Role of endothelin and nitric oxide imbalance in the pathogenesis of hypoxia-induced arterial hypertension

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Background. We have recently demonstrated that prolonged hypobaric hypoxia can lead to a hematocrit-independent sustained arterial hypertension (HTN) in genetically normotensive Sprague-Dawley rats. The rise in blood pressure in the hypoxic animals was accompanied by a marked but transient increase in plasma endothelin level. In addition, hypoxia has been shown to decrease nitric oxide (NO) production by cultured endothelial cells. This study was designed to test the hypothesis that hypoxia-induced HTN may be mediated by increased endothelin and/or decreased NO production.

Methods. Blood pressure, plasma endothelin and urinary NO metabolites (NOx) were monitored in rats during a 24-day exposure to hypobaric hypoxia (air pressure = 390 mm Hg). The results were compared with those obtained in animals maintained under normoxic condition (control group). To test the possible role of excess endothelin and depressed NO production, the studies were repeated using subgroups of animals treated with either an endothelin receptor ET-A/B blocker (L-754,142) or L-arginine.

Results. The untreated hypoxic group exhibited a threefold rise in plasma endothelin and a threefold fall in urinary NOx, prior to the onset of HTN. Endothelin receptor blockade led to a further fall in urinary NOx excretion and failed to mitigate HTN. In contrast, L-arginine supplementation improved the urinary NOx excretion and prevented HTN. Neither therapy affected the hypoxia-induced erythrocytosis.

Conclusions. We conclude that hypoxia-induced HTN is associated with depressed NO production and can be mitigated by L-arginine supplementation.

In an earlier study, we described a new model of sustained systemic hypertension (HTN) induced by prolonged hypobaric hypoxia in genetically normotensive Sprague-Dawley rats [1]. The observed HTN was not related to the rise in hematocrit, blood viscosity or blood volume since HTN occurred despite prevention of hypoxia-induced erythrocytosis by repeated phlebotomies. Moreover, the HTN persisted long after the restoration of normoxia and normal hematocrit and blood volume [1].

The rise in arterial blood pressure began within a few days after exposure to hypobaric hypoxia and was preceded by a threefold increase in plasma endothelin concentration [1]. This observation was consistent with the reported increase in plasma endothelin-1 in human volunteers following an ascend from a 490 to 4,559 meter altitude leading to acute pulmonary hypertension [2]. It is of note that hypoxia results in up-regulation of endothelin and ET-A and ET-B receptor gene expressions in the lung [3]. Moreover, hypoxia-induced pulmonary hypertension can be mitigated by endothelin-1 receptor blockade [4]. These observations point to the role of endothelin in the pathogenesis of hypoxia-induced pulmonary hypertension. In addition, intravenous infusion of endothelin-1 has been shown to cause sustained systemic HTN which can persist long after endothelin-1 administration.

Basal production of nitric oxide (NO) is essential for maintenance of the vasodilatory tone and normal blood pressure. Several studies have demonstrated a significant down-regulation of endothelial NO synthase (eNOS) expression with hypoxia in cultured endothelial cells [5, 6]. Based on the above observations, we hypothesized that hypoxia-induced HTN may be mediated by increased endothelin production and/or depressed NO generation. The present study was, therefore, designed to test this hypothesis.

METHODS

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN, USA) weighing 200 to 225 g were fed regular rat chow (Purina Rat Chow; Purina Mills Inc., Brentwood, MO, USA) and water ad libitum. The rats were randomly assigned to the following groups:

Group A. Rats assigned to this group were implanted with osmotic pumps (Alza Inc., Palo Alto, CA, USA) that were loaded with the endothelin receptor (ET-A/B) antagonist, L-754,142 (Merck Inc., Rahway, NJ, USA). The pump delivered the drug at a rate of 5 mg/kg/day throughout the 24-day hypoxic period. During this period animals were placed in a hypobaric chamber in which the air pressure was kept at 390 mm Hg using a continuous
vacuum pump and an adjustable inflow valve. The interior of the chamber was kept at the ambient temperature, and the normal interior light cycle was accommodated through a glass window in the chamber wall. The animals were kept within the chamber for 24 days. The chamber was briefly opened three times a week for routine animal care, measurement of tail arterial blood pressure and procurement of blood samples for hematocrit.

**Group B.** Rats in group B were implanted with unloaded osmotic pumps and received L-arginine (Sigma Co., St. Louis, MO, USA) supplementation in their drinking water (0.5 g/dl). These animals were exposed to hypobaric hypoxia in a manner identical to that described for group A.

**Group C.** Rats assigned to this group were implanted with unloaded osmotic pumps and were exposed to hypobaric hypoxia in a fashion similar to that of groups A and B animals.

**Group D.** Animals assigned to this group were placed in the chamber at normal atmospheric pressure.

Arterial blood pressure was measured using a tail sphygmomanometer (Harvard Apparatus, South Natick, MA, USA). Blood samples were obtained by orbital sinus puncture. Hematocrit was determined by microhematocrit method.

