Splenic contraction-induced reversible increase in hemoglobin concentration in intermittent hypoxia

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1Department of Medicine, Tokai University School of Medicine, Isehara, Kanagawa 259-1193, Japan; and 2Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160-7401

Kuwahira, Ichiro, Ueguri Kamiya, Tokuven Iwamoto, Yoshihiro Moue, Tetsuya Urano, Yasuyo Ohta, and Norberto C. Gonzalez. Splenic contraction-induced reversible increase in hemoglobin concentration in intermittent hypoxia. J. Appl. Physiol. 86(1): 181–187, 1999.—The effect of intermittent hypoxia (IHx) on blood hemoglobin concentration ([Hb]) and the underlying mechanisms were studied in rats exposed to 10% O2, 1 h/day, for up to 5 wk. IHx protocols with longer daily hypoxic exposure showed persistent polycythemia; however, it is unknown whether [Hb] increases transiently during hypoxia in protocols without polycythemia. Hypoxia produced a reversible [Hb] increase after 4 days of IHx but not in normoxic controls (NxC) or after shorter periods of IHx. Splenectomy abolished the phenomenon. Plasma epinephrine and norepinephrine levels during hypoxia were comparable in IHx and NxC groups, but the epinephrine-induced [Hb] increase was larger in IHx. The α1- and α2-adrenoreceptor blockade (phentolamine) and α2-blockade (yohimbine) abolished the [Hb] increase of IHx rats. Conversely, α2-receptor stimulation (oxymetazoline) increased [Hb] during normoxia in IHx but not in NxC. In conclusion, this IHx protocol results in reversible [Hb] increases during hypoxia via splenic contraction mediated by increased α2-adrenergic receptor response. This may protect O2 supply during hypoxia without the cardiovascular burden of polycythemia during normoxia.

α2-adrenoreceptor; Sprague-Dawley rat; acclimatization; oxygen delivery

ACUTE HYPOXIA is characterized by marked selective redistribution of blood flow that ensures adequate O2 supply to vital organs (1, 21). If hypoxia is maintained, the pattern of regional blood flow distribution returns toward that of normoxia (22), and organ O2 supply is maintained via an increase in blood hemoglobin concentration ([Hb]) secondary to the polycythemia of chronic hypoxia (22). The increased blood viscosity that accompanies polycythemia, however, increases the cardiovascular workload and contributes to the pulmonary (11) and systemic hypertension (28) of chronic hypoxia and tends to limit stroke volume and cardiac output during exercise (2, 13). There is evidence that excessive polycythemia is one of the contributing factors to chronic mountain sickness (26). There are conditions such as sleep apnea and bronchial asthma that are characterized by intermittent hypoxia; in these cases, arterial PO2 (PaO2) may be normal between episodes of hypoxia of variable duration and intensity. Long or frequent hypoxic episodes in animals and humans are associated with persistent polycythemia, pulmonary and systemic hypertension, and right ventricular (RV) hypertrophy (4, 10, 23, 24, 27, 29, 30, 32, 35, 36). On the other hand, transient elevations in [Hb] are observed in conditions such as breath-hold diving where the mechanism involves sympathetic-mediated splenic contraction (17, 18). Splenic contraction is also observed in conditions of increased sympathetic activity, such as exercise and physical restraint, in which hypoxia is not present (3, 5, 9, 12, 15, 31, 34). Accordingly, while intermittent hypoxia may lead to hematologic and cardiovascular changes comparable to those of sustained prolonged hypoxia, it is less clear whether intermittent hypoxic protocols are associated with a reversible increase in [Hb].

The purpose of the present experiments was to determine whether a well-defined intermittent hypoxic protocol, which is not associated with persistent polycythemia or other markers of chronic hypoxia, may result in a reversible increase in [Hb] during the hypoxic period. Pharmacological studies were carried out to determine the mechanism underlying the [Hb] increase.

A reversible increase in [Hb] in intermittent hypoxia would help maintain O2 delivery during hypoxia without the increased cardiovascular workload of polycythemia during the normoxic condition.

METHODS

Intermittent hypoxic chamber. Male Sprague-Dawley rats were housed in environmental Plexiglas chambers (30 × 30 × 30 cm), two rats in each, at a temperature of 23 ± 1 (SE)°C at a 12:12-h light-dark photoperiod. With the use of a timed solenoid valve, the gas flushing the chamber was automatically switched from compressed air to a mixture of 10% O2 in N2 and back to compressed air at a rate of 30 l/min. The O2 concentration in the chamber was monitored by an O2 analyzer (Beckman OM-11) and was stabilized within ~5 min after the gas composition had been changed. The rats assigned to the intermittent hypoxia group (IHx; see below) were exposed to 10% O2 for 1 h/day (1:00–2:00 PM) in the chamber. For the normoxic controls (NxC), the chamber was continuously flushed with compressed air. Standard rat chow and water were provided ad libitum.

