HEMATOLOGICAL, CARDIOVASCULAR AND RESPIRATORY PHYSIOLOGY AND PHYSIOPATHOLOGY

INTERMITTENT EXPOSURE TO HYPOBARIA OVERCOMES THE SUPPRESSING EFFECT OF TRANSFUSION POLYCYTHEMIA ON ERYTHROPOIETIN SECRETION

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RESUMEN: La exposición intermitente a hipoxia rebasa el efecto superior de la policitemia de transfusión sobre la secreción de eritropoyetina. Hemos comunicado previamente que la policitemia post-transfusional (TP) en ratones suprime la secreción de eritropoyetina (EPO) en respuesta a la exposición a hipobaria cuando el nivel de la policitemia, evaluada a partir del valor hematocrito, es lo suficientemente alto. Este efecto, sin embargo, no es aparente en ratones con policitemia inducida por exposición crónica a hipoxia hipobárica (PH), en los que la producción de EPO puede ser tan elevada como la observada en ratones normocitémicos. Como PH fue inducida mediante exposición intermitente a hipobaria, el presente estudio fue realizado con el objeto de explorar el efecto de la exposición diaria sobre la secreción de EPO en ratones y ratas TP, en los que la policitemia fue inducida mediante dos inyecciones consecutivas de eritrocitos homólogos. Los animales fueron expuestos a hipobaria en una cámara de altura simulada en forma continua o intermitente (16h/d). Se obtuvieron muestras de sangre a distintos tiempos de exposición para la determinación de la concentración de EPO inmunoreactiva (piEPO) mediante RLA. Los valores de piEPO fueron considerados como representativos de la tasa de producción de EPO. TP anuló el incremento de piEPO que se observa durante la exposición a hipobaria cuando fue continua o durante las primeras 34 exposiciones intermitentes. El efecto supresor de TP fue atenuado durante las exposiciones siguientes pese al mantenimiento del estado policitémico. Estos hallazgos sugieren que el mecanismo que controla la expresión del gene para EPO podría ser condicionado por la exposición intermitente a hipobaria de manera tal que se volvería insensible al efecto supresor de la policitemia sobre la producción de EPO oxígeno-dependiente.

Palabras Claves : Eritropoyetina, Hipoxia, Hipobaria, Policitemia.

RÉSUMÉ: L'exposition intermittente à l'hypobarie l'emporte sur l'effet suppresseur de la polyglobulie de transfusion sur la sécrétion d'érythropoïétine.

Nous avons préalablement montré que chez les souris, la polyglobulie de transfusion (TP) supprime la sécrétion d'érythropoïétine (EPO) en réponse à l'exposition à l'hypobarie, quand la valeur de l'hématocrite est suffisamment élevée. Cet effet n'est cependant pas observé chez les souris dont la polyglobulie a été induite par hypoxie (HP). Dans ces conditions, la sécrétion d'EPO peut être aussi importante que celle observée chez les souris normoglobuliques. La HP ayant été induite par exposition intermittente à l'air hypoxique, la présente étude a été réalisée dans le but d'explorer l'effet des expositions journalières à l'hypobarie sur la sécrétion d'EPO chez les souris et les rats à TP. Cette polyglobulie a été induite au moyen de deux injections consécutives d' unités homologues de globules rouges. Les animaux ont été exposés de façon continue ou intermittente (16h/j) à de l'air hypoxique dans une chambre de décompression. A partir du sang prélevé par intervalles on a mesuré la concentration plasmatique d'EPO immunoréactive (piEPO) par RIA. Les valeurs d'EPO ont été considérées comme taux de production d'EPO. La TP a inhibé la production d'EPO liée à l'hypoxie pendant l'exposition continue, et pendant les 3-4 premières expositions intermittentes. L'effet suppresseur de la TP sur la production d'EPO a diminué au cours des expositions intermittentes qui ont suivi. Ces résultats suggèrent que le mécanisme contrôlant la transcription du gène de l'EPO peut être conditionné par l'exposition intermittente à l'hypobarie, de telle sorte qu'il le rend insensible à l'effet suppresseur de la polyglobulie dans la production hormonale.

Mots-clés : Erythropoïétine, Hypoxie, Hypobarie, Polyglobulie.

SUMMARY: We have previously shown that transfusion polycythemia (TP) in mice suppresses erythropoietin (EPO) secretion in response to exposure to hypobaria when the hematocrit value is high enough. This effect, however, is not observed in mice with hypoxia-induced polycythemia (HP). In this condition, EPO secretion can be as high as that observed in normocythernic mice. Since HP was induced by intermittent exposure to hypobaric air, the present study was undertaken to explore the effect of daily exposures to hypobaria on EPO secretion in TP mice and rats, in which polycythemia was induced by two consecutive injections of packed homologous red cells. Animals were exposed continuously or intermittently (16 h/d) to hypobaric air in a decompression chamber. Blood was obtained at intervals and plasma immunoreactive EPO concentration (piEPO) determined by RLA. EPO values were taken as EPO production rates. TP blunted hypoxia-dependent EPO production during both continuous and the 3-4 first intermittent exposures. The suppressing effect of TP on EPO

production decreased during the next intermittent exposures. These findings suggest that the mechanism controlling EPO gene transcription could be conditioned by intermittent exposure to hypobaria in such a way that makes it insensitive to the suppressing effect of polycythemia on hormone production.

