Polycythemia and Changes in Erythropoietin Concentration in Rats Exposed to Intermittent Hypoxia

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Abstract It is not clear whether blood hemoglobin concentration ([Hb]) increases with an increase in the exposure period of intermittent hypoxia (IHx) and reaches a constant level. Furthermore, it is not known whether plasma erythropoietin concentration ([EPO]) also increases with an increase in the exposure period. Using a rat model, first, we evaluated changes in [EPO] every hour after single exposure of 10% O_2 for 120 min in order to determine a peak level of [EPO]. Second, we evaluated the effect of IHx of 10% O_2, 120 min/day for 0 (control), 1, 2, 3, 4, 6 and 8 weeks on [Hb], arterial blood pressure (BP), heart rate (HR), arterial blood gases (ABGs) and [EPO]. [EPO] increased after cessation of the single hypoxic exposure, reached a peak at 1 h, and decreased gradually to the control levels within 18 h. IHx of 10% O_2, 120 min/day, produced a time-dependent increase in [Hb], and [Hb] reached a constant level after the exposure for 6 weeks. BP increased after the exposure for 4 weeks and remained elevated. There was no significant difference in HR and ABGs. [EPO] increased significantly and remained elevated at the same level for 1–3 weeks, however, the peak level of [EPO] declined markedly after [Hb] reached a constant level.

1 Introduction

Conditions such as obstructive sleep apnea syndrome (OSAS) are characterized by intermittent hypoxia (IHx). Arterial O_2 pressure (Pao_2) is reduced, but its reduction and duration varies considerably depending on the severity of the disease. Accordingly, not all patients develop polycythemia. Using a rat model, we have previously shown that there is a threshold to elicit polycythemia and

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that repetitive exposure to 10% O_2 for 120 min/day produces polycythemia [1]. However, it is not clear whether blood hemoglobin concentration ([Hb]) increases with an increase in the hypoxic exposure period and reaches a constant level during IHx. Furthermore, it is not known whether plasma erythropoietin concentration ([EPO]) also increases with an increase in the exposure period of IHx. In the present study, we evaluated effects of IHx of 10% O_2, 120 min/day on [Hb] and [EPO] in the rat.

2 Methods

2.1 Production of Intermittent Hypoxia

Male Sprague-Dawley rats were housed in an 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 120 min/day for the desired exposure period in the chamber (see below). The experimental protocol had been reviewed by the Ethics Committee for Animal Experiments of our university.

2.2 Experimental Protocol and Statistics

First, we evaluated changes in [EPO] every hour after single exposure of 10% O_2 for 120 min in order to determine a peak level of [EPO]. Nine groups of male Sprague-Dawley (S-D) rats, 5 rats in each (weight 335 ± 4 g), were studied for the 0, 1, 2, 3, 4, 5, 6, 8 and 18 h after cessation of the hypoxic exposure. A PE-50 catheter was inserted 10–20 mm into the middle caudal artery under halothane anesthesia for withdrawal of blood samples. [EPO] was determined by radioimmunoassay (RIA).

Second, additional male S-D rats were divided into the following 7 experimental groups depending on the length (weeks) of hypoxic exposure – a 0-week (0-W, normoxic controls), a 1-week (1-W), a 2-week (2-W), a 3-week (3-W), a 4-week (4-W), a 6-week (6-W), and a 8-week (8-W) IHx groups, 6 rats in each (weight 347 ± 3 g). In each IHx group, the rats were exposed to 10% O_2, 120 min/day (1:00–3:00 P.M.) in the IHx chamber described above.