Experimental protocol. Thirty-eight rats were divided into two groups: an NxC group (n = 6, weight 395 ± 8 (SE) g) and an IHx group (n = 32, weight 387 ± 2 g). Within the IHx group, the rats were divided into five subgroups, depending on the length of hypoxic exposure: a 1-day (1-D, n = 7), a
4-days (4-D, n = 7), a 7-days (7-D, n = 6), a 21-days (21-D, n = 6), and a 35-days (35-D, n = 6) IHx groups. In addition to these IHx groups, six rats were maintained in the IHx chamber for 21 days, then returned to the home cage and kept under normoxia (Nx) for 60 days (IHX-Nx group). Because the spleen is a reservoir for red blood cells in the rat (7), before IHx exposure, splenectomy was performed under anesthesia in eight rats. Seven days after surgery, the rats were placed in the chamber for 21 days (Spn-IHX group).

At the end of the desired exposure length to intermittent hypoxia, a PE-50 catheter was inserted 10–20 mm into the middle caudal artery under halothane anesthesia for monitoring arterial blood pressure and heart rate and for withdrawal of blood samples. After completion of surgery, the rat was transferred to an accommodation box described before (20) and allowed to recover fully from anesthesia for 3 h. The box containing the rat was placed in an environmental chamber (50 × 30 × 15 cm), through which room air was circulated. When stable arterial blood pressure and heart rate were attained, the measurements under normoxia were started. After completion of the measurements under normoxia, hypoxia was induced. The measurements under hypoxia were carried out 30 min after the gas composition had been changed. The gas mixture was then switched to room air, and measurements were carried out 30 min after removal of hypoxia.

Arterial blood pressure and heart rate were recorded from the middle caudal artery by using a pressure transducer (Statham P23Gb) and a chart recorder (Nihon Kohden, J apan). The arterial blood gases in a 0.07-ml sample of arterial blood were measured with a pH/blood-gas analyzer (Instrumentation Laboratory, model 1304). Hemoglobin and oxyhemoglobin saturation were measured with 0.04 ml of arterial blood by using a Radiometer OSM3 hemoximeter.

At the end of the experiment, the rat was anesthetized with halothane, exsanguinated, and killed by an overdose of pentobarbital sodium. The heart was removed and placed in a normal saline bath at room temperature to prevent dehydration. The great vessels, atria, and atrioventricular valves were dissected from the ventricles. The RV free wall was separated by dissection at the line of reflection between the RV and the interventricular septum. Adherent blood clots were removed. After being blotted dry, the RV wall and the left ventricle (LV) plus interventricular septum (S) were weighed separately, and the ratio RV/(LV + S) was calculated. The ratio RV/(LV + S) was determined in the NxC group and in the 7-D, 21-D, and 35-D IHx groups.

Determination of circulating catecholamine levels. Because [Hb] may increase via catecholamine-induced splenic contraction (3, 8, 9, 15–18, 31, 33), circulating blood levels of epinephrine concentration ([Epi]) and norepinephrine concentration ([NE]) were measured by HPLC (37) in seven 21-D IHx rats and seven age-matched NxC rats. The rats were prepared as described above, and samples for [Epi] and [NE] were obtained at the end of the 30-min hypoxic challenge. Only one blood sample was obtained in each rat to minimize blood loss.

Epinephrine dose-response curve. To determine whether there is a difference between the splenic α-adrenergic-receptor reactivity in NxC and IHx rats, changes in [Hb] in response to the α-adrenergic agonist epinephrine were assessed. Epinephrine (Sigma Chemical, St. Louis, MO) was injected intravenously in doses ranging from 0.01 to 102 μg/kg, and a dose-response curve was constructed in thirteen 21-D IHx rats and thirteen age-matched NxC rats. Because it was difficult to keep conscious rats in the resting state, given the excitatory effects of epinephrine, the rats were anesthetized with halothane, intubated, and mechanically ventilated with O2 throughout the experiments. A PE-10 catheter was inserted 20–30 mm into the lateral tail vein for injection of epinephrine. Blood samples were obtained through a PE-50 catheter inserted into the middle caudal artery, and [Hb] was determined before and after each epinephrine dose. In preliminary experiments, we confirmed that repetitive blood sampling (0.04 ml/sample) did not influence final [Hb].

