# Sympathoadrenal Responses to Acute and Chronic Hypoxia in the Rat

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ABSTRACT The sympathoadrenal responses to acute and chronic hypoxic exposure at 10.5 and 7.5% oxygen were determined in the rat. Cardiac norepinephrine (NE) turnover was used to assess sympathetic nervous system (SNS) activity, and urinary excretion of epinephrine (E) was measured as an index of adrenal medullary activity. The responses of the adrenal medulla and SNS were distinct and dependent upon the degree and duration of hypoxic exposure. Chronic hypoxia at 10.5% oxygen increased cardiac NE turnover by 130% after 3, 7, and 14 d of hypoxic exposure. Urinary excretion of NE was similarly increased over this time interval, while urinary E excretion was marginally elevated. In contrast, acute exposure to moderate hypoxia at 10.5% oxygen was not associated with an increase in SNS activity; in fact, decreased SNS activity was suggested by diminished cardiac NE turnover and urinary NE excretion over the first 12 h of hypoxic exposure, and by a rebound increase in NE turnover after reexposure to normal oxygen tension. Adrenal medullary activity, on the other hand, increased substantially during acute exposure to moderate hypoxia (2-fold increase in urinary E excretion) and severe hypoxia (>10-fold). In distinction to the lack of effect of acute hypoxic exposure (10.5% oxygen), the SNS was markedly stimulated during the first day of hypoxia exposure at 7.5% oxygen, an increase that was sustained throughout at least 7 d at 7.5% oxygen. These results demonstrate that chronic exposure to moderate and severe hypoxia increases the activity of the SNS and adrenal medulla, the effect being greater in severe hypoxic exposure. The response to acute hypoxic exposure is more complicated; during the first 12 h of exposure at 10.5% oxygen, the SNS is not stimulated and appears to be restrained, while adrenal medullary activity is enhanced. Acute exposure to a more severe degree of hypoxia (7.5% oxygen), however, is associated with stimulation of both the SNS and adrenal medulla.

## INTRODUCTION

The sympathoadrenal system functions to maintain internal homeostasis in the face of a widely varying external environment. Hypoxia arising from any cause represents a serious threat to the mammalian organism because it reduces energy production and jeopardizes organ function. Indeed, since Cannon and Hoskins (1) demonstrated secretion of epinephrine (E)[1] into adrenal venous blood during asphyxia, it has been generally accepted that hypoxia stimulates a sympathoadrenal discharge to counter oxygen lack. The traditional view is of a concerted response to the stress of hypoxia, with the two limbs of the sympathoadrenal system, the sympathetic nervous system (SNS) and adrenal medulla, acting together to preserve homeostasis by increasing cardiac output, modifying blood flow distribution, and altering metabolism to improve oxygen delivery to vital tissues.

Investigations conducted over the past 70 years, however, have not provided a clear description of the sympathoadrenal response to hypoxia. In human subjects acutely exposed to hypoxia, for example, both increased (2, 3) or unchanged (4) catecholamine levels in plasma or urine have been observed. In animals,

This work was presented in part in abstract form at the Annual Meeting, Federation of American Society of Experimental Biologists, New Orleans, 1982.

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*Received for publication 25 June 1982 and in revised form 4 November 1982.*

[1] *Abbreviations used in this paper:* alpha-MT, DL-alpha-methyl-*p*-tyrosine methyl ester; Cr, creatinine; E, epinephrine; DHBA, dihydroxybenzylamine; *k*, fractional NE turnover rate; NE, norepinephrine; SNS, sympathetic nervous system.

evidence for and against sympathoadrenal activation in acute hypoxia has similarly been presented (5–10). Chronic hypoxia in man is associated with increased SNS activity (11, 12), although many relevant studies were conducted at high altitude and are, therefore, complicated by cold exposure and physical exertion. Recent studies (13, 14) from our laboratory indicating dissociation of SNS and adrenal medullary responses raised the possibility that the failure to distinguish clearly SNS from adrenal medullary activation contributed to the present uncertainty.

In light of these considerations, the studies reported herein examined separately the functional state of sympathetic nerves and adrenal medulla during acute and chronic exposure to both moderate (10.5% oxygen) and severe (7.5% oxygen) hypoxia in the rat. Measurements of norepinephrine (NE) turnover in rat heart served as the index of SNS activity, while urinary excretion of E provided an estimate of adrenal medullary secretion. Adrenal denervation was used as a further aid in determining the origin of the increased urinary catecholamine levels observed in hypoxia. These studies demonstrate that the sympathoadrenal response to hypoxia is complex and dependent on both the degree and duration of hypoxic exposure. While chronic hypoxic exposure or acute exposure to severe hypoxia increased SNS activity, acute exposure to moderate hypoxia was not associated with SNS activation and may have caused suppression. Adrenal medullary secretion was stimulated by acute exposure to moderate or severe hypoxia.

## METHODS

Female Sprague-Dawley-derived rats (150–200 g) were obtained from Charles River Breeding Laboratories, Inc. (CD rats; Wilmington, MA) or from Zivic-Miller Laboratories (Allison Park, PA). They were housed four to five animals per cage (except when placed in individual metabolic cages) at an ambient temperature of 23–25°C with a 12-h light-dark cycle and permitted access to Charles River chow (R-M-H 3,000; Agway, Inc., Syracuse, NY) and water ad lib. Bilateral splanchnic nerve section or adrenal demedullation was performed by the supplier (Zivic-Miller Laboratories) 2 wk before use.

