Effect of hypoxia on arterial baroreflex control of heart rate and muscle sympathetic nerve activity in humans

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Halliwill, John R., and Christopher T. Minson. Effect of hypoxia on arterial baroreflex control of heart rate and muscle sympathetic nerve activity by using the modified Oxford technique during both normoxia and hypoxia (12% O2). Compared with normoxia, hypoxia reduced arterial O2 saturation levels from 96.8 ± 0.3 to 80.7 ± 1.4% (P < 0.001), increased heart rate from 59.8 ± 0.9 to 79.4 ± 2.9 beats/min (P < 0.001), increased mean arterial pressure from 96.7 ± 2.5 to 105.0 ± 3.3 mmHg (P = 0.002), and increased sympathetic activity 126 ± 58% (P < 0.05). The sensitivity for baroreflex control of both heart rate and sympathetic activity was not altered by hypoxia (heart rate: −1.02 ± 0.09 vs. −1.02 ± 0.11 beats·min⁻¹·mmHg⁻¹; nerve activity: −5.6 ± 0.9 vs. −6.2 ± 0.9 integrated activity·beat⁻¹·mmHg⁻¹; both P > 0.05). Acute exposure to hypoxia reset baroreflex control of both heart rate and sympathetic activity to higher pressures without changes in baroreflex sensitivity.

METHODS

This study was approved by the Institutional Review Board of the Mayo Clinic and Foundation, and each subject gave his or her informed written consent before participation. We assessed baroreflex control of heart rate and muscle sympathetic nerve activity by using the modified Oxford technique during both normoxia and hypoxia.

Subjects

Sixteen healthy, nonsmoking, normotensive subjects (8 women, 8 men) between the ages of 20 and 33 yr participated in this study [height 175 ± 10 (SD) cm, weight 69.6 ± 13.7 kg, body mass index 22.6 ± 3.3 kg/m²]. None of the subjects was taking medications other than oral contraceptives, and none had been at altitude (>1,500 m) for at least 5 mo. All female
seven subjects, nerve recordings either were inadequate or (peroneal) nerve via microneurography. In the remaining responses. In nine of the subjects (3 women, 6 men), we of vasoactive substances for the purpose of assessing barore-
catheter was placed in an antecubital vein for administration of vasoactive substances for the purpose of assessing baroreflex responses. In nine of the subjects (3 women, 6 men), we recorded muscle sympathetic nerve activity from the fibular (peroneal) nerve via microneurography. In the remaining seven subjects, nerve recordings either were inadequate or were not stable during one or more of the trials.

During measurement periods, subjects breathed either room air (normoxia) or 12% O2 in N2 (hypoxia) via a two-way nonbreathing valve. Subjects breathed through a scuba mouthpiece while nasal breathing was prevented with a nose clip. Ventilation was measured via a pneumotach (model VMM-2a, Interface Associates, Laguna Niguel, CA), and end-tidal CO2 was measured at the mouth via an infrared CO2 analyzer (model 1260, Novametrix Medical Systems, Wallingford, CT). After instrumentation, subjects underwent two 18-min measurement periods (normoxia and hypoxia) separated by a 20-min rest period. We continuously recorded heart rate, arterial pressure, ventilation, and sympathetic activity during each measurement period. We assessed baroreflex control of heart rate and muscle sympathetic nerve activity during minutes 15–18. Our laboratory’s prior work has shown that repeated baroreflex trials separated by a 20-min rest period are reproducible (26, 37). Because hypoxia may have long-lasting effects (29), we did not randomize the order of trials between normoxia and hypoxia.

Muscle sympathetic nerve activity. Muscle sympathetic nerve activity was recorded via microneurography, as originally described by Sundlöf and Wallin (46). Multuniport ganglionic muscle sympathetic nerve activity was recorded from the fibular (peroneal) nerve posterior to the fibular head with a tungsten microelectrode. The recorded signal was amplified 100,000-fold, band-pass filtered (700–2,000 Hz), rectified, and integrated (resistance-capacitance integrator circuit, time constant 0.1 s) for analysis of muscle sympathetic nerve activity.

