Time-Dependent Changes in Catecholamine Turnover in Spontaneously Hypertensive Rats Exposed to Hypoxia (43870)

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Abstract. **In three separate experiments, 4 to 5-week-old spontaneously hypertensive rats (SHR) and normotensive controls (Wistar-Kyoto [WKy]) were exposed to hypobaric hypoxia (simulated altitude = 3658 m) for 3 hr, 3 days, or 3 weeks. Comparable groups were maintained in ambient laboratory conditions (normoxia). Hypoxia prevented the increase in blood pressure noted in 8-week-old normoxic SHR. Right ventricular hypertrophy first occurred after 3 days of hypoxia, and was found in both SHR and WKy. Catecholamine turnover was measured using the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine. In myocardium, both strains evidenced hypoxia-induced changes in norepinephrine (NE) turnover, which was increased at 3 hr, normalized at 3 days, and increased again at 3 weeks. Reduced basal NE concentration at 3 days indicated a temporary deficit in synthetic capacity, which would allow maintenance of a heightened neuronal output. Catecholamine turnover in right and left ventricles differed little in response to hypoxia, in spite of differential hemodynamic demands on SHR versus WKy or on right versus left ventricle. In contrast to findings in myocardium, significant interactive effects between strain and altitude exposure were noted for adrenal catecholamine turnover. Specifically, hypoxia exerted a suppressive influence in SHR that was not evident in WKy, and this may represent an important component of hypoxia-induced protection against the development of spontaneous hypertension.** [P.S.E.B.M. 1995, Vol 208]

The persistent reduction in adrenergic responsiveness that accompanies chronic hypoxia (1, 2) is difficult to explain in terms of neurotransmitter dynamics with currently available information. Transient increases in sympathetic neuronal activity have been reported in hypoxia (3, 4), yet hypoxic attenuation of adrenergic responsiveness is a long-lasting effect of continuing hypoxia (5, 6). While prolonged hypoxic activation of the sympathoadrenal system has been reported (7, 8), numerous experimental variables including severity and duration of hypoxic exposure, as well as potential species differences, complicate efforts to characterize the involvement of the sympathetic nervous system in acclimation to hypoxia.

The spontaneously hypertensive rat (SHR) offers a potentially informative model in which to study hypoxic adaptation of the sympathetic nervous system. The SHR, a supposedly hypersympathetic model of hypertension (9), undergoes a robust attenuation of α-adrenergic responsiveness in hypoxia (1). Additional findings in the hypoxic SHR that potentially reflect changes in sympathetic control included exaggerated reductions in metabolic rate and cardiac output (10, 11), and marked attenuation of hypertension development if exposure to the hypoxic environment occurs at a young age (10). If hypoxia-induced reductions in adrenergic responsiveness of target tissues are a simple result of increased norepinephrine release, then SHR exposed to hypoxia should have exaggerated norepinephrine release.

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Pulmonary vascular responses of SHR to hypoxia provide an additional adaptational problem. Thus, while the right ventricle is exposed to increased afterload associated with pulmonary hypertension (12), the left ventricle functions with decreased aortic pressure in a hypoxic environment (10). These differential demands on the right versus left side of the heart might also be expected to place differing demands on contractile support via activation of the sympathetic nervous system. If contractile demand is directly tied to sympathetic neuronal activity, sympathetic adaptation to hypoxia might differ markedly between right and left sides of the myocardium. Therefore, in the present study we investigated norepinephrine turnover in both left and right ventricles.

Based on measurements of urinary excretion and tissue concentrations of catecholamines, we had previously hypothesized that adaptations of sympathetic responsiveness to hypoxia were unlikely to be a simple result of sympathetic neuronal activation in hypoxia (4). The present study examined this hypothesis more directly by monitoring the decrease in catecholamine concentration following tyrosine hydroxylase inhibition ("catecholamine turnover"). Sympathetic neuronal activity was characterized in myocardium, as well as in adrenal glands, after three durations of exposure to hypoxia in both SHR and Wistar-Kyoto (WKy) rats. Thus, targets of the sympathetic nervous system presumed to be differentially challenged by hypoxia were studied after 3 hr, 3 days, or 3 weeks of hypoxic exposure. Myocardial data provided support for our hypothesis, in that little difference in hypoxia-induced alteration in norepinephrine (NE) turnover was evident between SHR and WKy. This lack of difference between strains was evident for both right and left ventricles, despite considerable differences in functional demand. Unexpectedly, evidence for a selective suppression of adrenal catecholamines in hypoxic SHR relative to similarly treated hypoxic WKy was found at all time points, suggesting that reduced adrenomedullary output is a potentially important mechanism for hypoxia-mediated attenuation of spontaneous hypertension.