Studies used both normobaric and hypobaric hypoxia because, as noted by Bert (15), physiological responses to hypoxia are independent of barometric pressure per se. In chronic experiments, a chamber maintained at barometric pressures of 380 torr (equivalent to 10.5% oxygen at sea level) or 272 torr (equivalent to 7.5% oxygen) was used; control animals were placed in a similar, but nonpressurized, enclosure for the duration of the study. In acute experiments, a clear vinyl chamber (Standard Safety Equipment Co., Palatine, IL) continuously perfused with gases of known oxygen and nitrogen concentrations was used. Gas concentrations within each chamber were measured with a mass spectrometer (Perkin-Elmer MGA 1100, Perkin-Elmer Corp., Aerospace Div., Pomona, CA). Flow through the chambers averaged 15–20 liter/min and maintained an $F_{ICO_2} < 0.005$.

In the measurement of [$^3$H]NE turnover, *l*-[(N)-7,8-$^3$H]NE (20–30 Ci/mmol sp act; New England Nuclear, Boston, MA) was purified before use by column chromatography with alumina as described below. The [$^3$H]NE was diluted to an appropriate concentration with isotonic saline and injected into the tail veins of unanesthetized animals in a total volume of 1.0 ml. The dose of [$^3$H]NE used in these studies varied between 50 and 125 μCi/kg (0.3–0.9 μg NE/kg). The rats were killed at preselected times by cervical dislocation. For each time point in the studies of NE turnover, four to six animals were killed from each experimental group. The tissues were rapidly removed, frozen on dry ice, and stored at −20°C for later processing (usually within 2 wk). For NE analysis, the organs were weighed and homogenized in iced, 0.4 N perchloric acid in a ground glass homogenizer (Kontes Glass Co., Vineland, NJ) to extract the NE and precipitate the proteins. After volume adjustment, the precipitated protein was removed by low-speed centrifugation.

Isolation of NE from the perchloric acid extract was achieved by column chromatography with alumina as previously described (16). NE was adsorbed onto the alumina column at pH 8.6 and eluted with 0.2 N acetic acid. The alumina (neutral, Fisher Scientific Co., Pittsburgh, PA) had previously been purified according to the method of Anton and Sayre (17). Recovery of added NE was usually >70% with a variation between columns of <10%. Standards were run with all batches of samples and results were corrected for recovery. Assay of NE (and of E where indicated) in the alumina eluates was performed by a modification of the spectrophotofluorometric method of Crout (18). Aliquots of the alumina eluates were counted for [$^3$H]NE by scintillation spectrometry in a Packard 460C liquid scintillation counter (Packard Instrument Co., Inc., United Technologies, Downers Grove, IL). Efficiency for $^3$H in this system is 30–35%. $^3$H-metabolites of [$^3$H]NE were determined as previously described (16, 19). In the estimation of NE turnover after inhibition of NE biosynthesis, DL-alpha-methyl-*p*-tyrosine methyl ester (alpha-MT; Sigma Chemical Co., St. Louis, MO) was dissolved in isotonic saline and injected in doses of 250 and 125 mg/kg i.p.

Quantitation of catecholamine content in 24-h urine samples used the spectrophotofluorometric method outlined above; for urine samples collected over shorter intervals, a more sensitive analytical procedure, a modification of the electrochemical method of Riggin and Kissinger (20), was used. After addition of dihydroxybenzylamine (DHBA; Aldrich Chemical Co., Inc., Milwaukee, WI) as internal standard, the urines were acidified to pH 2 and passed over a strong cation exchange resin (Dowex 50W × 4, 100–200 mesh; Bio-Rad Laboratories, Richmond, CA) that had previously been prepared by the method of Hirs et al. (21). NE, E, and DHBA were eluted in 1 N HCl, adsorbed onto alumina at pH 8.6 as described above, and eluted from the alumina with 0.1 M perchloric acid (Fisher Scientific Co.). 25–100-μl samples of the alumina eluate were injected onto a chromatographic system consisting of a pump (M-45, Waters Associates, Milford, MA), an automatic injector (WISP 710B, Waters Associates), reverse-phase 5-μm ODS column (Brownlee Labs, Santa Clara, CA), and glassy-carbon amperometric detector (LC-4A/17, Bioanalytical Systems, West Lafayette, IN). The mobile phase was composed of 0.07 M phosphate (Fisher Scientific Co.) at pH 5.8 containing 5% methanol, 2 mM sodium EDTA, and 0.7 mM sodium octylsulfonate (Eastman Kodak Co., Rochester, NY) flowing at a rate of 1.5–2.0 ml/min. Detector potential was set at +0.65 V vs. a Ag/AgCl reference electrode and the response quantitated by peak height with an integrating recorder

(Hewlett-Packard, Co., Avondale Div., Avondale, PA). Recovery of catecholamines averaged 60–70% and intraassay coefficients of variation for NE, E, and DHBA in standards averaged <5%. Creatinine was measured by using standard autoanalyzer techniques.

In the chronic hypoxia experiments, hypoxic and control rats were arbitrarily selected for morphological examination separate from the turnover studies. Under ether anesthesia, a median sternotomy was performed and left ventricular blood removed for hematocrit determination. The heart was excised and the free wall of the right ventricle was dissected from the left ventricle and interventricular septum and weighed. The ratio of the weights of the left ventricle plus septum to right ventricle $[(LV + S)/RV]$ served as a measure of right ventricular hypertrophy (22).