Baroreflex control of heart rate and sympathetic outflow. Baroreflex responses were assessed by measuring heart rate and muscle sympathetic nerve activity during arterial pressure changes induced by nitroprusside and phenylephrine as developed by Ebert and Cowley (5) and by Rudas et al. (37). During both normoxia and hypoxia, 100 µg sodium nitroprusside were given intravenously as a bolus, followed 1 min later by 150 µg phenylephrine HCl. This paradigm decreases arterial pressure ~15 mmHg below baseline levels and then increases it ~15 mmHg above baseline levels, over a short time course.

Data Analysis

Data were digitized at 250 Hz with signal-processing software (WinDaq, Datta Instruments, Akron, OH) and analyzed off-line. Each muscle sympathetic nerve activity recording was normalized by assigning the largest sympathetic burst under resting conditions an amplitude of 1,000 (13). All other bursts for that recording were calibrated against that value. The zero nerve activity level was determined from the mean voltage during a period of neural silence between sympathetic bursts. A period in which bursts were absent for >5 s was found in each tracing and used for this purpose.

Baroreflex control of sympathetic outflow was determined from the relation between muscle sympathetic nerve activity and diastolic pressure during vasoactive drug boluses (5, 14, 37). The slope of this relation is used as an index of reflex sensitivity. The operating point for the relation in terms of resting arterial pressure and nerve activity was determined as the average values over the 5-min period immediately preceding the nitroprusside bolus. Diastolic pressure was used because muscle sympathetic nerve activity correlates closely with diastolic pressure but not with systolic pressure (37, 46). To perform a linear regression between nerve activity and pressure, values for nerve activity were first signal averaged over 3-mmHg pressure ranges via custom software as described previously (13). A window of nerve activity that was 2.0 s in length and synchronized by the R wave of the electrocardiogram was signal averaged. The window was time shifted to account for the latency between R waves and sympathetic bursting. The duration of the shift was varied as needed from subject to subject, but it averaged 1.3 s. Subsequently, the extreme ends of the window, which represented sympathetic bursts occurring in the preceding and following heartbeats, were truncated, and the total integrated activity was determined as the area under the signal-averaged curve. This was done for each 3-mmHg pressure range. The points of truncation for all windows in a given trial were set at the obvious nadirs between the burst of interest and the preceding and following bursts, and the same timing was used for all windows in a given trial.

Baroreflex control of heart rate was determined from the relation between heart rate and systolic pressure during vasoactive drug boluses (5, 14, 37). The slope of this relation is used as an index of reflex sensitivity. The operating point for the relation in terms of resting arterial pressure and heart rate was determined as the average values over the 5-min period immediately preceding the nitroprusside bolus. Systolic pressure was used because heart rate correlates closely with systolic pressure but not with diastolic pressure (37, 46). To perform a linear regression between heart rate and pressure, values for heart rate were first pooled over 2-mmHg pressure ranges as described previously (5, 14, 37). The analogous regression between R-R interval and systolic pressure was also determined.

A hysteresis, in which the slopes of falling and rising pressure responses can differ, has been observed in cardiac baroreflex responses in some studies (37). We independently assessed the falling and rising pressure portions of our data, comparing normoxia and hypoxia trials. No differences in baroreflex sensitivity between the falling and rising responses were observed in the data. Furthermore, there were no differences in the effect of hypoxia on baroreflex sensitivity between the falling and rising portions of the response. Finally, independent analysis of the falling and rising portions of the response resulted in poorer fit of the linear regression (on the basis of lower correlation coefficients). Therefore, data and comparisons based on the combined falling and rising response are presented in detail. However, we also present slope comparisons for the falling and rising response in Table 1.

In animal models, it is often possible to assess baroreflex responses over a wider range of pressures so that response relations encompass the reflex from threshold to saturation.