In an attempt to determine urinary excretion of NO metabolites, four-hour urine collections were obtained at baseline and at various intervals during hypoxia (at 4 hr, 8 hr and on days 1, 2, 3, 7, and 14) and immediately after the end of the hypoxic period (day 24). On each occasion, animals were placed in individual metabolic cages within the hypobaric chamber, and urine was collected over crushed ice. Urinary concentration of total nitrates and nitrites (NOx) was measured using the Sievers Nitric Oxide Analyzer (Sievers Instruments Inc., Boulder, CO, USA) as previously described [7]. The values obtained were normalized against urinary creatinine excretion.

### Table 1. Hematocrit and body weight obtained prior to (initial) and after the conclusion (final) of the study in placebo-treated (Pl-Rx), endothelin-1 receptor blocker-treated (ERB-Rx), L-arginine-treated (Arg-Rx) hypoxic groups and normal control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight g</th>
<th>Hematocrit %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Control</td>
<td>221 ± 4.1</td>
<td>312 ± 5.7*</td>
</tr>
<tr>
<td>Pl-Rx</td>
<td>224 ± 2.8</td>
<td>284 ± 3.9*</td>
</tr>
<tr>
<td>ERB-Rx</td>
<td>219 ± 3.3</td>
<td>291 ± 4.2*</td>
</tr>
<tr>
<td>Arg-Rx</td>
<td>217 ± 3.5</td>
<td>288 ± 4.8*</td>
</tr>
</tbody>
</table>

Rx is treated.

* P < 0.05 vs. initial value.

* P < 0.05 vs. corresponding value in the control group.

samples were extracted with acetone, and the acetone extract was dried under nitrogen (extraction efficiency was 90%). The dried extract was reconstituted and assayed in an RIA using 125I-endothelin and rabbit anti-endothelin. The bound/free separation was achieved using second antibody separation procedure (donkey anti-rabbit gamma globulin). To avoid interassay variations, all samples were assayed in a single session. The intra-assay variation for this test was less than 8%. Sensitivity of the assay was 1 pg/ml for a 1 mL sample size. The specificity of the antibody was as follows: endothelin 1 to 100%, endothelin 2 to 52% and endothelin 3 to 96%. The assay had minimal crossreactivity with big endothelin (7%) and no cross reactivity with ANP, AVP, angiotensin II or ACTH.

### Statistical analysis

Repeated measure analysis of variance (ANOVA) and Duncan’s multiple range test were used in evaluation of the data that are presented as mean ± SEM. P values equal to or less than 0.05 were considered significant.

### Results

#### Effects of hypoxia

Data are shown in Table 1 and Figures 1 and 2. The placebo-treated hypoxic group showed a steady rise in hematocrit and arterial blood pressure beginning a few days following the onset of hypoxia. This was preceded by a marked rise in plasma endothelin concentration which peaked 48 hours after exposure to hypoxia. In contrast, urinary NOx excretion fell markedly early in the course of hypoxia reaching a nadir within 48 hours. Thereafter, urinary NOx gradually rose reaching normal values by day 7.

#### Effect of ET-A/B receptor blockade

Data are shown in Figures 3 and 4. ET-A and ET-B receptor blockade with L-754,142 led to a more severe and
longer lasting reduction of urinary NOx excretion when compared with the untreated hypoxic group. Moreover, the treatment did not mitigate the hypoxia-induced HTN. In addition, ET-A/B receptor blockade did not affect the hypoxia-induced erythrocytosis.

Effect of L-arginine supplementation

Data are illustrated in Figures 3 and 4. Compared to the untreated controls, L-arginine-treated rats showed a significant improvement in urinary NOx excretion. In addition, the rise in arterial blood pressure seen in the placebo-treated and ET-A/B blocker-treated animals did not occur in rats receiving L-arginine supplementation. Thus, L-arginine administration mitigated the fall in urinary NOx excretion and abrogated the development of HTN in rats subjected to prolonged hypobaric hypoxia. As with ET-A/B blocker (L-754,142), L-arginine did not affect hypoxia-induced erythrocytosis.

DISCUSSION

In a recent study we found that, under the given conditions, prolonged hypobaric hypoxia leads to a hematocrit-independent, severe sustained systemic HTN that persists long after restoration of normoxia. This was associated with a significant rise in plasma endothelin level preceding the onset of systemic HTN. Although the peak plasma endothelin concentration occurred before the onset of HTN, the endothelin level did not return to the baseline level until fourteen days after the onset of hypoxia [1]. This observation suggested that elevation of endothelin may have contributed to the associated HTN. If true, continuous endothelin receptor blockade would be expected to mitigate the associated HTN. Contrary to expectation, the study showed that treatment with endothelin-1 receptor antagonist did not mitigate the hypoxia-induced systemic HTN in this model. This is in contrast to the hypoxia-induced pulmonary hypertension that can be ameliorated or reversed by endothelin receptor blockade in rats exposed to acute or chronic hypoxia [4, 8]. The differential effects of endothelin-1 receptor blockade on hypoxia-induced systemic HTN and pulmonary hypertension may be, in part, due to the greater endothelin abundance and endothelin...
sensitivity of the pulmonary vasculature as compared to the systemic blood vessels [4].

