Fenoterol stimulates human erythropoietin production via activation of the renin angiotensin system

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Aims The present study assessed the hypothesis that the β_2 sympathomimetic fenoterol influences the production of erythropoietin (EPO) by activation of the renin angiotensin system (RAS), i.e. angiotensin II.

Methods In an open, parallel, randomized study healthy volunteers received i.v. either placebo (electrolyte solution), fenoterol or fenoterol in combination with an oral dose of the AT_1-receptor antagonist losartan.

Results Compared with placebo treatment $AUC_{EPO}(0,24\,h)$ was significantly increased after fenoterol application by 48% whereas no increase in the group receiving fenoterol and losartan could be detected. The rise of PRA was statistically significant under fenoterol and fenoterol plus lorsartan.

Conclusions Stimulation of EPO production during fenoterol infusion appears to be angiotensin II-mediated. Thus, angiotensin II may be considered as one important physiological modulator of EPO production in humans.

Keywords: erythropoietin, fenoterol, healthy volunteers, losartan, renin angiotensin system

Introduction

Our earlier experiments showed stimulation of erythropoietin (EPO) production in healthy volunteers receiving fenoterol i.v [1]. as well as in pregnant women during tocolysis with fenoterol [2]. Further hints for possible adrenergic control of human EPO production come from our finding that theophylline, which increases sympathetic tone by blocking of presynaptic adenosine receptors [3], is also able to increase EPO production [4, 5].

A common denominator in all studies in healthy volunteers was the increase in plasma renin activity (PRA) [1, 4, 5]. This finding led to the hypothesis that the increase of EPO production may have been mediated via an activation of the renin angiotensin system (RAS). Therefore, we investigated in the present study whether angiotensin II may be responsible for the increase in EPO production during fenoterol infusion by blocking angiotensin II effects using the AT_1 receptor antagonist losartan.

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Methods

Subjects

Thirty-six healthy male nonsmoking volunteers (mean age 25 years, range: 20–30 years; mean body weight 75 kg, range 62–95 kg) gave written informed consent to participate in the study. The conditions for participation were identical with those described for our earlier studies [1, 4, 5]. The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Göttingen.

Protocol

The study was conducted according to an open, parallel, randomized and placebo-controlled design. After inclusion, the subjects were randomly allocated to one of the following treatment groups: group 1 (placebo; physiological electrolyte solution: Sterofundin® 150 ml h^{-1} i.v.), group 2 (fenoterol: Partusisten®, 1.5 $\mu g\,min^{-1}$ i.v.) and group 3 (fenoterol and losartan: Partusisten®, 1.5 $\mu g\,min^{-1}$ i.v. and Lorzaar® 50 mg p.o.).

Fenoterol and placebo were administered for 6 h by an infusion pump (Perfusor®, Braun Melsungen AG, Germany). In group 3 losartan was administered 2 h before the start of the infusion. In order to adjust for volume administration in group 1, volunteers in groups

2 and 3 received 150 ml h^{-1} Sterofundin® i.v. in parallel with the infusion of fenoterol. The volunteers remained in a supine position for 6 h until the end of the infusion period.

Blood pressure and heart rate were recorded before, and 1, 2, 4, 6, 8 and 12 h after start of the infusion. Blood samples for erythropoietin concentrations were taken immediately before (0 h) and 2, 4, 6, 8, 12 and 24 h after start of the infusion. Plasma renin activity (PRA) was measured before the start of the infusion and at 2, 4, 6, 8, and 12 h thereafter. Serum potassium levels were measured before and 4 and 12 h after the start of the infusion.

Analytical methods

Clinical chemistry was carried out in the Department of Clinical Chemistry, University of Göttingen. Erythropoietin in serum was analysed using an ELISA (Kat.-Nr. 500, Medac GmbH, Hamburg, Germany) and plasma renin activity was measured with a [^{125}I] r.i.a. (RENK P2510, Sorin Biomedica, Saluggia, Italy) as in our earlier studies [1, 4, 5].

Calculations

The area under the erythropoietin serum concentration-time curve ($AUC_{(EPO)}$(0,24 h) using erythropoietin concentration data adjusted to percentage of baseline and the area under the PRA–time curve ($AUC_{PRA)}$(0,12 h)) were calculated by the linear trapezoidal rule.