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Hypoxia resets the baroreflex

Table 1. Rising vs. falling pressure and heart rate responses

<table>
<thead>
<tr>
<th>Heart rate</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rising pressure</td>
<td>Falling pressure</td>
</tr>
<tr>
<td>Slope, beat·min$^{-1}$·mmHg$^{-1}$</td>
<td>$-0.93 \pm 0.11$</td>
<td>$-1.16 \pm 0.13$</td>
</tr>
<tr>
<td>Intercept, beat/min</td>
<td>188 $\pm$ 14</td>
<td>222 $\pm$ 19</td>
</tr>
<tr>
<td>R-R interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope, ms·mmHg</td>
<td>13.5 $\pm$ 1.1</td>
<td>16.5 $\pm$ 1.7</td>
</tr>
<tr>
<td>Intercept, ms</td>
<td>$-890 \pm 151$</td>
<td>$-1,284 \pm 251$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. $P$ values are for rising vs. falling pressures. *$P < 0.05$ vs. normoxia.

Such data can often be expressed by a sigmoidal equation. However, with the use of bolus administration of nitroprusside and phenylephrine in humans, we have not been able to characterize the entire response relationship consistently, because the nonlinear threshold and saturation regions are variably present. As such, we have restricted our analysis to the linear region of the reflex response, which appears to be the region in which the human arterial baroreflex generally operates (9). This approach necessitates visual selection by the investigator to identify the linear region on each individual baroreflex trial. Our laboratory has used this approach consistently in the past (14, 26, 27).

Statistics. Because there were no discernable differences between men and women, data from the two groups were combined for statistical analysis. Baseline variables measured during normoxia and hypoxia were compared by paired $t$-tests. Differences were considered significant when $P < 0.05$. All values are reported as means $\pm$ SE unless otherwise indicated.

RESULTS

Cardiovascular Responses to Hypoxia

As expected, arterial $O_2$ saturation levels were lower during hypoxia ($80.7 \pm 1.4\%$) than during normoxia ($96.8 \pm 0.3\%; P < 0.001$), and this was associated with ventilatory responses characterized by a rise in ventilation from 4.9 $\pm$ 0.2 to 6.4 $\pm$ 0.4 l/min ($P = 0.001$) and a fall in end-tidal $CO_2$ from 5.8 $\pm$ 0.2 to 5.1 $\pm$ 0.2\% ($P < 0.001$). Hypoxia was associated with a rise in heart rate from 59.8 $\pm$ 2.4 to 79.4 $\pm$ 2.9 beats/min ($P < 0.001$), an increase in mean arterial pressure from 96.7 $\pm$ 2.5 to 105.0 $\pm$ 3.3 mmHg ($P = 0.002$), and an increase in muscle sympathetic nerve activity from 3,430 $\pm$ 800 to 6,230 $\pm$ 1,630 total integrated units/min (an increase of 126 $\pm$ 58\%) compared with normoxia ($P < 0.05$).

Cardiovascular Regulation During Hypoxia

We gave an intravenous bolus of 100 $\mu$g sodium nitroprusside, followed 1 min later by 150 $\mu$g of phenylephrine HCl to lower and raise arterial pressure. The fall in pressure produced by nitroprusside was similar during hypoxia and normoxia ($-16.2 \pm 1.4$ vs. $-17.5 \pm 1.4$ mmHg; $P > 0.2$). Similarly, the increase in pressure above baseline produced by phenylephrine was similar during hypoxia and normoxia ($15.5 \pm 2.0$ vs. $13.0 \pm 1.2$ mmHg; $P > 0.1$). Thus the range of pressures (maximum pressure $-$ minimum pressure) produced by the combination of sequential nitroprusside and phenylephrine was similar during hypoxia and normoxia ($31.7 \pm 2.8$ vs. $30.5 \pm 1.7$ mmHg; $P > 0.4$). Furthermore, hypoxia failed to alter the maximal rate of change in pressure produced by these compounds (rate of fall for hypoxia: $-2.6 \pm 0.3$ vs. normoxia: $-2.4 \pm 0.2$ mmHg/s, $P > 0.4$; rate of rise for hypoxia: $3.0 \pm 0.6$ vs. normoxia: $2.6 \pm 0.3$ mmHg/s; $P > 0.4$).

