Mechanism of the attenuated cardiac response to β-adrenergic stimulation in chronic hypoxia

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MAHER, J. T., J. C. DENNISTON, D. L. WOLFE, AND A. CYMERMAN. *Mechanism of the attenuated cardiac response to β-adrenergic stimulation in chronic hypoxia.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 44(5): 647–651, 1978. — A blunting of the chronotropic and inotropic responses of the heart to β-adrenergic stimulation occurs following chronic exposure to hypobaric hypoxia. To pursue the mechanism(s) involved, observations were made in six intact, conscious goats at sea level and in another six goats maintained in a decompression chamber at 445 Torr (~4,300 m) for 10 days ($\bar{P}a_{O_2} = 43$ Torr). No significant group differences in cardiac frequency and various indices of myocardial performance (peak dP/dt, time-to-peak dP/dt, V_{max}) were demonstrable either before or after cholinergic blockade with intravenous atropine methyl bromide, 1 mg/kg. Following hemodynamic studies, thoracotomies were performed and full-thickness biopsies were obtained from the free wall of each of the cardiac chambers. Neither monoamine oxidase activity nor norepinephrine level of any region of the heart was altered by chronic hypoxia. However, a twofold increase ($P < 0.001$) in catechol O-methyltransferase activity above sea-level values was found in both the atria and ventricles of the hypoxic animals. Thus, the attenuation in cardiac responsiveness to β-adrenoceptor stimulation in chronic hypoxia appears unrelated to the level of vagal activity, but may be attributable to enhanced enzymatic inactivation of catecholamines.

normoxia; hypobaric hypoxia; vagal blockade; catecholamine degradative enzymes; chronotropic and inotropic responses

ACUTE EXPOSURE TO HIGH ALTITUDE produces an adrenergic activation with increases in heart rate, cardiac output, and urinary and plasma catecholamines (3, 19). After several days of hypoxia heart rate returns to, while cardiac output falls below, preexposure values (19). Since these latter changes occur at a time when circulating catecholamines continue to rise (13), one may assume that sympathetic control of the cardiovascular system is altered by exposure to chronic hypoxia. Previous studies in our laboratory have established the validity of this assumption (14). These studies have shown that several days' exposure to hypobaric hypoxia reduces both the chronotropic (14) and inotropic (unpublished observations) responses of the heart to β-adrenergic stimulation (with isoproterenol) and hence reduces the functional reserve of the heart.

However, the mechanism of the attenuated cardiac responsiveness has not heretofore been elucidated. In this connection, three factors must be considered. First, chronic hypoxia may be associated with a more rapid rate of catecholamine inactivation. The two enzymes that are of major importance in the metabolic transformation of catecholamines in the mammal are catechol O-methyltransferase (COMT) and monoamine oxidase (MAO). Second, chronic hypoxia may be associated with a higher level of parasympathetic activity. The level of vagal activity in hypoxia can be assessed by blockade of this limb of the autonomic nervous system with atropine. Finally, one cannot exclude the possibility of a hyposensitivity or relative refractoriness of the cardiac β-receptors secondary to the elevated level of sympathetic activity that accompanies hypoxia.

The present study was designed to identify the causal factor(s) in the reduced responsiveness of the heart during chronic hypoxia. Understanding of the mechanisms responsible appeared to be a valuable basis for subsequent management of the depressed cardiac performance at high altitude.

METHODS

Twelve healthy domestic goats, conditioned for 3 mo and weighing 25–30 kg, were studied in two groups of six each. Two weeks prior to study the goats were anesthetized with pentobarbital sodium and catheters were implanted surgically in the carotid artery and jugular vein to facilitate pressure recordings, blood sampling, and intravenous drug administration. Observations were made in both groups of intact, conscious goats at sea level and in one group following 10 continuous days in a hypobaric chamber maintained at 445 Torr, equivalent to an elevation of 4,300 m. Temperature and relative humidity were maintained at 21°C and 50%, respectively, throughout the experimental period. Following hemodynamic studies in each environmental condition, the animals were rendered unconscious by the captive-bolt gun technique. Left thoracotomies were performed and full-thickness biopsies were rapidly obtained from the atria, ventricles and apex of the heart for determinations of norepinephrine (NE) levels, and monamine oxidase and catechol O-methyltransferase activities.