At 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 blood pressure (BP) and heart rate (HR) and for withdrawal of blood samples. After completion of surgery, the rat was transferred to an

accommodation box described before [2] and allowed to recover fully from anesthesia and surgery for 3 h. BP and HR were monitored continuously, using a pressure transducer (Statham P23Gb) and a chart recorder. When stable BP and HR were attained, arterial blood samples were obtained under normoxia. Arterial blood gases (ABGs) were analyzed by means of a pH/blood-gas analyzer (Instrumentation Laboratory Model 1304). [Hb] was measured with a Radiometer OSM3 hemoximeter. [EPO] was determined by RIA. At the end of the experiment, the rat was anesthetized with halothane and killed by an overdose of pentobarbital sodium.

All results are means ± SE. Comparisons of the data among the different length (weeks) of hypoxic exposure 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.

3 Results

Figure 1 shows changes in [Hb] in the IHx group. IHx of 10% O_2, 120 min/day, induced polycythemia in 1 week and produced a time-dependent increase in [Hb] from 13.7 ± 0.1 (0 week) to 17.4 ± 0.4 g/dl (6 weeks). There was no significant difference in [Hb] between 6 and 8 weeks, indicating that [Hb] reached a constant level after the exposure for 6 weeks. Fig. 2 shows changes in [EPO] after single exposure of 10% O_2 for 120 min. [EPO] increased markedly after cessation of the single hypoxic exposure, reached a peak at 1 h, and decreased gradually to the control levels within 18 h. Figure 3 shows changes in [EPO] in the IHx group. [EPO] increased significantly and remained elevated at almost the same level for 1–3 weeks (107.3 ± 10.0 ∼ 109.5 ± 9.4 mU/ml), but declined to 63.2 ± 3.5 (6 weeks) and 44.9 ± 5.4 mU/ml (8 weeks) even though

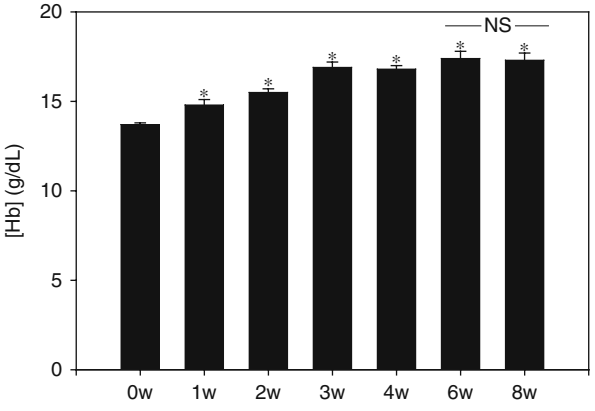

Fig. 1 Changes in [Hb] in the IHx group (*$P < 0.05$)

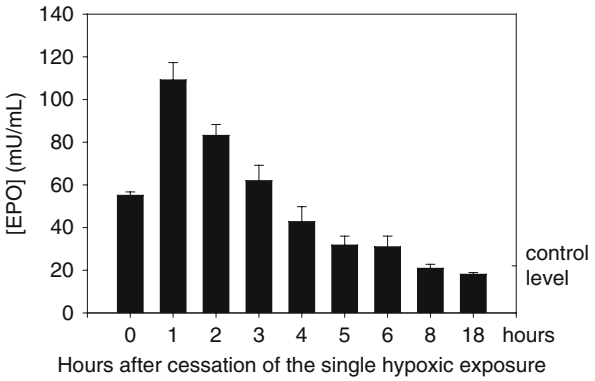

Fig. 2 Changes in [EPO] after single exposure of 10% O_2 in N_2 for 120 min

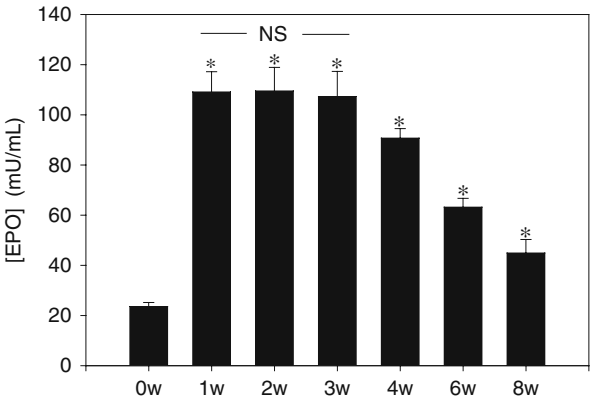

Fig. 3 Changes in [EPO] in the IHx group ($*P < 0.05$)