An example from one subject of the arterial baroreflex response relationships for heart rate and muscle sympathetic nerve activity, derived from these changes in arterial pressure, is shown in Fig. 1. Both relationships showed similar shifts upward and rightward, whereas slope was unchanged. Figure 2 shows the group average regressions between heart rate and systolic pressure and between sympathetic nerve activity and diastolic pressure. For both relationships, there was a shift in the baroreflex relationship upward and rightward as reflected by higher values for operating point pressure, heart rate, and sympathetic nerve activity in conjunction with no change in the slope of the response (Fig. 3, slope for heart rate: $-1.02 \pm 0.09$ vs. $-1.02 \pm 0.11$ beats·min$^{-1}$·mmHg$^{-1}$; for nerve activity: $-5.6 \pm 0.9$ vs. $-6.2 \pm 0.9$ integrated activity·beat$^{-1}$·mmHg$^{-1}$, both $P > 0.05$). Sample size analysis for nerve activity indicated that 58 subjects would need to be studied to demonstrate a difference in slope between normoxia and hypoxia. If we analyze our data in terms of R-R interval, we find that the slope for arterial baroreflex control of R-R interval is reduced by moderate hypoxia (slope for R-R interval: $11.2 \pm 1.5$ vs. $15.9 \pm 1.5$ ms/mmHg; $P < 0.05$). Table 1 shows an independent analysis of slope for heart rate and R-R interval during both the falling and rising pressure portions of the baroreflex response. No differences in baroreflex sensitivity between the falling and rising responses were observed in the data. Furthermore, there were no differences in the effect of hypoxia on baroreflex sensitivity between the falling and rising portions of the response.

DISCUSSION

The goal of the present study was to provide insight into cardiovascular regulation during hypoxia. We tested the hypothesis that acute hypoxia would alter the sensitivity of arterial baroreflex control of both heart rate and sympathetic vasoconstrictor outflow, resulting in augmented responses for a given change in pressure. Our key finding is that baroreflex control of both heart rate and muscle sympathetic nerve activity
is reset during hypoxia to higher pressures and higher levels of heart rate and sympathetic nerve activity. This resetting occurred without any discernable change in sensitivity of the arterial baroreflex.

**Hypoxia and Baroreflex Control of Sympathetic Nerve Activity**

To the best of our knowledge, this is the first study to assess the effects of hypoxia on baroreflex control of sympathetic nerve activity in humans. Our new findings build on prior reports of elevated muscle sympathetic nerve activity during hypoxia in humans (35, 40). However, our results contrast with the effects of hypoxia on control of sympathetic nerve activity in animal models. In several animal models, hypoxia has been shown to cause baroreflex resetting with an increase in sensitivity of sympathetic outflow to the kidney (18, 23, 31) and skeletal muscle vascular beds (31). It is unclear whether these differences are species related or are due to differences in study preparation. Some (18, 31) but not all (23) of these animal studies have relied on anesthetized, mechanically ventilated preparations.

It is interesting to note that changes in resting muscle sympathetic nerve activity do not obligue changes in sympathetic responsiveness. In fact, resetting of the arterial sympathetic baroreflex in the absence of changes in sensitivity has been observed after exercise in humans (14), and changes in sensitivity of the baroreflex can occur with (27) or without (26) concur-
Hypoxia and Baroreflex Control of Heart Rate

Several key studies have addressed the issue of whether or not hypoxia modifies baroreflex-heart rate responses in humans. The results of these studies suggest that baroreflex control of heart rate may be reduced by hypoxia (39), but results have varied (1, 6). Differences may be related to differences in data analysis methods (use of R-R interval vs. heart rate) or the degree or duration of hypoxic stress.