Materials and Methods

General. Spontaneously hypertensive and Wistar-Kyoto 4- to 5-week-old male rats were purchased from Charles River Laboratory. Rats were provided pelleted rat chow and water *ad libitum* throughout the experiments, and were maintained within temperature-controlled environments (22 ± 2°C) with 12:12-hr light:dark cycles. After a minimum of a 3-day acclimation period, baseline body weight and blood pressure measurements were made. Rats were then either exposed to simulated altitude (hypobaric hypoxia; 3658 m; 483 torr) or maintained under normoxic conditions (Ft. Collins, CO; 1520 m; 631 torr) in a room located close to the hypobaric facility. A large facility with an antechamber allowed all animal care and blood pressure measurements to be acquired without interruption of the hypoxic environment. In all three experiments, sympathetic activity was characterized using the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (AMPT, Sigma Chemical Co., St. Louis, MO) (300 mg/kg, ip; 2 ml/kg, saline as vehicle). Saline-injected animals (2 ml/kg ip) served as controls. In all three studies, some WKy rats were found to have grossly malformed and hypertrophied hearts, which made accurate dissections impossible. We assume the anomalies were inborn since the hypertrophy appeared to be treatment independent, and therefore all data from these rats were excluded from the study at the time of dissection.

***Experiment 1.* Acute (3-hr) Exposure to Hypoxia.** Twenty-four hours after taking baseline blood pressures, 16 SHR and 16 WKy were injected with AMPT, beginning at 9:00 AM. Eight SHR and eight WKy were then transferred to the altitude chamber. Due to the timed nature of injections, groups of three rats were injected over a 15-min period and then transferred. Thus, no more than 15 min elapsed between injection and exposure to hypoxia. The remaining eight SHR and eight WKy remained at laboratory altitude. An additional eight SHR and eight WKy were injected with saline and maintained at laboratory altitude as well. Rats were sacrificed 3.5 hr after injections. Because of gross myocardial hypertrophy, data from two hypoxic WKy rats in the AMPT-injected group are not reported. Hypoxic animals were sacrificed by decapitation inside the hypobaric chamber. Because of technical limitations, normoxic and hypoxic rats could not be sacrificed in an alternating manner. However, potential time-of-day effects were controlled by reversal of order for injections on two consecutive days.

***Experiment 2.* Sub-Chronic (3-day) Exposure to Hypoxia.** The experimental protocol was similar to that of Experiment 1. Exceptions included the following: additional SHR and WKy rats were included in this study so that a saline-injected reference group was obtained in hypoxia, as well as in normoxia; and AMPT or saline injections were given within respective altitude environments. As in Experiment 1, all rats were killed 3.5 hr after injection of either saline or AMPT. Due to the presence of gross myocardial hypertrophy, data from two normoxic, saline-injected, and one normoxic, AMPT-injected WKy were excluded.

***Experiment 3.* Chronic (3-week) Exposure to Hypoxia.** The experimental protocol was nearly identical to that in Experiment 2 with saline or AMPT injections given 3.5 hr prior to decapitation. An impor-

tant exception was the measurement of blood pressure at the end of experimentation in addition to the initial blood pressures. Due to the presence of gross myocardial hypertrophy, data from two hypoxic WKy, one saline- and one AMPT-injected, were excluded.

Techniques. *Blood pressure measurements.* Systolic blood pressures were obtained using the tail cuff technique from restrained rats that were warmed on a heating pad for approximately 10 min. Multiple pressure traces were obtained from each rat. Pressures reported are the means obtained from three adjacent tracings within 15 mm Hg of each other. This protocol is more rigid than typically recommended by scientific councils (see Spontaneously Hypertensive (SHR) Rats: Guidelines for Breeding, Care, and Use, Institute of Laboratory Animal Resources, National Academy of Sciences). With the adoption of this protocol, we have found inter-reader reliability regarding blood pressure interpretations to typically be greater than $r = 0.95$ and always >0.90.

Terminal procedures. Unanesthetized rats were decapitated, and blood was collected from the cervical wound for the measurement of hematocrit. Hearts were quickly removed, placed in ice-cold, heparinized saline, and blotted dry. On an ice-chilled petri dish, the atria were trimmed from the base of the ventricles, the right ventricle dissected at its juncture with the septal wall, and the wet tissue weight of the right ventricle (RV) and left ventricle plus septal wall (LV) obtained. The ventricles were then wrapped in tin foil, and quick-frozen on flasks containing frozen antifreeze (60% ethylene glycol). The left adrenal gland was rapidly removed and frozen in the same manner as were the ventricles. Tissues were stored at −80°C until assayed for catecholamines.