KEY WORDS: Erythropoietin, Hypoxia, Hypobaria, Polycythemia

INTRODUCTION

It has been repeatedly demonstrated (1-3) that erythropoietin (EPO) production in response to acute exposure to hypobaria (hypoxemic hypoxia) is very much higher in post-hypoxia (pH) than in hypertransfused (HT) polycythemic mice at equal levels of polycythemia. This finding, which does not fit with the feedback control theory of regulation of EPO production (4), has not been still adequately explained. However, it seems evident that the way in which polycythemia is an induced (chronic exposure to intermittent hypobaria vs. hypertransfusion) play an important role.

PH mice are usually made polycythemic by exposing them to about 456 hPA for 16-18 h/d for 2-3 weeks with return to sea level conditions for 6-8 h/d. This intermittent regimen of exposure to hypoxia determines that the EPO-synthesis machinery (EPO gene expression) and/or the regulatory mechanisms that controls it is turned on and off every day during a 2-3 week-period. This type of peak stimulation could be responsible, at least partially, for the unexpected high EPO production seen in PH than in HT mice during hypoxemic hypoxia.

Transfusion-polycythemia inhibits EPO production in response to acute exposure to hypobaria in a hematocrit-related fashion (1). The present investigation was thus designed to explore EPO production in response to continuous or discontinuous exposure to hypobaria in TP animals in an attempt to clarify the role of intermittent hypoxemic hypoxia on the genesis of the EPO-hypersecretory state seen in the PH mouse in several experimental conditions.

MATERIALS AND METHODS

Adult female mice and wistar rats were used in the experiments. Animals were made polycythemic by injecting them with 0.8 ml (mice) or 2.5 ml/100 g b. wt (rats) of a homologous red cell suspension (Hct = 0.80) on two consecutive days. Exposure to hypobaria was always started on the 4th day after the second transfusion. At this time, piEPO was very low and red cell production has almost disappeared. Hypoxemic hypoxia was induced by placing animals into a simulated altitude chamber, either continuously or intermittently (16 h/day). The pressure inside the chamber was maintained at 456 hPA. EPO production was estimated by determination of piEPO by a sensitive RIA (5). Results from animals with hematocrits below 0.58 were not considered. Results are presented as the arithmetic mean ± SEM. They were analyzed by one-way ANOVA and Newman-Keuls'test.

RESULTS AND DISCUSSION

No evidence exists that the clearance rate of EPO is subjected to physiologic regulation and, therefore, it can be assurned that piEPO directly reflects the EPO secretion rate. In addition, EPO is not stored in endocrine EPO-synthesizing cells and thus the rate of secretion is directly dependent on the rate of synthesis. Our results, expressed in terms of piEPO can thus be taken as representative of the EPO secretion rate.

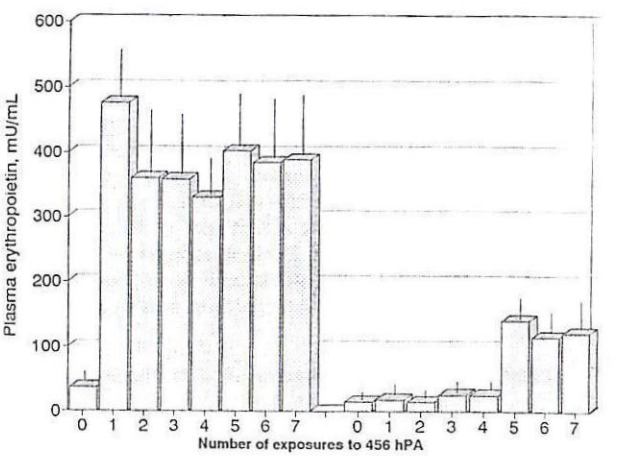

Figure 1. Effect of intermittent exposure to hypobaria on plasma erythropoietin concentration in both normocythemic and transfused polycythemic mice. Groups of 8 normocythemic and polycythemic mice were exposed to 456 hPA in a hypobaric chamber. Each exposure lasted 16 h. Number of exposures are shown in the abscissa. Bars and lines on top of bars indicate mean ± SEM. Normocythemic mice values are shown on the left side of the figure. Values for polycythemic mice are shown on the right side.