Changes in heart rate. A consistent confound in studies of baroreflex control of heart rate is the inverse relationship between R-R interval and heart rate. When baseline heart rate is increased, there is a disproportionate reduction in R-R interval responses because of the nonlinear relationship between R-R interval and heart rate. This issue has clouded many prior investigations on baroreflex control of heart rate and obstructed the understanding of the baroreflex resetting that occurs during exercise for many years (53). Because heart rate (and not R-R interval) is linearly related to cardiac output, heart rate relates to correction of a change in pressure by the baroreflex. Thus, when baseline heart rate is changed, it is reasonable to consider the change in heart rate (and not R-R interval) in response to changes in arterial pressure to provide insight into whether or not baroreflex function has changed. In the context of hypoxia, we found resetting of baroreflex control of heart rate occurs without alteration of the amplitude of the heart rate response to changes in pressure (i.e., no change in sensitivity). This is in agreement with prior work by Sagawa et al. (39). Under conditions of moderate hypoxia (arterial saturation of 76%, similar to the present study), however, under conditions of more severe hypoxia (arterial saturation of 65%), Sagawa et al. found blunting of heart rate responses. If we interpret our data in terms of R-R interval, we find that the slope for arterial baroreflex control of R-R interval is reduced by moderate hypoxia (slope for R-R interval: 11.2 ± 1.5 vs. 15.9 ± 1.5 ms/mmHg; P < 0.05). Unlike baroreflex control of sympathetic outflow, it appears that the sensitivity of arterial baroreflex control, when assessed in terms of R-R interval, is linked to changes in resting R-R interval such that a shortening of the resting R-R interval causes a reduction in R-R interval responses. However, heart rate responses appear analogous to the muscle sympathetic nerve activity responses. It is unclear whether this is a reflection of the dual innervation of the heart or simply a mathematical artifact.

In animal studies, hypoxia has usually been shown to attenuate baroreflex control of heart rate (18, 23, 25). However, one report in conscious rabbits found that hypoxia produced a decrease in heart rate without attenuation of baroreflex control (21), and another study in anesthetized dogs found higher heart rates with hypoxia (3). Some of these inconsistencies may be related to use of anesthetic agents or species differences.

Potential Pathways

We would presume that the effects of hypoxia on baroreflex function, if due to hypoxia per se, are via stimulation of the peripheral chemoreceptors. It is not thought that central chemoreceptors respond to modest hypoxia (2). Baroreceptor and chemoreceptor projections within the medulla often coincide, and the overlap of these medullary projections provides multiple locations in which interactions between these reflexes could occur (22). Studies in animals demonstrated that activating the baroreflexes by increasing arterial pressure could attenuate peripheral chemoreflex-mediated ventilatory (17) and vascular responses to hypoxia (16, 24). Miura and Reis (28) localized these interactive effects to the paramedian reticular nuclei. We can only speculate as to whether this location is involved in the baroreflex resetting that we have observed.

On the basis of descriptions of “classic” resetting described by Eckberg and Sleight (9), Korner (20), and Rowell (34), it would appear that the peripheral chemoreflex is causing changes in autonomic outflow via
pathways that are both baroreflex dependent and independent, meaning that some of the affected autonomic outflow tracts that are being activated are not under baroreflex control but that others are being activated via the baroreflex. This may be analogous to the classic baroreflex resetting observed during exercise (33).

Limitations

To simulate the physiological response to altitude, we did not interfere with the ventilatory response of our subjects. Ventilation increased with hypoxia, which led to hypocapnia. As such, the difference between trials cannot be attributed to hypoxia per se but could also be influenced by the concomitant hyperpnea and hypocapnia. The effects of changes in ventilation on muscle sympathetic nerve activity have been well documented in several studies (8, 41, 42, 45). During hyperpnea with increased tidal volumes, the inspiratory-expiratory differences in sympathetic outflow are enhanced because of feedback from the lung stretch receptors (42). Importantly, these changes in ventilation affect the within-breath modulation of muscle sympathetic nerve activity but do not alter the mean level of sympathetic activity, and these within-breath fluctuations do not alter the ability of the sympathetic nerves to be activated by other reflex mechanisms (e.g., unloading of cardiopulmonary receptors) (41, 42). A similar within-breath modulation of heart rate has been reported in animal (4, 11, 15) and human studies (7, 8) and reflects the respiratory gating of vagal outflow to the heart. Thus it is unlikely that our results are largely affected by hyperpnea. In contrast, hypocapnia is likely to reduce muscle sympathetic nerve activity and heart rate (12, 29, 43). Thus it is possible that the degree of resetting we observed would have been greater under isocapnic conditions. Therefore, although our results likely reflect the effects of hypoxia and may represent what happens during exposure to altitude, they may not reflect the responses produced by isocapnic hypoxia or hypercapnic hypoxia (e.g., during sleep apnea).