Neurochemical analyses. Tissues were homogenized in ice-cold, 0.1 *M* perchloric acid containing dihydroxybenzylamine (DHBA) as an internal standard, centrifugated at 10,000*g* in a refrigerated centrifuge, and the supernatant filtered through 0.2 μm filters. The filtrate was analyzed by HPLC (BAS, C_{18} stationary phase, isocratic delivery system, flowrate: 0.8 ml/min) with electrochemical detection (+0.7 v). The mobile phase, 0.05 *M* monochloroacetic acid (pH 3.1), included 120 mg/liter sodium octyl sulphate, 3% acetonitrile, and 2 m*M* Na_2EDTA.

Tissue content of catecholamines was calculated from comparison with internal and external standards, and expressed as concentration (μg/g tissue wet weight). "Turnover" was calculated by subtracting catecholamine values of AMPT-injected rats from mean values obtained from saline-injected rats sacrificed under the same conditions under which the injections were made. Since acutely exposed rats of Experiment 1 were injected with AMPT prior to exposure to simulated altitude, saline-injected rats in normoxia provided the baseline values for both normoxic and hypoxic rats.

Statistics. The primary statistical analysis used was two-way analysis of variance (ANOVA). Separate analyses were carried out for each duration of hypoxia. Statistical references throughout the text refer to conclusions based on ANOVA, except where stated differently. When significant interactions ($P < 0.05$) were obtained and when further analysis was needed, individual means were compared by the least significant difference (protected LSD) technique using the pooled variance from ANOVA. All statistics were performed with the Complete Statistical System (Statsoft, Tulsa, OK).

Results

***Experiment 1.* Acute (3-hr) Exposure to Hypoxia (Age 4.5 weeks).** Metabolic and neurochemical profiles for saline-injected, normoxic rats (SHR-N and WKy-N) are presented in Table I. Since all rats were injected under normoxic conditions in this experiment, normoxic saline-injected rats provided the baseline values for both hypoxic and normoxic AMPT-injected rats. No significant differences existed between SHR-N and WKy-N with the exception of adrenal norepinephrine content (NE-Adr), which was significantly higher in SHR.

Turnover of right ventricular NE was greater in WKy than SHR (ANOVA; $p < 0.05$), while hypoxia caused a significantly increased turnover in both ventricles irrespective of strain (Fig. 1). In adrenal glands (Fig. 2), there was an interactive influence between strain and altitude treatment on NE turnover (Fig. 2). Specifically, hypoxia led to attenuated NE turnover in SHR-H relative to SHR-N (LSD, $P < 0.05$), but en-

Table I. Metabolic and Neurochemical Profiles for Saline-Injected, Normoxic Rats from Experiment 1

	Group		ANOVA
	SHR-N	WKy-N	
n	8	8	
Bwt (g)	50 ± 4	54 ± 6	NS
SBP (mm Hg)	113 ± 19	114 ± 20	NS
Hct (%)	42 ± 3	42 ± 2	NS
RV-Wgt (mg)	56 ± 9	68 ± 16	NS
LV-Wgt (mg)	171 ± 16	185 ± 16	NS
NE-RV (μg/g)	1.20 ± 0.26	1.26 ± 0.17	NS
NE-LV (μg/g)	0.63 ± 0.11	0.70 ± 0.08	NS
NE-Adr (μg)	2.76 ± 0.46	2.08 ± 0.61	*
EPI-Adr (μg)	6.59 ± 0.79	7.77 ± 1.96	NS

Note. Values presented are means ± SD. Underlined values represent measurements obtained prior to the start of altitude exposure. The asterisk indicates significance ($P < 0.05$). N = normoxia; H = hypoxia; Wgt = weight; Bwt = body weight; SBP = systolic blood pressure; Hct = hematocrit; NE-RV = norepinephrine concentration in the right ventricle; Adr = adrenal gland; NS = not significant.

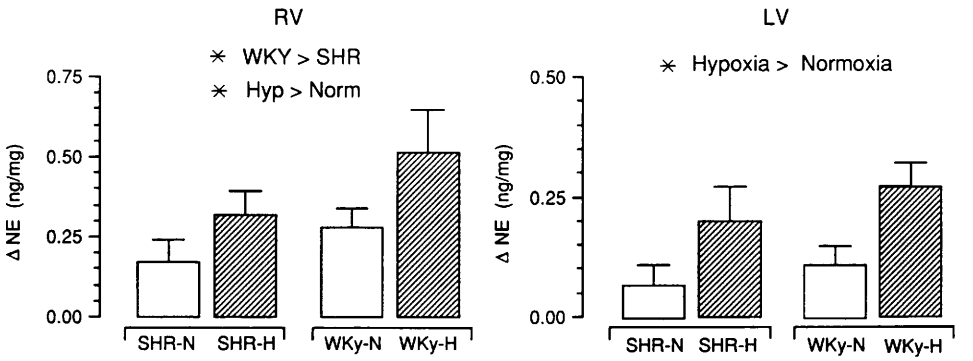

Figure 1. Norepinephrine turnover in right and left ventricles with acute exposure to hypoxia. Data presented are means ± SEM, and asterisks indicate significance as determined by ANOVA.

