Erythropoietin production in healthy volunteers subjected to controlled hypobaric hypoxia: further evidence against a role for adenosine

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Aims Objective of this study was to investigate whether adenosine modulates renal erythropoietin production.

Methods In the present study erythropoietin production was stimulated by hypobaric hypoxia by subjecting healthy volunteers to a simulated altitude of 4000 m in a low pressure chamber for 5.5 h. During exposure to hypoxia the subjects received i.v. in a randomized, single-blind, cross-over fashion the non-specific adenosine antagonist theophylline, the adenosine reuptake inhibitor dipyridamole and placebo.

Results Contrary to the working hypothesis, theophylline did not decrease and dipyridamole did not further boost erythropoietin concentrations.

Conclusions The results are in agreement with our earlier study using haemorrhage as a controlled physiological stimulus of erythropoietin production, and would question a major role for adenosine as a mediator of renal erythropoietin production.

Keywords: erythropoietin, theophylline, dipyridamole, hypobaric hypoxia, plasma renin activity, healthy volunteers

Introduction

There is some evidence from *in vitro* and animal experiments for the assumption that adenosine (ADO) might modulate renal erythropoietin (EPO) production [1]. According to this hypothesis, increasing renal ADO levels (e.g. under hypoxia [2]) would lead to an increased production of EPO and *vice versa*. Also, clinical data are available which may point towards such a role for ADO in humans. Bakris *et al.* [3] have shown that theophylline treatment in patients with post-transplant erythrocytosis (PTE) may reduce elevated EPO levels as well as haematocrit. However, this finding has not been unequivocally reproduced [4] and PTE is not considered a condition where the pharmacological control of EPO production can appropriately be assessed [5].

Our own animal experiments which assessed the ADO hypothesis systematically using selective adenosinergic compounds (not available for human use) in a model of functional hypoxia (by CO exposure) were unable to support this hypothesis [6]. Also, in a previous study we tried to assess this question in healthy volunteers who were subjected to a defined physiological stimulus of EPO production, namely haemorrhage. In this experiment the non-specific ADO antagonist theophylline did not inhibit the increase of EPO levels as predicted from the hypothesis [7]. The ADO reuptake inhibitor dipyridamole increases extracellular ADO levels. From theory and animal experiments it is expected to augment renal ADO effects [8]. In our previous experiment dipyridamole did not boost EPO levels [7] despite dipyridamole plasma levels which had been shown to inhibit ADO uptake in human platelets [9]. However, haemorrhage may not exert a signal to EPO

production that is fully comparable with *hypoxia*, e.g. as in some animal experiments [2]. Therefore, in a similar experimental design using theophylline and dipyridamole treatment, EPO production was stimulated by hypobaric hypoxia by subjecting healthy volunteers to a simulated altitude of 4000 m in a low pressure chamber.

Methods

Subjects

Seven healthy male non-smoking volunteers (mean age 25.1 years, range 20–31 years, mean body weight 75.3 kg, range 68–86 kg) participated in the trial. The conditions for participation were identical with the earlier study [7]. The study was approved by the Ethics Committee of the University of Göttingen and the Ethics Committee of the Board of Physicians Nordrhein, Düsseldorf, Germany. Written, informed consent was obtained from each participant.

Protocol

The volunteers were subjected to hypoxia in a hypobaric chamber according to the protocol of Eckardt *et al.* [8]. In brief, the atmospheric pressure was reduced to 460 Torr (4000 m above sea level) with an interruption for 15 min at 600 Torr during the ascent (2000 m above sea level). The subjects remained in the chamber for a total of 5.5 h. Thirty min before the subjects were exposed to hypoxia i.v. drug medication was started and administered for 6 h by an infusion pump (Perfusor®, Braun Melsungen AG, Germany) until the end of the stay in the chamber. The subjects received in a randomized, cross-over, single-blind design placebo (0.9 g dl⁻¹ NaCl), or theophylline (5 mg kg⁻¹

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Figure 1 Time course of erythropoietin serum concentrations expressed as percent of baseline values (mean $\pm$ s.e.mean). The period of exposure towards hypobaric hypoxia is indicated. ● theophylline; ■ dipyridamole; △ placebo.

loading dose over 20 min, followed by a continuous infusion of $0.5 \text{ mg kg}^{-1} \text{ h}^{-1}$ over the remaining 340 min; Euphyllin 200®), or dipyridamole ($0.21 \text{ mg kg}^{-1} \text{ h}^{-1}$; Persantin®). The treatments were separated by wash out periods of at least 2 weeks. In the chamber the subjects remained in the sitting position. Arterial haemoglobin oxygen saturation (SaO_2) was measured by a pulse oximeter with a fingerclip sensor. Haemoglobin and haematocrit were measured before hypoxia and 2, 6, 24, and 48 h thereafter. Blood pressure and heart rate were recorded before, and 2, 4, 6, 8 and 12 h after the start of hypoxia. Drug concentrations were measured before and after 4 h of the respective drug infusions. Blood samples for EPO concentrations were taken as shown in Figure 1. Plasma renin activity (PRA) was measured before hypoxia as well as 2, 4, 6, 8, and 12 h thereafter.