We did not randomize the order of normoxia and hypoxia trials. Therefore, there may be concern regarding the rise in blood pressure observed in the hypoxia trials. Previous studies have suggested that hypoxia causes either no change in pressure or a modest rise in pressure. For example, in Somers et al. (44), mean arterial pressure during hypoxia increased 3 mmHg above normoxic conditions. It is possible that the differences in the pressor response to hypoxia in various studies are due to the degree of hypoxia investigated, differences between hypocapnic and isocapnic hypoxia, whether or not subjects had been previously exposed to hypoxia or altitude, and differences in individual responsiveness to hypoxia.

In the context of baroreflex physiology, hysteresis has been historically defined as a change in sensitivity of the heart rate or R-R interval response to changes in pressure that is dependent on whether pressure is falling or rising (32, 37). One might argue that this is a long-standing misuse of the term hysteresis, because it is not entirely consistent with the more general definition of hysteresis. Regardless of the semantics, it is well documented but poorly understood that cardiac responses to falling and rising pressures may differ (37) and that this pattern is inconsistent across subjects (47). Thus, in the present work, we independently assessed the falling and rising pressure portions of our data, comparing normoxia and hypoxia trials. No differences in baroreflex sensitivity between the falling and rising responses were observed in the data. Furthermore, there were no differences in the effect of hypoxia on baroreflex sensitivity between the falling and rising portions of the response. However, the fact that average slopes do not differ between falling and rising responses does not obviate this issue within individuals. Thus we cannot exclude the possibility that subtle individual differences in the effect of hypoxia on baroreflex control of heart rate have been missed by this approach. In contrast to the literature regarding heart rate responses, to the best of our knowledge, no studies have documented differences in the response of muscle sympathetic nerve activity to falling vs. rising pressures.

In animal models, it is often possible to assess baroreflex responses over a wide range of pressures so that response relations encompass the reflex from threshold to saturation. Such data can often be analyzed by applying a sigmoidal model to the data. The modified Oxford method is not able to divulge the entire response relationship consistently in humans, because the nonlinear threshold and saturation regions are variably present within the pressure ranges achieved. As such, we have restricted our analysis to the linear region of the reflex response that was evident in the collected data. To do so, we have relied on visual selection by the investigator to identify the linear region on each individual baroreflex trial. Our laboratory has used this approach consistently in the past (14, 26, 27), but it may not be without limitation.

Perspectives

We are struck by the similarity between the arterial baroreflex resetting we have found to occur during acute exposure to hypoxia and that which occurs during exercise. Both hypoxia and exercise represent high-flow (high cardiac output), low-resistance states, in which local vasodilator mechanisms that attempt to secure adequate blood flow to match metabolic demand are in competition with neural vasoconstrictor reflexes attempting to maintain arterial pressure. It is possible that baroreflex resetting represents a stereotyped regulatory response that is beneficial in such states. Alternatively, arterial baroreflex resetting may be the final common pathway for numerous sympatoexcitatory reflexes such as peripheral chemoreflexes, muscle metaboreflexes, and others. In this context, baroreflex resetting causes the rise in sympathetic outflow.
Conclusions

Acute exposure to hypoxia resets baroreflex control of both heart rate and muscle sympathetic nerve activity to higher pressures and higher levels of heart rate and sympathetic nerve activity without changes in sensitivity of the arterial baroreflex.

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