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The Threshold of the Intermittent Hypoxic Exposure Period to Elicit Polycythemia in Rats

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It is not yet clear whether there is an intermittent hypoxia (IHx) threshold to elicit polycythemia and blood pressure elevation, and whether blood hemoglobin concentration ([Hb]) increases with an increase in the hypoxic exposure period. We have previously shown that repetitive exposure to 10% O_2 for 60 min/day for up to 5 weeks does not produce polycythemia. In the present study, we evaluated the effect of IHx of 10% O_2, 120 min/day for 1, 2, 3 and 4 weeks on [Hb], arterial blood pressure, heart rate and arterial blood gases in the rat. IHx of 10% O_2, 120 min/day induced polycythemia at 1 week and produced a time-dependent increase in [Hb] from 0 week to 4 weeks. Arterial blood pressure significantly increased during IHx exposure for 4 weeks probably due to a combination of an increased sympathetic activity as well as increased blood viscosity. The IHx threshold for polycythemia might exist between 60 min/day and 120 min/day in this level of hypoxia.

Key words: polycythemia, intermittent hypoxia, splenic contraction

INTRODUCTION

Conditions such as obstructive sleep apnea syndrome (OSAS) are characterized by intermittent hypoxia. Arterial O_2 pressure (Pao_2) is reduced, but its reduction and duration varies considerably depending on the severity of the disease. In patients with severe OSAS, polycythemia and hypertension were observed. We have previously shown that repetitive exposure to 10% O_2 for 60 min/day for up to 5 weeks, results in a reversible increase in hemoglobin concentration ([Hb]) only under hypoxic condition due to splenic contraction [6, 7] but does not produce polycythemia and hypertension. It is not yet clear whether there is an intermittent hypoxia (IHx) threshold to elicit polycythemia and blood pressure elevation, and whether [Hb] increases with an increase in the hypoxic exposure period.

The purpose of the present experiments was to evaluate the effect of IHx of 10% O_2, 120 min/day for 1, 2, 3 and 4 weeks on [Hb], arterial blood pressure, heart rate and arterial blood gases.

METHODS

Production of intermittent hypoxia

Male Sprague-Dawley rats were housed in environmental Plexiglas chamber (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% O_2 in N_2 and back to compressed air at a rate of 30 L/min. The O_2 concentration in the chamber was monitored by an O_2 analyzer (Beckman MO-11). The rats assigned to the IHx groups were exposed to 10% O_2 for the desired exposure time in the chamber (see below). For the normoxic controls (NxC), the chamber was continuously

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flushed with compressed air. Standard rat chow and water were provided ad libitum. The experimental protocol and all procedures had been reviewed by the Ethics Committee for Animal Experiments of our university.

Experimental protocol

Thirty-five male Sprague-Dawley rats were divided into two groups – a NxC group (0-W) (n=7, weight 358±7 (SE)g) and an IHx group (n=28, weight 364±3 (SE)g). Within the IHx group, the rats were divided into four subgroups, depending on the length (weeks) of hypoxic exposure – a 1-week (1-W), a 2-week (2-W), a 3-week (3-W), and a 4-week (4-W) IHx groups, 7 rats in each. In each IHx group, the rats were exposed to 10% O_2, 120 min/day (1:00-3:00 PM) in the IHx chamber described above.

One day after the end of the desired exposure length to IHx, 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 [8] and allowed to recover fully from anesthesia for 3 hours. The box containing the rat was placed in the environmental chamber described above, through which room air was circulated. Arterial blood pressure and heart rate were monitored continuously, using a pressure transducer (Statham P23Gb) and a chart recorder (Nihon Koden, Japan). When stable arterial blood pressure and heart rate were attained, arterial blood samples were obtained.

Arterial blood gases were analyzed by means of a pH/blood-gas analyzer (Instrumentation Laboratory Model 1304). [Hb] was measured with a Radiometer OSM3 hemoximeter. At the end of the experiment, the rat was anesthetized with halothane and killed by an overdose of pentobarbital sodium.

Statistics

All results are means ± SE. Comparisons of the data among groups were carried out by the following procedure. First, the Kruskal-Wallis rank test was used to determine whether a difference among the data was detected. If a difference was detected, the Mann-Whitney U-test was used to compare the data. A P value less than 0.05 was considered to indicate statistically significant differences.