A-Adrenergic-receptor antagonists administration. To evaluate the role of the splenic α-adrenergic-receptor subtypes, effects of the α1- and α2-adrenergic-receptor antagonist phentolamine (Phlm, CIBA-GEIGY, J apan) and the selective α2-adrenergic-receptor antagonist yohimbine (RBI, Natick, MA) on changes in [Hb] during hypoxia were assessed in six and eight 21-D IHx rats, respectively. Under halothane anesthesia, a PE-50 catheter was inserted into the middle caudal artery for monitoring blood pressure and heart rate and for blood sampling, and a PE-10 catheter was inserted into the tail vein for injection of the agents. Three hours after full recovery from anesthesia and surgery, Phlm was injected as a bolus of 5 mg/kg in the former group, and yohimbine was injected as a bolus of 2 mg/kg in the latter group, respectively. After stable arterial blood pressure and heart rate were attained, the measurements under three conditions (normoxia, hypoxia, and removal of hypoxia) were carried out as described in the Experimental protocol.

Selective α2-adrenergic-receptor agonist administration. The effect of the selective α2-adrenergic receptor agonist oxymetazoline (6) (Sigma Chemical, St. Louis, MO) on changes in [Hb] was assessed in six 7-D IHx rats and six age-matched NxC rats. The animals were studied under anesthesia by using the protocol described for the epinephrine dose-response curve. Oxymetazoline was injected as a bolus of 0.1 mg/kg through a PE-10 catheter inserted into the lateral tail vein. Blood samples were obtained through a PE-50 catheter inserted into the middle caudal artery, and [Hb] was determined before and 5–10 min after oxymetazoline injection. Preliminary experiments showed that this time was adequate to detect the peak [Hb] after oxymetazoline injection.

Statistics. All results are means ± SE. Comparisons of the data among three different conditions (normoxia, hypoxia, and removal of hypoxia) were carried out by the following procedure. First, the Friedman test was used to determine whether a difference among three conditions was detected. If a difference was detected, the Wilcoxon paired-sample test was used to compare the data between normoxia and hypoxia, between hypoxia and removal of hypoxia, and between normoxia and removal of hypoxia. Comparisons of the data among different groups were carried out by using the Mann-Whitney U-test. The Mann-Whitney U-test was also used to compare the data of catecholamine levels, and the data of a dose-response curve, between the NxC and IHx groups. The effect of epinephrine and oxymetazoline on [Hb] was assessed by paired-sample comparison with the control value by use of the Wilcoxon paired-sample test. A P value <0.05 indicates statistically significant differences.

RESULTS

Hemodynamic and arterial blood gas data obtained under normoxic conditions (Table 1 and Fig. 1) were within the normal range for conscious resting rats (20-22). The 30-min hypoxic challenge resulted in a significant increase in heart rate with no change in mean arterial blood pressure (Table 1). A significant decrease in Pao2 and oxyhemoglobin saturation and acute respiratory alkalosis, as shown by a significant
Circulating plasma [Epi] and [NE] levels obtained at the end of the 30-min hypoxic challenge in the IHx group (1,082 ± 176 and 648 ± 103 pg/ml, respectively) were not different from those seen in the NxC group under the same conditions (1,058 ± 248 and 541 ± 85 pg/ml, respectively).

Epinephrine injection at a lower dose resulted in a small but significant decrease in [Hb] in both IHx and NxC groups (Fig. 2). Above this level, epinephrine produced a dose-dependent increase in [Hb] in both groups; however, the increase was significantly larger in the IHx than the NxC groups.

The increase in [Hb] produced by the 30-min hypoxic challenge in the IHx groups was completely abolished by the combined $\alpha_1$- and $\alpha_2$-adrenergic-receptor antagonist Phlm (Table 3) as well as by the selective $\alpha_2$-adrenergic-receptor antagonist yohimbine (Table 3). On the other hand, the selective $\alpha_2$-adrenergic-receptor agonist oxymetazoline (6), administered under normoxic conditions, produced a significant increase in [Hb] in the IHx, but not in the NxC, groups (Fig. 3). The magnitude of the increase in [Hb] produced by oxymetazoline in the IHx group was comparable to that produced by the 30-min hypoxic challenge in the corresponding group (Table 2).