Statistics

Values are expressed as mean ± s.e.mean and were analysed by ANOVA (analysis of variance) followed by post *t*-tests with α adjustment according to Bonferroni-Holm. $P<0.05$ was considered statistically significant. 95% confidence intervals (CI) were calculated for differences of sample means where meaningful.

Results

Blood pressure and heart rate were similar in all treatment groups before intake of the study medication and remained unchanged in the placebo group. In groups 2 and 3 systolic blood pressure was unchanged whereas diastolic blood pressure fell significantly ($P<0.05$) by 10 mmHg during the infusion of fenoterol. Heart rate rose significantly ($P<0.05$) by 25 beats min^{-1} during fenoterol infusion (data not shown).

Basal serum potassium levels were equal in all groups (mmol l^{-1}; placebo: 3.7 ± 0.1, fenoterol: 3.8 ± 0.1, fenoterol + losartan: 3.7 ± 0.1). During infusion, potassium concentrations declined significantly in group 2 fenoterol (2.9 ± 0.1 mmol l^{-1}) and group 3 fenoterol + losartan (2.9 ± 0.1 mmol l^{-1}) compared with group 1 placebo (3.7 ± 0.1 mmol l^{-1}; $P<0.05$). 6 h after the end of infusion potassium concentrations had returned to baseline (group 1 placebo: 3.7 ± 0.1 mmol l^{-1}; group 2 fenoterol: 3.8 ± 0.1 mmol l^{-1}; group 3 fenoterol + losartan: 3.7 ± 0.1 mmol l^{-1}), no differences could be detected.

AUC_{PRA}(0,12 h) in group 2 fenoterol (27.8 ± 5.7 ng ml^{-1} h) was statistically significant larger than AUC_{PRA}(0,12 h) in the group 1 placebo (13.6 ± 1.9 ng ml^{-1} h); difference of means 14.2, 95% CI 1.7 to 26.7; $P<0.05$). AUC_{PRA}(0,12 h) in group 3 fenoterol + losartan (101.2 ± 17.6 ng ml^{-1} h) was four fold higher than in the group 2 fenoterol alone (difference of means 73.4, 95% CI 35.1 to 111.8, $P<0.05$) and also significantly different from placebo (difference of means 42, 95% CI 50.9 to 124; $P<0.05$).

Baseline EPO levels were 5.1 ± 1.0 mU ml^{-1} (placebo), 4.1 ± 0.9 mU ml^{-1} (fenoterol) and 4.2 ± 0.6 mU ml^{-1} (fenoterol + losartan) and were not significantly different. The time course of EPO concentrations is given in Figure 1. Fenoterol infusion increased AUC_{EPO}(0,24 h) by 48% compared with placebo. Statistically significant differences could be detected between the AUC_{EPO}(0,24 h) of fenoterol (4891 ± 449 h·% of baseline) *vs* placebo (3297 ± 281 h·% of baseline; difference of means 1594, 95% CI 495–2693; $P<0.05$) as well as *vs* AUC_{EPO}(0,24 h) of fenoterol + losartan (3315 ± 328 h·% of baseline; difference of means 1576, 95% CI 423 to 2730; $P<0.05$)

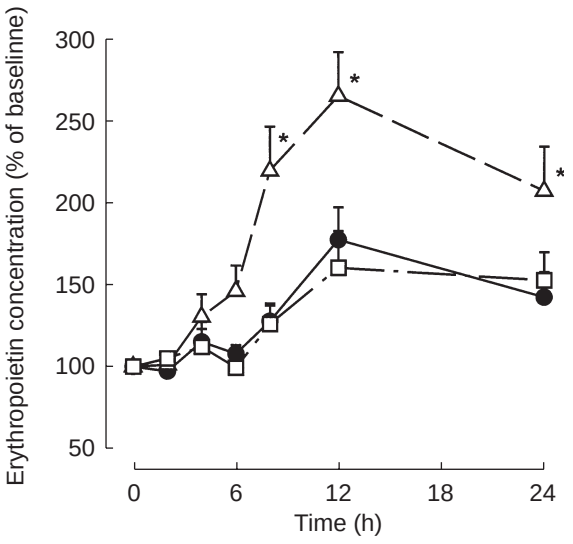

Figure 1 Time course of erythropoietin serum concentrations expressed in percentage of baseline values (mean ± s.e.mean; ★$P<0.05$ *vs* placebo) where ● is placebo, △ is fenoterol and □ is fenoterol + losartan.

whereas no differences could be demonstrated between placebo and the group fenoterol + losartan.