RESULTS

Figure 1 shows relationships between the IHx exposure period and [Hb]. There was a gradual increase in [Hb] from 0 week (NxC) to 4 weeks. In the 1-W IHx group, [Hb] was significantly higher than the control value. This indicates that polycythemia can be induced by IHx of 10% O_2, 120 min/day, even for 1 week.

Figure 2 shows changes in heart rate and arterial blood pressure. In the 4-W IHx group, arterial blood pressure was significantly higher than the control value (0-W, NxC). Heart rate was not different among all experimental groups.

Figure 3 shows changes in arterial blood gas data determined under normoxia. No significant difference was observed among the groups. This indicates that IHx does not produce changes in blood-gas response in these animals.

DISCUSSION

The results of the present study indicate that a significant increase in [Hb], that is polycythemia, is induced by IHx of 10% O_2, 120 min/day for 1 week, and that IHx produces a time-dependent increase in [Hb] from NxC to 4 weeks. Arterial blood pressure significantly increases during IHx exposure for 4 weeks.

The fact that repetitive exposure to 10% O_2 for 60 min/day for up to 5 weeks does not produce polycythemia [6, 7] suggests that the intermittent hypoxia threshold for polycythemia might exist between 60 min/day and 120 min/day in this level of hypoxia. Clinical conditions associated with IHx such as OSAS, often present permanent polycythemia. In these cases, the number of daily hypoxic episodes, and the total duration of hypoxia might reach the threshold to elicit permanent polycythemia. In previous studies, a relatively long period (days or weeks) of IHx has been shown to produce polycythemia in animals [10, 11]. It has been reported that rats exposed to IHx of 10% O_2, 60min/day for 4-6 weeks showed a significant increase in [Hb] [11]. However, in this previous study, the abdomen was opened

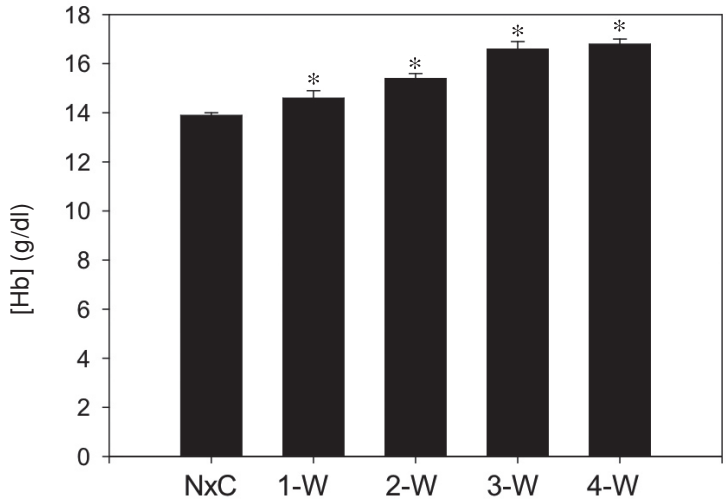

Fig. 1 [Hb] in the NxC (0 week) and IHx group. *Significantly different from the control value.

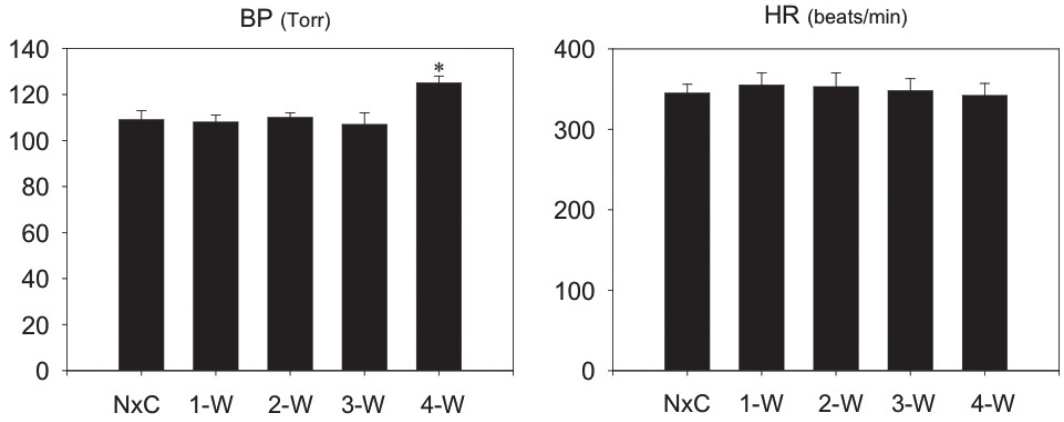

Fig. 2 Arterial blood pressure and heart rate in the NxC and IHx group. *Significantly different from the control value.