On the day of the study a microtip catheter pressure transducer (Millar Instruments) was inserted through the previously implanted carotid catheter into the left ventricle and a three-lead ECG system was appropriately positioned on the animal. The goats were studied without sedation while resting in a stanchion. Measurements included heart rate; left ventricular end-diastolic, developed, and peak pressures; peak dP/dt; time-to-peak

dP/dt; and V_{max}. Hemodynamic parameters were assessed according to methods described elsewhere (21). The data were collected in real-time by a PDP 11/40 computer, analyzed, and stored and results returned in real-time via printouts and plots. Analyses were based on 15 cardiac cycles and pressure measurements were sampled at the rate of 500/s.

Measurements were obtained before and 10 min after the intravenous administration of 1 mg/kg of atropine methyl bromide (Sigma Chemical Co.) to assess the parasympathetic contribution to heart rate and myocardial contractility. Atropine methyl bromide does not cross the blood-brain barrier and was used to avoid the CNS effects associated with high doses of atropine sulfate. This dose of atropine was tested previously and found to produce total blockade of the vagus to electrical stimulation in goats.

Arterial blood samples were drawn for analysis of Po_2, Pco_2, and pH with Radiometer electrodes and for oxygen saturation by oximetry (Instrumentation Laboratories Co-Oximeter). Blood gases and pH were measured at 37°C and later corrected to each animal's rectal temperature. Hematocrits were determined by high-speed centrifugation in microcapillary tubes, and hemoglobin concentration was measured by the cyanmethemoglobin technique. Cardiac and plasma norepinephrine were quantitated using the radioenzymatic assay described by Henry et al. (8) with minor modifications in the incubation time (90 min) and amount of phenylethanolamine-N-methyltransferase used (86 units). Tissue catechol O-methyltransferase and monoamine oxidase activities were measured by the radiometric methods of Griffiths and Linklater (6) and Jarrott (10), respectively. The protein content of tissues was determined using the Folin phenol method of Lowry et al. (12). Tissues from both groups of animals were analyzed concurrently to avoid circumstantial or artefactual differences.

A subject-by-treatment analysis of variance and, where appropriate, Student t-test were used for statistical evaluation. All values are reported as mean ± standard error. The level of significance was chosen as $P < 0.05$.

RESULTS

The blood gas, pH, and hematologic data are presented in Table 1. Arterial O_2 tension and saturation of the hypoxic group were dramatically lower during hypobaric hypoxia, as expected. Animals hyperventilated at 4,300 m as shown by the decrease in arterial Pco_2.

TABLE 1. *Arterial blood analyses in normoxic and chronically hypoxic goats*

Condition	n	Pa_{O_2}, Torr	Pa_{CO_2}, Torr	pH$_a$	O_2 Saturation	Hct, %	Hb, g/ 100 ml
Normoxia	6	98.2	39.0	7.47	94.9	29.3	10.2
		±1.6	±2.5	±0.01	±0.2	±1.2	±0.5
Chronic hypoxia	6	42.6	31.2	7.48	78.8	37.4	12.6
		±2.0	±1.6	±0.02	±1.9	±1.7	±0.6
P		<0.001	<0.05	NS	<0.001	<0.01	<0.01

The maintenance of pH at normoxic levels during chronic hypoxia probably reflects a compensatory excretion of bicarbonate ions. The hematocrit and hemoglobin concentration were higher by 28% and 24%, respectively, in the hypoxic as compared with the normoxic goats. Mean body weights and rectal temperatures of the two groups did not differ significantly. No difference in any measurement was observed between groups when studied in the normoxic, sea-level environment.