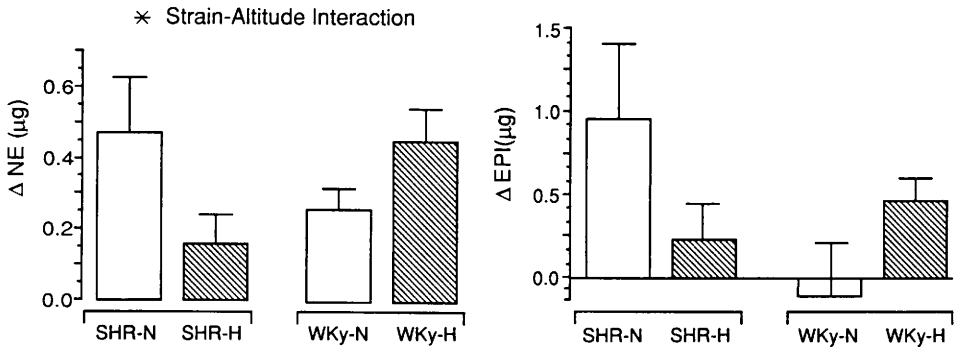

Figure 2. Catecholamine turnover in adrenal glands with acute exposure to hypoxia. Data presented are means ± SEM, and asterisks indicate significance as determined by ANOVA.

hanced NE turnover in WKy-H relative to its normoxic counterpart, although the latter difference did not achieve significance. A similar pattern for adrenal epinephrine turnover was noted, although, in this case, an interaction could not be statistically demonstrated (ANOVA, $P = 0.06$).

***Experiment 2.* Sub-Chronic (3-day) Exposure to Hypoxia (Age 5.5 weeks).** Metabolic and neurochemical profiles for saline-injected, normoxic and hypoxic rats are presented in Table II. After 3 days of hypoxia, significantly retarded growth and significant increases in hematocrit and right ventricular weight were noted, irrespective of strain (ANOVA, main effect of altitude). Left ventricular weight was higher in SHR relative to WKy, irrespective of altitude treatment (ANOVA, main effect of strain). No interactions were noted in these measurements. Systolic blood pressure, which was measured just prior to altitude treatment, did not differ among groups.

SHR had significantly higher NE concentration in right ventricle and adrenal relative to WKy (Table II). Hypoxia-induced reductions of NE in both ventricles and adrenal glands were also noted, irrespective of strain of rat. There was a significant interaction between strain and altitude in adrenal NE, due to a greater hypoxia-induced reduction in SHR than in WKy (LSD, SHR-H vs SHR-N, $P < 0.05$; WKy-H vs WKy-N, NS). Adrenal epinephrine (EPI) did not differ among groups.

NE turnover in right ventricle was significantly increased in SHR when compared with WKy, irrespective of altitude treatment (ANOVA, $P < 0.05$; Fig. 3). Although hypoxia appeared to cause a reduction in NE turnover in right ventricle of WKy, statistical validation of this trend was not obtained. There were no significant effects of strain or altitude treatment on NE turnover in left ventricle.

In adrenal glands, there was an interactive effect of strain and altitude treatment on NE turnover (Fig. 4). This interaction was due to an increased turnover in normoxic SHR relative to normoxic WKy, which was selectively suppressed by hypoxia (LSD, SHR-N vs WKy-N, $P < 0.05$; SHR-H vs WKy-H, NS). Hypoxia also significantly depressed EPI turnover in adrenal glands although this suppression did not differ between strains.

***Experiment 3.* Chronic (3-week) Exposure to Hypoxia (Age 8.5 weeks).** Metabolic and neurochemical profiles for saline-injected, normoxic and hypoxia rats are presented in Table III. Hypertension was manifest in SHR at this time (LSD, SHR-N > WKy-N, $P < 0.05$), while blood pressures of hypoxic SHR remained indistinguishable from normotensive WKy controls (LSD; SHR-H < SHR-N). Hypoxia had

Table II. Metabolic and Neurochemical Profiles for Saline-Injected Rats of Experiment 2

