Physiological and Genomic Consequences of Intermittent Hypoxia
Invited Review: Physiological consequences of intermittent hypoxia: systemic blood pressure

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Fletcher, Eugene C. Invited Review: Physiological consequences of intermittent hypoxia: systemic blood pressure. J Appl Physiol 90: 1600–1605, 2001.—One of the major manifestations of obstructive sleep apnea is profound and repeated hypoxia during sleep. Acute hypoxia leads to stimulation of the peripheral chemoreceptors, which in turn increases sympathetic outflow, acutely increasing blood pressure. The chronic effect of these repeated episodic or intermittent periods of hypoxia in humans is difficult to study because chronic cardiovascular changes may take many years to manifest. Rodents have been a tremendous source of information in short- and long-term studies of hypertension and other cardiovascular diseases. Recurrent short cycles of normoxia-hypoxia, when administered to rats for 35 days, allows examination of the chronic cardiovascular response to intermittent hypoxia patterned after the episodic desaturation seen in humans with sleep apnea. The result of this type of intermittent hypoxia in rats is a 10- to 14-mmHg increase in resting (unstimulated) mean blood pressure that lasts for several weeks after cessation of the daily cyclic hypoxia. Carotid body denervation, sympathetic nerve ablation, renal sympathectomy, adrenal medullectomy, and angiotensin II receptor blockade block the blood pressure increase. It appears that adrenergic and renin-angiotensin system overactivity contributes to the early chronic elevated blood pressure in rat intermittent hypoxia and perhaps to human hypertension associated with obstructive sleep apnea.

sleep apnea syndromes; anoxia; hypoxemia; hypertension; sympathetic nervous system; carotid chemoreceptors

NEARLY EVERY SCIENTIST IS familiar with the effects of chronic continuous hypoxia on the cardiovascular system. These include polycythemia, pulmonary hypertension, right ventricular hypertrophy, and right ventricular failure. However, in the early stages of many diseases, hypoxia is not continuous. For example, one of the earliest hypoxic manifestations of chronic obstructive as well as interstitial lung disease is intermittent hypoxia during exercise and sleep, especially rapid eye movement sleep (13). Cardiovascular manifestations of intermittent hypoxemia are less well characterized than continuous hypoxemia and are controversial (6). On the other hand, repetitive obstructive sleep apnea in humans and animals results in unequivocal acute elevation of systemic blood pressure with more prolonged elevation extending beyond the period of apneas (15). Very recent epidemiological data support the hypothesis that repetitive airway obstructive with oxyhemoglobin desaturation can lead to secondary systemic hypertension (20, 22). Indeed, repetitive intermittent hypoxia during sleep apnea may be a major cause of
hypertension in some patients previously labeled with essential hypertension.

Acute animal experiments in the dog, pig, and lamb have provided the opportunity to study hemodynamic and cardiovascular response to acute apnea in depth (4, 21). Because years of repetitive apnea with hypoxemia may be required to instigate sustained daytime hypertension, prospective studies of hypertension in animals and humans are difficult. Much hypertension research is conducted in rats and mice because of the many similarities of blood pressure control and cardiovascular response between rodents and humans. The technology of knockout mice and transgenic rats have made rodents even more important in such research. Given the short life span of rodents, many of the models of chronic hypertension paralleling human clinical disease can be studied in greater depth than is possible in humans or large animals. This minireview addresses the use of an intermittent hypoxia preparation in which rats are exposed to recurrent periods of intermittent hypoxia mimicking the recurrent desaturation of obstructive sleep apnea. Intermittent hypoxia can be administered with various cyclic durations, eight or more hours per day, for a variable number of days, to induce blood pressure elevation or potentially other manifestations of intermittent hypoxia.