Chronotropic response to parasympathetic blockade (Fig. 1). Base-line heart rates were not significantly different in the normoxic (102 ± 9 beats/min) and chronically hypoxic (117 ± 7 beats/min) groups. After the administration of atropine, 1 mg/kg iv, heart rates rose significantly, but to values that were not significantly different between groups (151 ± 10 vs. 147 ± 6 beats/min, normoxia vs. hypoxia). Assuming total blockade of the vagus in both conditions (normoxia and hypoxia), differences in heart rate would have reflected different levels of sympathetic activity. Since group heart rates were not dissimilar either before or after atropine, nor was sympathetic tone, the conclusion appears inescapable that chronic hypoxia did not alter the normoxic level of parasympathetic activity.

Inotropic response to parasympathetic blockade (Fig. 2). As with heart rates, postatropine values of peak dP/dt were significantly elevated in both groups, but group differences before (2,916 ± 342 vs. 3,269 ± 270 Torr/s,
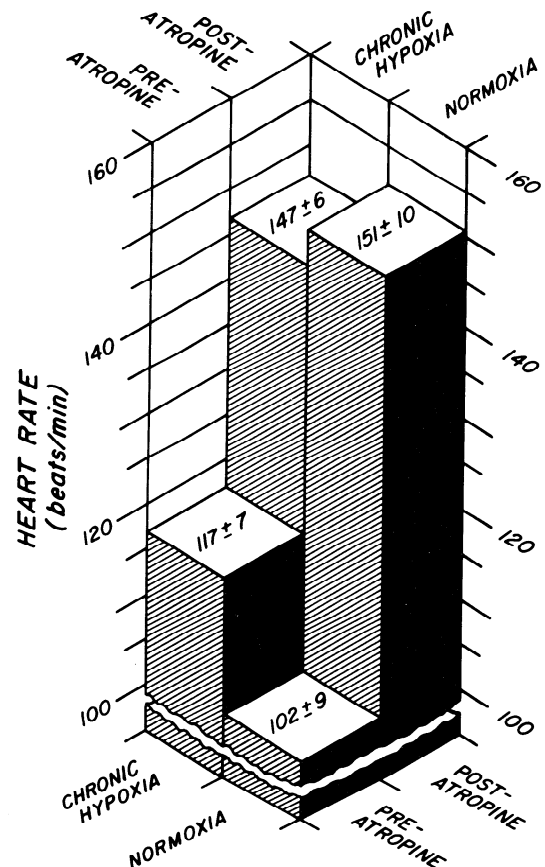

FIG. 1. Mean ± SE heart rates of the normoxic and chronically hypoxic goats before and 10 min after intravenous administration of 1 mg/kg of atropine methyl bromide.
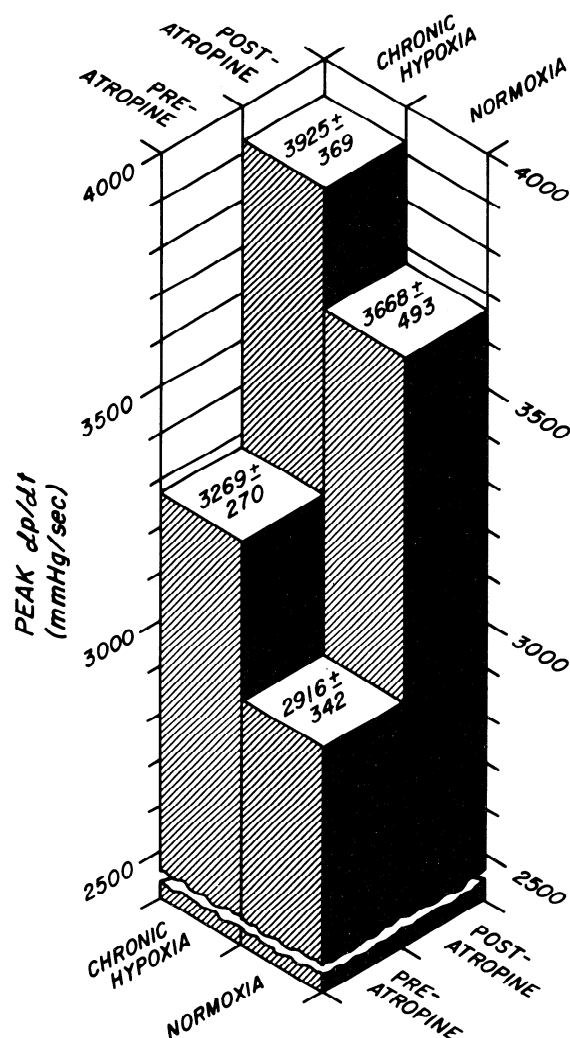
hearts of both groups were apparent with strikingly higher concentrations in the atria than in the ventricles. The concentration of norepinephrine in the right side of the heart was similar to that in the left side for