Data are presented as means±SEM unless otherwise noted. Statistical analyses were performed by using analyses of variance and of covariance and the Wilcoxon paired sample test (23). In experiments requiring multiple comparisons, the presence of statistically significant variation was established among all groups before individual comparisons were made between any two groups; individual comparisons used either the Newman-Keuls test (23) or repeat analysis of covariance. In studies of NE turnover, the data were plotted semilogarithmically. The slope (fractional NE turnover rate [$k$]) of the decline in NE specific activity over time after [$^3$H]NE administration was calculated by the method of least squares. In all measurements of NE turnover using [$^3$H]NE, no significant variation in endogenous NE was observed over the 24 h of the experiment unless otherwise noted. The statistical significance of each computed regression line was assessed by analysis of variance. Comparison of fractional turnover rates was made with analysis of covariance. NE turnover rates were calculated as the product of the fractional turnover rate and the endogenous NE concentration (5, 6). 95% confidence intervals were determined for the NE turnover rates as previously described (24). Statistical significance was accepted at the $P < 0.05$ level.

## RESULTS

*Chronic exposure at 10.5% oxygen.* The effect of 14 d exposure to hypobaric hypoxia (barometric pressure, 380 torr) on NE turnover in rat heart is graphically shown in Fig. 1. Data from this experiment and similar studies done at 3 and 7 d are given in Table I. After 14 d of hypoxia, $k$ in heart was greater in hypoxic rats (8.4±0.7% h) than control (3.2±0.5%/h; $P < 0.001$) and the calculated NE turnover rate increased by 134% from 20.2±4.2 ng NE/h (95% confidence intervals) to 47.3±6.0 ng NE/h. Similar elevations in the calculated cardiac NE turnover rate were observed at 3 and 7 d of hypoxic exposure (139 and 129%, respectively). Experiments reported elsewhere (25) demonstrating a more pronounced effect of ganglionic blockade on NE turnover in hypoxic animals than control animals were consistent with a primary increase in sympathetic nerve impulse traffic accounting for the acceleration of NE turnover in hypoxia. Thus, 10.5% oxygen increased SNS activity in rat heart by day 3 of exposure and this effect persisted through day 14.

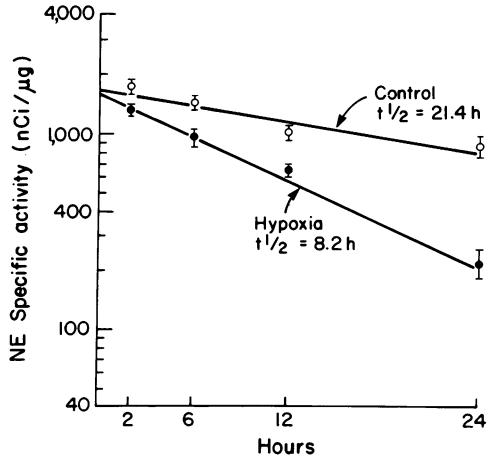


FIGURE 1 Effect of chronic hypoxia on NE turnover in rat heart. Animals were maintained at 10.5% oxygen (hypoxia) or 20.9% oxygen (control) for 14 d. On the final day of hypoxia all animals were injected with [$^3$H]NE (145 μCi/kg). Data are plotted as means±SEM for specific activity of heart from five animals in both groups at each time point. O, control; ●, hypoxic animals. Straight lines are significant approximations of data with $P < 0.001$. $t_{1/2}$ refers to half time of disappearance of NE. Summary of data appears in Table I.

Further evidence for activation of the SNS by chronic hypoxia was provided by the measurement of urinary catecholamine excretion (Fig. 2). Although not increased above a basal level of 139±14.8 ng NE/mg creatinine (Cr) per d on the first day of hypoxic exposure, urinary excretion of NE rose to 308±31.0 ng NE/mg Cr per d ($P < 0.01$ vs. basal) on day 3 of hypoxic exposure and remained elevated through day 14. Urinary output of E rose from a base-line value of 16.8±1.9 to 25.6±4.1 ng E/mg Cr per d over the period of hypoxia, an increase that was not statistically significant. Since the increment in urinary NE excretion exceeded that of E, the presumption was that the urinary NE originated from the SNS and not the adrenal medulla. To confirm this impression, the study was repeated in adrenally denervated rats. In these animals NE excretion increased from a basal level of 156±23.7 ng NE/mg Cr per d to 275±21.4 ($P < 0.01$) by day 3, whereas urinary E remained unchanged and barely detectable at 2.9±0.7 ng E/mg Cr per d, consistent with adrenal denervation. Thus, chronic 10.5% oxygen stimulated the SNS with activation demonstrable by day 3.

Animals exposed to 10.5% oxygen for 14 d demonstrated morphological changes characteristic of the response to hypoxia. Hematocrit was elevated from 43±1 in controls to 53±2 ($P < 0.01$) in hypoxic animals; right ventricular hypertrophy was also noted with a change in the index ratio of the left ventricle plus

TABLE I

*Effect of Chronic Hypoxia (10.5% Oxygen) on NE Turnover in Rat Heart*

| Duration of hypoxia (n) | $k$ | Endogenous NE | NE turnover rate | Change |
|---|---|---|---|---|
| | *%/h* | *ng/heart* | *ng/h* | *%* |
| 3 d (20) | 12.1±1.8* | 395.5±20.7‡ | 48.1±9.6 | 139 |
| Control (20) | 4.0±0.5 | 494.0±19.7 | 20.1±3.2 | |
| 7 d (21) | 11.8±1.6* | 407.4±19.2* | 48.6±8.9 | 129 |
| Control (21) | 3.5±0.5 | 597.3±24.0 | 21.2±3.5 | |
| 14 d (22) | 8.4±0.7* | 558.3±25.2 | 47.3±6.0 | 134 |
| Control (19) | 3.2±0.5 | 620.1±25.5 | 20.2±4.2 | |

* $P < 0.001$ hypoxia vs. control.