INTERMITTENT HYPOXIA RAT MODEL

In our laboratory, individual chambers (single animal per chamber) have been constructed in which rats are exposed to rapid swings in ambient oxygen concentration (the inspired oxygen fraction, \( F_{\text{IO}_2} \)), inducing changes in arterial oxygen saturation (\( S_{\text{aO}_2} \)) similar to that seen in humans with sleep apnea (Fig. 1). Other laboratories have used larger chambers in which multiple animals can be housed and fed for days, inducing repetitive intermittent (90–300 s) hypoxia cycles around the clock for weeks (16). The cylindrical Plexiglas chambers that we use are 28 cm in length with a diameter of 10 cm and a volume of 2.4 liters. A dampening device at the intake end of the chamber is used to dissipate the air stream so that no direct gas jets disturb the animal. The opposite end is covered with a snug-fitting lid in which holes are drilled to allow \( F_{\text{IO}_2} \) sampling and flow adjustment. A timed solenoid valve distributes pure nitrogen to each chamber for 12 s at a flow that is adjusted to reduce the \( F_{\text{IO}_2} \) to 3–5% for

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Fig. 1. Normobaric chamber, in which infusion of \( \text{N}_2 \) and \( \text{CO}_2 \) lowers inspired \( \text{O}_2 \) fraction (\( F_{\text{IO}_2} \)) or raises inspired \( \text{CO}_2 \) fraction (\( F_{\text{ICO}_2} \)), creating transient hypoxia or asphyxia. Infusion of gases lasts ~12 s (with a range of 6–14 s) and is followed by compressed air for the balance of each 30-s cycle. Tracing at the right shows a hypertensive response to acute hypoxia at a slow and at high speeds. The rat is conscious and unrestrained during these studies. bpm, Beats/min.
3–6 s. The suffusion of nitrogen is followed immediately by compressed air, allowing gradual return (over 15–18 s) to $F_{1O_2} = 20.9\%$. The cycle is repeated every 30 s diurnally (normal sleep period for rodents), for 6–8 h of the 24-h time period, on consecutive days. Multiple arterial blood samples as well as continuous arterial catheter oximetry monitoring during intermittent hypoxia have shown the average nadir level of $SaO_2$ in this system to be 70% (range 60–80%). For controls, compressed air at approximately the same liter flow is distributed to “sham cages,” which simulate the same noise and air disturbance. The tubing to the system can be manipulated to combine nitrogen with varying concentrations of CO$_2$ such that hypercapnia can be induced along with the hypoxia, simulating asphyxia. The rat behaviorally maintains its usual diurnal sleep habit (albeit restless) throughout the 35-day cycle. In the studies summarized below, unless otherwise noted, blood pressure was measured under resting, unrestrained, and unanesthetized conditions from 09:00 AM to noon, within 24 h preceding day 0 and again within 48 h following the end of the intermittent hypoxia period, via abdominal aorta catheter connected to Statham P23Db pressure transducers. Later studies, as noted in the text, use radiotelemetry devices to monitor intraarterial blood pressure weekly or daily.

**RESULTS: CHARACTERIZATION OF THE MODEL**

The first experiment that used the intermittent hypoxia rat model demonstrated that repetitive intermittent hypoxia can contribute to persistent mean arterial blood pressure (MAP) elevation (11). Wistar Thomae rats were subjected to intermittent hypoxia for 8 h/day over 35 days. There was a 13.7 mmHg increase in MAP in the 35-day intermittent hypoxia rats, whereas sham (compressed air) and unhandled controls showed no MAP change. Subsequently, we examined the role of peripheral chemoreceptors and the peripheral sympathetic nervous system in producing the diurnal blood pressure increase. Carotid body denervation was performed on two groups of male Wistar rats by severing both carotid sinus nerves (9). The sham-operated, non-denervated group exposed to intermittent hypoxia showed a 13-mmHg increase in MAP, whereas sham (compressed air) and unhandled controls showed no MAP change. Subsequently, we examined the role of peripheral chemoreceptors and the peripheral sympathetic nervous system in producing the diurnal blood pressure increase. Carotid body denervation was performed on two groups of male Wistar rats by severing both carotid sinus nerves (9). The sham-operated, non-denervated group exposed to intermittent hypoxia showed a 13-mmHg increase in MAP, whereas sham (compressed air) and unhandled controls showed no MAP change. Likewise, unhandled sham-operated rats and unhandled carotid body-denervated rats displayed no significant change in MAP from baseline. The study showed that intact peripheral chemoreceptors were involved in the blood pressure response to chronic intermittent hypoxia. Chemical sympathectomy using two injections (20 days apart) of the neurotoxin 6-OH dopamine was used to poison the peripheral sympathetic synapses (10). Rats injected with vehicle and exposed to intermittent hypoxia showed a 7.7-mmHg increase in MAP above baseline, whereas the 6-OH dopamine-injected, intermittent hypoxia, and unhandled control rats showed no change in MAP. Measurement of catecholamines in cardiac muscle homogenate confirmed sympathetic denervation in 6-OH dopamine animals. This study suggested that peripheral sympathetic nerves controlling vascular tone may play a major role in the diurnal blood pressure increase.