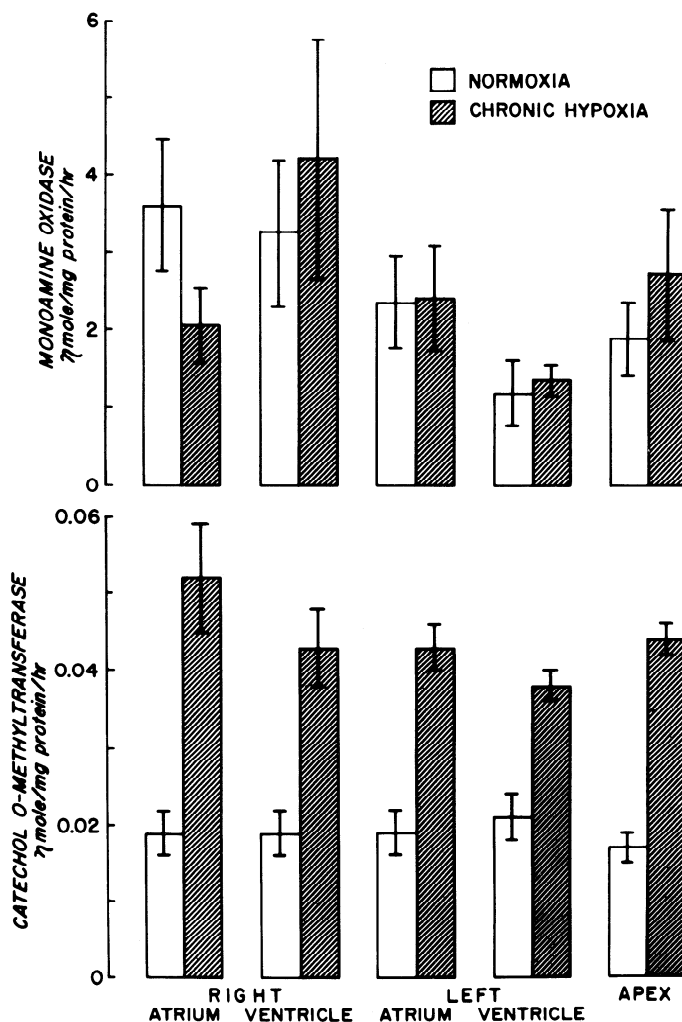FIG. 2. Mean ± SE values of peak dP/dt for the two groups before and after vagal blockade.

FIG. 3. Mean MAO (top) and COMT (bottom) activities of the various regions of the hearts of the normoxic and chronically hypoxic animals. Brackets indicate 1 SE.
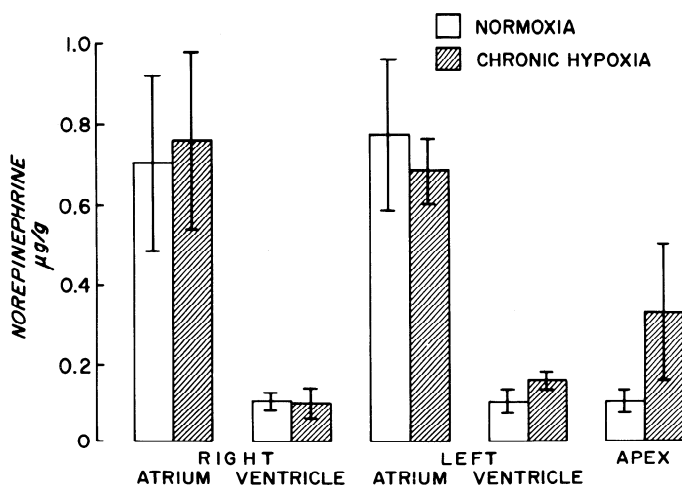
normoxia vs. hypoxia) and after (3,668 ± 493 vs. 3,925 ± 369 Torr/s) vagal blockade were not significant, thereby adding further support to the constancy of vagal activity following chronic hypoxic exposure. Other hemodynamic parameters including left ventricular end-diastolic, developed, and peak pressures, time-to-peak dP/dt, and V_{max} were similarly unaffected by hypoxia both prior to and following parasympathetic blockade.