	Group				ANOVA		
	SHR-N	SHR-H	WKy-N	WKy-H	Str	Alt	Str-Alt
n	8	9	7	9			
Bwt (g)	56 ± 3	54 ± 2	59 ± 3	60 ± 3	NS	NS	NS
SBP (mm Hg)	121 ± 12	114 ± 12	117 ± 16	111 ± 15	NS	NS	NS
Bwt (End) (g)	80 ± 7	71 ± 6	79 ± 6	73 ± 9	NS	*	NS
Hct (%)	44 ± 1	47 ± 1	43 ± 1	48 ± 2	NS	*	NS
RV-Wgt (mg)	83 ± 8	100 ± 12	80 ± 3	92 ± 9	NS	*	NS
LV-Wgt (mg)	254 ± 20	240 ± 19	231 ± 16	233 ± 15	*	NS	NS
NE-RV (μg/g)	1.4 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	0.7 ± 0.2	*	*	NS
NE-LV (μg/g)	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	NS	*	NS
NE-Adr (μg)	2.2 ± 0.3	1.7 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	*	*	*
EPI-Adr (μg)	4.6 ± 0.4	4.4 ± 0.5	4.6 ± 0.5	4.7 ± 0.6	NS	NS	NS

Note. Values presented are means ± SD. Underlined values represent measurements obtained prior to the start of altitude exposure. Asterisks indicate significance ($P < 0.05$). N = normoxia; Bwt = body weight; SBP = systolic blood pressure; Hct = hematocrit; NE-RV = norepinephrine concentration in the right ventricle; Adr = adrenal gland; Str = main effect of strain; Alt = main effect of altitude treatment; Str-Alt = an interaction; NS = not significant.

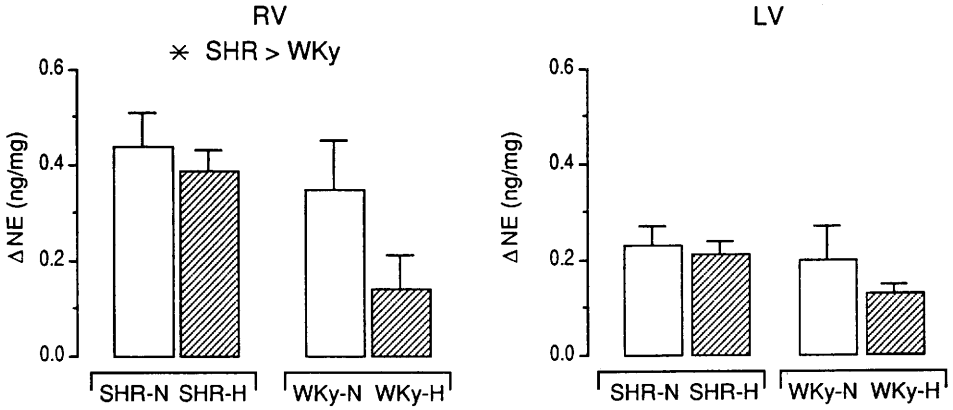

Figure 3. Norepinephrine turnover in right and left ventricles with sub-chronic exposure to hypoxia. Data presented are means ± SEM, and asterisks indicate significance as determined by ANOVA.

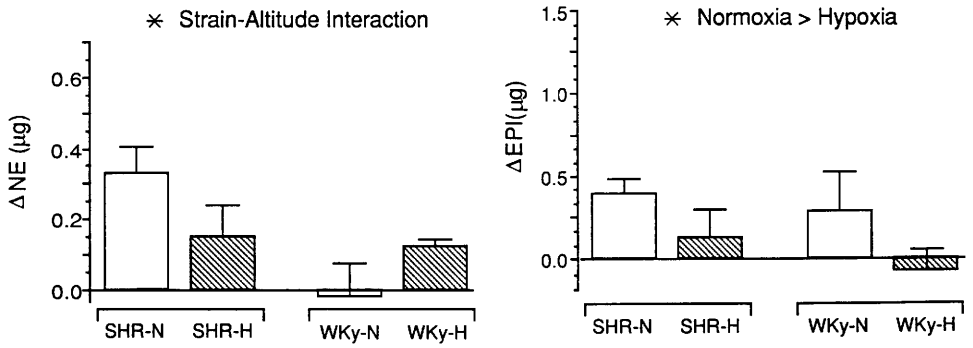

Figure 4. Catecholamine turnover in adrenal glands with sub-chronic exposure to hypoxia. Data presented are means ± SEM, and asterisks indicate significance as determined by ANOVA.

no effect on blood pressure of WKy. Hypoxia elicited a predictable right ventricular hypertrophy and polycythemia in both SHR and WKy. The polycythemic response to hypoxia was greater in WKy (LSD, WKy-H > SHR-H), while hematocrit of normoxic animals did not differ as a function of strain.