‡ $P < 0.01$ hypoxia vs. control.

Rats were maintained at a barometric pressure of 380 (hypoxia) or 760 (control) torr. During the final 24 h of each experiment, NE turnover was measured after intravenous injection of [$^3$H]NE. Values are means±SEM for k and endogenous NE; values for NE turnover rate are 95% confidence limits. Numbers in parentheses represent the number of animals.

septum to right ventricle from 3.46±0.13 in control animals to 2.63±0.05 after 14 d of hypoxia ($P < 0.01$).

*Chronic exposure at 7.5% oxygen.* To ascertain whether SNS activation increases with the severity of hypoxia, NE turnover was measured simultaneously in animals exposed for 7 d to 7.5, 10.5, and 20.9% (room air) oxygen (Fig. 3). Fractional NE turnover rates in heart were 4.3±0.7, 11.4±1.1, and 19.9±1.8%/h for animals exposed to 20.9, 10.5, and 7.5% oxygen, respectively ($P < 0.0001$ for all comparisons). Calculated cardiac NE turnover rates were accelerated by hypoxia, but because of the lower steady-state endogenous NE content in the hearts of rats exposed to 7.5% oxygen, calculated NE turnover rates were not clearly different in the two hypoxic groups (22.6±5.2, 38.8±5.8, and 43.8±9.4 ng NE/h for animals exposed to 20.9, 10.5, and 7.5% oxygen, respectively). The increase in cardiac NE turnover at 7.5% oxygen, however, was clearly sustained through at least 7 d of exposure.

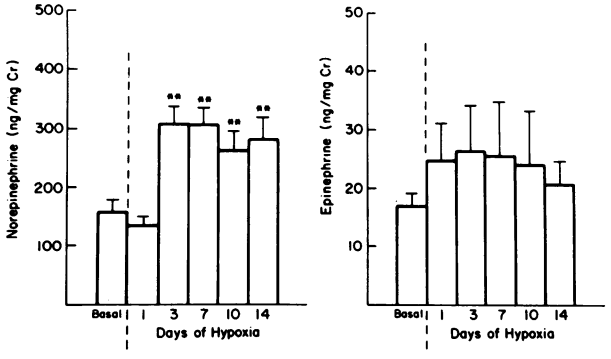


FIGURE 2 Effect of chronic hypoxia on urinary excretion of NE and E. Animals were maintained at 20.9% oxygen for basal and 10.5% oxygen for hypoxic measurements. 24-h collections of urine were made under oil by using individual metabolic cages. Data are means±SEM for eight animals. ** $P < 0.01$ for comparison between hypoxic and basal days.

*Acute exposure at 10.5% oxygen.* In contrast to the SNS response to chronic 10.5% oxygen, acute 10.5% oxygen did not stimulate SNS activity in the rat. As shown in Fig. 2, urinary NE excretion did not change during the first day of exposure to 10.5% oxygen, suggesting that SNS activity was not acutely increased by this degree of hypoxia. To examine this possibility more directly, cardiac NE turnover was measured during the first 10–24 h of hypoxic exposure. In a representative study (Fig. 4), animals were exposed to 10.5% oxygen for 11 h. In hypoxic rats the fractional NE turnover rate in heart was 7.8±1.3%/h and the calculated NE turnover rate was 36.3±8.0 ng NE/h (95% confidence intervals), values that did not differ from those observed in control animals (8.5±1.2%/h and 39.2±7.0 ng NE/h, respectively). Included in this experiment was a group of animals acutely exposed to cold (4°C) to provide a positive, internal control. Cold exposure, a known SNS stimulus, increased both fractional NE turnover in heart and calculated NE turnover to 22.4±1.7%/h ($P < 0.0001$) and 90.8±12.2 ng NE/h (by 132%), respectively, compared with ambient temperature controls. The slight difference between NE turnover in control and acutely hypoxic rats was not statistically significant in this experiment; in five additional consecutive experiments, however, a similar decrease in cardiac NE turnover was consistently obtained (Table II). Although the decrease was

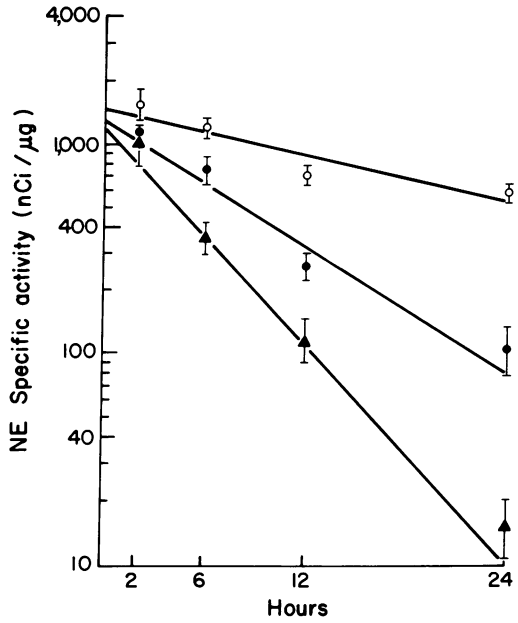


FIGURE 3 Effect of two levels of chronic hypoxia on NE turnover in rat heart. Animals were maintained at 7.5% oxygen (severe hypoxia), 10.5% oxygen (moderate hypoxia), and 20.9% oxygen (control) for 7 d. On the final day of hypoxia, all animals were injected with [$^3$H]NE (120 μCi/Kg). Data are plotted as means±SEM for specific activity of heart from five animals in each group at every time point. O, control (16.2 h); ●, moderate hypoxia (6.0 h); ▲, severe hypoxia (3.5 h). Numbers in parentheses represent half times of disappearance of NE. Straight lines are significant approximation of data with $P < 0.0001$.