Acute sympathetic activity has also been characterized in the intermittent hypoxia rat to see if asphyxic, hypocapnic, or eucapnic stimuli cause varying acute blood pressure response (1). Prazosin ($\alpha_1$-adrenergic blocker), yohimbine ($\beta_2$-adrenergic blocker), and atropine were used to block sympathetic and parasympathetic responses. Eucapnic intermittent hypoxia caused a threefold greater elevation in acute MAP and greater bradycardia than hypocapnic intermittent hypoxia. Asphyxia, however, is the strongest stimulus of the three to acute blood pressure change. Prazosin, but not yohimbine, blunted the blood pressure response and atropine blocked the hypoxia-associated bradycardia. Direct recording of splanchnic nerve activity in the awake, unrestrained rat showed that adding CO$_2$ to intermittent hypoxia caused a profound increase in sympathetic activity. One might suppose that, because intermittent asphyxia and intermittent eucapnic hypoxia invoke stronger acute blood pressure responses than intermittent hypocapnic hypoxia, chronic intermittent asphyxia or eucapnic hypoxia might also cause a greater increase in sustained, diurnal blood pressure than hypocapnia hypoxia. Hypocapnic hypoxia (no added CO$_2$), eucapnic hypoxia [7–10% inspired CO$_2$ fraction ($F_{1CO_2}$)], and hypercarbic hypoxia (11–14% $F_{1CO_2}$) were administered for 35 days to separate groups of rats with appropriate controls, as in previous studies (8). Chronic intermittent hypocapnic hypoxia raised MAP by 11 mmHg, but neither episodic eucapnic nor intermittent asphyxia had any additional effect beyond this. This suggests that the neurohumoral systems involved in the chronic diurnal blood pressure response to intermittent hypoxia are already maximally stimulated by hypocapnic hypoxia.

Arousal itself (startle reaction) is known to acutely increase sympathetic output, and some researchers feel that recurrent arousal with sleep disruption could contribute to chronic blood pressure changes in the setting of apnea. We exposed 12-wk-old ($n = 10$) Sprague-Dawley rats in individual chambers to recurrent buzzer noise (500 Hz, 100 dB) 6 of every 30 s for 8 h during a 24-h period for 35 days (3). Nine rats (sham) housed in identical cages but without noise stimulation served as controls. An infrared beam with detector was located at the end of each cage to quantify motion by registering the number of times in 8 h that the rat broke the beam. All animals showed a significant acute MAP response to noise that diminished after 30–60 min of noise exposure. Acoustic-stimulated rats showed higher movement activity throughout the 24-h period than did the nonstimulated rats, but there was no difference in MAP in either group before or after the respective 35-day experimental conditions.

In summary, these initial studies show that recurrent intermittent hypoxia during sleep causes MAP elevation after 35 days. The acute mechanism is repetitive chemoreceptor stimulation with increased periph-
eral sympathetic activity and vasoconstriction. Several means of sympathetic blockade diminish both the acute and chronic effects of intermittent hypoxia. Asphyxia is a more potent stimulus to acute hypertension than hypocapnic hypoxia but is no more potent on a chronic basis. Arousal alone, although it does elevate blood pressure acutely, has no chronic effect.