### DISCUSSION

Rats exposed repeatedly to 10% O₂ for 1 h/day showed a significant increase in [Hb] from 15.4 to 16.9 g/dl (mean value of the 7-D, 21-D, and 35-D IHx rats) during hypoxia. This increase was reversible, with blood [Hb] returning to normal levels during the normoxic intervals. The [Hb] increase was not observed when the NxC rats, not previously exposed to hypoxia, were challenged once with 10% O₂. There was no significant difference in hemodynamic and arterial blood-gas values between the IHx and NxC rats. The values observed during the 30-min hypoxic challenge are in good agreement with those obtained in our previous studies in conscious rats breathing similar gas mixtures (21, 22). These results show that, in contrast to other experimental protocols and clinical conditions of intermittent hypoxia, the regimen utilized in the present experiments results in an increase in [Hb] only during the hypoxic episode.

The source of the elevated [Hb] is primarily the spleen, as demonstrated by the fact that the reversible increase in [Hb] was abolished by splenectomy. The role of the spleen in storage and release of red blood cells has been described extensively (3, 5, 9, 12, 15–18, 31, 34). In the present study, neither the NxC nor 1-D IHx rats showed an increase in [Hb] during the hypoxic challenge; furthermore, the increase observed in the 4-D IHx rats was lower than that observed after a large number of exposures. This indicates that repetitive exposure to hypoxia is needed to produce splenic contraction and that after four such exposures the [Hb] increase does not reach its maximum value. Conversely, since there is no difference in the magnitude of the increase in [Hb] among the 7-D, 21-D, and 35-D IHx rats, it appears that the contribution of splenic
contraction to elevated [Hb] is the same once it is established. Interestingly, once splenic contraction in response to a hypoxic challenge is induced, it is maintained for at least 60 days after the intermittent hypoxic exposure is discontinued.

The role of catecholamines in effecting red blood cell release into the circulation via splenic contraction has been described in many different species (3, 8, 9, 15–18, 31, 33). Because hypoxia is associated with an in-

Table 2. Hemoglobin concentration in the NxC and IHx groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Normoxia [Hb], g/dl</th>
<th>Hypoxia [Hb], g/dl</th>
<th>Removal of hypoxia [Hb], g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>NxC</td>
<td>15.4 ± 0.1</td>
<td>15.2 ± 0.3</td>
<td>15.4 ± 0.2</td>
</tr>
<tr>
<td>1-D IHx</td>
<td>15.1 ± 0.2</td>
<td>15.4 ± 0.1</td>
<td>15.0 ± 0.2</td>
</tr>
<tr>
<td>4-D IHx</td>
<td>15.0 ± 0.2</td>
<td>15.7 ± 0.2*</td>
<td>14.9 ± 0.2</td>
</tr>
<tr>
<td>7-D IHx</td>
<td>15.5 ± 0.2</td>
<td>16.9 ± 0.1*</td>
<td>15.2 ± 0.2</td>
</tr>
<tr>
<td>21-D IHx</td>
<td>15.2 ± 0.4</td>
<td>16.9 ± 0.2*</td>
<td>14.9 ± 0.3</td>
</tr>
<tr>
<td>35-D IHx</td>
<td>15.3 ± 0.2</td>
<td>16.7 ± 0.2*</td>
<td>15.4 ± 0.2</td>
</tr>
<tr>
<td>IHx-Nx</td>
<td>15.1 ± 0.2</td>
<td>16.2 ± 0.3*</td>
<td>15.0 ± 0.2</td>
</tr>
<tr>
<td>Spln-IHx</td>
<td>15.4 ± 0.1</td>
<td>15.8 ± 0.1</td>
<td>15.5 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for hemoglobin concentration ([Hb]). *Significant difference between value observed in hypoxia and the corresponding values of normoxia and removal of hypoxia (P < 0.05). †Significant difference between value observed during hypoxia in the 4-D IHx group and the 7-D, 21-D, and 35-D IHx groups (P < 0.05).
increased sympathetic activity (19, 25), a role of the sympathetic nervous system in splenic contraction of intermittent hypoxia is reasonable to assume, and our data indicate that the sympathetic nervous system does play a central role in the elevated [Hb] observed in this model. The levels of both [Epi] and [NE] seen in the present experiments are considerably higher than those observed by us in normoxia (20), illustrating the well-established effect of acute hypoxia on plasma catecholamine levels (19, 25). However, the relationship among hypoxia, sympathetic stimulation, splenic contraction, and increased [Hb] observed in these experiments is not a simple one. First, as indicated above, exposure to several hypoxic episodes is necessary for maximal expression of this phenomenon; second, the plasma levels of catecholamines, which are an indirect indication of sympathetic activity, are comparable when the NxC and IHx rats are exposed to a hypoxic challenge, despite the fact that only the latter shows an increase in [Hb]. Accordingly, the increased [Hb] does not appear to be the result of an increased level of sympathetic activity in intermittent hypoxia.