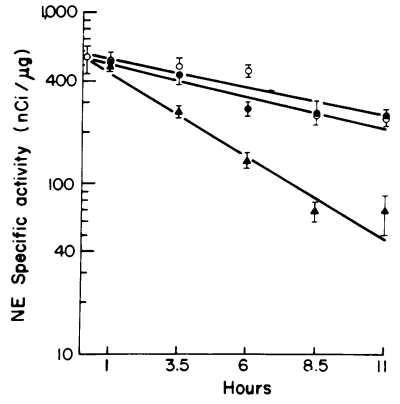


FIGURE 4 Effect of acute hypoxia and acute cold exposure on NE turnover in rat heart. All animals were injected with [$^3$H]NE (50 μCi/kg); five animals were killed 5 min after injection and were included in the estimation of each line as a common zero point. The remaining animals were randomly allocated to a cold room at 4°C (cold ▲; 3.1 h), an hypoxic chamber at 10.5% oxygen and 24°C (hypoxia O; 8.9 h), or an ambient chamber at 20.9% oxygen and 24°C (control ●; 8.2 h). Numbers after symbols refer to half times of disappearance of NE. Data are plotted as means±SEM for specific activity of heart from five animals in each group at every time point. Straight lines are significant approximations of data with $P < 0.0001$.

not statistically significant in any single experiment, taken as a group, the likelihood of six separate studies demonstrating the same directional change in NE turnover by chance alone is $P < 0.05$ (Wilcoxon paired sample test). This analysis suggests that acute exposure to 10.5% oxygen induces a small reduction in NE turnover.

Measurement of urinary catecholamine excretion over the initial 6 h of hypoxic exposure provided supporting evidence of SNS suppression at 10.5% oxygen. Urinary NE excretion fell from basal levels of 105.6±8.0 to 80.0±4.1 ng/mg Cr ($P < 0.05$). Urinary E excretion, on the other hand, increased from 28.9±3.7 to 40.2±5.4 ng/mg Cr ($P < 0.05$). These data support the NE turnover experiments that suggested SNS suppression during acute exposure to 10.5% oxygen and indicate that the adrenal medullary response to acute moderate hypoxia is different from the SNS response. Furthermore, the opposite changes in urinary excretion of NE and E argue against attributing the urinary findings solely to alterations in the renal handling of catecholamines.

To confirm a difference in SNS activity between acute and chronic 10.5% oxygen, a qualitative estimate of NE turnover was made in hypoxic and control animals using a nontracer method (Fig. 5). Alpha-MT inhibits tyrosine hydroxylase, the rate-limiting enzyme

TABLE II
*Effect of Acute Moderate Hypoxia on k*

| Experiment | k | | Change |
|---|---|---|---|
| | Control | Hypoxia | |
| | %/h | | % |
| 1 | 8.5±1.2 | 7.8±1.3 | −8 |
| 2 | 6.0±1.6 | 3.3±1.1 | −45 |
| 3 | 5.6±0.9 | 5.1±1.5 | −9 |
| 4 | 5.7±1.4 | 4.5±1.8 | −21 |
| 5 | 6.5±1.5 | 5.5±2.3 | −15 |
| 6 | 4.9±1.7 | 4.8±2.2 | −2 |
| | | | Mean −17 |

These six experiments, presented in the chronological order in which they were performed, were carried out as described for Fig. 4. The timing of the points at which cardiac NE specific activity was measured varied among experiments and the hypoxic severity ranged from 9 to 10.5% oxygen, but in all of them the measurement of NE turnover was made during the first 12 h of hypoxia. In all experiments, the data fit the monoexponential model as indicated. The difference in $k$ between control and hypoxia for the six experiments was statistically significant ($P < 0.05$) by Wilcoxon paired sample test.

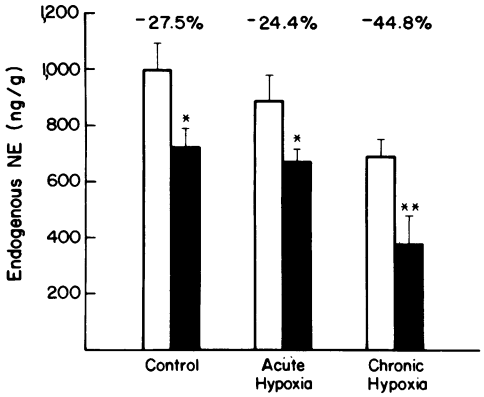


FIGURE 5 Effect of acute and chronic hypoxia on depletion of cardiac NE after alpha-MT administration. Animals were maintained for 8 h at 10.5% oxygen (acute hypoxia) or for 7 d at 10.5% oxygen (chronic hypoxia) or 20.9% oxygen (control). Alpha-MT or saline were given at time zero (250 mg/kg) and 4 h (125 mg/kg); animals were killed at 8 h. Data are represented as mean endogenous NE±SEM for groups of eight animals each. Open bars, mean NE content in heart of saline-treated animals; closed bars, alpha-MT-treated animals. Number over bar indicates percentage of decrease below saline control. $^{*}P < 0.05$ and $^{**}P < 0.01$ for comparisons between alpha-MT and saline treatment.

in NE biosynthesis, leading to depletion of tissue NE stores at a rate that reflects nerve impulse traffic. Within the same experiment, the extent of cardiac NE depletion over an 8-h interval was compared in animals maintained at 10.5% oxygen for 7 d, in those acutely exposed to the same hypoxic stimulus, and in control animals. In contrast to the 24.4% fall in endogenous NE levels in the hearts of the acutely hypoxic rats (similar to that seen in controls, a 27.5% decrease), cardiac NE content dropped 44.8% in the chronically hypoxic animals. The results of this experiment are consistent with the findings from previous studies using [$^3$H]NE in demonstrating acceleration of cardiac NE turnover by chronic, but not by acute, exposure to 10.5% oxygen.