RESULTS: SYSTEMIC MECHANISMS OF CHRONIC BLOOD PRESSURE ELEVATION

We have further investigated the specific role of adrenal and renal sympathetics in the chronic blood pressure response to intermittent hypoxia. Male Sprague-Dawley rats had adrenal medullectomy, bilateral renal artery denervation, or sham abdominal incision (2). Study and control rats were subjected to intermittent hypoxia or compressed air or remained unhandled for 35 days. Both adrenal demedullation and, separately, renal artery denervation eliminated the chronic diurnal blood pressure response to intermittent hypoxia. Systemic blood pressure in sham-operated rats exposed to intermittent hypoxia continued to elevate, indicating that the increase in diurnal MAP requires both intact renal artery sympathetics as well as an intact adrenal medulla. Circulating epinephrine (adrenal) appears to play an important role in blood pressure regulation in the setting of chronic intermittent hypoxia. The mechanism may be binding to presynaptic β2-receptors, where it is released as a cotransmitter with norepinephrine across the neural junction, facilitating neurogenic vasoconstriction (14).

Plasma renin activity is regulated by sympathetic nerves and can affect diurnal blood pressure through the renin-angiotensin system. Plasma renin activity was examined in 24 Sprague-Dawley rats exposed to 35 days of intermittent hypoxia (12). Half of the rats were treated with losartan [an angiotensin II receptor (AT1) blocker], and the other half received vehicle alone. Contrary to the previous studies, blood pressure was monitored unrestrained using implanted arterial telemetry sensors at baseline and every 7th day throughout the 35-day period. Intermittent hypoxia was associated with a 10-mmHg increase in MAP and a threefold rise in plasma renin activity compared with no increase in MAP or plasma renin activity in unhandled controls (Table 1). Losartan-treated animals exposed to intermittent hypoxia showed no blood pressure change. The results suggest that the renin-angiotensin system plays an important role in the blood pressure response to intermittent hypoxia, since AT1 antagonists prevent the rise in MAP. It is of interest that there are AT1 receptors on presynaptic sympathetic nerves that are believed to stimulate norepinephrine secretion across the sympathetic synapse, similar to sympathetic neurotransmission facilitation by circulating epinephrine.

Basal resting arterial tone is an extremely important concept in the chronic regulation of blood pressure. It is influenced by a host of factors, including sympathetic tone and endothelial vasodilator and vasoconstrictor substances acting on vascular smooth muscle. These include endothelin and endothelium-derived relaxing factor (or NO), prostaglandins (PGF2, PGE2), thromboxanes, and leukotrienes. Agents such as acetylcholine, bradykinin, and substance P work through the NO system by activating nitric oxide synthase, leading to generation of NO from L-arginine. NO also modulates sympathetic neurotransmission by decreasing both the release of and smooth muscle reaction to endogenous norepinephrine. Microvascular studies suggest an increase in the reactivity of arterioles to endogenous and exogenous vasoconstrictors as well as impairment in the action of endothelium-derived vasoactive factors in hypertensive animals (19, 24). Using in vivo video microscopy, we examined arteriolar reactivity in the cremaster muscle of male Sprague-Dawley rats exposed to 35 days of intermittent hypoxia (23). Cremaster muscles of intermittent hypoxia and control rats were exposed to varying doses of norepinephrine, acetylcholine, and endothelin-1. Intermittent hypoxia and control rats were also given one dose of a nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME). Intermittent hypoxia rats showed a 16-mmHg increase in MAP after the 35-day exposure, whereas control rats showed no change. Responses to norepinephrine and endothelin-1 were similar for intermittent hypoxia and control rats. Acetylcholine vasodilatation of arterioles in intermittent hypoxia rats was significantly attenuated compared with that of controls. The degree of vasoconstriction in response to blockade of the nitric oxide system by L-NAME was significantly less in arterioles of intermittent hypoxia rats compared with that in controls, implying lower basal resting nitric oxide release in the intermittent hypoxia rats.