Analytical methods and statistics

Haemoglobin, haematocrit, dipyridamole and theophylline, PRA and EPO were analysed as described previously [7]. The area under the concentration-time curve (AUC) was calculated by the linear trapezoidal rule. Values are expressed as mean $\pm$ s.e.mean and were analyzed by repeated measures ANOVA followed in the case of a significant finding by *t*-tests with Bonferroni-Holm correction.

Results

Oxygen saturation fell in all groups from 100% to about 85% during hypoxia. Within the 48 h observation period after the start of hypoxia, haemoglobin and haematocrit values were very similar in all groups and unaltered by treatment (data not shown). Four hours after the start of the i.v. drug infusion the mean plasma concentration of theophylline was $13.7 \pm 0.4 \text{ mg l}^{-1}$ and that of dipyridamole $1.5 \pm 0.2 \text{ µg ml}^{-1}$. In all treatment groups mean systolic

blood pressure fell during the first 2 h of hypoxia ($P > 0.05$), mean diastolic blood pressure was about 5 mmHg lower during infusion of theophylline during the first 2 h of hypoxia ($P < 0.05$). Otherwise there were no major changes of blood pressure during the entire experiment (data not shown). Heart rate increased significantly ($P < 0.05$) during hypoxia in all three treatment groups by about 15 beats min^{-1} (data not shown).

$\text{AUC}_{\text{PRA theophylline}} (0, 12\text{h}) (68.7 \pm 8.7 (\text{ng ml}^{-1} \text{ h}^{-1}) \cdot \text{h})$ was statistically significantly larger than $\text{AUC}_{\text{PRA placebo}} (0, 12\text{h}) (26.0 \pm 4.7 (\text{ng ml}^{-1} \text{ h}^{-1}) \cdot \text{h})$; difference of means $42.7 (\text{ng ml}^{-1} \text{ h}^{-1}) \cdot \text{h}$, 95% CI 22.4 to 63 ($\text{ng ml}^{-1} \text{ h}^{-1}) \cdot \text{h}$; $P < 0.001$). $\text{AUC}_{\text{PRA dipyridamole}} (0, 12\text{h})$ was not different from $\text{AUC}_{\text{PRA placebo}} (0, 12\text{h}) (24.5 \pm 3.8 (\text{ng ml}^{-1} \text{ h}^{-1}) \cdot \text{h})$. Baseline EPO levels were 3.5 ± 0.5 (theophylline), 5.4 ± 0.6 (dipyridamole) and $4.4 \pm 0.6 \text{ µu ml}^{-1}$ (placebo). The time course of EPO concentrations is given in Figure 1. There were no statistically significant differences between $\text{AUC}_{\text{EPO}} (0, 48\text{h}) [\text{h} \cdot \% \text{ of baseline}]$ of theophylline-treated (11653 ± 1885) or dipyridamole-treated subjects (7628 ± 641) in comparison with placebo (9711 ± 957).

Discussion

The results concerning the course of blood pressure and heart rate from the placebo group were in accordance with earlier observations using the same hypobaric protocol [10]; the verum treatments did not alter the obviously hypoxia-related changes. Theophylline and dipyridamole concentrations were comparable with those achieved in our first study [7]. Thus, theophylline plasma concentrations were in the range for the treatment of asthma and effective blockade of ADO receptors is assumed [11]. Plasma concentrations of this order of magnitude have been achieved in patients with effective control of PTE [3]. Dipyridamole plasma concentrations were of the order of magnitude where an inhibition of ADO reuptake was demonstrated in platelets of normal subjects after oral administration [9].

The increase of EPO during placebo treatment was similar to that in the study by Eckardt *et al.* [8]. The onset of EPO production was more rapid than after phlebotomy [7]. However, in agreement with this earlier study, there was no inhibition of EPO production by theophylline and no further boost by dipyridamole. In fact, we found—as in our earlier study—an increase of EPO production under theophylline; this increase was not statistically significant. Also, PRA values were of the same order of magnitude as in our previous study [7], confirming that hypoxia did not change the effect of the treatments on PRA. A clear increase during theophylline infusion was demonstrated. PRA is a variable that is modulated by sympathoadrenergic signals [12]. As outlined earlier, theophylline may block presynaptic adenosine receptors thereby increasing sympathetic signal transduction. This led us to conclude that the further increase of EPO levels under theophylline may be caused by an increased sympathetic tone. Another trial has addressed this hypothesis, and a clear stimulating effect was shown for the β_2 -adrenoceptor agonist fenoterol [13]. Taken together, these data are in agreement with those from our experiment using phlebotomy and do not support a role for adenosine in the control of erythropoietin production.

The authors wish to thank Dr M. Heer, DLR Institut für Luft- und Raumfahrtmedizin, for her many helpful contributions during this trial.

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(Received 3 January 1997,
accepted 26 March 1997)