Monoamine oxidase and catechol O-methyltransferase activities (Fig. 3). Though regional differences in MAO activity of the heart are apparent for both groups of animals, no significant group difference was found in any of the five areas examined. In contrast, the regional distribution of COMT was unremarkable in both groups although a very marked elevation in the activity of this enzyme was observed for each cardiac region examined in the hypoxic goats. The overall increase in COMT activity above the sea-level value was twofold, suggesting the possibility of a causal relationship between enhanced enzymatic inactivation of catecholamines and the attenuation in cardiac responsiveness to β-adrenergic stimulation.

Cardiac norepinephrine stores (Fig. 4). Regional differences in the endogenous norepinephrine levels of the

FIG. 4. Regional distribution of endogenous norepinephrine in the hearts of normoxic and chronically hypoxic goats. Values represent means ± SE.

both groups. No significant difference in the levels of norepinephrine was observed in any of these regions between the normoxic and hypoxic animals.

Plasma norepinephrine concentrations and heart rate (Fig. 5). Plasma norepinephrine concentration increased from 0.64 ± 0.13 ng/ml during normoxia to 0.76 ± 0.04 ng/ml after 24 h of hypobaric hypoxia and then to 1.25 ± 0.16 ng/ml after 10 days of continuous hypoxic exposure. Heart rate also rose with acute hypoxia from 102 ± 9 to 131 ± 8 beats/min. However, with chronic hypoxia, heart rate fell to a value (117 ± 7 beats/min) that was insignificantly different from the normoxic value despite a concurrent twofold increase in plasma norepinephrine.

DISCUSSION

Previous studies in intact, lightly sedated mongrel dogs have shown that the increase in heart rate following isoproterenol injection was significantly less following 10 days' exposure at 4,300 m than at sea level (14). The present study was designed to explore the mechanisms operative in the attenuated responsiveness to sympathomimetic stimulation following chronic hypoxia. Pilot studies in the goat established a similarity between species in the chronotropic response to isoproterenol after chronic hypoxic exposure and extended the previous findings in dogs to include a depression in inotropic responsiveness as well. An advantage of studying conditioned goats, unlike dogs (18), is that they need not be sedated to achieve a perfect resting situation.

The magnitude of the response of a target organ to catecholamines is determined by several factors: the level of parasympathetic activity opposing the stimulus, the ability of the organ to inactivate the material, and the number and sensitivity of receptor sites within the organ.

After parasympathetic blockade the resting heart rate and left ventricular peak dP/dt were not significantly different in normoxic and chronically hypoxic goats; this would argue for a similar level of resting sympathetic tone in both groups. Heart rate and peak dP/dt were not significantly different before atropine in normoxic and hypoxic groups. Since the resting level of sympathetic tone appeared equal in both groups these equivalent values for heart rate and peak dP/dt would indicate that vagal tone was not increased in the hypoxic group. That the level of parasympathetic activity can change with altitude exposure in response to maximal exercise has been shown by others (7). But our results suggest that the return of resting heart rate of altitude-exposed goats to sea-level values is not influenced by changes in parasympathetic activity.