*Posthypoxic normoxia.* Further evidence of SNS suppression during acute 10.5% oxygen was provided by measurement of cardiac NE turnover immediately after return to normoxia. Three groups of rats were studied by using the alpha-MT technique: (*a*) a group exposed to 10.5% oxygen for 5 h; (*b*) a group exposed to 10.5% oxygen for 2.5 h, then room air for 2.5 h; and (*c*) a control group. All animals were given alpha-MT (250 mg/kg) at 2.5 h and killed at 5 h, 2.5 h after alpha-MT administration. At this level of hypoxia, previous studies had demonstrated no change in endogenous NE levels in heart. In the continuously hypoxic animals, endogenous NE levels in heart after alpha-MT administration were 687.1±34.9 ng, not significantly different from control values of 674.0±25.7 ng. In rats returned to room air, cardiac NE content after alpha-MT treatment was reduced to 530.9±34.2 ng, a level significantly lower than either the controls or the treated hypoxic group ($P < 0.05$). This observation is consistent with increased cardiac NE turnover in animals returned to room air after brief exposure to 10.5% oxygen and suggests that the acute hypoxic exposure induced restraint of the SNS, with relief of restraint by normoxia.

*Acute exposure at 7.5% oxygen.* Since acute exposure to 10.5% oxygen did not stimulate cardiac NE turnover, the effect of more severe hypoxia (7.5% oxygen) was examined. In this experiment, the rate of disappearance of tritiated NE was markedly accelerated in hypoxic animals ($k$ = 13.8% in hypoxic and 3.8% in control animals); endogenous NE content, however, fell over 12 h to 67% of that found in control animals, a change in cardiac NE not seen at lesser degrees of hypoxia. Such a depletion in the endogenous pool of NE results in an underestimation of the NE turnover rate based on specific activity if steady-state kinetics are assumed to exist but do not prevail. NE turnover may be approximated under these circumstances by correcting for the fall in endogenous NE over the course of the experiment.[2] Applied to the present experiment as an approximation, the calculated NE turnover rate for animals acutely exposed to 7.5% oxygen was 57.6 ng NE/h; and for controls, 35.0 ng NE/h, a finding consistent with SNS activation.

To clarify further this apparent difference in the acute SNS response to 7.5 and 10.5% oxygen, urinary catecholamine excretion was measured over the initial 6-h period of exposure to 7.5% oxygen, as previously mentioned for 10.5% oxygen (Fig. 6). In the presence of 7.5% oxygen, both urinary NE and E excretion increased. Urinary NE rose from 85.2±9.8 ng NE/mg Cr to 393.9±57.6 ($P < 0.05$) and E from 25.2±2.4 ng E/mg Cr to 297.2±45.8 ($P < 0.05$). Since the adrenal medulla is a potential source of urinary NE, the marked elevation in urinary NE excretion cannot be taken as definitive evidence of SNS stimulation. The study was therefore repeated with animals subjected to bilateral splanchnic nerve section, a procedure that denervates the adrenal medulla (Fig. 6). When denervated animals were exposed to 7.5% oxygen, urinary E excretion remained at background levels (~10

[2] The average decline in endogenous NE during hypoxia was calculated as the difference in the mean endogenous NE for control animals minus the mean endogenous NE for hypoxic animals at the end of the study divided by the duration of the study in hours, ([567 − 378]/12 = 15.75 ng/h). This was added to the calculated NE turnover rate to determine the corrected turnover rate.

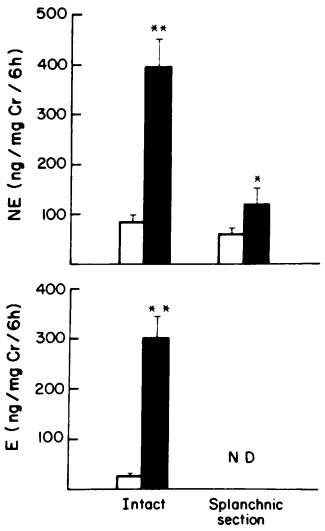


FIGURE 6 Effect of acute severe hypoxia on urinary excretion of NE and E. Animals were maintained at barometric pressure of 20.9% oxygen (control) or 7.5% oxygen (hypoxia) for 6 h; urine was collected under oil in individual metabolic cages. Data are means±SEM for groups. Open bars, control periods; closed bars, hypoxic periods. Splanchnic section refers to previous bilateral adrenal denervation; intact had undergone no denervation procedure. ND, none detected. $^{*}P < 0.05$ and $^{**}P < 0.01$ for comparisons between hypoxic and control periods.

ng E/mg Cr), an indication of satisfactory adrenal denervation. In contrast, urinary NE excretion doubled with 7.5% oxygen, increasing from basal levels of 60.0±9.8 ng NE/mg Cr to 123.5±29.1 ($P < 0.05$). Thus, as assessed by the urinary excretion of NE and E, acute 7.5% oxygen activates both limbs of the sympathoadrenal system, although the adrenal medulla appears to contribute the larger share of the increment in urinary NE excretion.

