miniature swine were selected from our swine colony for this experiment. Cardiac output, body weight, hematocrit, plasma volume, serum $[Na^+]$ and $[K^+]$, pulmonary arterial and aortic blood pressures, heart rate, urinary $[Na^+]$ and $[K^+]$, 24-hr urine volumes, and total T_3 and T_4 concentrations were determined prior to renal artery coarctation. From these measurements, stroke volume, 24-hr urinary Na^+ and K^+ excretion, whole blood volume, and red blood cell volume were calculated. All samples and measurements other than urine samples were obtained in unanesthetized pigs constrained by a canvas sling in a ventral recumbent position. Urine samples were obtained while the pigs were housed in cages especially de-

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signed for urine collection. A locally formulated swine ration was fed throughout the experiment. This diet provided 123 meq of potassium and 85 meq of sodium per day.

Following baseline measurements, all 11 pigs underwent unilateral renal artery coarctation. This technique consisted of suturing a 13-mm longitudinally split piece of tygon tubing (3 mm i.d.) around the left renal artery while the pig was under halothane anesthesia. The contralateral kidney was left intact.

The pigs were paired on the basis of body weight and from these pairings six of the pigs were randomly selected, placed in a hypobaric chamber within 3 days of surgery, and thereafter exposed to a simulated altitude of 4267 m. This exposure was continuous except for 1 hr a day when the chamber was pressurized for cleaning and feeding purposes. The other five pigs were housed in a similar manner after surgery with the exception that they remained in the "normoxic" laboratory environment (altitude = 1524 m). A coincidence of the treatment assignment procedure was that the three sib-pairs present in the 11 pigs had a representative in each treatment group.

After 9 weeks of hypoxic exposure the cardiovascular and metabolic measurements were repeated. In addition, systemic blood pressure measurements at 3, 5, and 7 weeks; urine samples at 1, 3, and 9 weeks; and serum for total circulating thyroid hormone measurements at 5- and 9-week postsurgical intervals were obtained.

Arterial blood pressures were obtained directly using a hubless, 15 cm long, 18-gauge needle which was inserted into the descending aorta. Prior to needle insertion, a local anesthetic block (2% lidocaine) was administered and a small incision made to facilitate the insertion. Entry was accomplished via the ninth intercostal space approximately 8 cm off the dorsal midline. The needle was advanced cranially and medially until the aorta was punctured. In most cases, aortic puncture was accomplished quickly. The needle was attached to a Statham p23Db pressure transducer, zeroed at heart level, and a 2-minute blood pressure tracing was recorded on a Brush Mark 280 recorder. This technique for arterial blood pressure determination has been routinely used in our laboratory. Measurements are repeatable and appear to involve little stress in the Yucatan miniature pig. A "resting" blood pressure can be obtained almost immediately after insertion. Stressful episodes cause small elevations in blood pressure that return to a plateau value within seconds.

Cardiac output was determined by dye dilution with a Waters XP 302 densitometer and cuvette. Catheters for cardiac output determinations (i.d. 0.023 in.) were placed in the pulmonary artery and the superior vena cava from an entry point immediately cranial to the sternum manubrium. Preparation of the entry point was the same as for the aorta. Pulmonary arterial pressure was recorded prior to cardiac output determinations.

Multiple dye curves were recorded on a Sanborn 4500 series recorder and area was determined by the method of Williams *et al.* (9). Peripheral resistance was calculated from mean aortic pressure divided by cardiac output. Pulmonary arterial pressure tracings were recorded as was described for aortic recordings. From this tracing, heart rate and mean pulmonary arterial pressures were de-

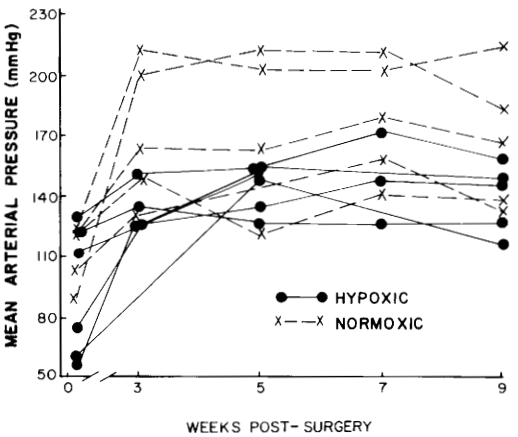

FIG. 1. Mean arterial blood pressure in normoxic and hypoxic miniature swine. Week 0 measurements were taken prior to renal artery coarctation and before separation into the respective altitude treatment groups. Treatment effects were significant ($P < 0.05$, repeated measures analysis of variance, SPSS, Weeks 3–9); however, effects of time on blood pressure were insignificant ($P > 0.5$) after 3 weeks of renal artery constriction (repeated measurements analysis of variance). Missing values are due to the inability to obtain aortic blood pressures.

termined. Stroke volume was calculated as cardiac output divided by heart rate.