To explore EPO production in both normocythemic (N) and transfused-polycythermic (HT) mice in response to intermittent exposure to hypobaria, N and HT mice were exposed to 456 hPA for 16 h/d for 7 days. Each consecutive day of the period, 8 mice from each group were selected at random,

anesthetized with ether, and exsanguinated by heart puncture. Hematocrits and piEPO were measured. Figure 1 shows piEPO in both groups of experimental mice as a function of the number of exposures to hypobaria. It rose in N mice to a level about 10 times the normal, nonhypoxic level during the first exposure, with a slight, non-significant decrease during the remaining exposures. This pattern of EPO response was opposed to that found in HT mice, in which the corresponding normal, nonhypoxic level of piEPO did not change during the first 4 exposures. Interestingly, piEPO titer rose to a level about 7 times higher than that of control, non-exposed HT mice. This occurred in spite of the persistence of the polycythemic state.

Analysis of the above results indicates that intermittent exposure to hypobaria counteracts the inhibitory effect of transfusion-polycythemia on EPO production when it is applied for enough time. The next experiment, therefore, was designed to know whether continuous exposure to hypobaria induces the same pattern of EPO response in HT animals. If this were really the case, then humans or animals living permanently at high altitude would secrete continuously increasing amounts of EPO with the concomitant increase in the erythrocyte production rate and the volume of the red cell mass. This does not occur in most high altitude inhabitants, in which high altitude polycythemia is, in general, a stationary condition and not a progressive process.

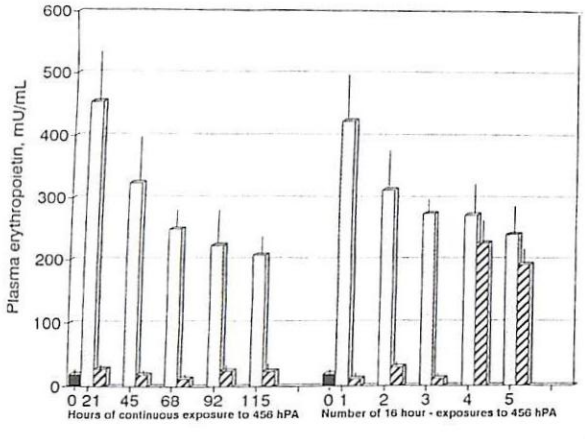

Figure 2. Effect of continuous or intermittent exposure to hypobaria on plasma erythropoietin concentration in both normocythemic and transfused polycythemic rats. Groups of 5 normocythemic and 5 polycythernic rats each were exposed to 456 hPA in a hypobaric chamber. Both types of animals were exposed either continuously (left side of the figure) or intermittently (right side of the figure). Each exposure to intermittent hypobaria lasted 16 h. Bars and lines on top of bars indicate mean ± SEM. Black bars = control, non-exposed rats; dotted bars = normocythemic, exposed rats, dashed bars = polycythemic, exposed rats.

N and HT rats were exposed to 456 hPA for 16 h/d for 5 days (intermittent hypoxia) or continuously for 115 h. Each consecutive day of the period (intermittent exposure group) or after 21, 45, 68, 92 and 115 h of continuous exposure, 5 rats from each group were selected at random and treated as explained above. Figure 2 shows piEPO in N and HT rats as function of either the time of continuous exposure or the number of exposures to hypobaria. N rats showed similar panerns of responses to both types of exposures, with a rapid rise in piEPO followed by a progressive decay with either time or nurnber of exposures. The responses of HT rats differed from those of N rats and could be summarized as follows: l) transfusion-induced polycythemia blunted the increased EPO production which occurred in N rats continuously exposed to hypobaria, and 2) transfusion-induced polycythemia blunted the increased EPO production which occurred in N rats every time they were exposed to hypobaria for 16 h. Blunting was, however, only effective during the first 3 exposures. It did not occur from the 4th exposure on, in spite of the persistence of a high degree of polycythemia in the animals.

The results presented herein confirm previously reported studies that indicate that transfusion-induced polycythemia depresses hypoxia-dependent EPO production (1, ó). These results, however, were obtained from experiments in which polycythemic animals were exposed to short periods of hypoxia (4-48 h) while in the present ones the exposure time was extended to 5-7 days.

Such prolonged exposition to hypobaria revealed that transfusion-induced polycythemia blunted the hypoxia-induced EPO production when the hypoxic stimulus was applied continuously. When it was applied intermittently, the blunting effect was only evident during the 24 exposures, disappearing thereafter.

Unfortunately, we do not have a valid explanation for the observed difference between continuous and intermittent exposure to hypobaria on EPO production in HT animals. EPO is synthesized by endocrine cells through the expression of an EPO gene apparently in response to transcription-regulating factors. The system, that include oxygen sensing by adequate receptors/sensors, intracellular signals, and regulation of genes expression, could be conditioned by intermittent exposure to hypobaria in such a way that makes it insensitive to

the suppressing effect of high cellular oxygen tension on hormone production. It is evident that we need to learn more about the mechanisms by which oxygen is sensed, the transduction of the hypoxic signal and the resulting impact of this signaling process on the regulation of the EPO gene in order to understand the meaning of the results presented.

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