Discussion

There is circumstantial evidence that adrenergic activity can modulate human EPO production. Our previous investigations have shown that the β_2 sympathomimetic fenoterol is able to increase EPO concentrations during tocolysis [2] and after physiological stimuli [1]. We observed a clear increase of plasma renin activity (PRA) [1]. As earlier animal experiments by Gould *et al.* [6] and Fisher *et al.* [7] demonstrated a correlation between components of the RAS and EPO production, we hypothesized that stimulation of EPO production may be mediated via a stimulation of the RAS, with angiotensin II as the final effector. Therefore, we investigated the influence of a blockade of angiotensin II AT_1-receptors by the selective AT_1 antagonist losartan on stimulation of human EPO production by fenoterol.

The infusion rates of fenoterol in this trial were similar to that commonly used for tocolysis in clinical practice. As expected [1, 2] serum potassium levels fell during infusion of fenoterol regardless of additional losartan intake whereas in the control group no changes could be detected. Increase of heart rate and decrease of diastolic blood pressure during fenoterol infusion did reflect the inodilatory response to fenoterol. The cardiac effects may reflect activation of the sympathetic nervous system. This would not allow a firm conclusion that β_2-receptor stimulation directly stimulates renin release. This may also be a β_1-adrenoceptor mediated effect or reflect a combination of β_1- and β_2- mediated effects. Renin release has been attributed to β_1-adrenoceptor [8], to β_2-adrenoceptor-mediated mechanisms [9, 10] or to both subtypes [11]. In the present trial and our previous study, PRA increased significantly during fenoterol infusion indicating stimulation of the RAS. A four fold higher increase of AUC_{PRA} was seen under coadministration of fenoterol with losartan. AT_1 receptor blockade has been demonstrated to antagonize angiotensin-mediated feedback inhibition of renin release in animals thus leading to several fold higher levels of PRA [12].

EPO concentrations in the placebo group increased slightly during the course of the trial. This effect may be explained by either a circadian rhythm of EPO production [13] or by haemodilution during infusion of the electrolyte solution and the blood samples taken. In comparison with the EPO concentrations and $AUC_{EPO}(0,24\ h)$ in the placebo group, EPO increased significantly after infusion of fenoterol. The increase of 48% *vs* placebo was even higher than that demonstrated in our earlier study during fenoterol infusion plus haemorrhage [1]. This demonstrates that haemorrhage may not have been the main factor for the stimulation of EPO in this circumstance.

Concomitant treatment with fenoterol + losartan did not lead to significantly different EPO concentrations or AUC_{EPO} compared with placebo. A previous investigation with a losrtan alone group showed that losartan alone led to a small and not statistically significant decrease of EPO concentrations in healthy volunteers in comparison with placebo treatment [14].

As treatment with the AT_1-receptor antagonist losartan prevented a fenoterol induced increase in EPO production it may be concluded that the stimulation of EPO production during fenoterol infusion is due to stimulation of the RAS with consecutive activation of AT_1-receptors. I.e., sympathetic control over EPO is most likely due to angiotensin II. Thus, angiotensin II appears to be an important modulator of EPO production in humans. Its place within the hierarchy of stimuli for physiological EPO production remains to be established.

Our finding of a link between the RAS and EPO production in humans is further supported by clinical observations of Vlahakos *et al.* [15] who showed in chronic haemodialysis patients that volume depletion by haemodialysis increases PRA followed by an increase of EPO concentration. Blockade of the RAS by captopril administration prior to haemodialysis resulted in an increase of PRA without an increase of EPO levels.

Finally, our findings may be one plausible explanation for the reduction of haematocrit, haemoglobin and EPO concentrations in patients with post transplant erythrocytosis receiving ACE-inhibitors [16, 17] or losartan [e.g. 18]. These reports support the clinical relevance of our data.

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