*Uptake and metabolism of [$^3$H]NE.* Since alterations in tissue handling of tracer NE could conceivably affect the measurement of NE turnover during acute or chronic hypoxia, the effect of hypoxia on neuronal uptake and intraneuronal metabolism of [$^3$H]NE was examined. After administration of tracer, the content of [$^3$H]NE and of $^3$H-metabolites was measured in plasma and various tissues 5 min after injection in control and hypoxic animals. Hypoxic animals had been exposed to 10.5% oxygen either for 1 h (acute exposure) or for 7 d (chronic). Content of [$^3$H]NE was similar in heart, liver, and kidney for all three groups and, in addition, quantities of [$^3$H]normetanephrine and of [$^3$H]vanillylmandelic acid in liver and kidney did not differ among the three groups. Levels of [$^3$H]NE in plasma, however, were ~50% greater in the chronically hypoxic rats than in either the control or the acutely hypoxic animals ($P < 0.05$). Since the tracer was administered in a dose based upon body weight, the higher plasma levels of [$^3$H]NE in the chronically hypoxic rats suggest a reduced volume of distribution of the tracer. Whether the equivalence of tissue content of [$^3$H]NE in the three groups despite the smaller distribution space in the chronically hypoxic animals reflects diminished neuronal uptake of tracer in this group cannot be determined from the current data. Apart from the elevation in plasma levels of [$^3$H]NE in the chronically hypoxic rats, no other parameters of tracer metabolism were altered in either acute or chronic hypoxia.

## DISCUSSION

The studies reported here demonstrate that the sympathoadrenal response to hypoxia in the rat is complex and dependent upon both the duration and severity of hypoxic exposure. Measurements of urinary E excretion indicate a prompt but modest increase in adrenal medullary activity (an ~40% increase over basal E excretion) during the first 6 h of 10.5% hypoxic exposure. At 7.5% oxygen, acute hypoxic exposure has a more marked stimulatory effect on the adrenal medulla as indicated by a 10-fold increase in E excretion (Fig. 6). Thus, acute exposure to an hypoxic environment stimulates the adrenal medulla, the secretory response being mild at 10.5% oxygen and vigorous at 7.5%. The adrenal medullary response to chronic exposure at 10.5% oxygen is modest and apparently sustained although in the experiment shown in Fig. 2 the increment in E excretion as compared with base line was not statistically significant.

Chronic exposure to moderate hypoxia (10.5% oxygen) increases SNS activity as indicated by increased cardiac NE turnover measured at 3, 7, and 14 d after hypoxic exposure (Fig. 1, Table I). The cardiac NE turnover data are supported by measurements of urinary NE excretion (Fig. 2), which demonstrate a twofold increase between days 3 and 14 of hypoxic exposure. The stimulatory effect of moderate hypoxia (10.5% oxygen) on the SNS does not, however, begin immediately upon exposure. During the first day of acute moderate hypoxia, NE turnover in heart is not increased (Fig. 4) in distinction to the definite increase at 3 d (Table I). This difference between the acute and chronic response was again demonstrated within a single experiment using the synthesis inhibition technique with alpha-MT (Fig. 5); 7 d of exposure to 10.5% oxygen increased the rate of cardiac NE depletion as compared with both control animals and those exposed acutely to hypoxia over an 8-h interval. The data suggest, moreover, that SNS activity was actually suppressed during exposure to acute moderate hypoxia. Although statistically significant suppression was not

demonstrated in any individual study, in six consecutive studies (Table II) the cardiac NE turnover was decreased by acute hypoxic exposure in every one, an unlikely occurrence by chance alone ($P < 0.05$). Urinary NE excretion during the early phases of acute hypoxic exposure at 10.5% oxygen supports the turnover data; a significant (25%) fall in urinary NE excretion was documented during the first six h of moderate hypoxia. Additional evidence in support of restraint or suppression of SNS activity during acute moderate hypoxia is provided by measurements of NE turnover immediately after return to normoxia after 2½ h at 10.5% oxygen; the degree of depletion induced by synthesis inhibition with alpha-MT was significantly greater in animals returned to normoxia after hypoxic exposure than in control animals or animals continued in an hypoxic environment.

These direct measurements of the component responses of the sympathoadrenal system to acute moderate hypoxia establish a primary role for the adrenal medulla and suggest a condition of SNS restraint. Previous indirect studies have reached similar conclusions about the primacy of the adrenal medulla in the response to acute hypoxia. By assessing hemodynamic parameters, Tucker (26), Hammill et al. (27), Lee et al. (28), and Nahas et al. (10), in separate studies, found that adrenalectomy was more effective than chemical sympathectomy in abolishing the cardiac response to hypoxia. Goldman and Harrison (8) did directly measure NE turnover by a synthesis inhibition technique but failed to demonstrate any significant change with acute hypoxia at 8% oxygen; they concluded that at this level of hypoxia either no increase occurs or it was too slight to establish with their technique. Our studies using direct measurements of NE turnover indicate that acute exposure to 10.5% oxygen may suppress SNS activity while stimulating the adrenal medulla. With continued exposure to an hypoxic environment SNS stimulation occurs, the latter being evident by 3 d at 10.5% hypoxia.

The acute SNS response to a more severe hypoxic exposure is, however, different from the response to moderate hypoxia. Studies involving cardiac NE turnover and urinary NE excretion (Fig. 6) both demonstrate SNS stimulation during the first 12 h of exposure to an hypoxic environment at 7.5% oxygen. Both the SNS and the adrenal medulla, therefore, are stimulated acutely by a more severe hypoxic exposure. Exposure to severe hypoxia at 7.5% oxygen for 7 d (Fig. 3) is also associated with a marked increase in cardiac NE turnover. These studies thus demonstrate that both the degree and duration of hypoxic exposure affect the SNS response.