Blood volume determinations were made using the Evan's blue dye technique with hematocrit as described by Chinard (10). Serum and urinary [Na^+] and [K^+] were obtained by flame photometry (Instrumentation Laboratory, Inc., Model 343).

Hormonal serum concentrations were determined by radioimmunoassay. Methodology utilized for triiodothyronine and thyroxin (11) determinations has been previously published.

Statistical analysis consisted of the paired *t* test, linear regression, one-way analysis of variance, or repeated measurements analysis of variance [Statistical Package for the Social Sciences (SPSS), 1975, McGraw–Hill]. Statistical significance is defined as $P < 0.05$.

Results. *Hypertension in the miniature swine.* Systemic blood pressure was significantly elevated in response to unilateral renal artery constriction in both the normoxic and hypoxic treatment groups ($P < 0.05$). Nine of the eleven pigs showed mean arterial pressure rises greater than 20 mm Hg (Fig. 1). These pressures reached a plateau by 3 weeks after surgery. No changes in systemic blood pressure were evidenced ($P > 0.5$) between this time period and blood pressure measurements throughout the rest of the experiment. A high correlation coefficient ($r = 0.77$ to 0.96) between week to week blood pressure measurements (weeks 3 through 9) attests to the reliability of obtaining "resting blood pressure" via aortic puncture.

It is unclear whether systemic blood pressure elevations are reflective of increased peripheral resistance (PR) and/or cardiac output (CO) (Table I). While both CO and PR increased, their concurrent increases appeared antagonistic as revealed by a negative relationship between change in CO and change in PR (slope = -0.0092 mm Hg kg^2 min^2/ml^2; $r = -0.670$; $P < 0.05$). A similar negative relationship between WBV and PR existed (Fig. 2).

Significant metabolic changes associated with the development of hypertension in the miniature pig, obtained from a grouping of normoxic and hypoxic pigs, include: elevated serum [Na^+] (Table I), decreased sodium excretion (Week 3), increased potassium ex-

TABLE I. CHANGES ASSOCIATED WITH THE DEVELOPMENT OF HYPERTENSION IN MINIATURE SWINE

Week	HR	CO	SV	PR	$U_{Na}V$	U_KV	UV	[Na^+]	[K^+]	BWT
0	82.2 ± 5.9	105 ± 10.6	1.28 ± 0.080	1.03 ± 0.12	36.4 ± 4.2	63.1 ± 4.3	470 ± 45	140.3 ± 0.81	4.07 ± 0.12	25.8 ± 1.2
	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$
3					20.2 ± 1.6	55.1 ± 3.9	458 ± 61	141.7 ± 0.58	4.25 ± 0.076	
					$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	
9	95.4 ± 5.6	132 ± 14.5	1.35 ± 0.099	1.33 ± 0.22	37.0 ± 6.4	77.6 ± 9.0	477 ± 57	145.6 ± 0.52	4.02 ± 0.11	33.6 ± 1.1
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$	$n = 11$
P^a	>0.1	>0.15	>0.5	>0.25	<0.025	<0.05	>0.5	<0.01	>0.25	<0.001

Note. Data represent the combined results for hypoxic and normoxic treatment groups. Values given represent means ± standard errors. Weak 0 measurements were taken prior to renal artery coarctation. *n*, The number of experimental subjects. Symbols and units of measure are as follows: HR, heart rate, beats/min; CO, cardiac output, ml/min/kg; PR, peripheral resistance, mm Hg kg min/ml; SV, stroke volume, ml/kg; $U_{Na}V$, 24-hr sodium excretion, meq; U_KV, 24-hr potassium excretion, meq; UV, 24-hr urinary volume, ml; [Na^+], serum sodium concentration, meq/liter; serum potassium concentration, meq/liter; and BWT, body weight, kg.

[a] One-way analysis of variance.