The ability of the heart to inactivate NE is dependent on the status of neuronal uptake mechanisms and the activity of intraneuronal MAO and extraneuronal COMT. Our finding of increased levels of COMT in the goat heart supports the thesis that the attenuated response to β-adrenergic stimulation we reported previously in dogs (14), and the return of heart rate to normal sea-level values reported herein, may be related to the enhanced inactivation of circulating NE by COMT. NE in the circulation is rendered inactive by the rapid uptake into peripheral stores, especially those within the heart (20). That this may not occur to as great an extent with chronic hypoxia is evidenced by the fact that MAO activity is not altered in the face of high levels of circulating NE and that endogenous NE levels in the heart are not increased. Exogenous NE, after uptake into the isolated canine heart, is principally metabolized to normetanephrine, the result of O-methylation by COMT (2). Thus, COMT may be of greater importance than MAO in the inactivation of cardiac NE.

Bassett and Cairncross (1) have suggested that repeated stress induced by foot shock results in an inhibition of neuronal NE uptake. Their results indicate an interference in the rate of uptake rather than a change in the affinity of NE for the uptake mechanism. They interpret their results as demonstrating a competition for the same uptake sites by compounds such as steroids, thus reducing the sites available for NE. If a similar mechanism is operative during chronic hypoxia, it would be expected to increase the availability of extraneuronal NE. An increase in substrate availability
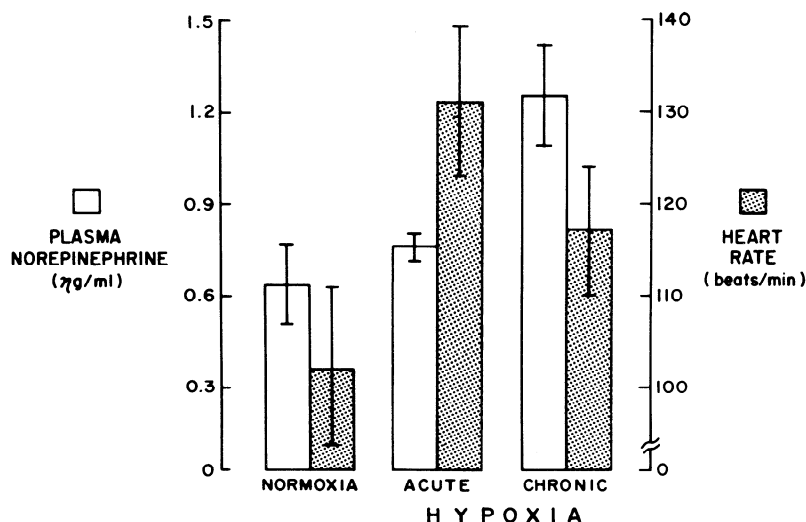

FIG. 5. Plasma norepinephrine concentrations and heart rates during normoxia and after acute (24 h) and chronic (10 days) hypoxia. Mean values ± SE are presented.

could thus account for an increase in COMT activity. It is noteworthy that a variety of steroids have been shown to inhibit the uptake of cardiac NE in the rat (9) and that hypoxia is known to increase plasma and urinary corticosteroids in man and animals (4, 17).

Our data do not exclude the possibility that the cardiac response observed with chronic altitude exposure may be due, at least in part, to a decrease in sensitivity and/or number of postsynaptic receptor sites. It is well known that chronic exposure of tissues to elevated concentrations of certain hormones and drugs leads to tolerance or desensitization to the effects of the particular agent. Such subsensitization has been described with catecholamines (16). When the β-adrenergic receptors in catecholamine-induced desensitized membranes were studied with (-)-[^{3}H]alprenolol, a high-affinity radioactively labeled β-adrenergic antagonist used to identify β-adrenergic receptors, a striking decrease in the number of functionally active sites was found (11).

It appears that the initial response to hypoxia results in a decrease in endogenous NE levels in heart (5, 15). This decrease was blocked by hexamethonium, indicating that it may be reflexly mediated (5). After this initial phase, which is also characterized by increased turnover of cardiac NE (15), normal endogenous levels are reestablished. Long-term adaptation is associated with an increase in the degradative enzyme COMT, probably induced by the persistently high level of circulating NE. This change may explain the attenuated cardiac response to exogenous sympathomimetic amines.

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