The concentration of NE in RV was elevated in SHR relative to WKy (ANOVA). Although hypoxia significantly reduced NE in RV irrespective of strain, this effect reflected primarily the hypoxia-induced ventricular hypertrophy since total right ventricular content of NE did not significantly differ as a function of altitude (data not shown). Adrenal content of NE was elevated, while EPI was reduced, in SHR relative

Table III. Metabolic and Neurochemical Profiles for Saline-Injected Rats of Experiment 3

	Group				ANOVA		
	SHR-N	SHR-H	WKy-N	WKy-H	Str	Alt	Str-Alt
n	8	9	8	7			
Bwt (g)	64 ± 2	63 ± 2	73 ± 1	73 ± 1	*	NS	NS
SBP (mm Hg)	137 ± 4	130 ± 4	131 ± 4	134 ± 4	NS	NS	NS
Bwt (End) (g)	168 ± 14	155 ± 12	180 ± 13	173 ± 13	*	*	NS
SBP (End) (mm Hg)	166 ± 13	132 ± 17	138 ± 14	132 ± 18	*	*	*
Hct (%)	46 ± 2	54 ± 1	46 ± 1	58 ± 2	*	*	*
RV-Wgt (mg)	141 ± 16	238 ± 21	142 ± 5	270 ± 42	NS	•	NS
LV-Wgt (mg)	432 ± 36	426 ± 33	440 ± 28	451 ± 26	NS	NS	NS
NE-RV (μg/g)	1.6 ± 0.1	0.9 ± 0.2	1.4 ± 0.2	0.8 ± 0.2	•	*	NS
NE-LV (μg/g)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	NS	NS	NS
NE-Adr (μg)	3.0 ± 0.2	3.5 ± 0.4	2.5 ± 0.2	3.0 ± 0.4	•	•	NS
EPI-Adr (μg)	8.0 ± 0.4	7.6 ± 0.4	8.5 ± 0.6	9.0 ± 1.4	•	NS	NS

Note. Values presented are means ± SD. Underlined values represent measurements obtained prior to the start of altitude exposure. Asterisks indicate significance ($P < 0.05$). N = normoxia; H = hypoxia; Wgt = weight; Bwt = body weight; SBP = systolic blood pressure; Hct = hematocrit; NE-RV = norepinephrine concentration in the right ventricle; Adr = adrenal gland; Str = main effect of strain; Alt = main effect of altitude treatment; Str-Alt = an interaction; NS = not significant.

to WKy (ANOVA). Hypoxia caused an increase in adrenal NE that was not specific to a particular strain.

In left ventricles, hypoxia caused significant increases in NE turnover irrespective of strain (Fig. 5). In right ventricles, NE turnover did not differ among groups when expressed on a tissue-weight normalized basis. This may again reflect right ventricular hypertrophy, since turnover of NE in right ventricle, when calculated on the basis of total content, followed the pattern found in left ventricle though not achieving statistical significance (data not presented).

In adrenal glands, there were no significant difference in NE turnover among treatment groups (Fig. 6). In contrast, a significant interaction between strain and altitude was noted for EPI turnover. This interaction was due to hypoxia-induced changes in opposite direction in SHR versus WKy (LSD, SHR-H vs WKy-H, $P < 0.05$; SHR-N vs WKy-N, NS).

Discussion

Blood Pressure. Attenuation of elevated blood pressure in the 8.5 week-old SHR in Experiment 3 is consistent with previous studies, which have shown that early exposure to moderate hypoxia prevents the development of spontaneous hypertension (10). The mechanism for this physiological adjustment is not known, although adaptations of the sympathetic nervous system have been implicated (1).

In the present study, blood pressures were not obtained within hypoxia in rats exposed for short periods of time (3 hr or 3 days). Logistical constraints, most notably the stress associated with the restraint required for blood pressure measurements, confounded these measurements, rendering them impossible or imprudent. Further, we have previously shown that hypoxic protection against the development of spontaneous hypertension is a progressive