Nitric oxide (NO), otherwise known as endothelium-derived relaxing factor (EDRF), plays a major role in regulation of vascular smooth muscle tone and hence arterial blood pressure in normal conditions [9]. Moreover, inhibition of NO production by NO synthase blockade leads to sustained systemic hypertension [10, 11]. It is of note that hypoxia leads to depressed L-arginine: NO pathway in the lungs. This is thought to play a role in the genesis of hypoxia-induced pulmonary hypertension [12, 13]. In fact, NOS blockade has been shown to worsen and NO administration (in the inhaled air) to ameliorate hypoxia-induced pulmonary hypertension [14]. In view of these considerations, we sought to determine the effect of prolonged hypobaric hypoxia and endothelin-1 receptor blockade on urinary excretion of NOx as a rough estimate of renal and systemic NO production. We found a sharp, early reduction in urinary NOx excretion followed by a gradual rise towards baseline values during the hypoxic period. The reduction in NO production in the hypoxic animals preceded the rise in arterial blood pressure. L-arginine supplementation mitigated the rise in arterial blood pressure and the fall in urinary NOx excretion in the hypoxic animals. This observation suggests that depressed NO production may play an important role in the pathogenesis of hypoxia-induced hypertension. The reduction in NO production with hypoxic hypoxia in rats employed here is consistent with the reported effect of hypoxia in cultured endothelial cells in vitro [5].

It is of note that the peak plasma endothelin-1 and the trough urinary NOx excretion preceded the rise in arterial blood pressure. In fact, the onset of hypertension coincided with the gradual improvements in plasma endothelin-1 and urinary NOx excretion. The available data do not allow a definitive explanation for the temporal discordance between the onset of systemic hypertension and the peak changes in endothelin-1 and NO production. However, it is tempting to speculate that acute pulmonary hypertension and myocardial depression associated with the rapid fall in oxygen tension may have masked the potential rise in systemic blood pressure expected to occur with the fall in NO and the rise in ET-1 production. It can be further speculated that amelioration of cardiopulmonary dysfunction with physiological adaptations may have been responsible for the delayed expression of systemic hypertension with continued hypoxia.

Interestingly, both plasma endothelin and urinary NOx values were normal during the chronic sustained phase of hypertension in this model. This observation suggests that, while the alterations of endothelin and NO productions may be involved in the induction of hypertension, they are not necessary for the maintenance of hypertension in this model. Accordingly, it appears that a combination of extended hypobaric hypoxia with the resultant transient disorders of endothelin and NO production among other factors is sufficient to induce sustained systemic hypertension in this model. Our data suggest that this phenomenon can be interrupted by augmentation of L-arginine: NO system but not by inhibition of ET-A/B receptors.

It is of interest that the magnitude and duration of decline in urinary NOx excretion was amplified by ET-A/B receptor blockade. This phenomenon can be readily explained by the loss of the stimulatory action of endothelin, through ET-B receptor, on NO and prostacyclin production [15–17]. The reduction in NO and prostacyclin production with ET-A/B blockade may account for the lack of discernible improvement of hypertension in this setting. This is because the vasodilatory action of ET-1 antagonist may have been offset by the concurrent reduction in prostacyclin and NO-mediated vasodilation.

In contrast to the present model of chronic hypobaric hypoxia, which is marked by systemic hypertension, most reported models of acute and chronic hypoxia-induced pulmonary hypertension have normal systemic blood pressures [8, 18]. The reason for the disparity in systemic blood pressure between these models is not entirely clear. However, several factors such as duration, severity and type of hypoxia (that is, normobaric vs. hypobaric) may account for this disparity. For instance, the short duration of studies of acute hypoxia precludes detection of systemic hypertension which as shown here occurs a few days after the onset of hypoxia. In addition, the severity of hypoxia is generally greater in the common models of chronic hypoxia-induced pulmonary hypertension and core pulmonale. The reduction in cardiac output due to severe pulmonary hypertension and core pulmonale can clearly offset the effect of elevated systemic vascular resistance and thereby obscure a potential systemic hypertension. Moreover, while uncertain, the normobaric or hypobaric nature of hypoxia may be a determining factor as well. Further studies are required to address these possibilities.

In conclusion, prolonged hypobaric hypoxia led to severe systemic hypertension in genetically normotensive Sprague-Dawley rats confirming our previous observations [1]. This was associated with a transient rise in plasma endothelin and a marked reduction in urinary excretion of NO metabolites. Continuous administration of ET-A/B receptor antagonist failed to improve the associated hypertension. However, L-arginine supplementation prevented the rise in blood pressure and mitigated the fall in urinary NOx excretion in this model. These findings point to the possible role of depressed renal and systemic NO production in the pathogenesis of arterial hypertension in this model.

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