Our data point to an alternative mechanism of sympathetic involvement, namely, an increased response of the α2-adrenergic receptors to their agonists. First, evidence that the splenic contraction of intermittent hypoxia is mediated by stimulation of α-adrenergic receptors is suggested by the fact that Phtm, a mixed α1- and α2-adrenergic-receptor antagonist, abolished the increase in [Hb] of the IHx rats. Yohimbine, a selective α2-adrenergic-receptor antagonist, had the same effect, suggesting that contraction of the spleen in the IHx rats is mediated by this receptor subtype. Second, evidence for an increase in the response of the α-adrenergic receptors to their agonists is suggested by the larger increase in [Hb] in response to epinephrine, which stimulates both α- and β-adrenergic receptors, and by the results of the administration of the α2-adrenergic-receptor agonist oxymetazoline. Epinephrine doses from 0.01 to 0.1 µg/kg actually showed a small decrease in [Hb], which was the same in both IHx and NxC rats. This is probably due to splenic vasodilatation induced by stimulation of the β-adrenergic receptors that occurs at low doses of epinephrine (33). However, at higher epinephrine doses, the increase in [Hb] was larger in the IHx rats, a result consistent with an increased response of the receptors to their agonists. Involvement of the α2-adrenergic-receptor subtype is suggested by the results of oxymetazoline, which increased [Hb] in the IHx rats during normoxia, whereas the same dose was ineffective in the NxC rats. Taken together, these data suggest that the intermittent hypoxic protocol utilized in these experiments results in an increased response of the α2-adrenergic-receptor subtype to its agonists, which leads to splenic contraction and a reversible increase in [Hb]. This phenomenon requires several exposures to achieve full expression, and its magnitude remains fairly constant for at least 35 days of intermittent hypoxia. Our results cannot determine which α2-adrenergic-receptor subtype is involved in this phenomenon; however, recent studies in the rat have shown a high concentration of α2A-subtypes in the spleen (14), suggesting a role for these receptors in this phenomenon. In addition, although three α2-receptor subtypes have been identified pharmacologically, only oxymetazoline shows preferential affinity for the α2A-adrenergic receptor (6). Although splenic contraction secondary to increased sympathetic activity has been

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### Table 3. Effect of phentolamine and yohimbine on hemoglobin concentration in the IHx group

<table>
<thead>
<tr>
<th>Condition</th>
<th>[Hb], g/dl</th>
<th>Removal of hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phentolamine</td>
<td>15.3 ± 0.4</td>
<td>15.0 ± 0.4</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>15.0 ± 0.2</td>
<td>14.9 ± 0.2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>15.0 ± 0.4</td>
<td>14.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. There are no significant differences among the 3 conditions.
demonstrated in other conditions such as exercise, this is the first demonstration that repetitive exposure to short-term hypoxia leads to an increased response of the α2-adrenergic receptors to the agonists and splenic contraction.

An important observation is that this intermittent hypoxic protocol did not result in persistent polycythemia or in the development of other chronic hypoxic markers, such as systemic and pulmonary hypertension and RV hypertrophy, which accompany experimental as well as clinical conditions of intermittent hypoxia (4, 10, 23, 24, 27, 29, 30, 32, 35, 36). These cases, however, are characterized by longer duration and frequency of hypoxia. This would suggest that duration of hypoxia has to reach a threshold for a given mechanism to be initiated and that the different hypoxic markers have different thresholds. In this view, it would appear that the threshold for an increased response of α2-adrenergic receptors to their agonists is lower than that for systemic and pulmonary hypertension and increased hematopoietic activity.

An increase in [Hb], which is reversed when normoxia is resumed, constitutes a useful mechanism that protects the supply of O2 to the tissues during hypoxic episode, without the cardiovascular burden imposed by persistent polycythemia. The increase in O2-carrying capacity produced by this mechanism is not negligible, as illustrated in Fig. 4, which shows the calculated values of O2 content of arterial blood (CaO2) that can be observed in the following conditions: acute hypoxia, IHx, chronic hypoxia, and Nx. The values shown in Fig. 4 were obtained from the present study and from a previous study from our laboratory (22). The lowest CaO2 is observed during acute hypoxia, in which the only compensatory mechanism protecting CaO2 is the hyperventilation that moderates the fall in PaO2 and leads only compensatory mechanism protecting CaO2 is observed during acute hypoxia, in which the

REFERENCES