Table 1. Resting plasma renin levels, losartan study

<table>
<thead>
<tr>
<th>Group</th>
<th>BP, mmHg</th>
<th>PRA, pg/ml</th>
<th>Day 14 BP, mmHg</th>
<th>Day 28 BP, mmHg</th>
<th>BP, mmHg</th>
<th>PRA, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>92.3</td>
<td>1.7</td>
<td>101.5*</td>
<td>105.1*</td>
<td>102.3</td>
<td>6.6*</td>
</tr>
<tr>
<td>Hypoxia + losartan</td>
<td>98.2</td>
<td>2.1</td>
<td>88.0</td>
<td>87.8</td>
<td>85.9</td>
<td>16.7*</td>
</tr>
<tr>
<td>Unhandled</td>
<td>94.0</td>
<td>1.8</td>
<td></td>
<td></td>
<td>94.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Unhandled + losartan</td>
<td>95.2</td>
<td>1.7</td>
<td></td>
<td></td>
<td>82.3</td>
<td>18.7*</td>
</tr>
</tbody>
</table>

Plasma renin activity (PRA) at baseline (day 0) and after cessation of hypoxia (day 36) along with baseline, day 14, day 28, and day 36 blood pressure (BP) in 4 groups of rats are shown. There are no day 14 or 28 BP values for the unhandled and unhandled losartan-treated animals because their BP was measured by indwelling catheters at the beginning and end of the study. BP of other rats could be monitored continuously throughout the 35-day period by telemetry transducers. *Values differ from day 0 levels at P < 0.05 level or greater.
hypoxia rats. Whole kidney messenger RNA endothelial nitric oxide synthase levels were not different between groups. This study suggests that chronic blood pressure elevation in this model involves increased peripheral resistance from decreased basal release or production of nitric oxide.

In summary, both the adrenal medulla and the renal sympathetic nerves are essential for the development of blood pressure elevation during chronic intermittent hypoxia. Plasma renin activity is elevated in rats exposed to chronic intermittent hypoxia. Angiotensin II receptor (AT1) blockade prevents the chronic blood pressure elevation from intermittent hypoxia. Higher basal vascular tone in chronic intermittent hypoxia exposed rats manifests itself by decreased acetylcholine responsiveness (NO-dependent vasodilatation). Decreased NO activity and increased angiotensin II receptor activity are two possible mechanisms to explain the link between daily intermittent hypoxia, recurrent elevated sympathetic activity, and increased vascular resistance following intermittent hypoxia.

The data thus far suggest the following scenario regarding blood pressure changes in response to chronic recurrent intermittent hypoxia in the rat. Acute and chronic hypoxia stimulate the peripheral chemoreceptors, causing increased sympathetic outflow to the adrenal medulla, heart, and resistance vessels. This is verified by observations of acutely increased splanchic nerve sympathetic activity and blood pressure and heart rate change in response to intermittent hypoxia as well as blockade of chronic diurnal blood pressure change by chemical sympathectomy. Also supporting this mechanism is the finding of increased plasma norepinephrine and right ventricular myocardial catecholamine levels. It appears that both the adrenal gland, via circulating epinephrine, and renal sympathetics, via the renin-angiotensin system, participate in the chronic diurnal blood pressure elevation. A common link between these two end organs of sympathetic activity may be binding of circulating (adrenal) epinephrine and angiotensin II to peripheral sympathetic synapses, potentiating sympathetic neural transmission. The link between repetitive intermittent activation of the sympathetic nervous system and the chronic increase in resting, unstimulated blood pressure may be 1) overactivity of the renin-angiotensin system with the increase in basal resting tone due to angiotensin receptor-facilitated increase in sympathetic activity and 2) changes in levels of basal NO activity. Two recent studies in humans support the theory of increased resting basal vascular tone through lack of NO activity in the sleep apnea patient. Carlson et al. (5) showed that forearm vascular relaxation to acetylcholine decreased in apnea subjects compared with nonapneic controls. Duchna et al. (7) found that bradykinin (endothelium-dependent NO) vascular relaxation was depressed compared with controls, whereas nitroglycerine (NO independent) vasorelaxation was unaffected. Two months of treatment with nasal continuous positive airway pressure allowed return of the bradykinin curves to control levels.