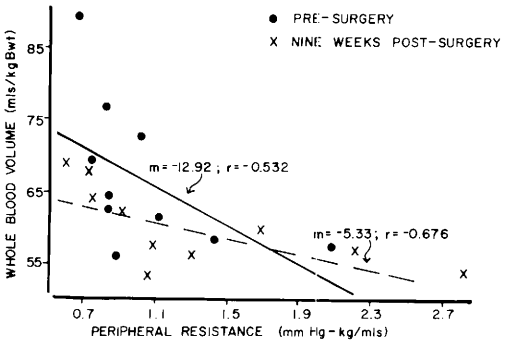

FIG. 2. Peripheral resistance versus whole blood volume/kilogram body weight presurgically and 9 weeks postsurgically. Slope (m) and correlation coefficient (r) are given. The 9-week postsurgical regression (dashed line) is significant ($P < 0.05$).

cretion (Week 9), and decreased circulating thyroxin concentration (Week 9) (Fig. 3). No significant differences existed between treatment groups for these variables.

Blood pressure responses in hypoxic and normoxic swine. Mean systemic arterial blood pressure (MAP) was higher in the normoxic treatment group (Fig. 1) than in hypoxic animals ($P < 0.05$). It appears that the lower MAP in the hypoxic treatment group resulted primarily from a diminution of CO (Table II) although statistical significance was not achieved. The apparent decrement in cardiac output was largely the result of a decreased stroke volume (SV; $P = 0.1$) in the hypoxic environment. Significant differences ($P < 0.05$) evident in the hypoxic group (Table II) were an elevated mean pulmonary arterial blood pressure, hematocrit, and red blood cell volume.

Familial response to renal insult. Our data suggested that much of the variability in our study was due to a diverse, familial response to renal artery constriction. A comparison of blood pressure responses to renal artery coarctation in three sib-pairs (Fig. 4) provides additional evidence that hypoxia moderated the rise in systemic blood pressure. In all three sib-pairs, the hypoxic sib had lower blood pressure, cardiac output, and stroke volume. The suggestion of unique, familial blood pressure responses is supported further by the hemodynamic mechanisms in which the blood pressure differences became evident. In addition to demonstrating a diverse response, these sib-pairings support the contention that hypoxia mitigates against hypertension through a diminished SV and CO.

Discussion. The miniature swine proved to be a suitable model for unilateral, two-kidney renal hypertension. Substantial blood pressure elevation in pigs following the renal artery coarctation technique mentioned in this study has been verified for up to 6 months. The sodium retention evidenced 3 weeks postsurgically is surprising only with regard to the timing of its occurrence. Blood pressures obtained immediately after the 3-week urine collection suggest that the blood pressure rise had already plateaued by this time.

Hypoxia moderated renal hypertension in the miniature swine as Fregly found it to moderate hypertension in rats. While the blood pressure mitigating effect of hypoxia was not as clearly demonstrated as in Fregly's rats, his choice of subjects (the extreme responders to bilateral renal encapsulation), undoubtedly provided a more homogeneous hypertensive model. The diverse, hypertensive response in the two-kidney Goldblatt model in swine has been evidenced in both dogs

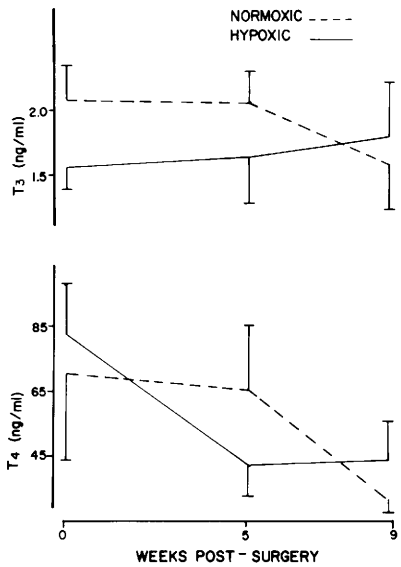

FIG. 3. Serum triiodothyronine (T_3) and thyroxin (T_4) values in normoxic and hypoxic treatment groups (mean values ± standard errors. Thyroxin concentration was significantly depressed at Week 9 when normoxic and hypoxic data are grouped and compared with Week 0 data.