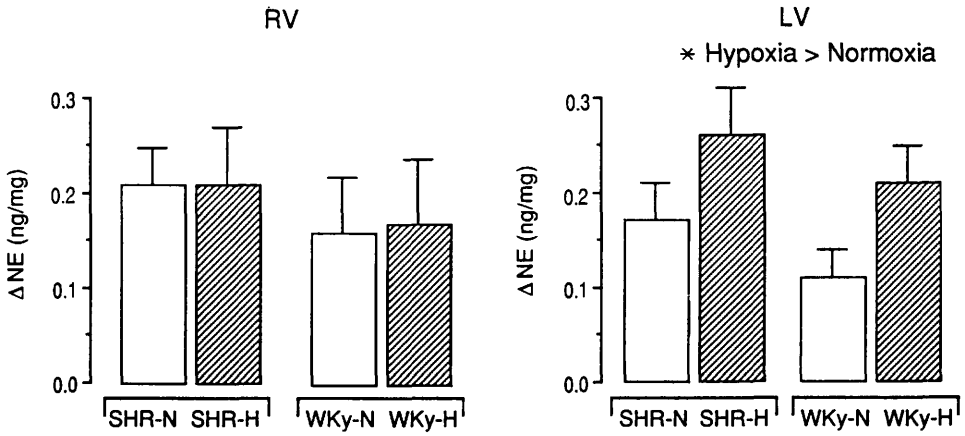

Figure 5. Norepinephrine turnover in right and left ventricles with chronic exposure to hypoxia. Data presented are means ± SEM, and asterisks indicate significance as determined by ANOVA.

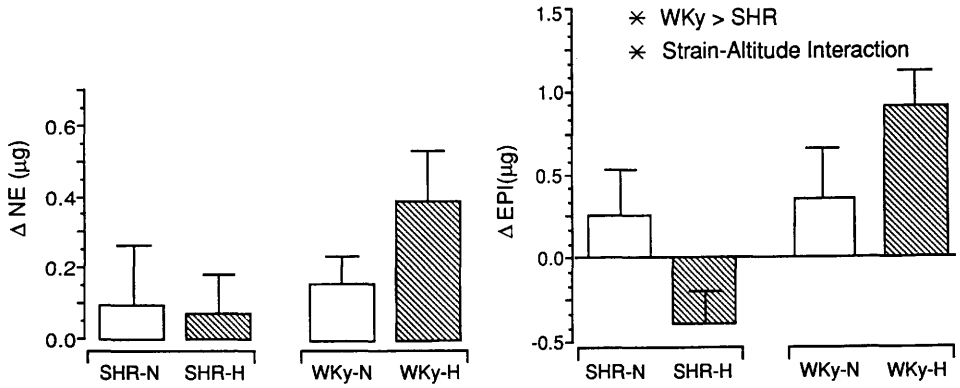

Figure 6. Catecholamine turnover in adrenal glands with chronic exposure to hypoxia. Data presented are means ± SEM, and asterisks indicate significance as determined by ANOVA.

phenomenon that requires several weeks to demonstrate with indirect blood pressure measurements (10).

While pulmonary arterial pressure was not measured in the present study, hypoxia-induced right ventricular hypertrophy has been closely associated with hypoxic pulmonary hypertension (13). The hypertrophy seen in the present study therefore provides reasonable documentation of the presence of pulmonary hypertension. Importantly, no difference in hypoxia-induced right ventricular hypertrophy (and by implication, hypoxia-induced pulmonary hypertension) was found between strains.

Although a reduction of left ventricular weight was not demonstrable in conjunction with hypoxic moderation of spontaneous hypertension in the present study, we have previously shown that such changes do occur with longer-term exposures (14). Thus, the hypoxic SHR undergoes differential adaptational changes in the right and left ventricle that might logically be associated with altered demands for sympathetic activation in the two ventricles.

Myocardial Turnover of NE. Ventricular turnover of NE was increased after 3 hr, unchanged after 3 days, and increased again after 3 weeks of hypoxic exposure. This triphasic change in NE turnover did not differ between the two strains of rats. Moreover, with only minor statistical deviations, this pattern of myocardial NE turnover was evident in both right and left ventricles. The depletion of neuronal stores of NE despite unchanged turnover after 3 days of hypoxia could be interpreted as a temporary deficit in synthetic capacity that interfered with heightened neuronal output. That is, a central drive to increase myocardial NE turnover persisted throughout this study (3 hr and 3 weeks), but was temporarily limited by reduced capacity to synthesize new transmitter at 3 days. Similarity of hypoxia-induced turnover changes in both ventricles, as well as in both strains of rats, contrasts with the markedly different functional demands placed on the two sides of the heart and in the two strains.

The present study complements a previous study from this laboratory in which indirect indices of sympathetic activity were measured (4). While absolute changes in NE concentration in the myocardium are similar between studies, we had initially interpreted the lack of difference in NE in hypoxic versus normoxic rats as a normalization of NE turnover that occurred with chronic hypoxia. In the present study, having both turnover and steady-state values for NE allowed a more accurate picture to emerge.

Findings from the present study are generally consistent with findings from another laboratory based on the use of the same tyrosine hydroxylase inhibitor (7). Important differences between their protocol and the present experiments include their use of adult, exclusively normotensive rats and a more severe hypoxic challenge (10% oxygen). Additionally, their assays were confined to total rather than partitioned ventricles, and assays were not performed earlier than after 2 days of altitude exposure. Still, these authors found that chronic hypoxia (28 days) caused an increase in myocardial turnover, although myocardial NE turnover was not statistically elevated subchronically (2 days), a time when a marked decrease in myocardial NE concentration was reported.