The changes in cardiac NE turnover reported here reflect predominantly changes in SNS activity. The evidence in favor of this conclusion includes the following: (*a*) under a variety of other physiological situations such as cold exposure (29), forced restraint (30), and changes in dietary intake (29, 31, 32) NE turnover has consistently and accurately reflected the state of activity of the SNS as assessed by other methods; (*b*) in a previous series of experiments, the connection between alterations in NE turnover and central sympathetic outflow during chronic exposure to hypoxia at 10.5% oxygen was demonstrated (25); (*c*) in the current study, changes in NE excretion (Figs. 2 and 6) were entirely consistent with measured changes in NE turnover; and (*d*) the uptake and metabolism of tracer NE by heart, liver, and kidney was unaffected by acute or chronic moderate hypoxia. Two possible confounding factors, however, require consideration: (*a*) the potential effect of adrenal medullary stimulation on SNS activity itself or on the assessment of SNS activity by measurement of NE turnover; and (*b*) the potential effect of hypoxia on the peripheral sympathetic nerve endings, particularly the energy-dependent amine uptake process of the axonal membrane.

Although the adrenal medulla is stimulated by hypoxia (1, 3, 6, 7, 9, 10, 33), increased circulating catecholamines of adrenal medullary origin cannot explain the changes in NE turnover demonstrated here. In the first place, no consistent relationship between the functional state of the SNS and the adrenal medulla was noted. Adrenal medullary stimulation occurred in association with both suppression of the SNS (acute exposure at 10.5% oxygen) and sympathetic stimulation (acute exposure at 7.5% oxygen). Secondly, changes in urinary NE excretion in adrenal demedullated animals paralleled changes in cardiac NE turnover. Finally, in other studies from our laboratory, adrenalectomy, adrenal demedullation, or splanchnic nerve section have failed to alter changes in NE turnover in a variety of physiological situations associated with sympathetic suppression and adrenal medullary stimulation, including fasting hypoglycemia (13), 2-deoxyglucose administration (14), and fasting during cold exposure (29). Thus, the changes in cardiac NE turnover reported here cannot be explained by alterations in adrenal medullary function.

The possibility that hypoxia alters peripheral sympathetic nerve function cannot be so easily dismissed. Although a simple direct effect of low oxygen tension on neuronal function can probably be excluded because of the differential effects of acute and chronic moderate hypoxia on cardiac NE turnover, a subtle effect on the neuronal uptake process in chronic moderate and acute and chronic severe hypoxia cannot be excluded with certainty. The abrupt fall in endogenous NE level during acute exposure at 7.5% oxygen and

the slightly lower steady-state endogenous NE level in chronically hypoxic animals are consistent with an effect on neuronal reuptake, although similar changes in endogenous NE levels may result from intense SNS stimulation (31). Furthermore, although tracer NE concentration and metabolism in heart, liver, and kidney at 5 min after intravenous injection was not altered by acute or chronic hypoxia at 10.5% oxygen, the tracer concentration in plasma was significantly higher in the chronically hypoxic group. The significance of the elevated plasma tracer concentration is uncertain since tracer levels were not altered in tissues reflecting neuronal (heart) and nonneuronal (liver and kidney) uptake. It is possible that the altered volume of distribution of tracer in chronically hypoxic animals does reflect, at least in part, an alteration in neuronal uptake. Such an alteration by diminishing NE uptake would increase NE turnover. It is, therefore, possible that a subtle defect in neuronal uptake induced by hypoxia contributes to the increase in NE turnover reported here. Such an alteration would have the same physiological impact as an increase in neuronally mediated NE release; indeed, to the extent that the physiological responses of released NE are augmented by impaired reuptake, feedback suppression of sympathetic neuronal outflow might be expected to restore NE turnover towards normal. Therefore, although altered reuptake cannot be excluded as contributing to the increase in NE turnover during hypoxia, it seems likely that increased central sympathetic outflow is the major factor accounting for the increased cardiac NE turnover reported here.

Alterations in SNS activity during hypoxic exposure may contribute to some of the physiological changes that accompany diminished oxygen supply. During acute hypoxic exposure, oxygen consumption and heat production fall (34–36) and hypothermia develops (34–40). This hypoxic depression of metabolism represents either the direct effect of oxygen lack at a cellular level, or the recruitment of a regulatory process that diminishes metabolic rate, or both (35). The suppression of SNS activity demonstrated here during acute exposure at 10.5% oxygen is consistent with the hypothesis that hypoxia evokes thermoregulatory mechanisms that diminish metabolic heat production in part through an effect on the SNS. Further evidence lending support to potential SNS participation in hypoxic hypothermia in rats is the reversal of the hypometabolic response to hypoxia by physical restraint (41), a well-known, potent stimulus of SNS activity (30). The rebound increase in sympathetic activity demonstrated here during return to normoxia also suggests that inhibition of SNS activity contributes to the hypothermia of acute hypoxic exposure. Thus, the sympathoadrenal response to hypoxia is complex; differential effects on the SNS and adrenal medulla may be noted depending upon the degree and duration of hypoxic exposure.

## ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance of Shawna Murray and the skillful secretarial assistance of Georgette Briguglio, Viki Fowler, and Edith Cusher.

This research was supported in part by U. S. Public Health Service grants AM 20378, AM 26455, HL 24084, and RR 76.

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