FUTURE RESEARCH DIRECTIONS IN THIS MODEL

The flexibility of the intermittent hypoxia model allows exploration of hypertensive mechanisms in many different directions. For example, the link between daily intermittent hypoxia and the resulting prolonged, nonhypoxic increase in vascular resistance may lie in the central nervous system from heightened sympathetic output or in the vascular endothelium driven by local factors responding directly to hypoxemia. In the central nervous system, Greenberg et al. (18) found evidence of increased Fos activity in various areas of the brain known to regulate central sympathetic neural activity in rats exposed to chronic (30 day) intermittent hypoxia compared with sham and unhandled controls. Increased c-fos expression was demonstrated in the nucleus of the tractus solitarius, medullary reticular formation (A1 noradrenergic cell area of the ventrolateral medulla), and the midline raphe (raphe pallidus and ventral area of the nucleus gigantocellularis). These areas are involved in the tonic and reflex control of sympathetic neural discharge, integrating peripheral afferent inputs (e.g., chemoreceptors) with sympathetic output to various target organs. Further work in this area might help to prove centrally driven, chronically increased sympathetic vascular tone in animals exposed to intermittent hypoxia.

Another important area where this model could be useful is in investigations of the function of vascular endothelium in regulating vascular smooth muscle growth and remodeling. One hallmark of sustained hypertension in humans and animals is vascular remodeling (capillary and arteriolar rarefaction; Ref. 17) and hypertrophy-hyperplasia of vascular smooth muscle cells and matrix in small resistance vessels (<100 μm). In chronic hypertension, the enhanced muscle mass and extracellular matrix increase contractile force, allowing vessels to withstand constantly increased intraluminal pressure. In normal adult species, vascular smooth muscle cells remain quiescent at a low mitotic state. During hypertension, especially that associated with increased renin-angiotensin activity, vascular smooth muscle cells show phenotypic modulation with hypertrophic-hyperplastic growth. Hemodynamic factors such as circumferential and axial stretch, shear stress (laminar flow), and platelet-derived mitogens can facilitate vascular remodeling. The pulsatile flow of blood is a powerful cell signal that can affect vascular structure and function. Increased pulse pressures can double the stretch stimulus, which would activate calcium and sodium channels, altering vascular tone, sodium pump activity, intracellular pH, and gene expression. Mechanical deformation of the extracellular matrix by pressure waves can stimulate vascular smooth muscle cell growth by integrin-associated tyrosine kinases and activation of gene promoter elements. It may be that shear stress and turbulent flow with endothelial cell signaling are greatly enhanced in cyclic vascular constriction-relaxation (vs.
continuous) seen during recurrent intermittent hypoxemia.

Science is making tremendous strides in understanding the relationship between systemic hypertension and sleep apnea. The intermittent hypoxia model gives us another tool to help solve this riddle. The development of hypertension in the setting of intermittent hypoxia probably involves a very slow time course. Thus it is extremely important to use animal preparations that can mimic some manifestations of sleep apnea over a reasonable time period, allowing study of likely scenarios in the relationship between sleep apnea and hypertension or other cardiovascular outcomes. The flexibility of the intermittent hypoxic rat model allows manipulation of stress factors (noise, CO₂, and nadir O₂ levels) and control of exposure periods. It also allows many invasive functional and anatomic studies not possible in humans, producing a better understanding of endocrine, neural, and renal mechanisms operating to elevate blood pressure. Such knowledge may enhance management of hypertension in patients with sleep-disordered breathing.

REFERENCES