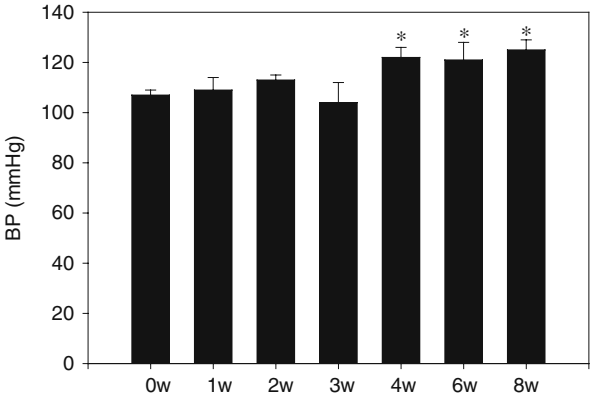

Fig. 4 Changes in BP in the IHx group ($*P < 0.05$)

the IHx exposure was continued. As shown in Fig. 4, BP increased significantly after the exposure for 4 weeks and remained elevated. There was no significant difference in HR and ABGs among all experimental groups.

4 Discussion

The results of the present study indicate that [Hb] increases with an increase in the hypoxic exposure period and reaches a constant level depending on the severity of hypoxia. It has been reported that repetitive exposure to 10% O_2 for 60 min/day for up to 5 weeks does not produce polycythemia [3, 4]. These results suggest that the intermittent hypoxia threshold for polycythemia might exist between 60 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. The magnitude of the increase in [Hb] may depend on the severity of the disease.

The IHx exposure is associated with a significant increase in [EPO]. [EPO] reaches a peak at 1 h after the daily hypoxic exposure, and decreases gradually to the control levels within 18 h. Cahan et al. examined the relationship between the duration of hypoxic exposure and EPO production in the rat [5]. They demonstrated that 1 h of hypobaric hypoxic exposure (0.5 atm, equivalent to 10% O_2) resulted in increased EPO levels 1 h after termination of hypoxia. Eckardt et al. found that [EPO] was significantly elevated 84 min after termination of hypoxic exposure in 4400 m for 5.5 h in humans [6]. Recently, it has been reported that EPO release in humans is increased as a result of 120 min of acute normobaric hypoxia, equivalent to 3100 m [7]. The results of the present study are in good agreement with these observations, although the duration and severity of hypoxia are variable between experiments. It has been also reported that mRNA for EPO is increased in rat kidneys after 1 h of acute hypoxia [8].

The peak level of [EPO] is markedly reduced after [Hb] reaches a constant level even though the IHx exposure is continued. The results suggest a marked reduction in EPO production in kidneys despite continued IHx. Although there might be a negative feedback control in EPO formation, mechanisms mediating the decline of [EPO] could not be determined from the present study.

An increase in [Hb] results in hypertension. The cause of hypertension is probably a combination of an increased sympathetic activity during IHx as well as increased blood viscosity derived from polycythemia. It has been reported that repetitive episodic hypoxia causes elevations of systemic blood pressure in rats [9]. It has been also demonstrated that the increased blood viscosity due to polycythemia is only partially responsible for the systemic hypertension [10]. In the present study, hypertension occurred after 4 weeks of IHx, although a significant increase in [Hb] was induced at 1 week of IHx. An increased sympathetic activity during IHx may play an important role on the systemic

hypertension. In summary, the results of the present study indicate that [Hb] increases with an increase in IHx and reaches a constant level. IHx is associated with an increase in [EPO]. However, the peak level of [EPO] is markedly reduced after [Hb] reaches a constant level despite continued IHx.

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