Considered together, these studies provide consistent evidence that both adolescent and adult rats, and both normotensive and hypertensive rats, respond to hypoxia with acute and chronically maintained increases in myocardial NE turnover that are relatively homogeneous between right and left ventricles. Depletion of myocardial NE after sub-chronic exposures (2 to 3 days) appears to reflect temporary impairments in synthetic capacity in conjunction with heightened central drive.

Studies in both humans (6) and rats (2, 15) have shown that myocardial β-adrenergic responsiveness, as well as receptor number, is reduced with chronic hypoxic exposure. While the present findings do not rule out a role for sustained neuronal activation as a

mechanism for these reductions, the similarity of NE turnover in hypoxic SHR and WKy provides no insight into either the anomalous cardiac function that occurs only in SHR in response to hypoxia (11) or the impaired autonomic control previously reported in SHR relative to WKy (16).

Other data also tempers enthusiasm for a simple hypothesis linking heightened myocardial neuronal tone and reductions in β-adrenergic responsiveness. First of all, the hypoxic SHR has been characterized as undergoing an exaggerated drop in responsiveness to adrenergic stimuli, based on findings in isolated aortic rings (1). While aortic NE turnover has never been assessed in the hypoxic SHR to our knowledge, the limited sympathetic innervation of the rat aorta (17) makes it less likely that this drop in responsiveness would be neuronally mediated. Another report indicates that the membrane of papillary muscle from SHR is uniquely sensitive to hypoxia (18). The finding in papillary muscle persists in spite of a propranolol administration, an intervention that would interfere with sympathetic neurotransmission. Thus, cellular changes can be induced throughout the cardiovascular system of the hypoxic SHR that are likely to have significant functional repercussions, and these changes appear to be independent of sympathetic mediated events. Lastly, cultured cardiac myocytes have been shown to undergo decreases in β-adrenergic receptor number and adenylate cyclase responsiveness to isoproterenol with exposure to acute hypoxia (19). Data on receptor and postreceptor mechanisms will be necessary to provide a complete characterization of myocardial adaptations to hypoxia in SHR.

Adrenal Profiles. The present study, as well as a previous report from this laboratory utilizing a nearly identical protocol (4), provides a consistent pattern of hypoxia-induced changes in adrenal catecholamine concentration; only modest differences can be noted. The tyrosine hydroxylase inhibitor used in the present study has been widely used (7, 20) and allowed the extension of previous findings to include estimates of chromaffin cell activity.

The pattern of hypoxia-induced adrenal activation in WKy is consistent with previous results, but these earlier studies examined only acute responses (20) or used a more severe hypoxic stimulus (7) than used in the present study. Hypoxia had a unique influence on adrenal catecholamine turnover in SHR. Thus, in contrast to findings in the WKy, hypoxia tended to decrease adrenal catecholamine turnover in SHR at all three time points used in the present study.

Selective suppression of adrenal catecholamine turnover in SHR represents an important mechanism whereby hypoxia could elicit a potent antihypertensive influence. The heightened adrenal medullary activity that is typically elicited by hypoxia in normotensive animals has been clearly linked with peripheral chemoreceptor stimulation and is associated with a roughly equivalent efflux of norepinephrine and epinephrine from the adrenal gland of the rat (20, 21). As such, selective suppression of adrenal catecholamine turnover in hypoxic SHR provides substantial implication of CNS involvement in this potentially antihypertensive influence of hypoxia.

The findings of a selective attenuation of adrenal catecholamine turnover in hypoxic SHR are consistent with the reported ability of adrenal medullectomy to block the development of spontaneous hypertension (22). The failure of this procedure to reverse established hypertension strengthens the hypothesis that altered adrenal responsivity in early development represents a substrate for modulation of blood pressure. Just as medullectomy is only effective in young SHR, we have shown that hypoxia is more effective at mitigating the development of hypertension than reversing established hypertension. While the present study assessed adrenal medullary activity only in resting animals, the SHR is known to be a hyperresponsive animal (23, 24). As such, it may be that hypoxia would provide an even greater protective influence during stressful events that have a cumulative impact on the cardiovascular system.

In summary, young normotensive and hypertensive rats were found to respond to hypoxia with similar acute and chronically maintained increases in myocardial NE turnover that were relatively homogeneous between right and left ventricles. In contrast to myocardium, the adrenal medulla was selectively suppressed in the hypoxic SHR, thus pointing to an important role for adrenal catecholamines in hypoxic moderation of spontaneous hypertension.

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