TABLE II. HEMODYNAMIC VARIABLES BEFORE AND AFTER THE DEVELOPMENT OF RENAL HYPERTENSION

	Week 0		Week 9	
	Normoxia (n = 5)	Hypoxia (n = 6)	Normoxia (n = 5)	Hypoxia (n = 5)
CO	118 ± 30	94 ± 38	145 ± 21	111 ± 20
HR	92.1 ± 19.4	73.9 ± 6.8	94.9 ± 7.0	91.2 ± 10.0
SV	1.30 ± 0.12	1.25 ± 0.11	1.49 ± 0.14	1.21 ± 0.12
PR	0.99 ± 0.12	1.07 ± 0.21	1.31 ± 0.38	1.24 ± 0.24
PAP	26.0 ± 2.9	27.3 ± 1.3	27.0 ± 2.0	36.8 ± 1.6*
WBV	68.7 ± 3.0	64.8 ± 4.8	58.4 ± 3.6	60.2 ± 2.0
PV	42.6 ± 2.6	41.2 ± 1.5	37.8 ± 3.2	34.7 ± 2.2
Hct	40.2 ± 1.7	37.4 ± 2.6	37.4 ± 2.3	46.0 ± 1.7**
RBCV	26.2 ± 1.4	23.6 ± 3.5	20.6 ± 1.3	25.5 ± 0.5*

Note. Week 0 measurements were taken prior to renal artery coarctation and prior to initiation of the hypoxic exposure. Symbols and units of measure are as follows: HR, heart rate, beats/min; CO, cardiac output, ml/min/kg; PR, peripheral resistance, mm Hg kg min/ml; SV, stroke volume, ml/kg; PAP, mean pulmonary arterial blood pressure, mmHg; WBV, whole blood volume; ml/kg; PV, plasma volume, ml/kg; Hct, hematocrit, %; RBCV, red blood cell volume, ml/kg. Values given are means ± standard errors. Variable n in the hypoxic treatment group is due to inability to obtain MAP from one hypoxic pig on Week 9.

and rats (12, 13) as well. Our findings suggest that part of this diverse response is familial in origin.

Thyroid hormone measurements failed to support the prior findings that hypoxic moderation of hypertension was mediated via the thyroid. However, sampling time utilized in this experiment may preclude a definitive interpretation (14).

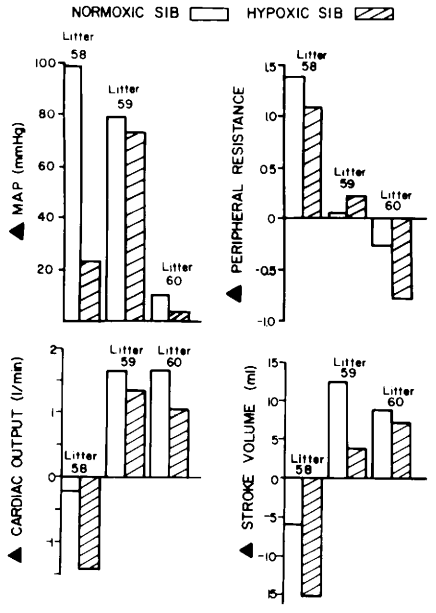

FIG. 4. Familial differences are suggested by sib-pair responses to renal artery constriction. Each of the three litters illustrated had a single representative in the two treatment groups.

While the hemodynamic basis for the mitigation of renal hypertension in this study is unclear, a diminution of SV seems the most substantive. Certainly the reduction in stroke volume associated with pulmonary hypertension following mild hypoxic exposure is well documented in man and cattle (15, 16). However, decreased SV normally occurs in the absence of acute systemic blood pressure change. The mild pulmonary arterial hypertension evidenced in this study seems unlikely to present a significant restriction to blood flow although decreased myocardial contractility in the hypoxic environment (17) may cause the restriction to be more severe.

The effect of hypoxia-induced increased pulmonary vascular resistance and systemic venoconstriction, and the resulting stimulation of blood volume receptors may be a more fruitful area of speculation. This seems to be a likely point of interaction for hypoxia and hypertension since central blood volume in the hypertensive patient has been linked to body fluid and hemodynamic perturbations (18). While central blood volumes were not determined, the significant negative relationship between peripheral resistance and either cardiac output or whole blood volume suggests a close relationship between volume

and blood pressure control in this model of hypertension.

The potential blood pressure modifying effects of hypoxic adaptations such as polycythemia, respiratory alkalosis, venoconstriction, decreased aldosterone levels, decreased plasma volume, and electrolyte and intercompartmental fluid shifts await further scrutiny.

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