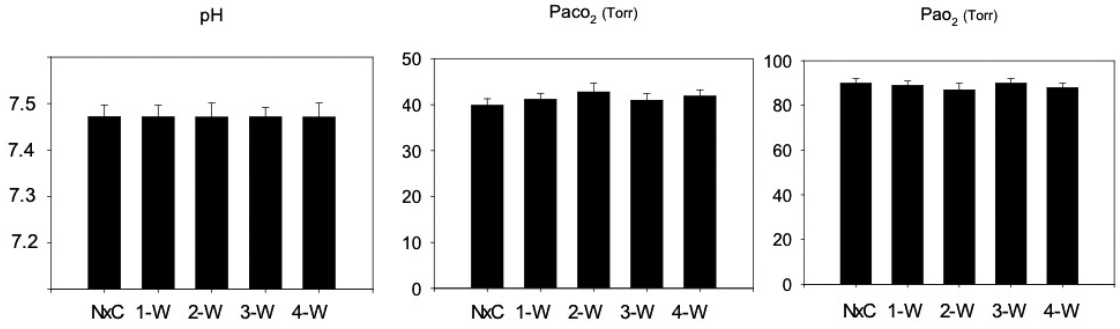

Fig. 3 Arterial blood gas data in the NxC and IHx group. There was no significant difference between the NxC group and the IHx group in arterial blood gases.

under anesthesia and the abdominal aorta was cut to obtain arterial blood samples. Transient elevations in [Hb] are observed in conditions such as circulatory shock where the mechanism involves sympathetic-mediated splenic contraction. The source of the elevated [Hb] might be the spleen since the role of the spleen in storage and release of red blood cells has been described extensively [8]. The difference of the results between the previous and the present study might be due to the experimental settings. In addition, a time-dependent increase in [Hb] was not demonstrated in the previous study, which may support the notion that the elevated [Hb] is produced by the splenic contraction in these animals. To our knowledge, the results of the present study demonstrated the shortest period to elicit permanent polycythemia in the rat in this level of IHx.

Arterial blood pressure increased significantly during IHx exposure for 4 weeks. It has been reported that repetitive episodic hypoxia causes elevations of systemic blood pressure in rats [3]. The cause of hypertension is probably a combination of an increased sympathetic activity during IHx as well as increased blood viscosity derived from polycythemia. Gonzalez *et al*. showed that the increased blood viscosity due to the high hematocrit of prolonged hypoxia is only partially responsible for the systemic hypertension [4]. In the present study, hypertension occurred after 4 weeks of the IHx exposure, although polycythemia was induced at 1 week of IHx. An increased sympathetic activity during IHx may play a more important role on the systemic hypertension rather than polycythemia [4, 5, 9, 12].

The mechanism mediating polycythemia during IHx was not determined in the present study. Cahan *et al*. examined the relationship between the duration of hypoxic exposure and serum erythropoietin (EPO) production in the rat [1]. They demonstrated that one hour of hypobaric hypoxic exposure (0.5 atm, equivalent to 10% O_2) resulted in increased EPO levels 1 hour after termination of hypoxia and further increased levels 2 hours after termination of hypoxia. Eckardt *et al*. found that EPO was significantly elevated 84 min after termination of hypoxic exposure in 4400m for 5.5 hours in humans [2]. From these observations, it would be possible that increased EPO production is induced by IHx of 10% O_2, 120 min/day, and results in polycythemia in the rat.

In summary, we evaluated the effect of IHx of 10% O_2, 120 min/day for 1, 2, 3 and 4 weeks on [Hb], arterial blood pressure, heart rate and arterial blood gases in the rat. IHx of 10% O_2, 120 min/day for 1 week, induces polycythemia and produces a time-dependent increase in [Hb] from NxC to 4 weeks. Arterial blood pressure significantly increases during IHx exposure for 4 weeks probably due to a combination of an increased sympathetic activity as well as increased blood viscosity. The IHx threshold for polycythemia might exist between 60 min/day and 120 min/day in this level of hypoxia.

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